MECHANISMS OF DOPAMINE RELEASE FROM RAT STRIATUM AND NUCLEUS ACCUMBENS SLICES: THE ROLE OF TRANSPORTERS, RECEPTORS AND MEMBRANE DEPOLARIZATION

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ABSTRACT

The striatum (STR) and nucleus accumbens (NACC) are major terminal fields of dopaminergic neurons originating in the midbrain. In rat brain, most studies of the characteristics of dopamine (DA) neurons have utilized the striatum (STR) because of its greater size. However, the STR and NACC subserve different functions. In this dissertation, the STR and NACC were compared directly with respect to the release of [^{3}H]DA and endogenous DA from superfused slices *in vitro* in response to elevated potassium, excitatory amino acids, serotonin (5-HT), and D-amphetamine. To assist in evaluating the contribution of intrinsic neuronal circuitry on overflow of [^{3}H]DA, the effects of alterations in cation concentration, antagonists selective for various relevant receptors, and an inhibitor of interneuronal activity were tested. The validity of measuring overflow of [^{3}H]DA was tested by measuring overflow of endogenous DA under some of the same conditions.

Under basal conditions, [^{3}H]DA overflow was greater in STR than in NACC. Blockade of DA re-uptake enhanced overflow by 31% in NACC and 55% in STR. Removal of Ca reduced overflow by >50% in both regions. In the NACC, tetrodotoxin (TTX) reduced, and Mg-free buffer increased overflow of [^{3}H]DA.

Release stimulated by elevated potassium (K) had characteristics consistent with exocytotic release. K-stimulated release was strongly reduced (80%) by removal of Ca and enhanced by removal of Mg in both STR and NACC. Elevated K released more [^{3}H]DA in NACC (9.0%) than in STR (4.8%). TTX enhanced (33%) K-stimulated release in STR but reduced (82%) release in NACC. Activation of glutamate or $GABA_A$ receptors was not an important contributor to K-stimulated release. K-stimulated release was not affected by

transporter blockade except for inhibition of release observed in the NACC by GBR 12909.

Release stimulated by L-glutamate had characteristics consistent with exocytotic release. L-Glutamate-stimulated release was strongly reduced (80%) by removal of Ca and enhanced by removal of Mg in both STR and NACC. TTX reduced release in STR but not in NACC. In the presence of Mg, release stimulated by L-glutamate, as well as by the glutamate analogs AMPA and kainate, was reduced by the non-NMDA glutamate antagonist CNQX but not by the NMDA-selective antagonist MK-801.

In the absence of Mg, NMDA-stimulated release from STR and NACC had characteristics consistent with exocytotic release. TTX and MK-801 reduced NMDA-stimulated release. Release was greatly enhanced by blockade of the DA transporter in STR.

Release stimulated by D-amphetamine had characteristics consistent with transporter-mediated release. D-Amphetamine-stimulated release was not strongly affected by TTX or by removal of Ca or Mg. D-Amphetamine released more [^{3}H]DA in STR (21%) than in NACC (14%) and release from both tissues was greatly reduced by blockade of the DA transporter.

Release stimulated by 5-HT also had characteristics consistent with transporter-mediated release. 5-HT-stimulated release was not strongly affected by TTX or by removal of Ca. 5-HT released less [^{3}H]DA in STR (3.8%) than in NACC (5.5%). Antagonists selective for various 5-HT receptor subtypes did not reduce 5-HT-stimulated release. Removal of Mg reduced 5-HT-stimulated release in STR and NACC and release in both tissues was greatly reduced by blockade of the DA transporter.

In summary, regulation of DA release was different in STR and NACC. These differences were mostly quantitative. 5-HT was able to stimulate release

of DA by a transporter-dependent mechanism in both STR and NACC. Factors affecting release of [^{3}H]DA affected release of endogenous DA in a similar manner.

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by

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DEDICATION

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List of Abbreviations

5-CT, 5-carboxyamidotryptamine

5-HT, 5-hydroxytryptamine, serotonin

8-OH-DPAT, 8-hydroxy-2-(di-n-propylamino)tetralin

[^{3}H]DA, tritiated dopamine

[^{3}H]5-HT, tritiated serotonin

AMPA, α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid)

aMpT, α-methyl-para-tyrosine

APV, D-(-)-2-amino-5-phosphonopentanoic acid

ATP, adenosine trisphosphate

Ca, calcium

CCK, cholecystokinin

CNQX, 6-cyano-7-nitroquinoxaline-2,3-dione

CPP, 3-((±)-2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid

DA, dopamine

DOI, 1-(2,5-dimethoxy-4-iodophenyl)-2-amino-propane

DOI, 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane

EGTA, ethylenebis(oxyethylenenitrilo)tetraacetic acid

FLX, fluoxetine

GABA, γ–aminobutyric acid

GAD, glutamate decarboxylase

GBR 12909, 1-(2-(bis(4-fluorophenyl)methoxy)ethyl)-4-(3-phenyl-propyl)piperazine

ICS 205930, endo-8-methyl-8-azabicyclo[3.2.1]oct-3ol indol-3-yl-carboxylate

K, potassium

List of Abbreviations (continued)

MDL 72222, endo-8-methyl-8-azabicyclo[3.2.1]oct-3-ol 3,5-dichlorobenzoate

MFB, medial forebrain bundle

Mg, magnesium

MK-801, (+)-5-methyl-10,11-dihydro-5H-dibenz[a,d]cyclohepten-5,10-imine

n., nucleus

NACC, nucleus accumbens septi

NMDA, N-methyl-D-aspartic acid

NOM, nomifensine

NT, neurotensin

SN, substantia nigra

STR, striatum (caudate/putamen)

TH, tyrosine hydroxylase

TTX, tetrodotoxin

VTA, ventral tegmental area

STATEMENT OF PURPOSE

The goals of this project were to determine if there were differences in the local regulation of dopamine (DA) release in the striatum (STR), a structure largely concerned with motor function, and the nucleus accumbens (NACC), a region critical to several forms of behavioral reinforcement including that induced by drugs of abuse. Both areas are targets of dopaminergic projections from midbrain nuclei and the similarities of the properties of the dopaminergic neurons in the two regions have led many to study only the larger striatal region and generalize those findings to the nucleus accumbens. My approach was to identify, by direct comparison, potential differences in the sensitivity of the two areas to various stimuli and uptake inhibitors, the contribution of interneuronal regulation, and the effect of removal of regulatory cations.

In the background, I summarize anatomical evidence for functional roles of the striatum and nucleus accumbens. Since my preliminary experiments suggested differences in serotonergic regulation of dopamine release between the two regions, I also include a separate section on anatomy and reuptake properties of serotonin neurons, as well as serotonin release in striatum and nucleus accumbens. I then compare what has been reported to date on various release mechanisms in the two areas.

My own work compares the roles of monoamine transporters and receptors as regulators of dopamine release in striatum and nucleus accumbens. In the discussion, I attempt to explain the relative importance of release mechanisms in the two regions as potential contributors to the different functions of the striatum and nucleus accumbens.

The primary objective of this project was to test the hypothesis that serotonin (5-HT) can act as a local facilitator of (DA) release in STR and NACC

and to determine the mechanisms by which 5-HT induces DA release in each brain region. More generally, I suggest that release of DA is regulated differently in the STR and NACC and that endogenous 5-HT, released by activation of the 5-HT input to STR and NACC from the raphe nuclei, may play a role as a modulator of extracellular DA concentration in the STR and NACC. Full evaluation of this more general hypothesis is beyond the scope of this project. However, the studies proposed should provide some information on the potential role of 5-HT in regulating DA release.

INTRODUCTION: THE ROLE OF DOPAMINE IN REINFORCEMENT

1. DOPAMINE IN THE BRAIN

The neurotransmitter function of DA, independent of its role as a precursor of norepinephrine, was first proposed by Von Euler and Lishajko (1957) and supported by Bertler and Rosengren (1959). Since that time, its role as a major inhibitory neurotransmitter in ascending catecholaminergic pathways has become better appreciated with the increasing understanding of the function of brain areas that are targets for these projections. Its characterization as an inhibitory neurotransmitter was originally based on evidence of the production of inhibitory post-synaptic potentials (IPSP) when it was applied to target cells. Now there is evidence that at some synapses the result of dopaminergic transmission may be a net excitation.

There appear to be at least three types of DA receptors (D1, D2, and D3) in the brain, each linked to more than one transduction system. Some D1 receptors, located in the striatum, frontal cortex, and nucleus accumbens, are positively linked to adenylyl cyclase. D1 receptor activation can also increase phosphoinositide turnover. D2 receptors, also located in striatum and limbic areas, can activate potassium (K) channels, can decrease phosphoinositide hydrolysis, can inhibit calcium (Ca) channel activity, and can either be not coupled to or negatively coupled (inhibitory) to adenylyl cyclase (for review see Andersen, et al., 1990). The distribution of D3 receptors overlaps, but differs from that of D2 receptors and, so far, the signal transduction pathway associated with the D3 receptor has not been identified. The D3 receptor may be a target for drugs currently used to alleviate major neurological or psychiatric symptoms (Sokoloff et al., 1990).

DA constitutes over 50% of the catecholamine content of the brain (Cooper et al., 1986) and most of the DA in the central nervous system is found in the basal ganglia. The forebrain DA system modulates many aspects of motivated and motor behavior (Fibiger and Phillips, 1986), and malfunction may play a role in schizophrenia (Andreasen, 1985).

The regulation of the release of DA has been a target of investigation into the mechanism of reinforcement and motor behaviors. In my work, I concentrate on comparison of the release of DA from the striatum (STR) and nucleus accumbens (NACC). Models for DA function have been based on studies in the striatum because of the relatively greater amount of tissue; however, the NACC should be examined, as it is this area that is critical for reinforcement. The role of DA in the NACC with respect to reinforcement is established by several lines of evidence. DA can regulate as well as initiate a variety of physiological responses in the NACC by a complex combination of actions via activation of its receptors at multiple synaptic sites (Mogenson et al,1988).

It has been hypothesized that DA neurons, particularly those which project to the NACC region, are the critical substrate for both the motor-stimulating and rewarding properties of drugs like D-amphetamine and cocaine that act as indirect DA agonists by inhibiting DA (and other monoamine) uptake (both cocaine and D-amphetamine), stimulating DA release (D-amphetamine), and inhibiting MAO (D-amphetamine) (Roberts and Koob, 1982; Mogenson et al,1988).

2. LESION STUDIES

Functional integrity of the mesolimbic dopamine pathway from the ventral tegmental area (VTA) to the nucleus accumbens appears to be critical to the

reinforcing actions of drugs such as cocaine or D-amphetamine. Lesion of the DA mesolimbic neurons with 6-hydroxydopamine abolishes D-amphetamine self-administration (Lyness et al., 1979) as well as D-amphetamine-evoked motor stimulation (Kelly and Iverson, 1976). Cocaine self-administration is disrupted in animals in which the ability of the mesolimbic system to release DA is substantially impaired by lesions (Roberts and Koob, 1982).

3. EFFECTS OF INCREASED EXTRACELLULAR DOPAMINE LEVELS

Agents that increase the extracellular level of DA or activate DA receptors are reinforcing. For example, direct dopamine receptor agonists having activity at D2 receptors (apomorphine, piribedil, propylbutyldopamine and bromocryptine) are reported to be reinforcing in the monkey (Woolverton et al., 1984). Although the selective D1 agonist SKF 38393 is not self-administered under the same conditions, D1 receptors are thought to play a significant role in reinforcement by drugs such as cocaine although the precise nature of that role has yet to be established (for review, see Woolverton and Johnson, 1991). Psychomotor stimulants, such as cocaine or amphetamine, which are thought to act as indirect agonists by increasing the concentration of catecholamines in CNS synapses, are reinforcing. Rats will self-administer D-amphetamine into the nucleus accumbens (Hoebel et al., 1983), consistent with reinforcement via increased synaptic DA. DA or D-amphetamine injected onto the NACC stimulates locomotor activity. Injection of the DA antagonist haloperidol into the NACC blocks the locomotor activation produced by D-amphetamine (for review, see Le Moal and Simon, 1991). D1 and D2 receptor antagonists inhibit reinforcement of intravenous self-administration of cocaine

or amphetamine (Koob, 1991; Hubner and Moreton, 1991). Recent microdialysis studies have provided direct evidence that both cocaine and D-amphetamine induce large increases in DA efflux in the NACC (Sharp et al., 1987; DiChiara and Imperato, 1988; Hernandez and Hoebel, 1988; Kelley and Delfs, 1991).

4. EFFECTS OF SUSTAINED OR REPEATED ACTIVATION OF DOPAMINERGIC SYSTEMS

Although elevated DA seems to be a factor in reinforcement, the mechanisms underlying the stimulant and reinforcing effects of addictive drugs such as D-amphetamine and cocaine remain uncertain. Elevated DA at the synapse could be a result of increased synthesis. The stimulation of tyrosine hydroxylase (TH), the enzyme that catalyzes the rate-limiting step in catecholamine synthesis, has been investigated as a possible target of reinforcing drugs. Cocaine regulation of tyrosine hydroxylase could represent part of the biochemical basis of cocaine addiction and craving (Beitner-Johnson and Nestler, 1991). Although cocaine slightly upregulates TH activity, this change in TH activity is not sufficient to explain all of the actions of reinforcing drugs.

Continuous cocaine administration has persistent effects on neurochemical systems different from those that are altered during continuous D-amphetamine treatment. In several DA-rich regions, continuous treatment with D-amphetamine induces large increases in D2 receptor density, as measured by [^{3}H]spiperone binding, and decreases in D1 receptor density, as measured by [^{3}H]SCH23390 binding. Chronic cocaine administration produces a completely different pattern of receptor density changes with

increased [^{3}H]flunitrazepam (benzodiazepine) binding in DA-rich areas, cortex, and amygdala and decreased [^{3}H]QNB (muscarinic cholinergic) binding in DA-rich areas, hippocampus, and amygdala (Zeigler et al., 1991). Administration of cocaine once a day for three days decreases DA uptake in the NACC but not in STR when measured 24 hours after the final injection (Izenwasser and Cox, 1990). The effect in the NACC is due to an increased K_m. Repeated administration of cocaine for ten days causes an enhanced maximal rate of reuptake (V_{max}) of DA in the NACC (Ng et al., 1991). These changes are no longer evident after ten days of abstinence.

5. ROLE OF SEROTONIN

A large number of behavioral, electrophysiological and biochemical studies support an influence of 5-HT on the DA system (for review see Soubrie et al., 1984) and therefore, the factors that affect the release of 5-HT in the STR and NACC are of interest. 5-HT is implicated in pain, feeding, sleep, sexual behavior, cardiac regulation, and cognition. Abnormalities in 5-HT are well documented in Alzheimer's disease and there is evidence that changes in 5-HT systems occur in association with non-disease aging (McEntee and Crook, 1991). 5-HT has a stimulatory effect on basal release of DA in both STR and NACC (Ng, et al., 1972; de Belleroche and Bradford, 1980; Westfall and Tittermary, 1982; Blandina et al., 1989; Nurse et al, 1988). There are multiple 5-HT receptor subtypes in the brain (Nelson et al., 1980). Antagonism of the 5-HT_2 receptor with ritanserin, which has low affinity for D2 receptors, exerts significant therapeutic effects in several neuropsychiatric disorders. Among these effects are reduction of negative symptoms in chronic schizophrenia and reduction of extrapyramidal side effects associated with neuroleptic treatment

as well as beneficial effects on Parkinson's disease itself. 5-HT_2 receptors have also been ascribed an important role in major effects of hallucinogens (Ugedo et al., 1989).

AFFERENT AND EFFERENT CONNECTIONS OF THE STRIATUM AND NUCLEUS ACCUMBENS

The purpose of this section is to review some of the anatomical evidence for similarities and differences in the neuronal afferents and efferents of the STR and NACC. Emphasis is given to the dopaminergic and serotonergic neurons which innervate these regions. Most of the studies cited below are from rat. I have incluced schematic representations of some of the major pathways related to the DA projections to the STR (Appendix 1) and NACC (Appendix 2).

1. STRIATUM

The major function of the STR is thought to be motor control (Cohen and Sherman, 1988). The influence on motor behavior is primarily through pathways, such as the corticospinal tract, that arise in the motor cortex. It is believed that the primary role of the STR is early in the initiation of movement; it does not merely have a feedback relationship with the motor cortex but controls motor output to the spinal cord. The first correlation of a neurotransmitter deficiency with a neurological disease was the finding of degeneration of the dopaminergic input to the STR from the substantia nigra (SN) in patients with Parkinson's disease (Hornykiewicz, 1971).

a. Neuronal inputs

(i) Dopaminergic

The STR is a terminal field of dopaminergic neurons arising in the SN pars compacta, also known as the A9 cell group. DA neurons from the SN project to the STR (globus pallidus, caudate, and putamen) forming the

nigrostriatal pathway. The projections to the caudate/putamen arise predominantly from the ventral and intermediate sheets of DA neurons in the SN and ventral tegmental area (VTA). The ventromedial striatum also receives input from the VTA (Domesick,1988).

The gross anatomy of the ascending dopaminergic projections from the midbrain involves predominantly ipsilateral DA projections with a one to five percent contralateral (but not bilateral) component. Examination of the fine structure of these ascending projections using combined histochemical, biochemical, anterograde autoradiographic tracing, and HRP retrograde tracing techniques indicates that the A9 and A10 neurons form a continuum, with both groups contributing to the nigrostriatal and mesocortical systems. It appears that there exists a reversed dorsal-ventral organizational topography which segregates the nigrostriatal and mesocorticolimbic projections. Greater levels of innervation complexity are becoming apparent within limbic structures (NACC, amygdala, and olfactory tubercle) containing striatal components and striatal structures containing limbic component inputs.

Individual neurons can contain two or more biologically active compounds, each a potential or accepted transmitter substance. This suggests that single neurons may simultaneously release more than one transmitter. The SN-VTA is composed of unique subpopulations of DA, DA/cholecystokinin (CCK), neurotensin (NT)/CCK, and DA/NT/CCK neurons whose neurochemical characteristics are unique but whose projections follow the basic topographical patterns (Fallon, 1988).

(ii) Non-dopaminergic

The STR is innervated by projections originating in all cortical areas. The caudate/putamen can be subdivided into patch and matrix compartments with the patch compartment receiving a prelimbic cortical input and the matrix compartment receiving a neocortical input (Heimer et al., 1985). The dorsolateral striatum receives its major cortical input from the motor cortex (Domesick,1988). The striatum also receives substantial input from the midline thalamic nuclei (Kornhuber and Kornhuber, 1986). The "extrapyramidal" motor circuit leads from the motor cortex to the striatum, through the pallidum, ventral thalamus, and back to the motor cortex (Domesick,1988). The frontal neocortex distributes glutamatergic fibers both to the whole ipsilateral caudate/putamen and also to the dorsal parts of the contralateral caudate/putamen. The caudal neocortex probably also sends such fibers to the dorsal ipsilateral caudate/putamen (Walaas, 1981). The excitatory nature of these glutamatergic inputs to the striatum has been demonstrated (Cordingley and Weight, 1986).

The nigrostriatal neurons form symmetrical synapses onto cell bodies, dendritic shafts, and dendritic spines of the predominant output cells of the striatum. Spines that have a mesostriatal synapse also receive a second input, from excitatory glutamatergic corticostriatal fibers, with an asymmetrical synapse nearer the spine head (Strange, 1990). Twenty to forty percent of the striatal D2 receptors are localized on glutamatergic corticostriatal terminals. D1 terminals are mainly localized on intrinsic striatal cells (Kornhuber and Kornhuber, 1986).

Several neurotransmitters other than glutamate have been found in the STR. Cholecystokinin-immunoreactive (CCK-I) neurons of the ventral midbrain supply a broad innervation of forebrain regions, particularly in the pars

compacta of the substantia nigra. Sparse CCK-I cell bodies were localized in the caudate/putamen (Seroogy and Fallon, 1989). Receptors for both subtypes of γ-aminobutyric acid ($GABA_A$ and $GABA_B$) have been demonstrated in the STR (Bowery et al., 1987). The striatum is innervated by NT terminals which do not appear to contain DA. In the NACC, NT and DA are co-localized in some neurons (Bean et al., 1989). Intracerebroventricular injections of NT have been found to augment DA efflux in the NACC but not in the STR (Blaha et al., 1990). The STR and globus pallidus contain few β-adrenergic receptors (Wanaka et al, 1989). 5-HT is present in relatively high concentrations in the caudate/putamen. The B7 (dorsal raphe) neurons seem to be the major source of the striatal 5-HT. Stimulation of B7 (dorsal raphe) neurons inhibited unit activity of cells in the caudate/putamen. Microiontophoretically-applied methysergide, a 5-HT antagonist, to individual caudate/putamen cells blocked this effect (Olpe and Koella, 1977).

b. Efferent pathways

The principal outputs of the dorsal striatum are channeled via the striato-pallido-thalamic and the striatonigral pathways. The caudate/putamen projects to both sections of the dorsal pallidum and to the thalamus. The efferents of the internal pallidum are channelled via the habenular nucleus, providing innervation to wide areas of the mesencephalic reticular formation, locus ceruleus, SN, hypothalamus and areas of the ventral forebrain. The striatonigral projection forms a direct connection from the caudate/putamen to the substantia nigra (Bjorklund and Lindvall, 1984). Measurements of glutamic acid decarboxylase (GAD), a measure of neurons containing γ-aminobutyric acid (GABA), indicate that the highest GAD activities in the brain are found in

the substantia nigra in the terminals of cells originating in the striatum (Brownstein and Palkovits, 1984). The function of GABA appears to be inhibitory. The GABAergic projection from the globus pallidus and ventral pallidum to the ventral mesencephalon utilizes GABA to increase the membrane potential and reduce the firing frequency of A9 as well as A10 dopamine neurons (Olpe et al, 1977; Pinnock, 1984; Grace, 1987). There is evidence that suggests that substance P and enkephalin are also transmitters in this region.

2. NUCLEUS ACCUMBENS

Anatomical connections of the NACC place it strategically between limbic and motor structures, suggesting a major role in the limbic-motor integration that underlies certain complex, adaptive behaviors (Mogenson et al,1988). The NACC is a target for a large number of limbic afferent inputs (Phillipson and Griffiths, 1985) but sends efferents to only a small number of sites (Nauta et al., 1978). It receives partially overlapping projections from a variety of limbic structures in the forebrain (hippocampal formation, basolateral nucleus of the amygdala, cingulate cortex and prefrontal cortex), and from dorsal raphe (Domesick,1988). A circuit parallel to the "extrapyramidal" motor circuit involves limbic counterparts: frontal cortex to ventral STR/NACC, ventral pallidum, thalamic nucleus and back to the frontal cortex. There also exist projections from the ventral pallidum to all the limbic structures (frontal cortex, NACC, amygdala) which project back to the VTA via the medial forebrain bundle. The NACC also projects back to the VTA while the ventromedial striatum does not. The VTA is thus involved in several complex feedback loops (Domesick,1988).

a. Neuronal inputs

(i) Dopaminergic

The NACC is a terminal field of DA neurons arising from the VTA, also known as the A10 group. DA neurons of the VTA also project to the frontal cortex. In addition, the NACC receives input from the medial SN (Fallon, 1988). The input from the A10 region is a major afferent pathway to the NACC. In the NACC, DA is thought to alter the relay of limbic outputs to motor structures and thus may significantly modulate the influence that limbic structures exert on the extrapyramidal motor system (Mogenson et al,1988).

(ii) Non-dopaminergic

The frontal neocortex is the origin of glutamatergic fibers to the NACC. The caudal allocortex sends such fibers through the fimbria/fornix to the NACC (Walaas, 1981). Axons arising in the hippocampal formation form asymmetric synapses with glutamate decarboxylase-immunoreactive (GAD-I) neurons in NACC, suggesting that the input from the hippocampal formation exerts a strong and direct influence on output pathways of NACC (Meredith et al., 1989b). High GAD activities, suggesting the presence of GABA as a transmitter, are found in the NACC (Brownstein and Palkovits, 1984). Both $GABA_A$ and $GABA_B$ receptors have been demonstrated in the NACC (Bowery et al., 1987).

Cholinergic neurons are differentially distributed within the NACC (Meredith et al., 1989a). Muscarine acts, in a TTX-insensitive manner, at M1 receptors in the NACC (Uchimura and North, 1989). The NACC is a major site of nicotine action, in contrast to the STR (Lapin et al., 1989). In doses which result in moderate plasma levels, nicotine has selective stimulant actions on nerve terminals of the mesolimbic system (Carr et al., 1989). Corticotropin-

releasing factor (CRF) is thought to be involved in coordinating responses to stress. Intracerebroventricular CRF administration decreased glucose utilization in prefrontal cortex and NACC (Sharkey, et al., 1989). The NACC appears to receive NE innervation predominantly from subceruleus nuclei of the pons-medulla while the locus ceruleus neurons project to more rostral areas (Russell et al., 1989). NACC has some nerve terminals in which DA and NT are co-localized (Bean et al., 1989). Intracerebroventricular injections of NT augmented DA efflux in the NACC but not in the STR (Blaha et al., 1990). There is evidence for vasopressin receptors in the NACC (Gerstberger and Fahrenholz, 1989). CCK regulates DA efflux from NACC nerve terminals via a direct presynaptic action on receptors (Vickroy and Bianchi, 1989). Sparse CCK-immunoreactive (CCK-I) cell bodies are localized in the NACC, olfactory tubercle, and bed nucleus of the stria terminalis (Seroogy and Fallon, 1989). CCK-8 modulates mesolimbic DA activity by functionally opposing the postsynaptic effects of DA in the region of the NACC (Weiss et al., 1989).

b. Efferent pathways

The efferent pathways from the NACC are similar to those from the STR. There is a prominent projection from the NACC to the VTA and the medial part of the SN (Bjorklund and Lindvall, 1984). The NACC also projects to the ventral pallidum and entopeduncular nucleus (Austin and Kalivas, 1989; Heimer et al., 1991). The descending pathway continues further caudally to innervate dorsal and medial parts of the mesencephalic reticular formation, including the raphe nuclei. Thus, the various descending pathways from the STR and NACC all feed into the dorsal and medial areas of the mesencephalic reticular formation. As in the STR, the principal neurotransmitter of these

efferents from the NACC is thought to be GABA. Also, the arcuate nucleus of the hypothalamus and its efferent β-endorphinergic fibers are involved in the descending pathway from the NACC to the periaqueductal gray area (Yu and Han, 1989).

SEROTONERGIC PROJECTIONS TO FOREBRAIN REGIONS

5-HT neurons are less topographically organized and have a less restricted terminal field in the cerebral cortex than DA neurons (Lindvall and Bjorklund, 1984). Serotonergic cell bodies are concentrated in the raphe nuclei. The dorsal raphe (DR) is the largest raphe nucleus and contains the highest density of serotonergic cell bodies in the brain. It is located in the rostral part of the tegmentum pontis and in the caudal part of the tegmentum mesencephali (rostral B7 and caudal B5-B4). The density in the medial raphe (MR; B8) is much lower (Steinbusch, 1984; Hillegaart, 1991).

The serotonergic neurons of the raphe nuclei project to a large number of brain structures. Midbrain raphe nuclei have both ipsilateral and contralateral projections to cerebral cortex, with DR projecting predominantly ipsilaterally and MR projecting either bilaterally in a symmetrical fashion or contralaterally. Three major ascending and two major descending serotonergic pathways can be described. The dorsal ascending pathway arises from the medial and rostral parts of the DR. Its fibers are situated dorsolaterally to the medial forebrain bundle (MFB), terminating predominantly in the caudate/putamen complex, although some fibers reach the NACC and the globus pallidus. The medial ascending pathway originates from the DR and projects to the SN with collaterals projecting to the caudate/putamen complex (Steinbusch, 1984). The ventral ascending pathway arises from the mesensephalic raphe nuclei and project to the VTA and then the MFB. Before entering the MFB, some fibers, originating from the DR, innervate the posterior hypothalamus. Rostrally, 5-HT fibers leave the MFB to enter the fasciculus retroflexus, to distribute to several areas, among which are the dorsomedial thalamus and the medial habenula and primarily terminate into the lateral habenula. Further rostrally, 5-HT fibers

leave the MFB to innervate many structures, including: the subthalamic nucleus, the hypothalamic nuclei (medial, lateral, anterior, paraventricular), the median eminence , the suprachiasmatic n., the supraoptic n., and the septum, the olfactory bulb, and the amygdala complex by means of the striae terminalis. A small number of serotonergic fibers terminate in the head of the caudate n. Some 5-HT fibers, originating almost exclusively from the dorsal and medial raphe nuclei, traverse the cingulate bundle to reach the cerebral cortex and, in particular, the prefrontal cortex, the neocortex, the visual cortex, the entorhinal cortex, and the hippocampus. 5-HT axons have a uniform pattern of distribution throughout all areas of the lateral neocortex and distribute to practically all cortical layers. High densities of 5-HT innervation are observed at all levels in the gray matter. There are descending pathways such as the one which runs from the DR to the locus ceruleus, some continuing to the dorsal tegmental n. (Lindvall and Bjorklund, 1984; Steinbusch, 1984; Hillegaart, 1991).

As indicated above, the STR is the major target for ascending DR fibers (Hillegaart, 1991). The substantia nigra receives a direct monosynaptic inhibitory input from the dorsal raphe and medial raphe nuclei and these pathways use 5-HT as a neurotransmitter serving to tonically inhibit dopaminergic neurons (Dray et al., 1976; 1978). There is not an homogeneous distribution of serotonergic terminals in the neostriatum of the rat. Terminals are mainly localized in the ventral part of the caudal area of the neostriatum (Ternaux et al., 1977).

There are pronounced MR projections to the VTA, NACC, and hippocampus (Vertes and Martin, 1988; Hillegaart, 1991). Some 5-HT-like immunoreactive neurons in the midbrain periaqueductal gray of the rat also send their axons to the NACC (Li et al., 1989). The ventromedial part of the

caudate nucleus and the ventrolateral NACC and ventrolateral caudate/putamen show a dense innervation of 5-HT-positive fibers.

The wide distribution of the mesencephalic raphe projections suggests highly collateralized axon systems. For instance, single neurons in the DR are in position to exert control over both ends of the massive nigro-striatal and striato-nigral pathways. Neurons in the DR send collaterals to the caudato-putamen as well as to the SN. Single DR cells also have been shown to project to the medial thalamus and the olfactory cortex as well as to the septum. A major population of DR cells with cortical projections also innervates other forebrain areas. The raphe system shows little variation across species, facilitating cross-species comparisons (Lindvall and Bjorklund, 1984; Hillegaart, 1991).

Thus, although the innervation of the STR and NACC is quite similar, both having inputs from similar regions, using similar neurotransmitters, and having outputs to the same structures, there exist intriguing differences with respect to innervation of these regions. It is also apparent from the above discussion that the serotonergic neurons arising primarily from the raphe nuclei, are in a position to influence the activity of dopaminergic neurons in both the STR and NACC.

REGULATION OF EXTRACELLULAR DOPAMINE AND SEROTONIN IN STRIATUM AND NUCLEUS ACCUMBENS

1. ROLE OF MONOAMINE REUPTAKE SYSTEMS

Neurotransmitter transport plays an important role in the process of synaptic transmission by monoamines. Released neurotransmitters cross the synaptic cleft by diffusion and then interact with receptors located at the post-synaptic membrane, resulting in changes in the post-synaptic cell. Termination of the neurotransmitter signal occurs in most cases through uptake of transmitter back into the pre-synaptic terminal or into glial elements (Iversen, 1971; Kuhar, 1973; Bennett et al., 1974). Re-uptake is an active process which assures both constant and high levels of neurotransmitter in the neuron and low concentrations in the cleft (Hedgvist and Stjarne,1969). During depolarization, which favors reversal of the sodium-dependent re-uptake process, release may occur via the transporters (Levi and Raiteri, 1978; Nelson and Blaustein, 1982; Kanner, 1983).

There are also transporters located in the membranes of storage organelles in the presynaptic terminals. After uptake of neurotransmitter from the synaptic cleft into the cytoplasm by the tricyclic antidepressant-sensitive, sodium-dependent transporter, cytoplasmic amine is further concentrated into a specialized organelle (vesicle) by a reserpine-sensitive proton-coupled process that is not dependent on sodium (Iversen, 1975; Njus et al., 1981). This mechanism protects accumulated amine from loss by leakage or intraneuronal metabolism, reincorporates released material into the storage pool, lowers the concentration gradient across the neuronal membrane to enhance uptake, and the acidic pH of the vesicle's interior serves to stabilize the neurotransmitter (Kanner, 1983).

a. Mechanism of monoamine transport

Neurotransmitter transport across the neuronal plasma membrane occurs as a consequence of coupling the process to ions which move down their electrochemical gradients. The transport systems can achieve high concentration gradients (10^4) by utilizing pre-existing ion gradients, created and maintained by ion pumps (Njus et al., 1981; Pastuszko et al., 1982). High-affinity uptake appears to be an active process in that it is observed in the presence of metabolic energy provided by glucose and is inhibited by agents such as cyanide or dinitrophenol that interfere with ATP synthesis. Ouabain, which inhibits Na/K-ATPase to abolish the Na gradient (Iversen and Neal, 1968; White and Keen, 1970), also inhibits uptake. The function of the Na/K-ATPase is to convert the energy of cellular ATP into ionic gradients. When artificial Na gradients are applied, the ATPase is not needed and ouabain does not affect the process (Kanner, 1983). Biogenic amines are transported into nerve endings by a process that has an absolute requirement for Na (Bogdanski and Brodie,1969) and Na may be co-transported. This dependence on Na indicates that sodium ions and possibly the potassium ion gradient provide the driving force for active neurotransmitter uptake achieved by co-transport with ions moving down their electrochemical gradients into the cell. The uptake mechanism exhibits the kinetic properties of a two-substrate sequential reaction in which both amine and Na (1:1) must bind to the carrier for transport of amine to occur, and in which amine and Na act as mutually cooperative co-substrates. (Sammet and Graefe, 1979.)

Magnesium participates in the operation of the Na/K ATPase of animal cells both as an essential co-substrate at low concentrations and as an inhibitor at higher concentrations (Sachs, 1988). The interaction is complex. Higher

than physiological concentration of Mg might reduce the activity of the Na/K-ATPase which, in turn, would potentiate the increase in cytoplasmic Na concentration (e.g. induced by glutamate uptake and NMDA receptor stimulated depolarization), promoting the reverse transport of DA (Lonart and Zigmond, 1991).

The original model of neuronal amine uptake (Kanner, 1983) excludes any involvement of extracellular ATP in the reuptake mechanism even though ATP is co-stored and co-released with catecholamine and is present in the synapse with catecholamine during the uptake process. Some evidence suggesting a more active involvement of ATP has recently been generated. Uptake of [^{3}H]DA and [^{3}H]NE (and possibly 5-HT) into synaptosomes has been reported to be potentiated (higher K_m and V_{max}) by ATP in a dose-dependent manner. Uptake is faster and relatively insensitive to temperature in the presence of extracellular ATP while in the absence of ATP uptake showed temperature dependence. It is proposed that in addition to the carrier-mediated (neuronal) uptake of catecholamine, there is neuronal uptake that is regulated by ATP and inhibited by cocaine which may be more relevant for terminating the synaptic action of catecholamine because of its faster rate of uptake and greater capacity. It is possible that, in addition to the neuronal carrier-mediated uptake, there is an ATP-regulated, Na-dependent, temperature-independent, Mg-independent reserpine-insensitive uptake across the plasma membrane into synaptosomes which has much faster kinetics and larger capacity (Cao et al., 1990a,b)

b. Significance of dopamine reuptake systems in controlling extracellular dopamine levels

Justice et al. (1988) have attempted to model the dopaminergic nerve terminal in a quantitative manner. This resulting model of the DA nerve terminal includes three intraterminal pools of DA. Figure 1 is a modification of the Justice model. The DA which is newly synthesized and positioned for release by nerve stimulation (rDA) is in equilibrium with two other pools. The pool representing long-term vesicular storage of DA (vDA) is depleted by reserpine and slowly depleted after inhibition of DA synthesis by treatment with α-methyl-para-tyrosine (aMpT). The rDA pool is replenished from vDA if the release rate exceeds the synthesis rate for the rDA pool. The third pool, cDA, is the one into which the uptake carrier places DA from the extracellular space. This pool is not vesicle-bound and is degraded by monoamine oxidase. The regulation of DA levels in the extracellular fluid by the interaction of these pools of DA (or other monoamine) with various agents that stimulate release will be addressed in this dissertation after a discussion of uptake and release.

The neuronal monoamine uptake systems in STR and NACC play a major role in the removal of released DA and 5-HT from the extracellular space. Blockade of uptake has been shown to significantly potentiate the post-synaptic actions of DA and 5-HT in NACC (Uchimura and North, 1990). Drugs that apparently act at site(s) different from that at which cocaine and GBR 12909 inhibit transport may modify DA transport in STR and NACC (Izenwasser et al., 1991). However, treatment for 10 days with DA uptake blockers (D-amphetamine, cocaine, GBR 12909, mazindol, nomifensine) produced no significant increases of apparent maxima for uptake (V_{max}) or binding (B_{max}) or consistent changes in ligand affinity for the transporter. This evidence suggests

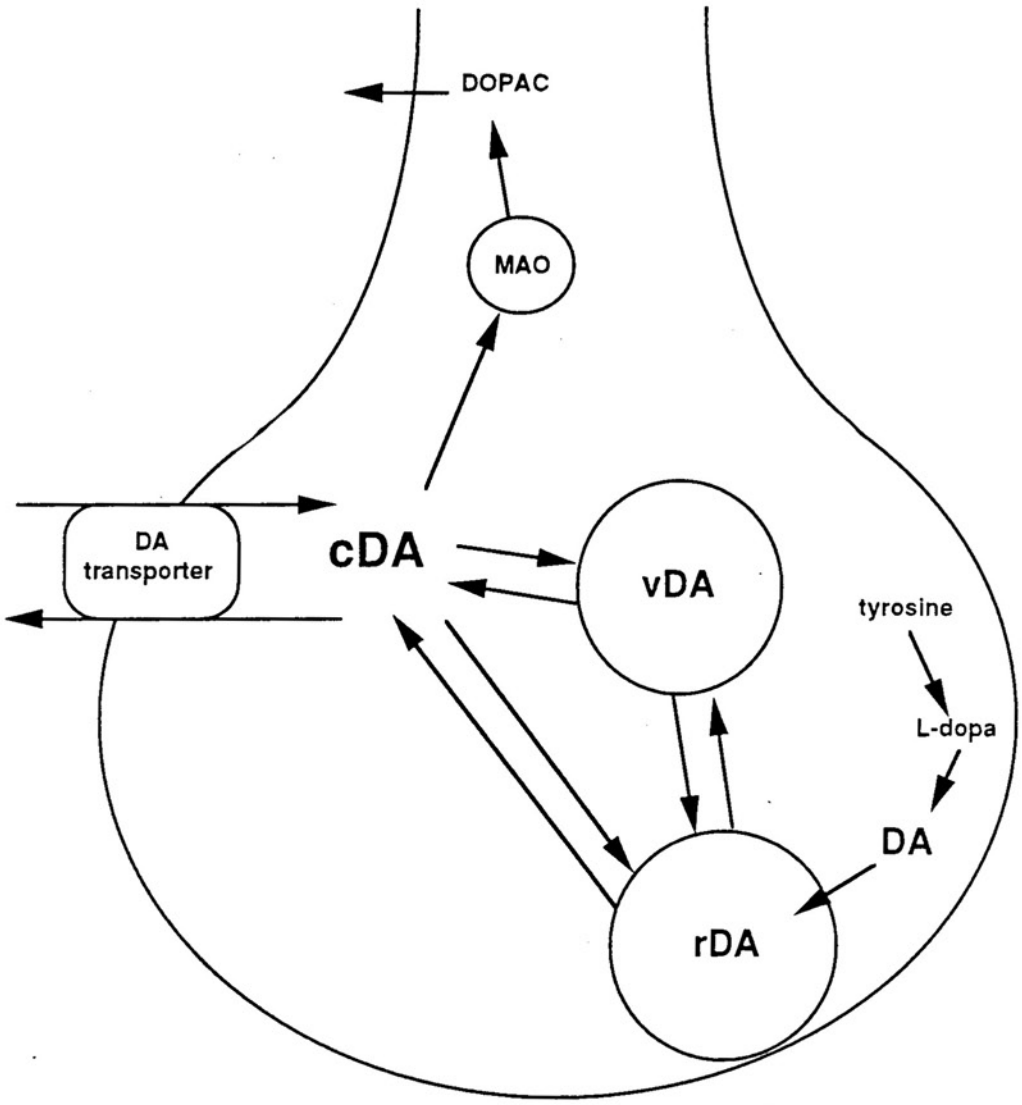


Fig. 1. Pools of Dopamine. Synthesis provides DA for a pool that is releasable immediately (rDA), via exocytosis, by electrical stimulation or membrane depolarization by elevated K. The cytoplasmic pool (cDA) is the source of DA released by reversal of the transporter. Long-term storage is represented by the vesicular pool (vDA). It is likely that the three pools exist in equilibrium. Figure is modified from Justice et al., 1988 and Arbuthnott et al., 1990b.

transporters are not regulated by changes in local neurotransmitters in the same way as receptors, nor influenced similarly with respect to upregulation and supersensitization by repeated treatment with antagonists (Kula and Baldessarini, 1991). Uptake sites have a highly heterogeneous distribution in the dorsal and ventral STR in rat and man (Donnan et al., 1991), suggesting that some functional effects of psychoactive drugs, such as cocaine, that bind to the DA uptake complex could be related to the distribution of specific uptake sites (Graybiel and Moratalla, 1989). As mentioned above, DA uptake has been shown to be partially dependent on ATP apparently acting through an extracellular site (Hardwicke et al., 1989; Cao et al., 1990a,b).

There is a good correlation between the potencies of substances that inhibit the neuronal uptake of [^{3}H]DA in preparations of STR *in vitro* and their relative potencies as behavioral stimulants or in maintaining self-administration behavior (Ritz et al., 1987). An intact dopaminergic pathway from the VTA to the NACC is required for cocaine to be self-administered (Roberts and Koob, 1982). Cocaine inhibits DA uptake with an IC_{50} in the range 400-600 nM (Ritz et al, 1987; Izenwasser et al., 1990a). Brain concentrations of cocaine in rats following intravenous administration of stimulant doses are in the range 1 to 10 μM (Misra et al., 1976; Nicolaysen et al., 1988) and plasma concentrations of cocaine in humans after the intravenous administration of a dose of cocaine inducing subjective effects are also about 1 μM (Javaid et al, 1978). It therefore seems likely that inhibition of dopamine uptake occurs when reinforcing doses of cocaine are administered. Cocaine also inhibits DA reuptake in the STR; however, integrity of the nigrostriatal DA pathway is not essential for cocaine self-administration. Although DA transporters from the STR and NACC have different apparent molecular weights (Lew et al., 1991), the DA transporter in STR appears similar to that in NACC with respect to sensitivity to inhibitors

(Berger et al., 1990; Izenwasser et al., 1990a). GBR 12909, a potent and selective inhibitor of DA uptake (K_i =1 nM) with a more than 100-fold lower affinity for the NE and 5-HT uptake complexes (Andersen, 1989), inhibits DA uptake with equal potency in NACC and STR. On the other hand, [^{3}H]DA uptake into crude synaptosomes was 2-3 fold higher in caudate/putamen than NACC although concentrations of DA in rat dorsal caudate putamen relative to NACC were equivalent. [^{3}H]Mazindol binding was also 2-3 fold higher in the caudate/putamen. The differences were due to density changes (V_{max} or B_{max}) not to differences in apparent affinity. These differences suggest that the dopaminergic fibers of the ventral striatum, especially the medial NACC, may either be relatively lacking in their capacity for uptake of released DA (Marshall et al., 1990a) or that the density of the DA fibers is lower in NACC than in STR.

Some psychoactive compounds are substrates for the transporter. For example, D-amphetamine is transported by the neuronal uptake system and subsequently induces release of catecholamine. This is apparently achieved by reversal of the monoamine transporter, since cocaine and desipramine, two inhibitors of the transporter, block D-amphetamine-induced catecholamine release (Azzaro et al., 1974).

c. Serotonin reuptake systems

Serotonergic neurons are known to exhibit a high affinity reuptake system, predominantly located in the nerve terminals, that is capable of taking up serotonin from extracellular concentrations of 10^{-8} M or less. At higher concentrations, serotonin is also transported into neurons by a different uptake mechanism that is also found to accumulate 5-HT in NE- and DA-containing

terminals (Steinbusch, 1984). For example, in the rat neostriatum, [^{3}H]5-HT at relatively low extracellular concentrations ($4.4x10^{-8}$) was taken up not only by serotonergic terminals but also to a significant extent by dopaminergic terminals (Ternaux et al., 1977).

5-HT transporters in rat brain were found to be highly concentrated in dorsal and medial raphe nuclei, the central gray, the superficial layer of the superior colliculus, the lateral septal nucleus, and the paraventricular nucleus of the thalamus. High concentrations were found in brainstem areas containing DA (SN and VTA) and NE (locus coeruleus) cell bodies. Moderate concentrations were found in frontal parietal cortex, olfactory tubercle, regions of the basal ganglia, septum, amygdala, thalamus, hippocampus. Lower densities are apparent in other areas of the neocortex with low to medium levels in the caudate/putamen, moderate levels in globus pallidus, and low to medium density in the nucleus accumbens (De Souza and Kuyatt,1987).

Cocaine inhibits the neuronal uptake of 5-HT as well as DA (Koe, 1976; Ritz et al., 1987; Uchimura and North, 1990). In Fawn-Hooded rats, high ethanol consumption is associated with high 5-HT uptake (Daoust et al., 1991). Ethanol may inhibit the Na/Ca antiporter (Alexi and Azmitia, 1991), suggesting a role for 5-HT in alcohol abuse.

ß-Adrenergic antagonists show stereoselective affinity for 5-HT receptors in the CNS and have been shown to competitively inhibit 5-HT transport, with no stereoselectivity, in cultured pulmonary vascular endothelial and smooth muscle cells. The (+)-isomer and the (-)-isomer of propranolol similarly inhibit 5-HT uptake in a dose-dependent manner (Lee and Fanburg, 1991). Compounds that exhibit moderate to high affinity for the 5-HT_{1b} (TFMPP, Ru 24969, mCPP) binding site also exhibit moderate to high potency for inhibiting 5-HT uptake. In addition to inhibiting 5-HT uptake and competing for the

[^{3}H]paroxetine binding to the 5-HT uptake recognition site, the compounds tested also displayed varying abilities to increase 5-HT efflux (Wolf and Kuhn, 1991b).

The preceding data suggest that uptake mechanisms are important in the modulation of the concentration of both DA and 5-HT at the synapse. Although modest differences in properties such as distribution and V_{max} exist in the STR and NACC, no direct evidence for divergent roles of the two areas regarding reinforcement has been reported. However, as will be explained in the following sections, transporters can also play a direct role in the release of transmitter.

2. RELEASE MECHANISMS

a. Exocytotic (vesicular) release

Exocytotic release of neurotransmitter has been shown to be dependent on extracellular Ca. Membrane depolarization is necessary for opening calcium channels and increasing internal calcium concentration near membrane transmitter-release sites (Mulkey and Zucker, 1991). Neurons and other excitable tissues possess several different types of voltage-sensitive Ca channels that differ considerably in their biophysical and pharmacological properties. L-type Ca channels are located on the soma (Sanna et al., 1986) and N-type Ca channels are located on presynaptic nerve terminals (Miller, 1987). In many cases, the evoked release of a neurotransmitter appears to be dependent on Ca influx through N-type Ca channels whereas L channels in the same neurons do not seem to contribute under normal physiological conditions.

T-type Ca channels, which provide transient currents at small depolarizations, do not appear to play a significant role in transmitter release. Increased intraneuronal Ca triggers a transient release of neurotransmitter, apparently by facilitating the fusion of synaptic vesicles with the presynaptic plasma membrane, followed by release of transmitter from the vesicle into the extracellular fluid (Cooper et al., 1986). The action of TTX on the Na channels of the nerve terminal can inhibit the voltage-sensitive Na currents responsible for Ca entry and exocytotic release. Both K and veratrine induce DA release by an exocytotic mechanism (Arbuthnott et al., 1990a). In terms of the Justice model described above, exocytotic release is primarily from the vesicular storage pool (vDA) via the monoamine pool replenished by synthesis (rDA).

Presynaptic inhibition of neurotransmitter release is thought to involve the receptor-mediated regulation of ion channels in the nerve terminal. The Ca channels responsible for the Ca influx that triggers neurotransmitter release may be directly regulated by presynaptic receptors. Alternatively, the depolarization of the nerve terminal produced by an action potential may be modified so that the depolarization spike becomes less effective in activating Ca channels. Opening K or Cl channels stabilizes the membrane potential, resulting in a briefer action potential which is less effective in depolarizing the terminal and results in a smaller Ca influx. All these mechanisms appear to be used in the regulation of neurotransmitter release. Receptor activation by inhibitory neurotransmitters appears to induce a large shift in the voltage dependence of channel activation which means that, for small to medium depolarizations, the Ca current is reduced. However, at very strong depolarizations, all the Ca channels are available for activation and the inhibitory effect of the transmitter is lost. The mechanism by which presynaptic Ca and K channels are regulated is thought to involve the participation of G

proteins and second messengers. It is likely that the G protein, G_0, is the normal transducer of the inhibitory signal. Ca flux through N-type Ca channels appears to be the major trigger for neurotransmitter release. N channels can be inhibited by presynaptic receptors, acting directly through the activation of G proteins that alter the voltage dependence of the channels so that fewer of them open during the brief depolarization. Some L- and T-type Ca currents also seem to be inhibited by neurotransmitters and, in some instances, second messengers (diacylglycerol, cGMP, arachidonic acid) may modulate neuronal Ca channels.

Many of the receptors that produce inhibition of neuronal Ca currents also regulate neuronal K channels. Activation of presynaptic K channels could produce presynaptic inhibition. Some neurotransmitters appear to produce neuronal excitation by inhibiting K channels. These inwardly-rectifying K channels may be subject to modulation by more than one type of G protein. It appears that presynaptic inhibition in the nervous system may often be produced by simultaneously regulating Ca and K channels, both effects mediated by G proteins. Some component of presynaptic modulation may be exerted by neurotransmitter directly on the intracellular release mechanism in addition to effects on presynaptic ion channels (Miller, 1990).

b. Exchange diffusion

Exocytotic release has been shown to be dependent on extracellular Ca. Both tyramine and D-amphetamine induce DA release *in vitro* and *in vivo* by a Ca-insensitive mechanism. Releasing amines such as D-amphetamine, p-OH-D-amphetamine, and tyramine share a common facilitated diffusion system for uptake into striatal homogenates. Fischer and Cho (1979) have shown that

release of monoamine from synaptosomes by indirectly acting amines is temperature dependent, whereas uptake of D-amphetamine is not. This release is saturable, sensitive to Na and K concentrations, and inhibited by competitive and noncompetitive agents such as cocaine and phenoxybenzamine. Release by these amines is stereoselective (D-amphetamine is more potent than L-amphetamine). There is a linear relationship between the ability of a compound to inhibit DA uptake and its ability to induce release, which demonstrates sensitivity to structural modification of the substrate . Collectively, this evidence suggests a carrier-mediated release process. Fischer and Cho (1979) propose a mechanism of exchange diffusion for this process in synaptosomes defined as the movement of one substrate into a compartment stimulating the efflux of a second substrate from that compartment. The transport of releasing amines into the synaptosomes following their concentration gradients accelerates the efflux of internal monoamine out of the nerve terminals, following its concentration gradient, by increasing the probability of exchange via the plasma membrane transporter. Once the monoamine has been released, the high concentration of the releasing amine outside the synaptosome prevents its reuptake. Non-exocytotic release, or exchange diffusion, of DA is further defined as efflux that persists in reduced Ca and that is blocked by nomifensine (Arbuthnott et al., 1990b).

Properties similar to those observed in synaptosomes have been demonstrated in nerve terminals. There is evidence that D-amphetamine and other sympathomimetic amines enter the nerve terminal by a Na-dependent carrier mechanism which is presumably the monoamine uptake system (Azzaro et al., 1974; Bartholow and Rutledge, 1977). Upon entering the nerve ending, sympathomimetic amines are thought to displace biogenic amines from intraneuronal binding sites. Most psychostimulants are weak bases and DA

uptake into synaptic vesicles uses an interior-acidic pH gradient. D-Amphetamine, fenfluramine, tyramine, and similar compounds reduce the pH gradient, resulting in reduced uptake and increased release of neurotransmitter into the cytoplasm (Sulzer and Rayport, 1990). The displaced amine is then released from the cytoplasm by a reversal of the carrier mediated transport system (Paton, 1973a, b). Transmitter release induced by reduced external Na utilizes a carrier-dependent mechanism *in vitro*. Thus, the efflux of biogenic amine by the exchange diffusion process is Na dependent and the rate of efflux would by a function of: (1) monoamine gradient, (2) Na gradient, and (3) availability or activity of carrier.

Ouabain, tyramine, D-amphetamine or elevated potassium produce a concentration-dependent release of biogenic amines. Nomifensine, which blocks monoamine transport, reduces both tyramine and D-amphetamine induced release. K-induced release is apparently increased in the presence of nomifensine, probably due to reduced re-uptake of released amine. It appears that D-amphetamine, tyramine, and ouabain all have access to the carrier-dependent release mechanism *in vivo*. Although D-amphetamine is known to be an inhibitor of MAO (Green, 1971), this cannot account for the magnitude of the observed release. Disruption of DA synthesis by α-methyl-p-tyrosine (aMpT) clearly discriminates between releasing agents. *In vivo* pretreatment with aMpT for a period of 2 hr depletes DA release by all agents and also abolishes resting release. However, given shortly before releasers, when aMpT can be thought to be acting principally on the newly synthesized store of DA, aMpT is still able to strongly reduce the actions of D-amphetamine, although the release stimulated by K was slightly reduced and that stimulated by tyramine was unchanged. This implies that D-amphetamine release has access to newly

synthesized pool while tyramine releases exclusively from the long-term store which is sensitive to reserpine (Arbuthnott et al., 1990b).

Reduction of extracellular Na to 50 mM, with osmolarity maintained by choline or lithium replacement, induces an excessive release of DA that is blocked by nomifensine (Hurd and Ungerstedt, 1989), indicating that a reduction of the normal sodium gradient is sufficient to enhance release. Metabolic inhibitors enhance nerve-stimulated release of NE, [^{3}H]DA and [^{3}H]5-HT from sympathetic nerves. The potentiation by metabolic inhibitors of D-amphetamine- and potassium-induced release of monoamine from isolated brain tissue is not simply due to depletion of tissue ATP levels, but may be related to Na or K transport. The Na/K-ATPase Na pump is inhibited either: (1) when ATP levels are reduced by metabolic inhibitors, or (2) in the presence of inhibitors such as ouabain. This potentiation by metabolic inhibition is consistent with amine and Na being co-transported across the membrane during D-amphetamine-induced release of the amine. Elevation of the intraneuronal Na concentration by inhibition of Na/K-ATPase would provide increased levels of the co-substrate for transport and could account for the potentiation of release produced by metabolic inhibitors and ouabain (Rutledge, 1978).

Superfusion with aMpT to block DA synthesis abolishes the efflux of endogenous DA and DOPAC from slices or synaptosomes. D-amphetamine produces a concentration-dependent increase in the basal efflux of endogenous DA and a concomitant decrease in endogenous DOPAC efflux. The ability of D-amphetamine to increase DA efflux is potentiated by the monoamine oxidase inhibitor pargyline and blocked by aMpT or by blockade of the DA neuronal uptake carrier with 10 μM nomifensine (Parker and Cubeddu, 1986a, b). Pargyline had little effect on endogenous DA efflux but abolished

DOPAC efflux and increased tissue DA levels. These data are consistant with the exchange diffusion model and extend the model to include the following elements: (1) synthesis provides a continuous supply of DA which is metabolized rapidly within the neuron and is lost as DOPAC; (2) D-amphetamine facilitates the synthesis-dependent efflux (Na-dependent) of DA, probably by an accelerated exchange diffusion mechanism (which may explain the inability of reserpine pre-treatment to abolish the behavioral stimulant effects of D-amphetamine since reserpine depletes vesicular stores of DA); (3) newly-synthesized cytoplasmic DA does not contribute to impulse-induced DA release; and (4) D-amphetamine does not restore electrically evoked DA release. D-Amphetamine does not distinguish newly synthesized and older stored pools of DA but rather accelerates DA efflux from a single cytoplasmic pool, and this pool is maintained by both vesicular and recently synthesized transmitter (Parker and Cubeddu, 1986a,b).

c. A model for regulation of transmitter release

The previously described data were consolidated by Justice et al. (1988) and expanded by Arbuthnott et al. (1990b) into a model whereby all three storage pools of DA or other monoamine exist in equilibrium (Fig. 1): cytoplasmic (cDA), releasable (rDA), and vesicular "long-term" storage (vDA). Synthesis supplies rDA. D-Amphetamine releases from rDA and vDA via cDA and the transporter. Similarly, since vDA is also in equilibrium with cDA, tyramine, by inhibiting the storage of dopamine in vDA, can also make dopamine available for release via cDA. Exocytotic releasers, which induce release from rDA, become sensitive to inhibition of release by nomifensine, a transporter inhibitor, if the animal has been pretreated with reserpine which

depletes vDA (Arbuthnott et al., 1990a,b). Nomifensine does not reduce DA release induced by K under control conditions but markedly inhibits DA efflux when the animals are reserpine pretreated. This suggests that inhibition of storage into vDA diverts release from newly synthesized stores of DA to pools accessible for exchange diffusion. Membrane depolarization allows Na into the terminal, thereby facilitating release by exchange diffusion from cDA. By making the assumption that all these DA pools interact and that depolarization can release, in a dynamic fashion, from both cDA and rDA, the model can explain short and long term changes in DA metabolism and release while being consistent with data concerning the effects of metabolic inhibitors and transport inhibitors on releasing amines (Arbuthnott et al., 1990a, b).

Extracellular levels of HVA have been shown to be sensitive to extracellular Na and not directly related to extracellular levels of either DA or DOPAC. This suggests the possibility of a Na-sensitive, carrier-mediated process involved in the formation/transport of HVA, and the compartmentalization of DA metabolism (Hurd and Ungerstedt, 1989).

This model consolidates hypotheses of vesicular and transporter-mediated mechanisms in regulation of monoamine release. As was observed for the properties of the transporter as a terminator of neurotransmitter action at the synapse, there do not appear to be differences reported between transporter-mediated mechanisms of release in STR and NACC.

3. RELEASE OF DOPAMINE FROM STRIATUM AND NUCLEUS ACCUMBENS

a. Electrical stimulation

Electrical current applied to preparations of nervous tissue is thought to simulate release of neurotransmitter in a manner similar to release induced by action potentials. Release of endogenous DA from the STR or NACC elicited by electrical stimulation is not accompanied by an increase in overflow of DOPAC. Inhibition of neuronal uptake by GBR 12909 markedly increases the apparent release (overflow) of DA from electrically-stimulated STR by decreasing re-uptake. The rate of formation of DOPAC is greater in NACC than STR, however, the levels of DOPAC do not produce a reliable index of the release or utilization of DA (Soares-DA-Silva and Garrett, 1990). In rabbit striatal slices, reserpine pretreatment reduces tissue DA by 95%. Basal efflux of DA and DOPAC (the large majority of the basal efflux of endogenous compounds), was slightly greater than in untreated slices. After reserpine, electrical stimulation evoked no overflow of DA. These data suggest that in the absence of vesicular transmitter stores, synthesis provides a continuous supply of DA which is metabolized rapidly within the neuron and is lost as DOPAC. Newly-synthesized cytoplasmic DA does not appear to contribute to impulse-induced dopaminergic transmission (Parker and Cubeddu, 1986a).

b. Elevated potassium (membrane depolarization)

Another commonly used model for stimulation of neurotransmitter release utilizes elevated K and concomitant reduction of Na to depolarize neuronal tissue preparations. With respect to release elicited by elevated K,

axonal and somatodendritic DA release are similarly regulated: both are Ca-dependent and inhibited by D_2 agonists. However, somatodendritic release is less influenced by action potential generation (Kalivas and Duffy, 1991). In cultured neurons, K-evoked [^{3}H]DA release is blocked by ω–conotoxin, an inhibitor of N-type Ca channels (Grilli et al., 1989). Elevated K-induced release of DA is greatly reduced when DA synthesis is inhibited by aMpT (Herndon et al., 1985). Nomifensine does not depress DA release stimulated by high K (Raiteri et al., 1979). Release by K becomes sensitive to nomifensine blockade when the normal vesicular release process is modified by pretreatment with reserpine, suggesting that the mechanism of release may be changed to a carrier-dependent mechanism following inhibition of the long-term storage of amine (Arbuthnott et al., 1990a, b). K-stimulated release of DA is suppressed by CCK-8S in a concentration-dependent manner in the anterior NACC (Voigt and Wang, 1984; Vickroy and Bianchi, 1989; Marshall et al., 1991). In contrast, in the medial posterior NACC CCK-8 facilitates K-stimulated release from NACC. CCK does not appear to affect K-stimulated DA release from STR (Vickroy and Bianchi, 1989). Release stimulated by elevated K is Ca-sensitive (i.e. substantially reduced at concentrations of Ca below 0.4 mM) but not completely abolished even when all Ca is theoretically chelated by EGTA. K-stimulated release consists of unmetabolized [^{3}H]-amine even in the absence of MAO inhibition (Raiteri et al., 1979).

c. D-Amphetamine

D-Amphetamine is a reinforcing drug and an effective stimulator of monoamine release. It may therefore be useful to compare stimulation of release by D-amphetamine with more standard methods in an attempt to

understand the mechanism of action of drugs of abuse. D-Amphetamine releases DA from DA nerve terminals, inhibits neuronal uptake of DA, stimulates DA synthesis, and inhibits MAO. D-Amphetamine produces a concentration-dependent increase in the basal efflux of endogenous DA and a concomitant decrease in endogenous DOPAC efflux (Parker and Cubeddu, 1986a). Systemic D-amphetamine increases DA in the NACC and medial prefrontal cortex to the same degree (Moghaddam and Bunney, 1989). D-Amphetamine releases DA from terminal fields (NACC) as well as dendrites (A10). In general, axonal and somatodendritic release of endogenous DA from rat NACC and A10 region is regulated by similar factors, although somatodendritic release is less responsive to D-amphetamine (Kalivas and Duffy, 1991).

As previously discussed, D-amphetamine releases DA by exchange diffusion. Indirectly acting sympathomimetic amines such as D-amphetamine and p-OH-D-amphetamine are substrates for uptake into neuronal tissue (Fischer and Cho, 1976; Cho et al., 1977). The DA transporter (Fig. 1) appears to be utilized at low D-amphetamine concentrations (10^{-7} M) but passive entrance into neurons and displacement of [^{3}H]DA from vesicular binding sites occurs at high concentrations (10^{-4} M) of D-amphetamine (Liang and Rutledge, 1982). Although D-amphetamine can attain its equilibrium concentration inside synaptosomes by diffusion, blocking the carrier inhibits D-amphetamine-induced release of [^{3}H]DA (Fischer and Cho, 1979). Even in the absence of MAO inhibition, the radioactivity released by D-amphetamine is almost entirely unmetabolized [^{3}H]DA (Raiteri et al., 1979). The ability of D-amphetamine to increase DA efflux is blocked by inhibiting DA synthesis or by blockade of the DA uptake carrier with nomifensine, and is potentiated by pargyline. Pargyline abolishes DOPAC efflux (Parker and Cubeddu, 1986a). D-Amphetamine might release DA from pools which do not contribute to the K-induced release of DA.

A parallel between release of radiolabeled and endogenous transmitter by D-amphetamine cannot necessarily be assumed (Herndon et al., 1985).

The mechanism by which D-amphetamine releases biogenic amines in STR is not entirely understood. There is evidence that D-amphetamine-stimulated release is not entirely Ca-independent. In rat striatal synaptosomes pre-loaded with [^{3}H]DA, D-amphetamine induces a large increase in release of [^{3}H]DA 30 to 60 seconds after introduction. This release is blocked by NOM. D-Amphetamine-stimulated rapid release is reduced by 60% in the absence of Ca. In contrast, D-amphetamine-induced release after a 6 min exposure is not affected by the absence of Ca. The initial release of DA by D-amphetamine may therefore be Ca dependent, suggesting an exocytotic process. After several minutes, the release of DA induced by D-amphetamine, which has declined considerably from the first 60 sec, is less sensitive to changes in Ca levels (Bowyer et al., 1984). D-Amphetamine does not exert its action via a voltage-dependent Ca channel sensitive to nifedipine nor via depolarization of the voltage dependent Na channels since it is not inhibited by tetrodotoxin. Reserpine treatment inhibits the Ca-dependent release by D-amphetamine which implies that vesicular stores are specifically released during Ca-dependent D-amphetamine-evoked release. In comparison to release from synaptosomes, Ca does not appear necessary for D-amphetamine-evoked release in striatal slices. D-Amphetamine may act to release Ca from synaptosomal stores to evoke the release of [^{3}H]DA (Bowyer et al., 1987).

Reserpine pretreatment does not abolish the behavioral stimulant effects produced by D-amphetamine. The central stimulant properties of D-amphetamine in lab animals are preserved even after pretreatment of the animals with reserpine (Rech and Stolk, 1970). However, the behavioral actions of D-amphetamine are blocked by aMpT which blocks DA synthesis

(Weissman et al., 1966). The action of D-amphetamine is most likely indirect (mediated by endogenous DA and not by direct action of D-amphetamine on specific receptors) because its ability to inhibit electrically evoked [^{3}H]ACh release in rabbit STR is blocked by the D_2 receptor antagonist sulpiride and by destruction of striatal dopaminergic nerve terminals with 6-hydroxydopamine (Parker and Cubeddu, 1986a).

d. Excitatory amino acids

Descending glutamatergic tracts into STR, NACC, VTA, and SN have been identified. It is therefore of interest to compare release induced by glutamatergic stimulation with other releasers of DA. This type of stimulation may most closely reproduce physiological stimulation since excitatory amino acids activate specific receptors on neurons. Electrophysiological evidence from rat midbrain slices indicates that both NMDA-sensitive and non-NMDA-sensitive glutamate receptors may play a critical role in the control of excitability, pacemaker firing, and dendritic DA release of DA neurons (Mereu, et al., 1991). These receptors are also thought to play a critical role in neuronal death in Parkinson's disease, in psychosis, and in the mechanism of action of drugs of abuse related to DA neurons. KA and/or QUIS receptors make a significant but modest contribution to this response. The L-glutamate action is modulated by agents such as SKF 10,047 or TCP, known to bind to the phencyclidine site, located within the NMDA-receptor-gated cation channel. TTX (0.1 μM) partially blocks release stimulated by KA or QUIS but not that by L-glutamate or NMDA (Clow and Jhamandas, 1989). Excitatory amino acids do not appear to have a tonic excitatory effect on striatal dopamine release (Moghaddam and Gruen, 1991).

L-Glutamate increases DA release predominantly by activation of the NMDA receptor located presynaptically on dopaminergic afferents (Krebs et al., 1991). NMDA stimulates release of [^{3}H]DA from the striatum of rat and guinea pig in a concentration-dependent manner (Werling et al., 1990). Release is inhibited by the competitive NMDA-selective antagonists 3-((±)-2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid (CPP) and D-(-)-2-amino-5-phosphonopentanoic acid (APV), the non-competitive antagonists (+)-5-methyl-10,11-dihydro-5H-dibenz[a,d]cyclohepten-5,10-imine (MK-801) and phencyclidine, and by physiological concentrations of Mg which block the NMDA receptor-channel complex. Release is facilitated by the co-agonist glycine. *Mu-* and *delta*-selective opioids (DAMGO and DPDPE) are without effect on NMDA stimulated release. *Kappa*-opioid-selective U50-488H inhibits NMDA-stimulated release and the inhibition is reversed by naloxone or the *kappa* antagonist nor-BNI. This suggests that some of the terminals of the nigrostriatal dopamine neurons may bear receptors of both the NMDA and *kappa*-opioid type. NMDA-evoked release is partially sensitive to TTX (Krebs et al., 1991). TTX-resistant release is blocked by Mg or MK-801 and potentiated by glycine. These results suggest that at least a portion of the NMDA receptors involved in the facilitation of DA release are located on DA nerve terminals. NMDA also stimulates Ca-dependent release of [^{3}H]DA from NACC slices. Release is inhibited completely by physiological concentrations of Mg and by APV and phencyclidine (Jones et al., 1987).

In an effort to elucidate the contributions by various release processes, release of [^{3}H]DA was elicited by combinations of glutamate and methamphetamine (Bowyer et al., 1991). Methamphetamine is a D-amphetamine analog thought to have a similar mechanism of action. The release of [^{3}H]DA by methamphetamine and low concentrations of glutamate is

additive. Methamphetamine-evoked release of [^{3}H]DA is not affected by 5 μM MK-801, a noncompetitive NMDA antagonist. Methamphetamine-evoked DA release, with or without Mg blockade of the NMDA-gated cation channel, is independent of NMDA or other glutamate receptors and is additive with DA release produced by exogenously applied glutamate. This indicates that there are essentially separate mechanisms involved in glutamate- and methamphetamine-evoked DA release.

Concentrations of glutamate above 1 mM, however, may stimulate the efflux of DA from rat striatal slices superfused with buffer containing a physiological concentration of Mg (1.2 mM) via a mechanism which appears to involve the monoamine transporter. The release is not affected by the kainate/quisqualate antagonist 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) and only partially reduced by APV or MK-801. Release is not affected by TTX, absence of Ca (+EGTA), or systemic application of reserpine. Nomifensine reduces efflux to 15% of control and removal of NaCl blocks glutamate-evoked efflux (Lonart and Zigmond, 1991). Thus, glutamate can release DA by two mechanisms. Under some circumstances, it may be possible for glutamate to induce DA release by reversal of the DA transporter. At lower concentrations of glutamate, receptor-mediated release is the principle mechanism.

e. Acetylcholine

The excitatory neurotransmitter acetylcholine (ACh) was the first neurotransmitter identified. Cholinergic inputs to the STR were originally defined due to their role in the pathology of Parkinsons disease. Acetylcholine stimulates release of several other transmitters, including DA. Acetylcholine-stimulated release of [^{3}H]DA in STR is calcium-dependent. The presence of

cholinergic presynaptic receptors of the nicotinic and muscarinic types on striatal dopaminergic terminals has been demonstrated (Giorguieff et al., 1977). The muscarinic agonist oxotremorine induces a slight but significant release of DA which is completely blocked by the muscarinic antagonists atropine and scopolomine, but not affected by the nicotinic antagonist pempidine. Atropine or scopolomine also reduces release stimulated by acetylcholine. Nicotine stimulates release of DA in the NACC but not the caudate/putamen. After a delay, DOPAC and ascorbic acid levels increase in both areas, indicating increased metabolism. These effects are antagonized by pretreatment with the central nicotinic antagonist mecamylamine, indicating involvement of nicotinic receptors (Brazell et al, 1990). Application of nicotine to either the substantia nigra (Lichtensteiger et al., 1982) or the VTA (Calabresi et al., 1989) increases the firing rate and alters the firing pattern of dopaminergic neurons. Nicotine induces release of [^{3}H]catecholamines from hippocampal slices (Arqueros et al., 1978). Taken together, these findings suggest roles for both muscarinic and nicotinic receptors in regulation of dopaminergic pathways in the brain.

f. Serotonin

5-HT has an inhibitory effect on most cerebral DA-mediated functions. This negative regulation involves interactions at or beyond the post-synaptic DA receptor (Jenner et al., 1983). However, the mechanism of action is complex and 5-HT can act presynaptically to influence DA release. The SN receives a direct monosynaptic inhibitory input from the DR and MR and these pathways use 5-HT as a neurotransmitter to tonically inhibit dopaminergic neurons (Dray et al., 1978). However, in the NACC the main effect of 5-HT on medium spiny

neurons is to depolarize the neurons by selectively reducing the inward rectifier K conductance (North and Uchimura, 1989).

There exist multiple receptors for 5-HT in the rat brain (Nelson et al., 1980) which may modulate a variety of functions. These subtypes have been grouped pharmacologically into the following categories: 5-$HT_{1\text{-like}}$ (linked to adenylyl cyclase), 5-HT_2 (involved in inositol phosphate metabolism), 5-HT_3 (fast ion channel), and, recently, 5-HT_4 (for review see Radja et al., 1991).

Feeding behavior is an example of 5-$HT_{1\text{-like}}$ receptor-mediated effects associated with DA. Low-dose 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT), a 5-$HT_{1\text{-like}}$-selective agonist, stimulates feeding by a 5-HT_{1a} autoreceptor-mediated inhibition of 5-HT release and consequent disinhibition of dopaminergic transmission (Montgomery et al., 1991). The NACC is involved in the expression of the feeding resulting from dorsal and medial raphe injection of 8-OH-DPAT but the caudate nucleus is involved only in the effect derived from the dorsal raphe (Fletcher, 1991).

5-HT exerts an inhibitory control of zona compacta and VTA DA cell activity through 5-HT_2 receptors (Ugedo et al., 1989). This inhibitory action at the cell bodies is in contrast to the effect of 5-HT in the NACC, the terminal region for VTA neurons. Intracellular recordings from medium spiny neurons in the NACC, reveal that 5-HT (1-100μM) depolarizes a majority of neurons and causes generation of action potentials (North and Uchimura, 1989). The depolarization is competitively antagonized by 5-HT_2-selective antagonists. 5-$HT_{1\text{-like}}$- and 5-HT_3-selective agonists as well as 5-HT_3-selective antagonists are without effect (North and Uchimura, 1989). Selective 5-HT_2 receptor antagonism elicits decreased tissue DA and 5-HT levels in NACC. This treatment increases DA and 5-HT in extracellular fluid measured by microdialysis (Devaud and Hollingsworth, 1991). 5-HT liberated by electrical

stimulation of dorsal raphe neurons activates a 5-$HT_{2/1c}$ receptor (sensitive to metergoline) on nigral somadendrites which triggers a local dendritic release of DA (Trent and Tepper, 1991).

Much attention has been given to the effects of 5-HT_3 receptor-mediated effects on dopaminergic neurons in the brain. 5-HT and 5-HT_3-selective agonists have been reported to release DA in STR and NACC (Jiang et al., 1990; Blandina et al., 1989; Chen et al., 1991b). The ability of 5-HT or the 5-HT_3-selective agonist 2-methyl-5-HT to elicit DA release is antagonized by the 5-HT_3-selective antagonists granisetron and ICS 205-930 but not by antagonists selective for 5-HT_1/5-HT_2 (metergoline), 5-$HT_{1\text{-like}}$ (methiothepin), or 5-HT_2 (methysergide) receptors. The 5-$HT_{1\text{-like}}$- or 5-HT_2-selective agonists 5-CT, 8-OH-DPAT or DOI do not alter DA release. Release stimulated by 2-methyl-5-HT is Ca-dependent and partially sensitive to TTX (Blandina et al., 1989; Jiang et al., 1990). However, blockade of central 5-HT_3 receptors by GR38032F (ondansetron) does not result in alterations in metabolism of DA or 5-HT in caudate nucleus, NACC or SN (Koulu et al., 1989). Another 5-HT_3-selective agonist, 1-phenyl-biguanide (0.1-1.0 mM), dose-dependently enhances extracellular DA content in NACC. This effect is antagonized by the 5-HT_3-selective antagonists zacopride and GR38032F (Chen et al., 1991b).

Mechanisms involving the monoamine transport system (Figure 1) also appear to be operative. In superfused striatal synaptosomes, 5-HT stimulates both the spontaneous and Ca-evoked efflux of radioactivity in a concentration dependent manner but LSD, a 5-HT_2 partial agonist, does not. The 5-HT_3-selective antagonists MDL72222 and GR38032F do not reverse the stimulation, but cocaine or nomifensine completely inhibits 5-HT-stimulated DA release. This indicates that 5-HT induces the release of DA from striatal nerve terminals via a mechanism involving the transport of 5-HT into the DA terminals, rather

than by activation 5-HT_3 receptors (Yi et al., 1991). In STR or NACC slices, 5-HT enhances basal overflow of radioactivity from prelabeled tissue and reduces K-induced release of [^{3}H]DA. This effect was not altered by methysergide or the 5-HT uptake blocker chlorimiprimine but was reversed by the DA transport inhibitors nomifensine and benztropine (Nurse et al., 1988). Results reported later in this dissertation confirm this observation. The various receptor types and tissue distribution of 5-HT receptors indicate a potentially important role in the mediation of DA release in the STR and NACC.

g. Other releasers of dopamine

Although less is known about other neurotransmitters that may depolarize DA neurons and therefore regulate release of DA, some studies deserve mention to illustrate the complexity of the regulation of DA release. Most of the spontaneously released DA in STR is not Ca-dependent (Blandina et al., 1989). Intranigral administration of substance P produces a long-lasting increase in DA levels in the STR (Reid et al., 1990b). GABA and dynorphin A decrease, but neurokinin A increases DA levels (Reid et al., 1990a). NT releases endogenous and [^{3}H]DA from STR or NACC (Hetier et al., 1988). Fenfluramine also induces DA release. Fenfluramine is thought to enter the neuron by passive diffusion at all concentrations and displace [^{3}H]DA from vesicles (Liang and Rutledge, 1982). CCK-8S induces the release of DA in the NACC but not in the STR (Marshall et al., 1990b).

A difference begins to emerge in the sensitivities of DA release from the STR and NACC to various stimuli. For example, as mentioned above, nicotine stimulates release and CCK-8 facilitates K-stimulated release in NACC but not

in STR. These findings suggest that there might also be differences in the release stimulated by other agents.

4. RELEASE OF SEROTONIN IN STRIATUM AND NUCLEUS ACCUMBENS

As stated earlier, serotonin initially appeared to produce disparate results in my preliminary experiments. The projections to STR and NACC from the raphe nuclei are the source of serotonin in these areas. It seemed a reasonable hypothesis that regulation of serotonin release itself could be critical to differential regulation of release of DA from STR and NACC as well as important in the role of DA release in NACC in reinforcement.

The release of 5-HT is controlled by autoreceptors of the 5-HT_{1a} and 5-HT_{1b} type, inhibitory heteroreceptors of the $GABA_B$, muscarinic, α_2, and D_1 type, and stimulatory heteroreceptors for somatostatin. Regulation is also afforded by receptors that are acted upon by co-localized neurotransmitters. Examples are the receptor for the inhibitory transmitter adenosine and the stimulatory transmitter substance P (Middlemiss, 1988). As is the case for DA, most spontaneous release is not Ca-dependent (Gray and Green, 1987). As mentioned previously, selective 5-HT_2 receptor antagonism alters 5-HT and DA neurotransmission in the NACC of the rat (Devaud and Hollingsworth, 1991).

Similar to the model for release of DA, the model for release of 5-HT assumes that the rate of release is dependent on the amount of 5-HT in a releasable pool and that this pool may be resupplied during depolarization by newly synthesized 5-HT (Auerbach and Lipton, 1985). Release of 5-HT obeys first-order kinetics from a releasable pool that can be replenished during the release process. The second phase (steady-state), which is postulated to occur at a rate equal to the rate of pool replenishment, is regulated both by the net

rate of 5-HT synthesis and by the level of membrane depolarization. This suggests that regulation of 5-HT metabolism at the site of the nerve terminal could affect 5-HT release during prolonged discharge of the neuron.

Electrical stimulation of dorsal raphe neurons induces 5-HT release from the hippocampus. Similar mechanisms may be involved in the STR and/or NACC. Depolarization-evoked release of 5-HT is mediated by N-type rather than L- or T-type voltage sensitive Ca channels (Sharp et al., 1990). In rabbit hippocampal slices, N- and L-type voltage sensitive calcium channels differ in their contribution to regulation of 5-HT release. L-type channels are operative when a weak and sustained depolarization is applied or when N-type channels are blocked or when the cytoplasmic transmitter pool is expanded by inhibition of MAO (Feuerstein et al., 1991).

In the rat hypothalamus, the release of [^{3}H]5-HT elicited by depolarization by elevated K is inhibited by 5-HT in a dose-dependent manner. The inhibition was abolished by the 5-$HT_{1\text{-like}}$-selective antagonist methiothepin, but not by antagonists selective for 5-HT_2 or 5-HT_3 receptors such as methysergide, cyproheptadine or mianserin, respectively (Cerrito and Raiteri, 1979; Martin and Sanders-Bush, 1982). Systemic 8-OH-DPAT inhibited 5-HT release in globus pallidus, hippocampus, frontal cortex, NACC and medial septum. 5-HT_{1a} autoreceptor-mediated control of 5-HT release is functional in all of these brain regions (Hjorth and Sharp, 1991).

D-Amphetamine-stimulated release of 5-HT is concentration dependent, attenuated but not abolished by absence of Ca or by reserpine pretreatment and antagonized by the D2 antagonist haloperidol or the non-selective DA antagonist cis-flupenthixol. The effects of D-amphetamine on [^{3}H]5-HT release are probably mediated, in part, by presynaptic DA receptors located on the 5-HT terminals and this response may depend upon the release of DA from adjacent

terminals. DA stimulates release of [^{3}H]5-HT in a concentration-dependent manner. DA-stimulated release is reduced by removal of Ca and antagonized by haloperidol and flupenthixol. Neither DA receptor antagonist has any effect on K-evoked [^{3}H]5-HT release (Balfour and Iyaniwura, 1985). The D-amphetamine analog MDMA (3,4-methylenedioxy-methamphetamine, Ecstasy) induces a biphasic reduction in brain 5-HT levels and causes marked degeneration of 5-HT nerve terminals in cerebral cortex, hippocampus and striatum. It induces release of [^{3}H]5-HT and inhibits uptake of [^{3}H]5-HT. The 5-HT transporter plays a significant role in the release of 5-HT induced by MDMA and related agents (Hekmatpanah and Peroutka, 1990). Thus, a role for both DA and monoamine transport in 5-HT release is suggested.

The effects of cocaine on 5-HT release have not been extensively investigated. The electrophysiological effects of cocaine on identified 5-HT neurons indicate that the mechanism of cocaine action is through inhibition of the uptake of 5-HT, but do not consider a 5-HT releasing effect (Wolf and Kuhn, 1991a). Specific binding sites for cocaine exist on 5-HT terminals and cocaine inhibits 5-HT uptake by directly interacting with the transporter (Koe, 1976; Reith et al., 1983; Reith et al., 1985). It has been suggested that 5-HT uptake inhibitors can interfere with the ability of 5-HT autoreceptor agonists to suppress 5-HT release. Many 5-HT autoreceptor agonists that suppress release also bind to the 5-HT transporter and inhibit 5-HT uptake. Thus, cocaine could influence the regulation of 5-HT release like other 5-HT uptake inhibitors (Wolf and Kuhn, 1991a).

Cocaine modifies 5-HT synaptic activity and interacts with 5-HT neurons in a manner similar to its interaction with DA systems (Reith et al., 1983; Reith et al., 1985). Several compounds, including cocaine, that increase the synaptic availability of 5-HT, decrease the firing rate of 5-HT cells of the dorsal raphe

(Aghajanian et al., 1987). The inhibition of 5-HT cells of the DR by cocaine is more pronounced than that observed for DA cells of the VTA (Pitts and Marwah, 1987, 1988). Cocaine displaces [^{3}H]paroxetine from the 5-HT transporter with a greater affinity than it displaces [^{3}H]mazindol from the DA transporter (Ritz et al., 1987).

The release of 5-HT can be modulated by a number of other pharmacologic agents. Nicotinic receptor activation by dimethylphenyl piperazinium iodide releases [^{3}H]5-HT as well as DA. Dimethylphenyl piperazinium iodide-evoked release of [^{3}H]5-HT was decreased by (Met^5)enkephalin, (Leu^5)enkephalin or morphine. These compounds did not alter spontaneous release, release stimulated electrically via field stimulation, or release stimulated by elevated K (Westfall et al., 1983). DR cells are excited by CCK-8 in a concentration-dependent manner and the excitation is blocked by CCK-A type antagonists (Boden et al., 1991). Histamine inhibits electrically-stimulated release of [^{3}H]5-HT (but not release of [^{3}H]DA) in neostriatum, frontal cortex and hypothalamus via histamine H_3 receptors (Smits and Mulder, 1991). Inhibitory α_2 modulation of [^{3}H]5-HT release develops supersensitivity following long-term deprivation of the noradrenergic input (Benkirane et al., 1985). Release of 5-HT from frontal cortex may be controlled by an inhibitory $GABA_B$-receptor located presynaptically (Gray and Green, 1987). While most data implicating opioid regulation of 5-HT release has been performed in the hippocampus, opioid receptors are present on rat striatal 5-HT neurons (Parenti et al., 1983) and the same mechanism may be applicable. In the hippocampus, selective opioid agonists [D-Ala^2,N-methyl-Phe^4,Gly^5-ol]enkephalin (μ) and [D-Pen^2,D-Pen^5]enkephalin (δ) inhibits K-evoked [^{3}H]5-HT release while U50,488H (κ) is less effective. Naloxone reverses this inhibition. Both receptors exert control on 5-HT release by interacting with a G protein which is

sensitive to pertussis toxin (Passarelli and Costa, 1989). In the ground squirrel, the δ/μ opioid agonist DADLE elicited a dose-related inhibition of [^{3}H]5-HT release from hippocampal slices that was completely reversed by naloxone (Kramarova et al., 1991).

5. SUMMARY

In conclusion, several mechanisms may regulate release of DA or 5-HT from presynaptic terminals. (1) Vesicular release normally occurs when an action potential invades the nerve terminal; this release is absolutely dependent upon calcium (Frankenhaeuser and Hodgkin, 1957; Dodge and Rahamimoff, 1967; Mulkey and Zucker, 1991). (2) Ligand-gated ion channels can admit Ca which induces vesicular release (MacDermott and Dale, 1987). (3) Some releasing agents such as D-amphetamine (Raiteri et al., 1979, Liang and Rutledge, 1982) act by being taken up into the terminal via the dopamine transporter, or by passive diffusion, and displacing stored dopamine from vesicles into the cytosolic pool. This cytosolic dopamine is then transported into the synaptic cleft by a reversal of the transporter (Figure 1). It might be expected that agents structurally similar to dopamine or D-amphetamine could also induce release via the dopamine transporter.

The NACC is critical to reinforcement produced by cocaine and other drugs of abuse. Because of the relatively greater volume of the STR, this structure is often used as a general model for dopaminergic function. Indeed, many properties of dopaminergic neurons in the two areas are comparable (e.g. pharmacological profile of the dopamine uptake system; Izenwasser et al., 1990a). However, increasing evidence indicates that some characteristics of

the two regions differ, as might be expected of two regions whose physiological functions are divergent. Among the differences characterized so far are density of DA transporter (Marshall et al., 1990a; Izenwasser et al., 1990b), predominance of DA receptor subtypes (Sokoloff et al., 1990), and sensitivity to neuroleptic-induced depolarization block (Chen et al., 1991a). There are also significant differences in the neural connections of these areas as discussed.

My research concentrated on further elucidating the differences in regulation of transmitter release in the STR and NACC, with the goal of understanding differences in DA transmission in the NACC, which produces reinforcement, and in the STR which is not important in reinforcement. I reasoned that the differential contribution of neurotransmitters, reflecting differing neuronal inputs to STR and NACC, to vesicular or non-vesicular release might be helpful in this regard. I decided to use several agents known to stimulate release of transmitter in *in vitro* brain preparation to evaluate the potential contribution to release in both areas. I also performed experiments under various conditions that enhanced or inhibited certain mechanisms of release (e.g. low Ca to assess vesicular release, low Mg to assess NMDA-receptor type glutamate-regulated release). Finally, I used antagonists at several 5-HT receptor subtypes to evaluate the contribution of 5-HT receptors to the observed release of DA.

METHODS

1. REAGENTS

Reagents used in this study were procured from the following sources: cocaine hydrochloride, D-amphetamine sulfate, ethylenebis(oxyethylenenitrilo)-tetraacetic acid (EGTA), serotonin (5-HT), tetrodotoxin (TTX), picrotoxin, muscimol, L-ascorbic acid, and L-glutamic acid from Sigma Chemical Co. (St. Louis, MO), 1-(2-(bis(4-fluorophenyl)methoxy)ethyl)-4-(3-phenyl-propyl)piperazine (GBR 12909), domperidone, metoclopramide, 1-(1-napthyl)piperazine, nomifensine, (+) and (-)propranolol hydrochloride, endo-8-methyl-8-azabicyclo[3.2.1]oct-3ol indol-3-yl-carboxylate (ICS 205930), ritanserin, ketanserin tartrate, endo-8-methyl-8-azabicyclo[3.2.1]oct-3-ol 3,5-dichlorobenzoate (MDL 72222), methiothepin, (+)-5-methyl-10,11-dihydro-5H-dibenz[a,d]cyclohepten-5,10-imine (MK-801), and pargyline hydrochloride from Research Biochemicals Inc. (Natick, MA), N-methyl-D-aspartic acid (NMDA) from Cambridge Research Biochemicals (Atlantic Beach, NY), [^{3}H]dopamine ([^{3}H]DA) (specific activity approximately 50 Ci/mmol) from Amersham Corp. (Arlington Heights, IL), and fluoxetine hydrochloride from Lilly Pharmaceuticals Corp. (Indianapolis, IN), α-amino-3-hydroxy-5-methyl-4-isoxazole proprionic acid (AMPA) and 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) from Tocris Neuramin (Buckhurst Hill, Essex, England), sodium metabisulfite, NaH_2PO_4, and H_3PO4 from Aldrich Chemical Co. (Milwaukee, WI), 1-octane sulfonic acid from Kodak (Rochester, NY), acetonitrile from Mallincrodt Specialty Chemicals (Paris, KY).

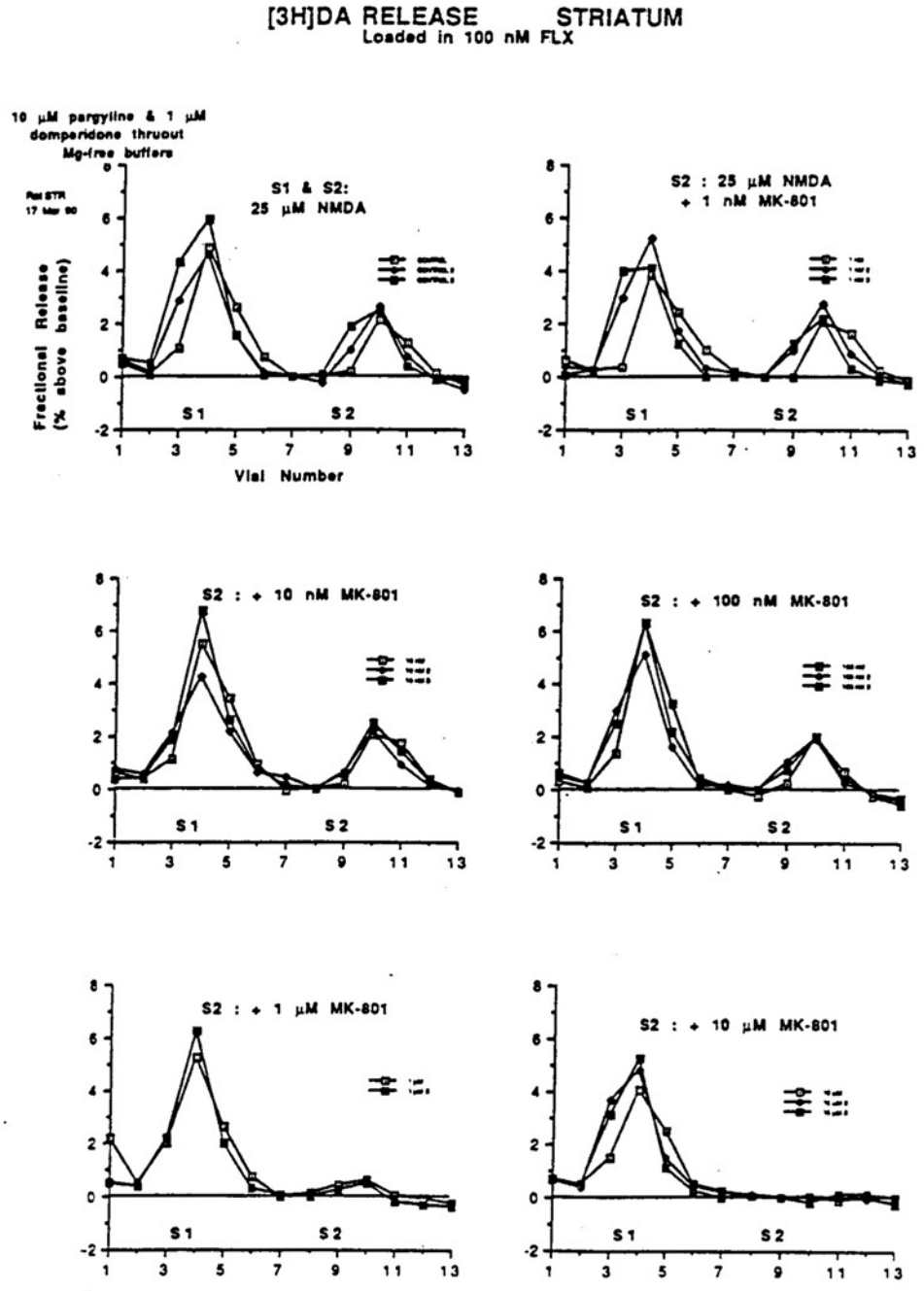
2. MEASUREMENT OF OVERFLOW OF PRELOADED [^{3}H]DOPAMINE

Male Sprague-Dawley rats (Taconic, Germantown, N.Y.) weighing approximately 250-350 g were kept on a 12 hr light/dark cycle with food and water available *ad libitum*. Animals were sacrificed by decapitation without anesthesia. Brains were removed to ice immediately. Tissue from STR or NACC was dissected and chopped into 225 x 225 µM strips and suspended in modified Kreb's-HEPES buffer (MKB: in mM; 127 NaCl, 5 KCl,1.3 NaH_2PO_4, 2.5 $CaCl_2$, 1.2 $MgSO_4$, 15 HEPES, 10 glucose, 100 µM ascorbic acid, 1 µM domperidone, 10 µM pargyline; pH adjusted to 7.4 with NaOH) by trituration through a plastic transfer pipette. Following three washes in MKB, tissue was resuspended in MKB and incubated with 15 nM [^{3}H]DA for 20 min. Tissue was always loaded with [^{3}H]DA in buffer containing 100 nM FLX (to inhibit uptake of [^{3}H]DA into serotonergic terminals) and physiological concentrations of Ca and Mg. In some experiments in which effects of Ca or Mg were tested, the appropriate ion was omitted from the buffer after the tissue was loaded with [^{3}H]DA. Tissue was resuspended a final time in MKB and distributed in 275 µl aliquots between glass fiber filter discs into chambers of a BRANDEL (Gaithersburg, MD) superfusion apparatus. MKB, with or without added transporter inhibitor, as appropriate, was superfused over the tissue at a flow rate of 0.5 ml/min. When a transporter inhibitor was to be tested, it was present throughout the experiment after the tissue was loaded with [^{3}H]DA. After 25 min of superfusion, to establish a low, stable baseline release (typically less than 1.0%), tissue was stimulated (S1) by a 2 min exposure to MKB containing 20 mM K with a commensurate reduction in Na. The inflow was returned to MKB (5 mM K) for a period of 10 min. The tissue was stimulated a second time (S2) by a 2 min exposure to L-glutamate, NMDA, D-amphetamine, or 5-HT. Again, the

inflow was returned to MKB (5 mM K) for 10 min to allow release to return to baseline levels. Tissue was then exposed to 0.2 N HCl for 45 min to extract the remainder of the radioactivity from the sample. Superfusates were collected at 2 min intervals into scintillation vials. The tissue itself was collected into the final vial with the filter discs. Radioactivity was measured by liquid scintillation spectroscopy. Data for baseline calculations were expressed as radioactivity collected in the vial expressed as a fraction of total radioactivity contained in the tissue at the beginning of the collection interval (baseline overflow). Data from stimulated release were expressed as Fractional Release (%) above baseline, or as the percent of radioactivity released by the control stimulus (% Control Release), after baseline overflow was subtracted. Under these conditions, the radioactivity released is predominantly [^{3}H]DA (Werling et al., 1988). Figure 2 illustrates the data from a typical experiment where released radioactivity is expressed as % Fractional Release (above baseline). After determining % Fractional Release, data from chambers receiving identical treatments were averaged.

Analysis of variance (ANOVA) was performed on each data set as appropriate. For comparisons of data in Tables 3 and 4 and Figs 8 and 9, ANOVA was performed for each stimulator (K, NMDA, glutamate, amphetamine, and 5-HT). Comparisons of STR to NACC were performed on "Control" values only. Where ANOVA indicated significant treatment differences ($p < 0.05$), further analysis was performed using *post hoc* Dunnett's Tests ($q'_{0.05,v,\rho}$). Where differences in response to uptake inhibitors or antagonists were indicated, ANOVA was performed on the data set for each uptake inhibitor (GBR 12909, fluoxetine, nomifensine, cocaine) or antagonist (ICS 205-930, metoclopramide, ketanserin, 1-(1-napthyl)piperazine) and, where significant ($p < 0.05$) treatment differences were present, Scheffe F-tests ($S_{0.05}$) performed for

Fig. 2. Time course of [^{3}H]DA release from slices of STR of rat brain. Radioactivity detected in vials converted to Fractional Release (% above baseline) per collection vial. Each vial represents a superfusate collection period of 2 min. Similar results were obtained from NACC (data not shown). Release was stimulated by introduction of 25 μM NMDA into superfusing buffer for a period of 2 min. Because of the physiological antagonism at the NMDA receptor-channel complex, Mg was omitted from the buffer. Each line represents analysis of radioactivity in superfusate from a single superfusion chamber. Each graph illustrates results from superfusion chambers in which increasing concentrations of MK-801, as indicated in the heading for each panel, were introduced in the interstimulus interval and maintained throughout the remainder of the collection periods. In this example, S1 and S2 were 25 μM NMDA. Release for S1 or S2 was calculated as the average of release from three individual superfusion chambers calculated over the appropriate collection period.


[3H]DA RELEASE STRIATUM
Loaded in 100 nM FLX
10 µM pargyline & 1 µM domperidone thruout
Mg-free buffers
Rat STR
17 Mar 90
S1 & S2:
25 µM NMDA
S2 : 25 µM NMDA
+ 1 nM MK-801
S2 : + 10 nM MK-801
S2 : + 100 nM MK-801
S2 : + 1 µM MK-801
S2 : + 10 µM MK-801
Fractional Release
(% above baseline)
Vial Number
S1
S2

comparisons between pairs of releasers (K, NMDA, D-amphetamine, 5-HT) for each concentration of uptake inhibitor.

3. MEASUREMENT OF OVERFLOW OF ENDOGENOUS DOPAMINE

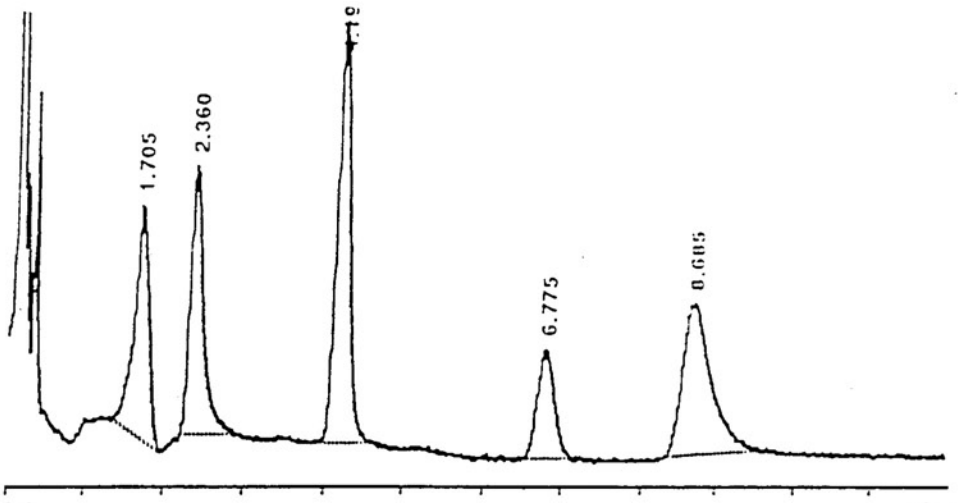
Endogenous dopamine, collected under the superfusion conditions described above, was measured by high performance liquid chromatography with electrochemical detection (HPLC/EC). With the electrochemical detector, the analyte is measured by the amount of oxidation or reduction current that develops at a specific applied potential as the analyte passes the electrode surface. Some selectivity is obtained by choosing an applied potential which will not result in responses from interfering compounds. As the analyte is detected, its chemical form is changed by oxidation or reduction reactions. Selectivity is enhanced by the use of multiple electrodes. The first electrode, the Guard Cell, is positioned after the HPLC pump but before the analytical column. The potential set at the Guard Cell oxidizes all potential interfering compounds before the mobile phase reaches the analytical column. As the mobile phase is recirculated, some interfering compounds are irreversibly oxidized and the mobile phase is continuously reconditioned, resulting is a very low background current (approximately 0.01 picoamps). The second electrode (detector 1) and third electrode (detector 2) are housed together in the Analytical Cell and positioned immediately after the column. The second electrode eliminates interference from compounds which might oxidize at lower potentials than dopamine. Quantitation is achieved by monitoring the current at the third electrode (detector 2). The electrochemical detector was an ESA Coulochem 5100A utilizing a 5011 Analytical Cell and 5020 Guard Cell. The potentials were set as follows: -0.04 (detector 1), +0.32 (detector 2), and +0.40

(Guard Cell). The current at detector 2 was monitored and data collected (at 0.3 sec intervals) by an Apple Macintosh Plus computer utilizing Rainin Dynamax HPLC Method Manager software.

Superfusate was collected into chilled vials. Samples were immediately filtered (Corning syringe filter, nylon membrane, 0.2 μm pore) into amber autosampler vials which were then sealed and kept at 3°C until analysis. The sample injection volume was 100 μl. The HPLC mobile phase consisted of an aqueous solution of NaH_2PO_4 (75 mM, 99.999%), 1-octane sulfonic acid sodium salt (1.4 mM, HPLC grade) and 10% (v/v) acetonitrile (HPLC grade) brought to pH 3.1 with H_3PO4 (85 wt%, 99.99%,) and filtered through a Nylon-66 membrane (0.2 μM pore, Rainin). Mobile phase was recirculated at a flow rate of 1 ml/min at ambient temperature. The column was Hypersil ODS C-18 (150 mm, 4.6 mm i.d., 3 μm, Alltech). Protein was determined by the method of Lowry (Peterson, 1977).

Figure 3 illustrates the detection of DA, NE, 5-HT, and their metabolites achieved using the HPLC/EC conditions described above. Data collection started at three minutes after injection in order to allow interference from the solvent front to pass, and continued for 12 minutes in order to monitor the peak generated when 5-HT was used to effect release of DA. When a relatively large amount of 5-HT was present in the sample to be analyzed, as when 10 μM 5-HT was used to stimulate release of DA, an interfering peak (Fig. 4) appeared after the sample had remained in the autosampler vial more than 4 hours and increased with increasing time. This peak, presumably a product from the decomposition of 5-HT, chromatographed close enough in time to DA to make analysis of these samples difficult. This difficulty was circumvented by altering

the order of samples in the autosampler such that all samples with large amounts of 5-HT present were analyzed first and by the inclusion of sodium metabisulfite and $HClO_4$ (to achieve a final concentration of 100 μM and 1 mM, respectively) in all the collection vials to retard decomposition.



Analysis: Channel A

Peak Name	Peak No.	Time	Type	Height(μV)	Area(μV-sec)	Area%
DOPAC	1	1.705	N	34879	447413	17.928
dopamine	2	2.360	N	40381	459006	18.393
5-HIAA	3	4.190	N	63543	721060	28.894
HVA	4	6.775	N	16226	241044	9.659
5-HT	5	8.685	N	22861	626991	25.124

Fig. 3. Chromatogram of HPLC standards. Stock solutions of norepinephrine, dopamine, dihydroxyphenylacetic acid, homovanillic acid, 5-hydroxyindoleacetic acid, and serotonin were combined and diluted to a final concentration of 10^{-9} M each in pre-oxygenated superfusion buffer containing sodium metabisulfite and $HClO_4$ (to a final concentration of 0.1N and 1 mM, respectively). The solution was then filtered into an autosampler vial and analyzed as described in the Methods section. Current (pA) is plotted on the y-axis and time (min) is represented on the x-axis. Norepinephrine eluted within the first minute and was not resolved from the solvent peaks under these conditions.

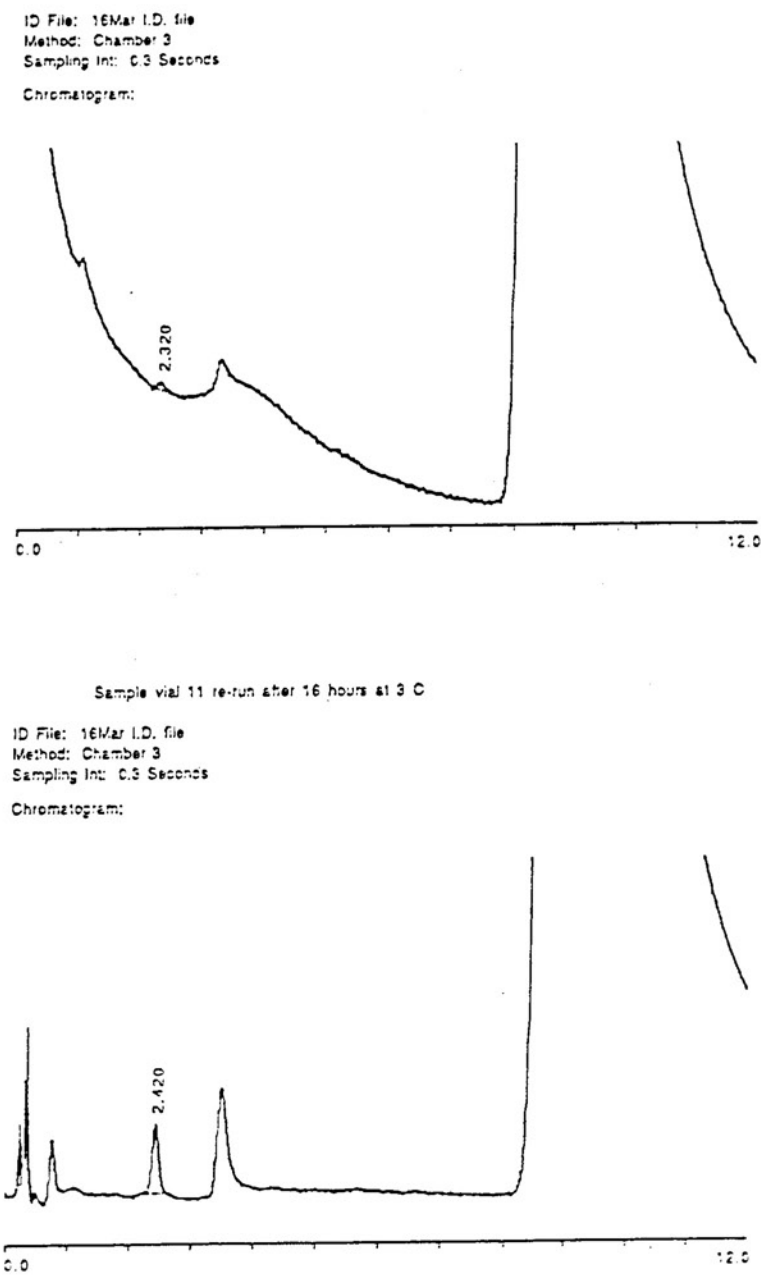


Fig. 4. Chromatogram of a presumed decomposition product from 5-HT detected in HPLC analysis of DA. Sample is from superfusion experiment performed as described in text except that no tissue was present in the superfusion chamber and no preservative (sodium metabisulfite or $HClO_4$) was present in the collection vial. Panel A shows results of immediate analysis of sample (vial number 12) with large 5-HT peak (t >7 min) but no peak with retention time similar to DA (2.6 min). Panel B is results of analysis of the same sample after 16 hours at 3°C.

RESULTS

1. RATIONALE

In the experiments described below, I have compared regulation of overflow of pre-loaded radiolabeled dopamine ([^{3}H]DA) from NACC and STR. I have used releasers that work through receptors to activate vesicular release, and others that work via receptor-independent mechanisms. To confirm the interpretation regarding the mechanism of action employed, I have also examined the effects of cations critical to individual processes. I have used Ca-free buffer containing EGTA to confirm release by vesicular mechanisms. Mg-free buffer and the selective antagonists MK-801 and CNQX were employed to help elucidate the contribution of excitatory amino acids to release stimulated by various agents. To evaluate the contribution of interneuron activity to my observations, I have investigated the effects of tetrodotoxin (TTX), an inhibitor of action potential propagation, on the stimulated release of [^{3}H]DA. To investigate the possible contribution of GABAergic inhibitory inputs, I have employed picrotoxin and muscimol. In order to confirm that the effects observed under some of the experimental conditions above were not artifacts resulting from the use of tissue which had been pre-loaded with radiolabel, I have measured overflow of endogenous DA from STR and NACC in a limited number of experiments. Endogenous overflow was stimulated by K and 5-HT in control and Mg-free buffers, with and without GBR present, as well as in Ca-free buffer. Overflow was also simulated by NMDA in Mg-free buffer with and without GBR present.

2. MEASUREMENT OF [^{3}H]DOPAMINE AND ENDOGENOUS DOPAMINE

Chromatography of representative samples of superfusate (and, where appropriate, liquid scintillation spectroscopy of the separated fractions) indicated that the peak of radioactivity co-chromatographed with dopamine, suggesting that the primary species measured by liquid scintillation spectroscopy was [^{3}H]DA and not a metabolite. Representative experiments performed with only one stimulation period (S2) indicated that the presence of a previous period of K-stimulated release (S1) had no significant effect on the subsequent release of [^{3}H]DA by K or the other releasers. In order to compensate for possible differences among the 18 superfusion channels, the chambers designated as "Control" were varied on a systematic basis, and the ionic conditions or concentration gradients of tested compounds were routinely altered with respect to their position in the superfusion apparatus.

a. Baseline (spontaneous) overflow

Baseline (spontaneous) overflow of pre-loaded [^{3}H]DA was considered to be the radioactivity detected in the absence of applied releaser. The overflow under the conditions employed in this study was stable throughout the experiment. Overflow under control conditions (expressed as % of total radiolabel remaining) was 0.81 $\pm$ 0.03 in STR and 0.60 $\pm$ 0.02 in NACC in each fraction (Table 1). The difference between spontaneous release in STR and NACC was significant by ANOVA ($p < 0.01$). Ca-free buffer (containing EGTA) reduced spontaneous overflow by approximately 60% in STR and 50% in NACC . Mg-free buffer enhanced and 1 μM TTX decreased baseline overflow in NACC. The DA transport inhibitor GBR and the non-selective transport

	STRIATUM		NUCLEUS ACCUMBENS	
Control	0.81% ± 0.03% (N = 35)		0.60% ± 0.02% (N = 33)	
	Average % Control	N	Average % Control	N
+GBR (1 μM)	155 ± 10 *	(7)	131 ± 8 *	(7)
+FLX (1 μM)	97 ± 3	(7)	103 ± 3	(7)
+NOM (1 μM)	127 ± 3 *	(7)	114 ± 6 *	(7)
+cocaine (10 μM)	125 ± 6 *	(7)	102 ± 8	(7)
Ca-free	38 ± 4 *	(9)	50 ± 6 *	(4)
+TTX (1μM)	96 ± 8	(11)	77 ± 4 *	(10)
Mg-free	127 ± 18	(6)	154 ± 13 *	(7)
Mg-free + TTX	103 ± 8	(4)	98 ± 18	(5)
+MK-801 (1 μM)	(83 &150)	(2)	(143 & 79)	(2)
Mg-free + MK-801	(110 & 198)	(2)	(124 & 97)	(2)
+picrotoxin (50 μM)	103 ± 18	(4)	(103 & 136)	(2)
+CNQX (10 μM)	98 ± 6	(3)	103 ± 1	(3)

Table 1. Baseline (spontaneous) overflow of [^{3}H]DA from slices of STR or NACC of rat brain in various buffers. Control data are expressed as average percent of radioactivity collected in each fraction (vial), where no stimulator was present, relative to that contained in the tissue at the beginning of the collection interval (%). Reported values of overflow (% Control) for each different buffer are means from N independent experiments ± s.e.m. * indicates significant difference ($p < 0.05$) from Control. Values in parentheses indicate values for $N < 3$ experiments. "Control" buffer indicates 2.5 mM Ca and 1.2 mM Mg. Ca-free indicates the presence of 1 mM EGTA. Mg-free indicates no $MgSO_4$ in buffer. "+TTX" indicates the presence of 1 μM TTX and "+MK-801" indicates the presence of 1 μM MK-801.

inhibitor NOM (1 μM) significantly enhanced overflow in both STR and NACC. The enhancement of overflow mediated by the non-selective transport inhibitor cocaine (10 μM) was significant only in the STR. FLX, the transport inhibitor selective for 5-HT, at 1 μM had no effect on spontaneous overflow in either STR or NACC. Picrotoxin (50 μM), CNQX (10 μM), or MK-801 (1 μM) had no significant effect on spontaneous overflow of [^{3}H]DA.

Under control conditions, an overflow of endogenous DA is evident (Table 2) which appears stable throughout the superfusion period. DA overflow under control conditions (expressed as pmol/mg protein/fraction) was 0.53 ±.08 in STR and 0.19 ± 0.06 in NACC. The difference between spontaneous release in STR and NACC was significant by ANOVA ($p < 0.05$). This spontaneous DA overflow parallels the release of [^{3}H]DA under several conditions. Baseline DA overflow is reduced significantly by removal of Ca in both STR and NACC (Table 2). The spontaneous overflow is enhanced by the presence of 1 μM GBR. This suggests that, under conditions employed in these studies, DA released spontaneously into the extracellular environment is partially recovered by the transporter.

b. Comparison of [^{3}H]dopamine and endogenous dopamine release by various stimulating agents

Release of [^{3}H]DA was effected by including (in different samples) the following in the stimulation buffers: 20 mM K (with a commensurate reduction of Na), D-amphetamine (10 μM), L-glutamate (25 μM, 100 μM and 1 mM), NMDA (25 and 100 μM), and 5-HT (10 and 100 μM). These compounds stimulated the release of [^{3}H]DA in a concentration-dependent manner at the concentrations tested under the conditions employed in this study. The concentrations used were chosen such that the release effected was reliably measurable and within

Spontaneous Overflow

	AVG (pmol/mg protein/vial)				N
STRIATUM					
Control	0.5	±	0.1		(5)
Ca-free	0.2	±	0.1	*	(3)
+GBR (1μM)	4.7	±	0.4	*	(3)
Mg-free	0.6	±	0.1		(8)
Mg-free + 1 μM GBR	5.3	±	1.4	*	(4)
NUCLEUS ACCUMBENS					
Control	0.2	±	0.1		(4)
Ca-free	0.1	±	0.0		(3)
+GBR (1μM)	0.8	±	0.3	*	(3)
Mg-free	0.3	±	0.2		(3)
Mg-free + 1 μM GBR	1.0	±	0.6		(3)

Table 2. Baseline (spontaneous) overflow of endogenous DA from slices of STR or NACC of rat brain in various buffers. Data are expressed as average pmol/mg protein/fraction collected in each vial during periods when no stimulator was present (baseline). Reported values of overflow for each different buffer are means from N independent experiments ± s.e.m. * indicates significant difference ($p < 0.05$) from Control. "Control" buffer indicates 2.5 mM Ca and 1.2 mM Mg. "Ca-free" indicates the presence of 1 mM EGTA. "Mg-free" indicates no $MgSO_4$ in buffer.

the ascending portion of the dose-response curve. The effects of Mg-free buffer, and TTX (1 μM) in buffers both with and without Mg, were determined as was the effect of buffer containing 1 mM EGTA and no exogenous Ca ("Ca-free buffer") (Tables 3 and 4). ANOVA indicated significant treatment differences for each stimulator except 100 μM 5-HT. The amount of [^{3}H]DA released by this high concentration of 5-HT was not influenced by these changes in ionic composition of the superfusing medium or by TTX.

Release of endogenous DA was effected, as above, by including the following in the stimulation buffers: 20 mM K (with a commensurate reduction of Na), 5-HT (10 μm), NMDA (25 μM), and L-glutamate (1 mM) (Tables 5, 6, and 7). I determined that these compounds, as well as GBR (1 μM), Ca-free buffer (+1 mM EGTA), or Mg-free buffer, did not interfere with the measurement of DA by HPLC/EC under the chromatographic conditions employed. The potential problem of measuring DA in the presence of 10 μM 5-HT is discussed above.

(i) Elevated Potassium

Elevated K (20 mM) released about twice as much [^{3}H]DA from NACC as it did from STR (Table 3) ($p < 0.01$). The release was reduced by at least 80% in Ca-free buffer in both STR and NACC. The presence of 1 μM TTX, which inhibits voltage-sensitive Na channels and thus inhibits the propagation of action potentials, appeared to enhance K-stimulated release in the STR but the difference did not attain statistical significance over all experiments. However, in 5 of 5 experiments where direct comparison of control release with release in the presence of TTX was possible, paired t-statistic indicated a significant increase ($p < 0.05$). In contrast to STR, TTX significantly reduced K-stimulated release (by 72%) in the NACC. Removal of Mg increased release stimulated by

Table 3. Stimulation of [^{3}H]DA release from slices of STR or NACC of rat brain: effect of Ca removal and TTX addition. Data are expressed as percent of radioactivity released by the stimulating agent relative to that contained in the tissue at the beginning of the stimulus interval (Fractional Release, %). Reported values of fractional release (%) are means from N independent experiments, each of which was performed in duplicate, ± s.e.m. * indicates significant difference ($p<0.05$) from Control.

	Control			Ca-free (1 mM EGTA)			1 μM TTX		
	Frac rel(%)		N			N			N
STRIATUM									
20 mM K	**4.8**	± *0.2*	(12)	**1.0**	± *0.1*	(8) *	**6.4**	± *0.5*	(8)
glutamate (1 mM)	**0.78**	± *0.07*	(6)	**0.10**	± *0.04*	(5) *	**0.16**	± *0.07*	(3) *
amphetamine (10 μM)	**21**	± *2*	(10)	**12**	± *2*	(7) *	**23**	± *4*	(5)
5-HT (10 μM)	**3.8**	± *0.3*	(13)	**4.6**	± *0.8*	(6)	**4.1**	± *0.5*	(7)
5-HT (100 μM)	**15**	± *1*	(10)	**19**	± *2*	(5)	**14**	± *2*	(5)
NUCLEUS ACCUMBENS									
20 mM K	**9**	± *0*	(15)	**1.1**	± *0.1*	(10) *	**2.5**	± *0.4*	(7) *
glutamate (1 mM)	**1.3**	± *0.1*	(8)	**0.23**	± *0.06*	(7) *	**0.91**	± *0.34*	(4)
amphetamine (10 μM)	**14**	± *1*	(10)	**9.7**	± *0.7*	(6) *	**11**	± *2*	(4)
5-HT (10 μM)	**5.5**	± *0.5*	(15)	**4.6**	± *0.8*	(9)	**4.9**	± *1.3*	(6)
5-HT (100 μM)	**16**	± *3*	(4)	**19**	± *3*	(4)	**13**	± *3*	(3)

	Mg-free			1 μM TTX (-Mg)		
	Frac rel(%)	N		Frac rel(%)	N	
STRIATUM						
20 mM K	7.9 ± 0.8	(9)	*	11 ± 1	(10)	*,†
glutamate (25 μM)	1.4 ± 0.2	(3)	a	0.85 ± 0.10	(3)	†
glutamate (100 μM)	3.5 ± 0.4	(3)	a	1.2 ± 0.2	(3)	†
glutamate (1 mM)	4.3 ± 0.4	(5)	*	2.4 ± 0.3	(3)	*,†
NMDA (25 μM)	2.4 ± 0.2	(9)	a	0.67 ± 0.28	(6)	†
NMDA (100 μM)	5.3 ± 0.3	(5)	a	2.1 ± 0.6	(5)	†
amphetamine (10 μM)	22 ± 0	(3)		29 ± 3	(4)	
5-HT (10 μM)	1.0 ± 0.2	(4)	*	1.4 ± 0.3	(6)	*
5-HT (100 μM)	19 ± 2	(3)		16 ± 2	(3)	
NUCLEUS ACCUMBENS						
20 mM K	15 ± 1	(8)	*	5.1 ± 0.6	(9)	*,†
glutamate (25 μM)	2.1 ± 0.2	(4)	a	1.0 ± 0.2	(3)	†
glutamate (1 mM)	8.9 ± 0.8	(5)	*	4.0 ± 0.7	(4)	*,†
NMDA (25 μM)	3.3 ± 0.3	(10)	a	0.59 ± 0.13	(3)	†
NMDA (100 μM)	5.0 ± 0.7	(4)	a	1.9 ± 0.4	(4)	†
amphetamine (10 μM)	11 ± 1	(4)		10 ± 3	(3)	
5-HT (10 μM)	1.5 ± 0.6	(5)	*	4.3 ± 0.5	(5)	
5-HT (100 μM)	20 ± 5	(3)		24 ± 4	(3)	

Table 4. Stimulation of [^{3}H]DA release from slices of STR or NACC of rat brain: effect of Mg removal in the absence and presence of TTX. Data are expressed as percent of radioactivity released by the stimulating agent relative to that contained in the tissue at the beginning of the stimulation interval (Fractional Release, %). Reported values of fractional release (%) are means from N independent experiments, each of which was performed in duplicate, ± s.e.m. * indicates significant difference ($p < 0.05$) from Control (Table 1 values). † indicates significant difference ($p < 0.05$) from Mg-free values (Table 2). [a] Control experiments in the presence of Mg were not performed with these treatments because release above background was not detected reliably in the presence of Mg.

K stimulated release

	AVG (pmol/mg protein/vial)				N
STRIATUM					
Control	2.6	±	0.4		(5)
Ca-free	0.4	±	0.3	*	(3)
+GBR (1μM)	7.9	±	2.5	*	(4)
Mg-free	3.2	±	0.6		(8)
Mg-free + 1 μM GBR	12.5	±	2.8	*	(3)
NUCLEUS ACCUMBENS					
Control	1.2	±	0.6		(4)
Ca-free	0.1	±	0.1	*	(3)
+GBR (1μM)	1.8	±	0.7		(3)
Mg-free	1.2	±	0.7		(3)
Mg-free + 1 μM GBR	3.1	±	1.6		(3)

Table 5. Stimulation of release of endogenous DA from slices of STR or NACC of rat brain by elevated potassium. Data are expressed as pmol/mg protein/fraction collected in each vial following stimulation by buffer containing 20 mM K (S1). Reported values of overflow under each condition are means from N independent experiments ± s.e.m. * indicates significant difference ($p < 0.05$) from Control. "Control" buffer indicates 2.5 mM Ca and 1.2 mM Mg. "Ca-free" indicates the presence of 1 mM EGTA. "Mg-free" indicates no $MgSO_4$ in buffer.

	NMDA-stimulated release				
	AVG (pmol/mg protein/vial)				N
STRIATUM					
Mg-free	6.5	±	0.9		(4)
Mg-free + 1 μM GBR	75.3	±	21.0	†	(4)
NUCLEUS ACCUMBENS					
Mg-free	1.6	±	0.7		(3)
Mg-free + 1 μM GBR	8.6	±	3.4	†	(3)

Table 6. Stimulation of release of endogenous DA from slices of STR or NACC of rat brain by N-methyl-D-aspartate. Concentration of NMDA was 25 μM. Data are expressed as pmol/mg protein/fraction collected following stimulation (S2). Reported values of overflow for each condition are means from N independent experiments ± s.e.m. † indicates significant difference ($p < 0.05$) from release in "Mg-free" buffer.

5-HT-stimulated release

	AVG (pmol/mg protein/vial)				N
STRIATUM					
Control	4.0	±	1.1		(5)
Ca-free	8.0	±	4.5		(3)
+GBR (1 μM)	0.5	±	0.3	*	(3)
Mg-free	2.7	±	0.6		(3)
NUCLEUS ACCUMBENS					
Control	1.1	±	1.0		(3)
Ca-free	1.2	±	0.5		(3)
+GBR (1 μM)	0.0	±	0.0	*	(3)

Table 7. Stimulation of release of endogenous DA from slices of STR or NACC of rat brain by serotonin. Concentration of 5-HT was 10 μM. Data are expressed as pmol/mg protein/fraction collected following stimulation (S2). Reported values of overflow for each condition are means from N independent experiments ± s.e.m. * indicates significant difference ($p < 0.05$) from Control. "Control" buffer indicates 2.5 mM Ca and 1.2 mM Mg. "Ca-free" indicates the presence of 1 mM EGTA. "Mg-free" indicates no $MgSO_4$ in buffer.

K in both STR and NACC (compare Table 3 with Table 4). The effect of 1 μM TTX on K-stimulated release in the absence of Mg (Table 4) was similar to its effects in the presence of Mg, specifically, an increased release in STR and decreased release in NACC relative to Mg-free control.

In order to determine the possible contribution of glutamate receptors to K-induced release, MK-801, a noncompetitive antagonist of the NMDA receptor/channel complex, and CNQX, a non-selective antagonist of compounds acting at non-NMDA EAA receptors (Honore et al. 1988) were tested. Neither 1 μM MK-801 (± Mg) (data not shown) nor 10 μM CNQX (Fig 5) had a significant effect on K-stimulated release in either STR or NACC. These data are consistant with the hypothesis that activation of glutamate receptors does not contribute significantly to K-stimulated release.

The possible contribution of $GABA_A$ receptor activation to the K-stimulated release of [^{3}H]DA was tested using picrotoxin, a $GABA_A$ antagonist, and muscimol, a $GABA_A$ agonist. Picrotoxin alone (50 μM) appeared to enhance K-stimulated release in STR but not in NACC (Table 8). Picrotoxin had no additional effect on K-stimulated release in the presence of 1 μM TTX in either tissue. Similarly, muscimol (1 μM) alone had no significant effect on K-stimulated release in either STR or NACC and did not alter the TTX-induced inhibition of K-stimulated release. These data suggest that $GABA_A$ receptors do not modulate DA release under the experimental conditions employed.

Elevated K increased overflow of endogenous DA (Table 5). Ca-free buffer greatly diminished background release as well as K-stimulated release in both STR and NACC. Table 5 represents release corrected for the weight of protein in the superfusion chamber at the end of the superfusion period. Removal of Ca reduces the amount of DA released per mg of protein. K-stimulated release of endogenous DA is also enhanced by removal of Mg and

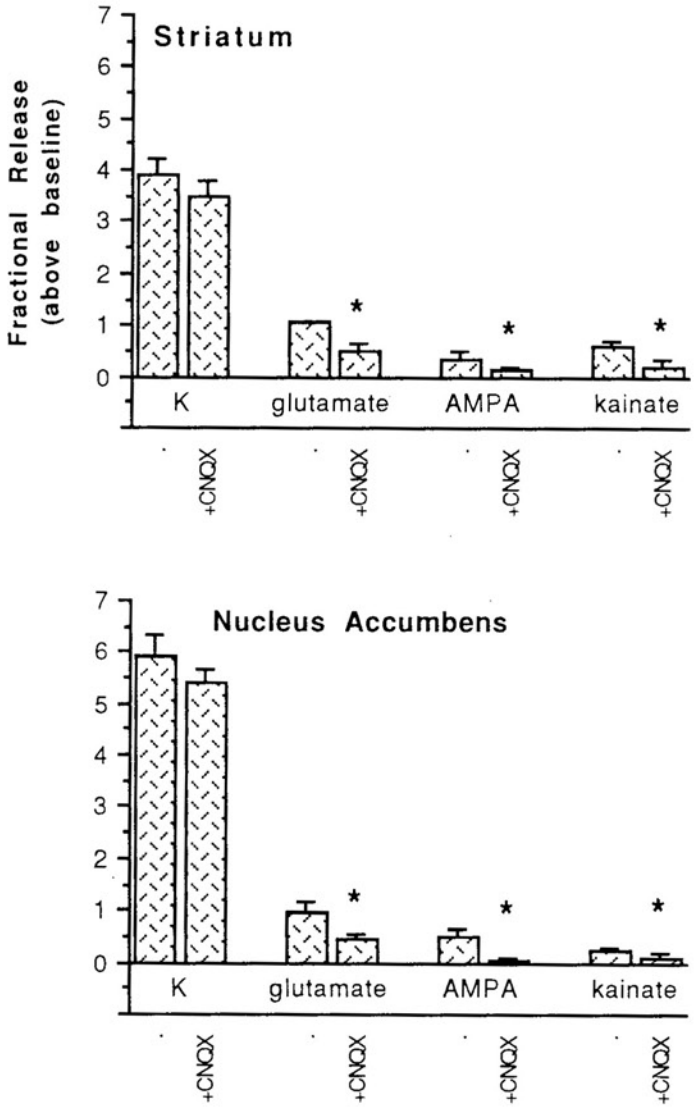


Fig. 5. Effect of CNQX on stimulated release of [^{3}H]DA from slices of STR or NACC of rat brain. Data are expressed as Fractional Release above baseline. Release was stimulated by application of buffer containing elevated K (20 mM), L-glutamate (1 mM), AMPA (10 μM), or kainate (10 μM) for a period of 2 min. Bars represent means ± s.e.m. from triplicate determinations in each of three independent experiments. "+CNQX" indicates chambers where CNQX (10 μM) was present in buffers from the beginning of superfusion. Tissue not exposed to CNQX was exposed to buffer containing 0.1% (v/v) DMSO which was the vehicle for CNQX. * indicates significant difference ($p < 0.05$) between EAA and EAA + CNQX (ANOVA).

	STRIATUM			NUCLEUS ACCUMBENS	
	Average % Control		N	% Control	
picrotoxin (50 μM)	117	± 22	(4)	(107 & 103)	
muscimol (1μM)	120	± 15	(4)	(112 & 121)	
Release in the presence of TTX (1 μM)					
+TTX	125	± 8 *	(4)	(70 & 49)	#
picrotoxin + TTX	117	± 13	(4)	(65 & 78)	#
muscimol + TTX	125	± 15	(4)	(77 & 64)	#

Table 8. Effect of $GABA_A$-selective compounds on K-stimulated release of [^{3}H]DA from slices of STR or NACC of rat brain. Reported values of overflow (% Control) for each different buffer are means from N independent experiments ± s.e.m. * indicates significant difference ($p < 0.05$) from Control (paired t-statistic). # indicates significant difference ($p < 0.05$) from Control (ANOVA). Release was stimulated by introduction of elevated K (20 mM final concentration) into the buffer for a period of 2 min (S1). "Picrotoxin" indicates chambers where picrotoxin (50 μM) was present in buffers from the beginning of superfusion. "TTX" indicates chambers where TTX (1 μM) was present in buffers from the beginning of superfusion. "Muscimol" indicates chambers which were exposed to 1 μM muscimol only in the stimulation buffer with 20 mM K. All tissue was allowed to accumulate [^{3}H]DA in the absence of picrotoxin, TTX or muscimol (Control buffer).

by addition of the DA uptake inhibitor GBR 12909 in both STR and NACC. The effect of 1 μM GBR on K-stimulated release is discussed in section **d**(i), concerning uptake inhibition by GBR 12909.

(ii) L-glutamate and NMDA

L-Glutamate stimulated a slightly greater percentage of release of [^{3}H]DA in NACC than in STR (Table 3). The release was reduced by at least 80% in Ca-free buffer in both STR and NACC. Release of [^{3}H]DA was reduced by TTX (1 μM) in STR (80%) but not in NACC. In Mg-free buffer, 1 mM L-glutamate-stimulated release was enhanced by 550% in STR and 680% in NACC (compare Table 3 and Table 4). In the absence of Mg, TTX (1 μM) reduced L-glutamate-stimulated release (at all concentrations tested) by approximately 60% in both STR and NACC (Table 4). Even with the reduction caused by TTX, release in Mg-free buffer was significantly greater than release in the presence of Mg. MK-801 (1 μM), a noncompetitive NMDA receptor-channel blocker, had no effect on L-glutamate-stimulated release in the presence of Mg (Fig. 6). In the absence of Mg, release stimulated by L-glutamate was reduced by MK-801 to a level comparable to release stimulated in the presence of Mg but not to as low a level as that stimulated in Ca-free buffer. CNQX (10 μM) is a non-selective antagonist of compounds acting at non-NMDA EAA receptors (Honore et al. 1988). CNQX consistently reduced the values for the mean fractional release induced by L-glutamate, AMPA, and kainate in the presence of Mg in both tissues (Fig 5). ANOVA performed on the data set for all glutamate agonists indicated a significant effect ($p < 0.05$) of CNQX on release of [^{3}H]DA.

All NMDA-stimulated release reported was performed in Mg-free buffers because NMDA produced negligible release in the presence of Mg (data not

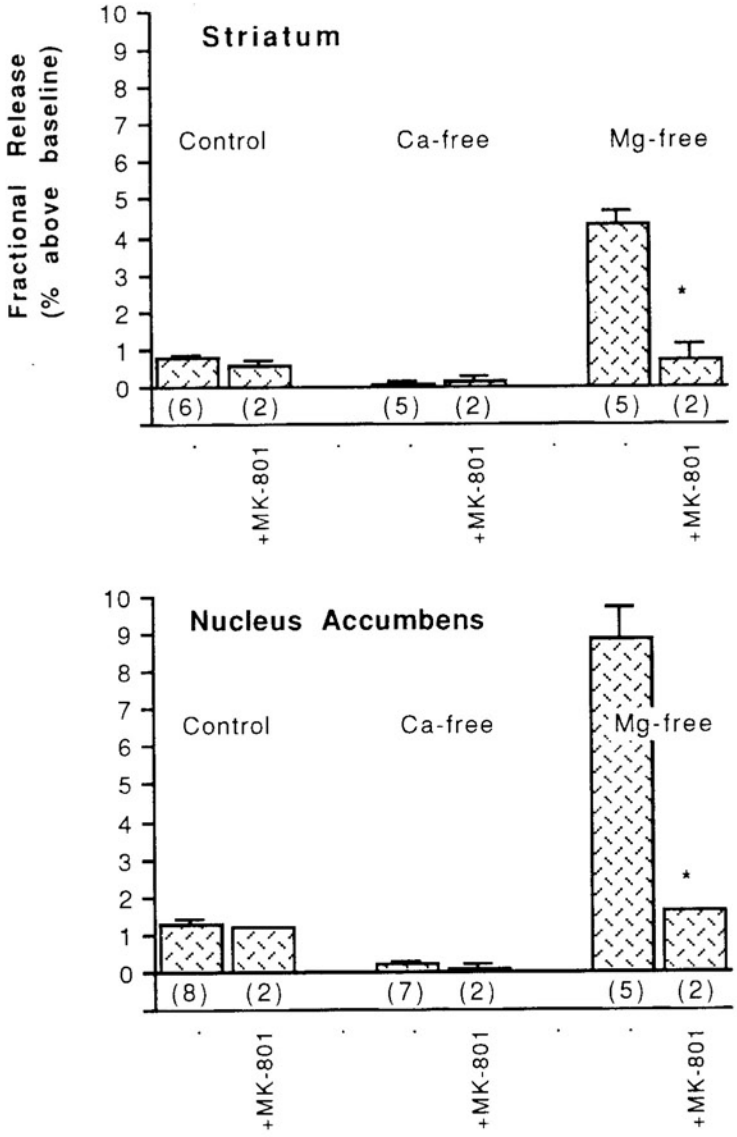


Fig. 6. Effect of MK-801 on L-glutamate-stimulated release of [^{3}H]DA from slices of STR or NACC of rat brain. Data are expressed as Fractional Release above baseline. Release was stimulated by introduction of L-glutamate (1 mM) into the superfusing buffer for a period of 2 min. Bars labeled "+MK-801" represent means ± standard deviation from triplicate determinations in each of two independent experiments. Values reported in the absence of MK-801 are from Table 4. "+MK-801" indicates chambers where MK-801 (1 μM) was present in buffers from the beginning of superfusion. "Ca-free" indicates buffer which contained 1 mM EGTA and no exogenous Ca. "Mg-free" indicates buffers with no exogenous Mg present. All tissue was allowed to accumulate [^{3}H]DA in the presence of Ca and Mg and in the absence of EGTA (conditions identical to "Control"). * indicates significant difference ($p < 0.05$) between treatment and treatment in the presence of MK-801 (ANOVA).

shown). In both STR and NACC, removal of Mg (Table 4) increased release stimulated by NMDA to reliably measurable levels. TTX reduced NMDA-stimulated release significantly in both STR and NACC. MK-801, at concentrations of 1 μM and above, greatly reduced NMDA-stimulated release in both tissues (Fig 2).

L-Glutamate was able to stimulate release of endogenous DA. This release was abolished in Ca-free buffer (Fig. 7). NMDA (25 μM) was also able to release endogenous DA (Table 6) in Mg-free buffer. The effect of GBR 12909 on the ability of NMDA to stimulate release of DA (in Mg-free buffer) is discussed in section **d**(i).

(iii) D-amphetamine

D-Amphetamine released significantly more [^{3}H]DA ($p < 0.05$) from STR than from NACC (Table 3). Ca-free buffer reduced D-amphetamine-stimulated release by at least 30% in both tissues. TTX did not reduce or enhance release stimulated by D-amphetamine in either STR or NACC. Release stimulated by D-amphetamine was not affected by Mg removal (compare Table 3 and 4). In Mg-free buffer, TTX had little effect on D-amphetamine-stimulated release. The effect of D-amphetamine on endogenous DA release was not investigated.

(iv) serotonin

5-HT (10 μM) released more [^{3}H]DA from NACC than from STR ($p < 0.05$) (Table 3). In contrast, 100 μM 5-HT was equally efficacious in both tissues. Similarly, release of [^{3}H]DA stimulated by either 10 μM or 100 μM 5-HT was not significantly reduced or enhanced in either tissue by removal of Ca (Table 3).

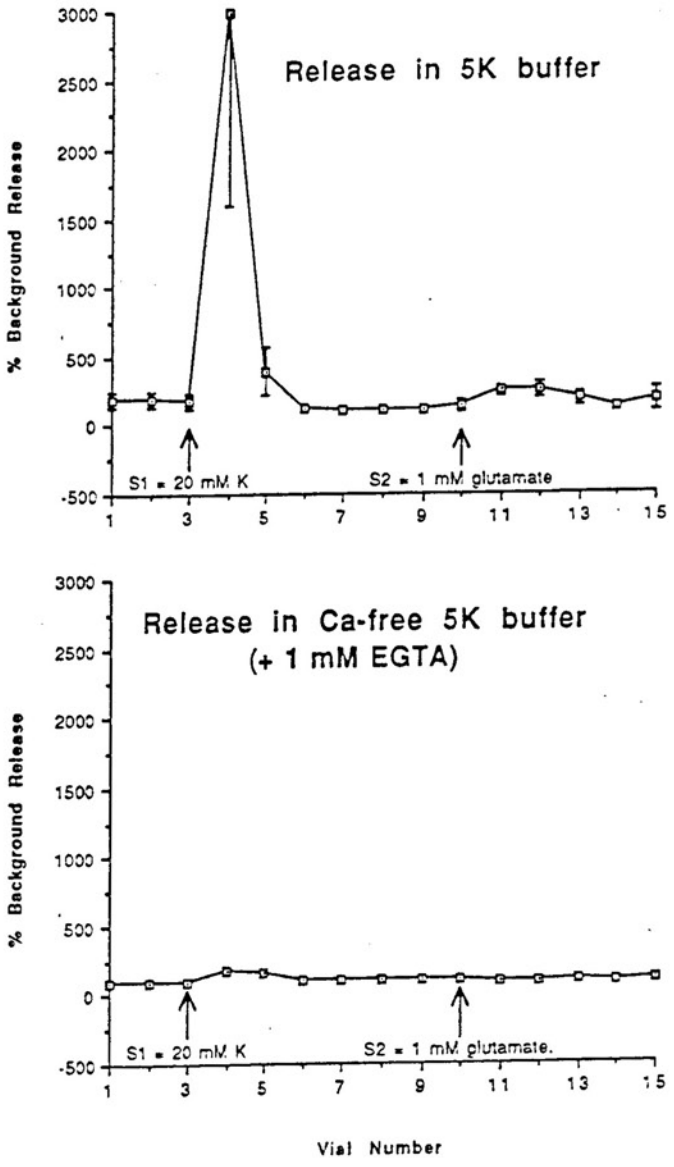


Fig. 7. Stimulation of release of endogenous DA from slices of STR of rat brain. Release of endogenous DA was stimulated by 20 mM K at t = 3 min (S1) and 1 mM L-glutamate at t = 10 min (S2). The experiment was performed in buffer containing Mg. Each point represents the average ± s.e.m. from three independent experiments.

TTX did not reduce or enhance release of [^{3}H]DA stimulated by 5-HT in either STR or NACC. Release stimulated by 100 μM 5-HT was not affected by removal of Mg (Compare Table 3 to Table 4) but release of [^{3}H]DA stimulated by a lower concentration of 5-HT (10 μM) was reduced significantly (by at least 70%) in either STR or NACC. In Mg-free buffer, TTX had little effect on 100 μM 5-HT-stimulated release of [^{3}H]DA and potentiated 10 μM 5-HT-stimulated release.

5-HT (10 μM) also released endogenous DA from both STR and NACC (Table 7). As was the case for release of [^{3}H]DA, release of endogenous DA by 10 μM 5-HT was not reduced by Ca-free buffer in either tissue. The effect of 1 μM GBR 12909 on 5-HT stimulated release will be described in section **d**(i).

c. Effect of serotonin receptor antagonists on serotonin-induced dopamine release

It is possible that 5-HT releases [^{3}H]DA by activating one or more forms of 5-HT receptor. It has been suggested that activation of 5-HT_3 receptors induces DA release in NACC (Chen et al., 1991b) and STR (Blandina et al., 1989). To evaluate the role of 5-HT receptors in the release of [^{3}H]DA under my experimental conditions, the effects of 5-HT receptor agonists and antagonists have been tested.

The selective agonists at 5-HT_1 receptors (TFMPP and 8-OH-DPAT) or 5-HT_3 receptors (1-phenylbiguanide and 2-methyl-5-HT) did not evoke release at concentrations up to 10 μM. The 5-HT_2-selective agonist 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI) did not stimulate release at concentrations up to 10 μM. The 5-HT_2-selective agonist α-methyl-5-HT evoked release in a similar manner to 5-HT. However, this release was not antagonized by the 5-HT_2-selective antagonists ketanserin, ritanserin or spiperone but was reduced

by inhibition of the DA transporter. Preliminary results suggest that α-methyl-5-HT evokes release in the same fashion as 5-HT (see below).

The selective antagonists at 5-HT_3 receptors, ICS 205930 or metoclopramide, failed to affect the release of [^{3}H]DA induced by 10 μM 5-HT in either STR or NACC (Fig. 8) at concentrations up to 1 μM (i.e. concentrations 60-fold and 20-fold higher, respectively, than their apparent affinities at 5-HT_3 receptors; Murphy, 1990, Fozard et al., 1977). MDL 72222 at 1 μM also failed to modify 5-HT-mediated [^{3}H]DA release (data not shown). These results suggest that under our experimental conditions, the release-inducing effects of 5-HT were not dependent on activation of 5-HT_3 receptors.

Antagonism of 5-HT_2 receptors was also without measurable effect on release stimulated by 10 μM 5-HT. The 5-HT_2 receptor-selective antagonists ketanserin and 1-(1-napthyl)piperazine did not inhibit 5-HT-mediated release of [^{3}H]DA in either STR or NACC (Fig. 9) at concentrations more than 50-fold higher than their apparent affinities at 5-HT_2 receptors (Murphy, 1990; Glennon, 1987). Similar results were obtained with 1 μM ritanserin (data not shown). ANOVA indicates no significant difference between control release and release stimulated in the presence of either 5-HT_2- or 5-HT_3-selective antagonists. Concentrations of (-)-propranolol, a 5-HT_1 receptor antagonist, in the range 1 nM to 1 μM sometimes increased release of [^{3}H]DA induced by 10 μM 5-HT in STR and NACC (Fig. 10). However, similar effects were observed with the same concentrations of (+)-propranolol, the enantiomer with little affinity for 5-HT_1 or beta-adrenergic receptors (Nahorski and Willcocks, 1983; Middlemiss, 1984). Similarly, methiothepin, a 5-HT_1 antagonist, also had no significant effect on 5-HT-stimulated release of [^{3}H]DA in either tissue (data not shown). It seems unlikely that the observed effects of propranolol on 5-HT-induced

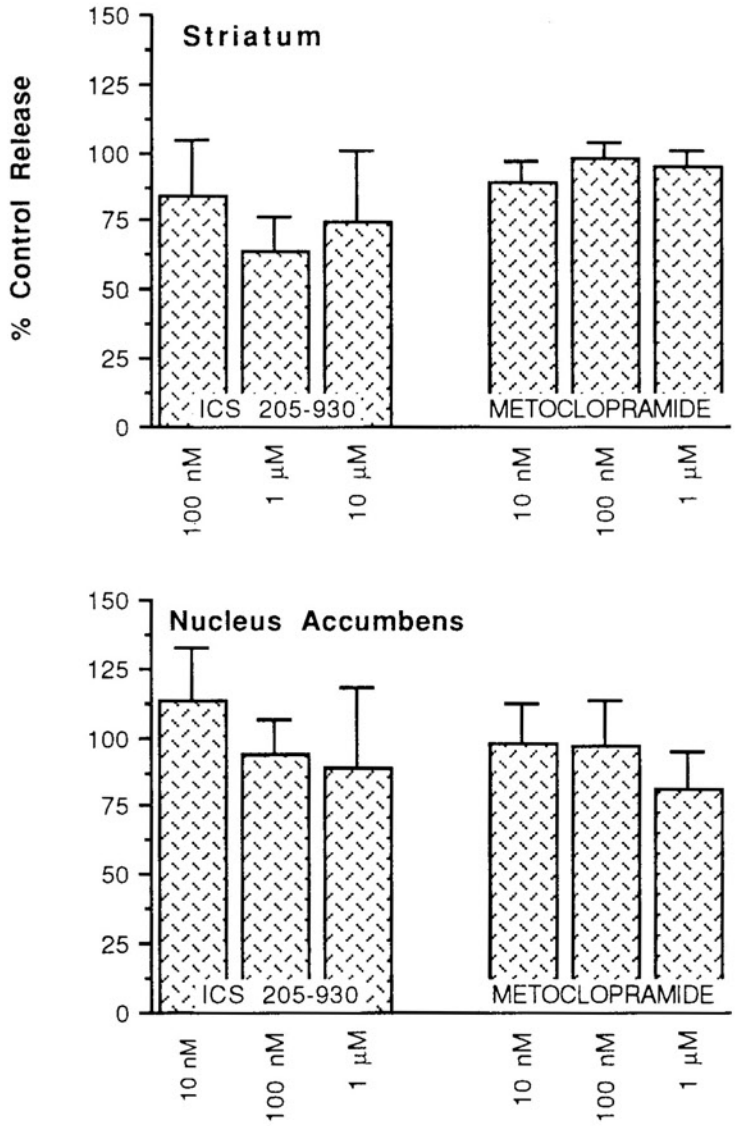


Fig. 8. Lack of effect of 5-HT$_3$ antagonists on 5-HT-stimulated release of [^{3}H]DA from slices of STR or NACC of rat brain. Data are expressed as percent of control release stimulated by 10 µM 5-HT. Bars represent average values ± s.e.m. from duplicate determinations in each of three or more independent experiments.

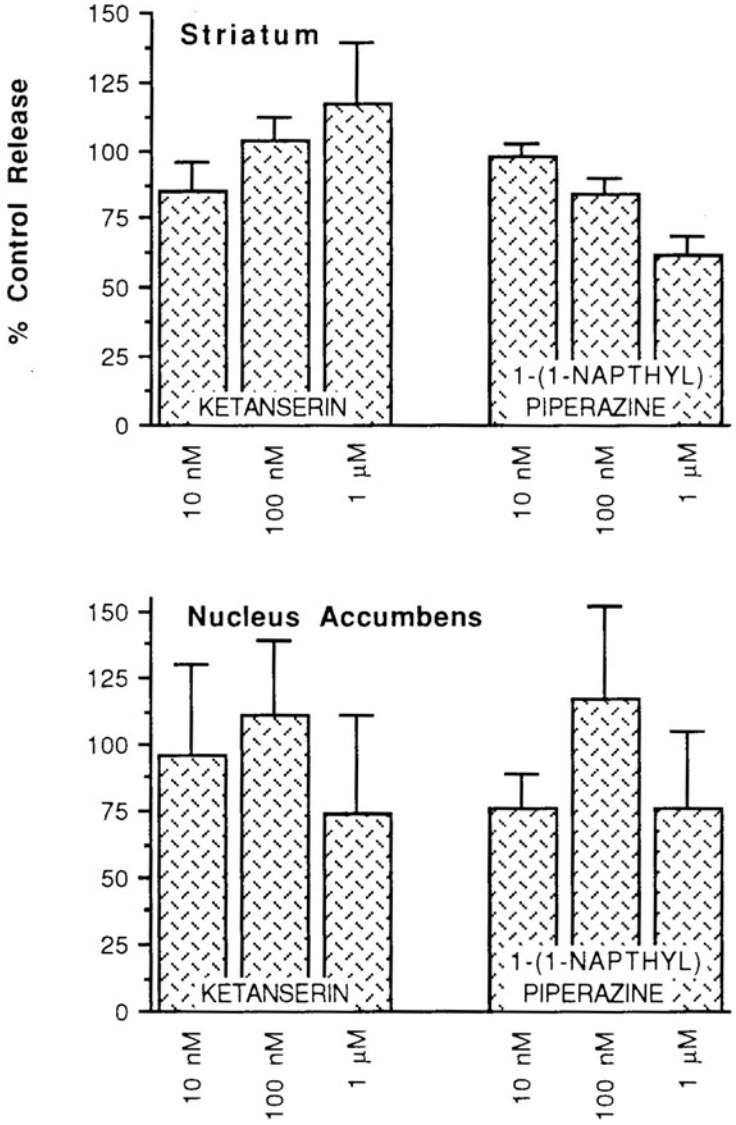


Fig. 9. Lack of effect of 5-HT$_2$ antagonists on 5-HT-stimulated release of [^{3}H]DA from slices of STR or NACC of rat brain. Data are expressed as percent of control release stimulated by 10 µM 5-HT. Bars represent average values ± s.e.m. from duplicate determinations in each of three or more independent experiments.

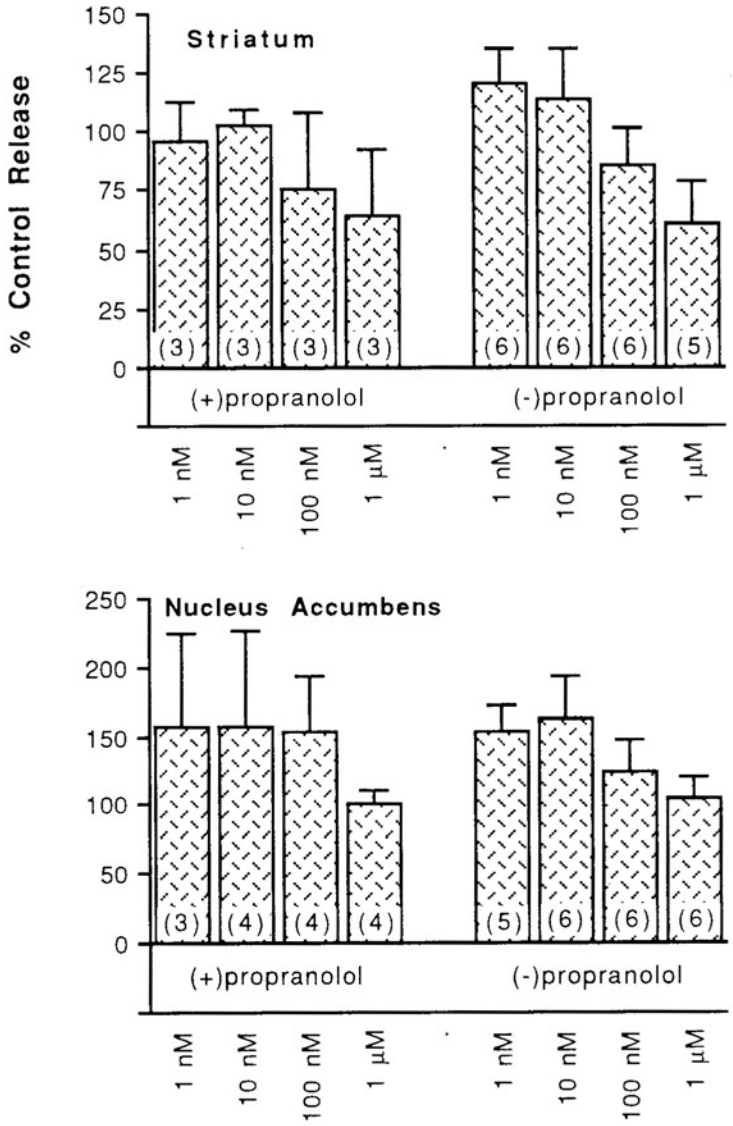


Fig. 10. Effect of the 5-HT$_1$ antagonist propranolol on 5-HT-stimulated release of [^{3}H]DA from slices of STR or NACC of rat brain. Data are expressed as per cent of control release stimulated by 10 μM 5-HT. Bars are average values ± s.e.m. from duplicate determinations in each of N independent experiments (N values are in parentheses at the base of each bar).

release were mediated through a specific receptor mechanism or that release is affected by 5-HT_1 receptor antagonism.

d. Effects of dopamine and serotonin transport inhibitors

In view of the failure of 5-HT-receptor antagonists to reduce the 5-HT-mediated release of [^{3}H]DA in STR and NACC and the insensitivity of this release to the removal of extracellular Ca (see above), I suggest that, under my experimental conditions, 5-HT does not induce DA release by acting on 5-HT receptors to activate an exocytotic release process. More probably, 5-HT acts through a carrier-mediated mechanism that might be similar to the process by which D-amphetamine induces [^{3}H]DA release. To test this hypothesis, the effects of inhibitors of monoamine re-uptake systems have been evaluated.

(i) GBR 12909

The selective DA transporter-blocker GBR 12909 (Andersen, 1987) inhibited D-amphetamine- and 5-HT-stimulated release of [^{3}H]DA in a concentration-dependent manner in both STR and NACC (Fig 11). ANOVA indicated significant differences in effects of GBR 12909 among stimulators of [^{3}H]DA release in both STR and NACC. However, in STR and NACC there was no significant difference between the effect of GBR 12909 on D-amphetamine- and 5-HT-stimulated release at any concentration of GBR 12909 tested. This suggests that, under these conditions, both 5-HT and D-amphetamine require the DA transporter to effect release of pre-loaded [^{3}H]DA. Release stimulated by elevated K, a non-specific, depolarizing release stimulator, was unaffected by the same concentrations of GBR 12909 that completely inhibited D-amphetamine- or 5-HT-stimulated release in the STR. In contrast, K-stimulated

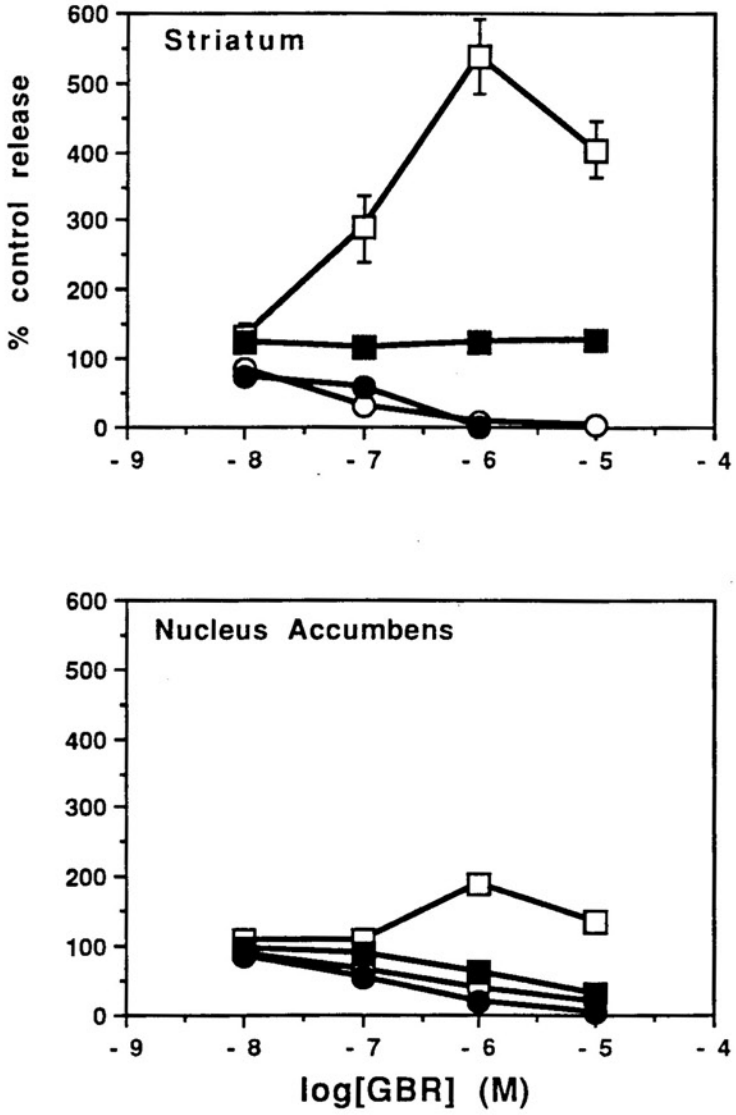


Fig. 11. Effect of GBR 12909 (GBR) on stimulated release of [^{3}H]DA from slices of STR or NACC of rat brain. Data are expressed as percent of fractional release elicited by the stimulating agent in the absence of GBR 12909. These values are reported in Tables 1 & 2. Release was stimulated by 10 μM D-amphetamine (open circles), 10 μM 5-HT (filled circles), 25 μM NMDA (open squares), or 20 mM K (filled squares). Points are means ± s.e.m., expressed as percent of control release, from duplicate determinations in each of 3 independent experiments for D-amphetamine, 5-HT, and NMDA and from 10 experiments for K. (In this and subsequent figures, the s.e.m. ranges sometimes fall within the symbol used to indicate the mean value.) Mg was deleted from the medium in all experiments using NMDA as a stimulator.

release in the NACC was reduced in a concentration-dependent manner by GBR 12909. There was no significant difference between K-stimulated release and 5-HT- or D-amphetamine-stimulated release at any concentration of GBR 12909 tested in the NACC. This is in contrast to all other uptake inhibitors tested (see below) where there were significant differences between the effects of uptake inhibitors on K-stimulated release compared to the effects of uptake inhibitors on release stimulated by 5-HT, D-amphetamine, or NMDA. Release stimulated by NMDA was enhanced by greater than five-fold in STR but only slightly in NACC.

In both STR and NACC, GBR 12909 (1 μM) enhanced baseline overflow and K-stimulated release of endogenous DA (Tables 2 and 5). In contrast to the release of [^{3}H]DA described above (Fig 11), GBR 12909 did not significantly decrease K-stimulated release in the NACC (Table 5). NMDA-stimulated release of endogenous DA was greatly enhanced in STR and enhanced in NACC (Table 6), paralleling the results described above for release of [^{3}H]DA (Fig 11). Also similar to the above results (Fig 11), GBR 12909 (1 μM) completely inhibited 5-HT-stimulated release of endogenous DA in both STR and NACC (Table 7).

(ii) Fluoxetine

At concentrations below 1 μM, FLX is a potent and selective inhibitor of the 5-HT transporter. At higher concentrations, FLX can inhibit the DA and NE transporters as well (Izenwasser et al., 1990a). ANOVA indicated significant differences among stimulators with respect to FLX effects in both STR and NACC. FLX had no significant effect on release of [^{3}H]DA stimulated by any releaser tested in the STR or NACC (Fig 12) at concentrations <1 μM. At 1 μM

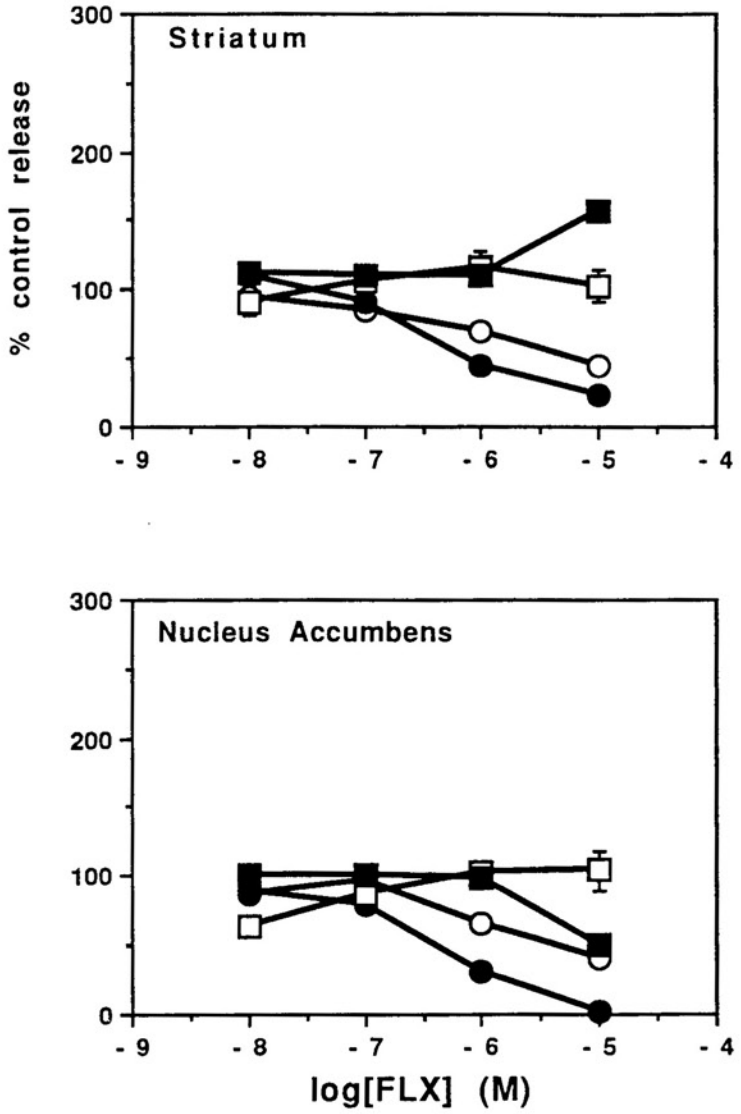


Fig. 12. Effect of fluoxetine (FLX) on stimulated release of [^{3}H]DA from slices of STR or NACC of rat brain. Data are expressed as percent of fractional release elicited by the stimulating agent in the absence of FLX. These values are reported in Tables 1 & 2. Release was stimulated by 10 μM D-amphetamine (open circles), 10 μM 5-HT (filled circles), 25 μM NMDA (open squares), or 20 mM K (filled squares). Points are means ± s.e.m., expressed as percent of control release, from duplicate determinations in each of 3 independent experiments for D-amphetamine, 5-HT, and NMDA and from 10 experiments for K. Mg was deleted from the medium in all experiments using NMDA as a stimulator.

and above, FLX inhibited both D-amphetamine- and 5-HT-stimulated [^{3}H]DA release in both tissues, presumably by blocking the DA and/or other monoamine transporters. In STR, there was no significant difference between the effect of FLX on D-amphetamine- and 5-HT-stimulated release at any concentration of FLX tested. At concentrations above 1 μM, there was a significant difference between the effect of FLX on 5-HT- and D-amphetamine-stimulated release in NACC. NMDA-stimulated release was unaffected in both tissues. At the highest concentration, K-stimulated release was modestly increased in STR and decreased in NACC.

(iii) Nomifensine

NOM is a non-selective blocker of monoamine transport. Although it is most potent at the DA transporter, it produces significant blockade of NE and 5-HT transporters (Tuomisto, 1977). The effects on release in both STR and NACC (Fig 13) were generally similar to those of the selective DA transport inhibitor GBR 12909. ANOVA indicated significant differences among stimulators with respect to NOM effects in both STR and NACC. NOM reduced the release of [^{3}H]DA stimulated by D-amphetamine and 5-HT in a concentration dependent manner, further indication that both compounds require the monoamine transporter to effect release. In both STR and NACC, there was no significant difference between the effect of NOM on D-amphetamine- and 5-HT-stimulated release at any concentration of NOM tested. Also similar to the effect of GBR 12909, NOM greatly enhanced release stimulated by NMDA in the STR but had little effect in the NACC. K-stimulated release was not reduced, but slightly enhanced, in both tissues. This finding was in contrast to the reduction of release effected by GBR 12909 in the NACC.

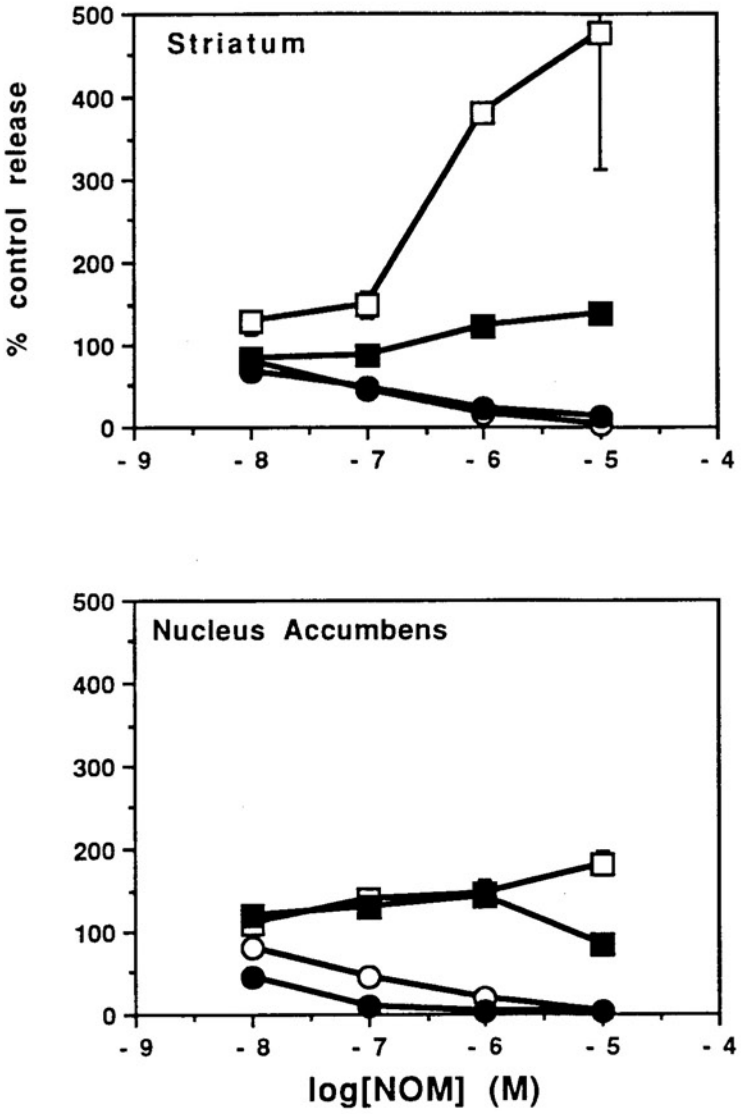


Fig. 13. Effect of nomifensine (NOM) on stimulated release of [^{3}H]DA from slices of STR or NACC of rat brain. Data are expressed as percent of fractional release elicited by the stimulating agent in the absence of NOM. These values are reported in Tables 1 & 2. Release was stimulated by 10 μM D-amphetamine (open circles), 10 μM 5-HT (filled circles), 25 μM NMDA (open squares), or 20 mM K (filled squares). Points are means ± s.e.m., expressed as percent of control release, from duplicate determinations in each of 3 independent experiments for D-amphetamine, 5-HT, and NMDA and from 9 (STR) or 10 (NACC) experiments for K. Mg was deleted from the medium in all experiments using NMDA as a stimulator.

(iv) Cocaine

Cocaine, like NOM, is a non-selective inhibitor of monoamine transport (Koe, 1976). The concentrations of cocaine tested ranged from 100 nM to 100 μM. ANOVA indicated significant differences among stimulators with respect to cocaine effects in both STR and NACC. The effects on release of pre-loaded [^{3}H]DA (Fig 14) were very similar to those of NOM (Fig. 13) and somewhat similar to that of GBR 12909 (Fig.11) in both STR and NACC. D-amphetamine- and 5-HT-stimulated release were reduced in a concentration-dependent manner in both STR and NACC, but the difference between the two stimulators was more pronounced than in the presence of NOM or GBR 12909. In STR, there was no significant difference between the effect of cocaine on D-amphetamine- and 5-HT-stimulated release at any concentration of cocaine tested. At concentrations above 1 μM, there was a significant difference between the effect of cocaine on 5-HT- and D-amphetamine-stimulated release in NACC. Also, K- and NMDA-stimulated release in STR were enhanced by cocaine, as by NOM, but at the highest concentration of cocaine tested (100 μM) NMDA induced less release than in the presence of 10 μM cocaine. The effect on K- and NMDA-stimulated release in NACC was minimal.

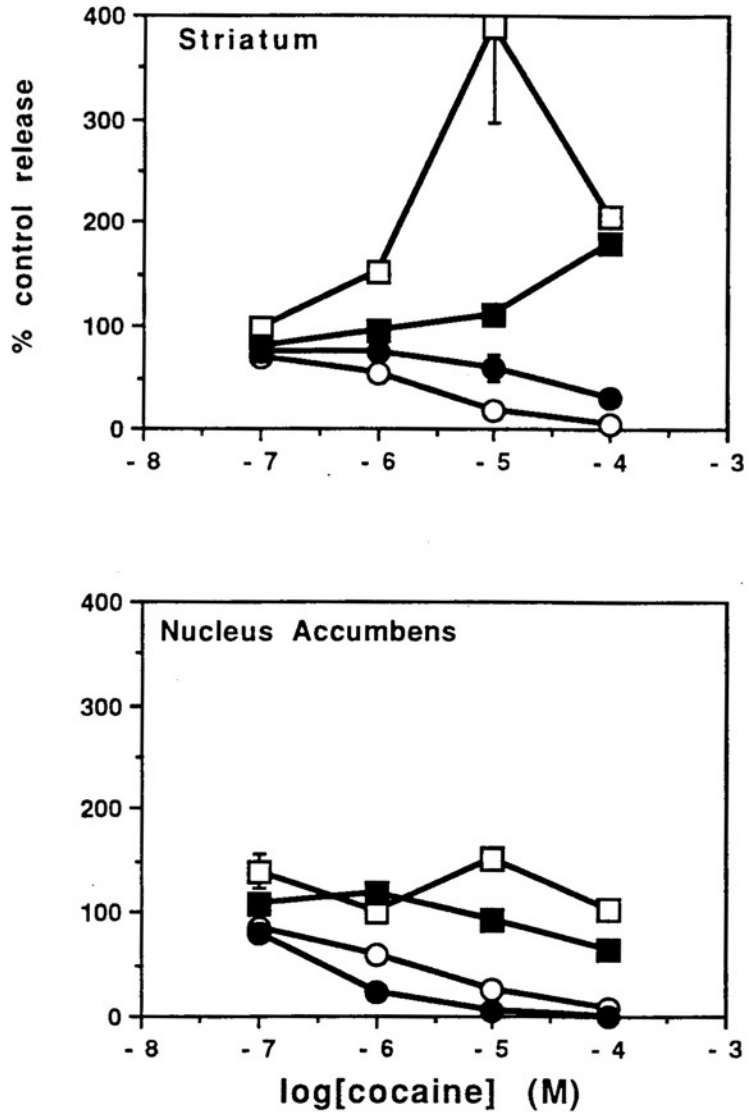


Fig. 14. Effect of cocaine on stimulated release of [^{3}H]DA from slices of STR or NACC of rat brain. Data are expressed as percent of fractional release elicited by the stimulating agent in the absence of cocaine. These values are reported in Tables 1 & 2. Release was stimulated by 10 μM D-amphetamine (open circles), 10 μM 5-HT (filled circles), 25 μM NMDA (open squares), or 20 mM K (filled squares). Points are means ± s.e.m., expressed as percent of control release, from duplicate determinations in each of 3 independent experiments for D-amphetamine, 5-HT, and NMDA and from 9 (STR) or 10 (NACC) experiments for K. Mg was deleted from the medium in all experiments using NMDA as a stimulator.

DISCUSSION

I have examined several agents as releasers of pre-loaded [^{3}H]DA from rat STR and NACC, comparing agents that demonstrated transporter-dependent (exchange diffusion) as well as those which induced transporter-independent (vesicular) release. In order to compare the mechanism of action of the various releasers, I have also examined the sensitivity of the effects of these releasers to changes in Ca and Mg concentrations and to modulation by agonists and antagonists at various receptors and by TTX. In order to verify that the observed results are not artifacts of using tissues which have been pre-loaded with radiolabel, I have measured release of endogenous DA under some of the same conditions employed for release of [^{3}H]DA. Figures 15 and 16 summarize diagramatically the various mechanisms proposed to be operative under conditions employed in these studies.

1. SPONTANEOUS OVERFLOW

In the absence of releasing stimulators, tissue from STR or NACC expressed overflow of [^{3}H]DA (Table 1) or endogenous DA (Table 2) at a rate which remained stable throughout the superfusion period. The nature of this overflow was such that removal of Ca reduced the rate of overflow appreciably but not completely (Tables 1 and 2). The dependence on extracellular Ca suggests that the process whereby a significant fraction of pre-loaded transmitter escapes the neuron is exocytotic (vesicular). That the labeled transmitter escapes the neuron via a vesicular process and at a relatively constant rate indicates that the [^{3}H]DA which enters the cytoplasm via the plasma membrane transporter has distributed to vesicles which can be

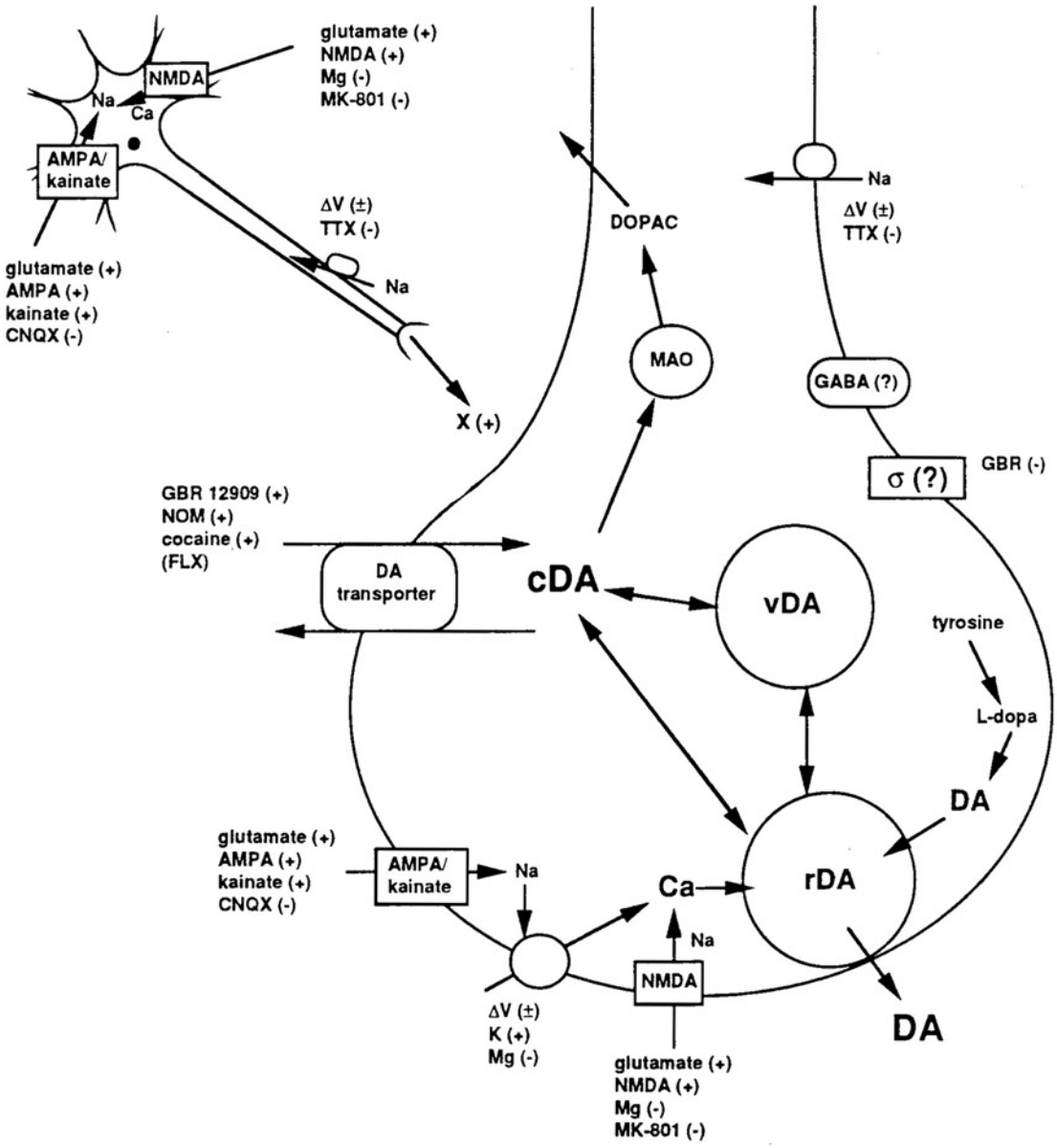


Fig. 15. Diagram of Ca-dependent transmitter release from dopaminergic nerve terminal. Idealized representation of dopaminergic terminal. Action potentials propagate along the axon by voltage-gated Na channels which can be blocked by TTX. Voltage-gated Ca channels admit Ca ions in response to depolarization of the membrane. Membrane potential can be altered by the modulation of Na entry through ligand-gated Na channels, increased external K, or the action of inhibitory transmitters acting at their receptors. Ligand-gated Ca channels admit Ca in response to receptor activation by excitatory transmitters. Ca triggers fusion of the vesicle with the plasma membrane and release of transmitter. Released transmitter is recovered by the transporter. Compounds or conditions that enhance release of DA are denoted by (+) and those that diminish DA release by (-).

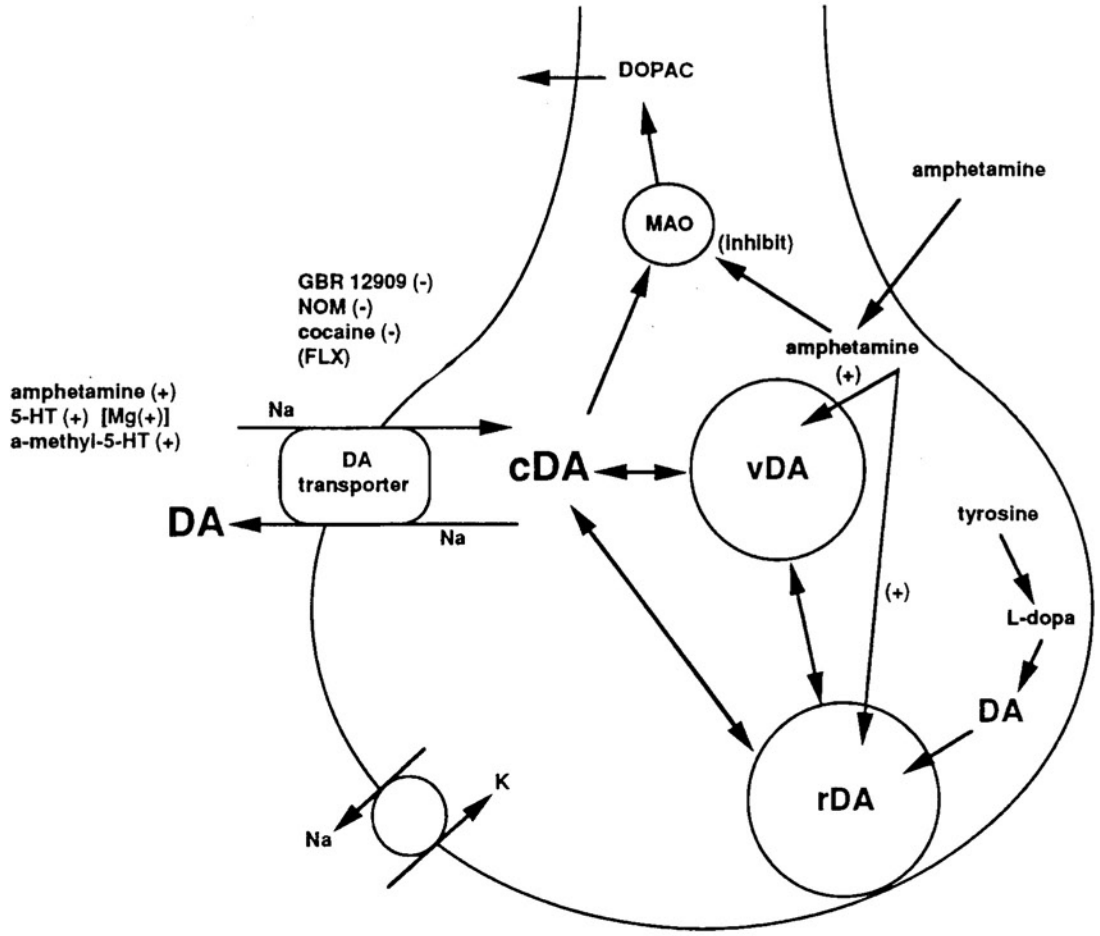


Figure 16. Diagram of transporter-mediated transmitter release from dopaminergic nerve terminal. Releasing substances are transported into or diffuse into the terminal and displace DA from vesicular stores (vDA and rDA) into the cytoplasmic pool (cDA). Reverse transport is facilitated by increased internal Na (co-substrate for transport) and increased cDA. Reverse transport is inhibited by blockade of the transporter. Compounds or conditions that enhance release of DA are denoted by (+) and those that diminish DA release by (-).

released under basal conditions and is in relative equilibrium, at least with respect to the time course of these experiments.

Low external Ca may also diminish reverse (outward) transport of DA. The two plasma membrane Ca pumps require energy to transport Ca outside the cell under normal conditions (Kutchai, 1988). In the absence of an outside-to-inside Ca gradient, less Ca would be entering the neuron from the extracellular environment. The plasma membrane Ca-ATPase would be less active, possibly increasing the ATP content of the cytoplasm, enhancing the operation of the Na/K ATPase, increasing the Na gradient, decreasing the concentration of Na in the neuron and perhaps hyperpolarizing the neuron. The other transporter thought to maintain low intracellular Ca, the Ca/Na exchange transporter, would be operating at a reduced rate in the absence of Ca to transport from the outside, decreasing the cytoplasmic Na concentration, which would diminish the outward transport of monoamine by reducing the availability of co-substrate for transport.

It is generally accepted that sensitivity to removal of external Ca is characteristic of an exocytotic process. This Ca sensitivity also suggests that part of the spontaneous overflow could be due to activation of receptors at the nerve terminal which, under control conditions, facilitates entry of Ca from outside the neuron, triggering release of [^{3}H]DA. If this is the case, the above results suggest that 50-60% of spontaneous overflow may be due to activation of receptors by the presence of releasing substances (basal stimulation of release).

Spontaneous overflow appears to be enhanced in STR and is significantly increased in NACC by Mg-free buffer (Tables 1 and 2). Reduction of the extracellular Mg concentration is known to enhance neuronal excitation in many neuronal systems (Hamon et al., 1987; Mody et al., 1987). Mechanisms

proposed for this excitation include (1) removal of antagonism by Mg on pre- and post-synaptic Ca entry, (2) decreased membrane surface charge screening and associated facilitation of inward currents, and (3) removal of the voltage-dependent Mg block of the NMDA receptor/channel complex. In order to determine if the activation of glutamate receptors contributes to the overflow under basal conditions, the effects of antagonists selective for NMDA- and non-NMDA-type glutamate receptors (MK-801, CNQX) have been investigated (Table 1). Neither MK-801 nor CNQX reduced the basal release of [^{3}H]DA, which argues against a role for glutamate in basal release. This suggests that the enhancement of baseline overflow in the absence of Mg is due to a non-specific charge screening effect or specific relief of a specific Mg blockade of some non-NMDA Ca channels.

The effect of several inhibitors of monoamine transport on the spontaneous overflow of [^{3}H]DA was investigated (Table 1). The concentrations chosen (10 μM cocaine, 1 μM GBR or NOM) represent concentrations that produced significant effects on stimulated release of [^{3}H]DA (as discussed in sections below). The concentration of 1 μM FLX was chosen because, although FLX becomes non-selective at this concentration (Izenwasser et al., 1990a), it was well above the reported IC_{50} for inhibition of 5-HT uptake (Wong et al., 1990). The spontaneous overflow of [^{3}H]DA was significantly enhanced by the presence of the non-selective monoamine transport inhibitor NOM in both STR and NACC. The DA-selective DA transport inhibitor GBR 12909 also significantly increased spontaneous overflow in both tissues. This suggests that the spontaneous overflow is not dependent on the DA transporter. Cocaine, the other non-selective monoamine transport inhibitor tested, had a significant effect only in STR. FLX, the 5-HT-selective transport inhibitor, had no effect on baseline overflow. These data suggest that, under

conditions employed in these studies, [^{3}H]DA is released spontaneously into the extracellular environment by a mechanism which is not dependent on the DA transporter and is partially recovered by the DA transporter, but not by the 5-HT transporter.

Spontaneous overflow of [^{3}H]DA was significantly reduced by TTX (1 μM) in NACC but not in STR (Table 1). Since TTX inhibits action potential propagation by inhibiting voltage-sensitive Na channels, a net excitatory input to NACC terminals is indicated. This suggests that part of the baseline overflow in NACC under control conditions is due to activation of receptors distal to the [^{3}H]DA-containing neuron terminal, perhaps suggesting activation via interneurons. As discussed above, MK-801, a non-competitive NMDA receptor-channel blocker, failed to reduce baseline overflow significantly, either with or without Mg present (Table 1). These data suggest the possibility of a significant excitatory non-glutamatergic input to the NACC.

Neither picrotoxin (50 μM) nor CNQX (10 μM) had a significant effect on spontaneous overflow of [^{3}H]DA (Table 1). This suggests that neither $GABA_A$ nor non-NMDA glutamatergic receptor activation has a significant effect on baseline overflow.

Spontaneous overflow of endogenous DA (Table 2) paralleled the overflow of [^{3}H]DA (Table 1) from pre-loaded tissue in both STR and NACC. Baseline overflow of endogenous DA was strongly reduced by removal of Ca in both tissues. The reduction by Ca-free buffer did not reach significance in NACC due, in part, to the relatively high variance in measurement of the extremely low level of overflow of DA from this tissue. As discussed above, the Ca sensitivity suggests that part of the spontaneous overflow could be due to activation of receptors at the nerve terminal. The spontaneous overflow of endogenous DA also appears to be enhanced in STR and NACC by Mg-free

buffer, although this did not attain statistical significance. The Ca (and possibly Mg) data strengthen the case presented above for baseline release of [^{3}H]DA which suggests that a significant portion of spontaneous overflow may be due to activation of receptors on DA neurons by releasing substances (basal stimulation of release). The presence of GBR (1 μM) enhanced spontaneous overflow as above, suggesting that, under conditions employed in these studies, endogenous DA, as well as [^{3}H]DA, released spontaneously into the extracellular environment is partially recovered by the DA transporter in both STR and NACC.

2. EXOCYTOTIC RELEASE

a. Release of [^{3}H]dopamine

Potassium, L-glutamate, and NMDA stimulated release in a manner consistent with an exocytotic mechanism in STR and NACC. L-Glutamate and the glutamate analogs NMDA, AMPA, and kainate act on tissues by action at specific receptors. In contrast, elevated K indiscriminately depolarizes all neurons present. Release stimulated by K showed a strong Ca dependence in that removal of Ca reduced release by at least 80% in both STR and NACC (Table 3). In the presence of Mg (see below), release stimulated by 1 mM L-glutamate was difficult to measure above background. That slight release, however, was almost completely suppressed by removing Ca. The residual release evident in the presence of Mg and absence of Ca could indicate a small contribution from a transporter-dependent, Ca-independent mechanism proposed to operate at high L-glutamate concentrations (Lonart and Zigmond, 1991). However, the strong Ca dependence of K- and L-glutamate-stimulated release argues for a primarily vesicular release mechanism.

Glutamatergic inputs from the cortex have been demonstrated for the STR (Hassler et al., 1982, Cordingly and Weight, 1986) as well as the NACC (Walaas, 1981; Jones, et al., 1987). Release of [^{3}H]DA stimulated by L-glutamate was greatly enhanced in both STR and NACC by the removal of Mg (compare Table 3 and Table 4). This is consistent with the presence of glutamatergic excitatory inputs that synapse on local NMDA-type receptors. Activation of the NMDA receptor-channel complex results in the opening of a cation channel, allowing entry of Ca (MacDermott and Dale, 1987), possible subsequent activation of voltage-sensitive Ca channels, and release of neurotransmitter. NMDA (100 μM) was unable to produce reliably measurable release in the presence of Mg. The Mg blockade is characteristic of NMDA receptor function. In a physiologic setting, it has been suggested that this blockade is relieved by progressive depolarization of the neuron, possibly through the interaction of the EAA with the non-NMDA quisqualate/AMPA (α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid) and kainate receptors (MacDermott and Dale, 1987). Activation of non-NMDA glutamatergic receptors depolarize the neuron by opening a Na/K cation channel.

The presence of AMPA and kainate receptors in STR and NACC is suggested by the stimulation of release by these agents (Fig. 5). CNQX, at the concentration employed (10 μM), is a non-selective antagonist at EAA receptors of the non-NMDA type (i.e.. AMPA and kainate). CNQX significantly ($p < 0.05$) reduces EAA stimulated release in both the STR and NACC. The reduction of L-glutamate-, AMPA-, and kainate-stimulated release by CNQX suggests that these receptors may be involved in the release stimulated by L-glutamate in the presence of Mg. That the release stimulated by L-glutamate in STR and NACC is due to activation of the NMDA receptor-channel complex has been suggested (Jones et al., 1987; Werling et al., 1990) and is supported by findings in this

study of greatly enhanced release stimulated by L-glutamate in the absence of Mg (compare Table 3 with Table 4). However, L-glutamate stimulated release in the presence of Mg is unaffected by MK-801 (Fig. 6) but the greatly enhanced release in the absence of Mg is reduced by MK-801 to the level of release seen in the presence of Mg. Combined with the reduction of release by CNQX, these data suggest that the release stimulated by L-glutamate seen in the presence of Mg is not due to activation of the NMDA receptor-channel complex.

TTX blocks voltage-sensitive Na channels, thereby inhibiting action potential propagation (Narahashi et al., 1964). As release of [^{3}H]DA may be modulated by action of neurotransmitters at sites removed from the nerve terminal, either trans-synaptic or trans-axonal, the presence of TTX would be expected to eliminate all effects except those at the terminal. TTX reduced L-glutamate-stimulated release in the STR but not in the NACC. This suggests that the excitatory glutamatergic inputs in the NACC act directly at the terminal while in the STR they may act in a manner dependent on action potential propagation.

In order to optimize my ability to isolate the direct contribution of NMDA receptors in L-glutamate-mediated release, release was stimulated in the absence of Mg and the presence of TTX (Table 4). In the absence of Mg, L-glutamate- and NMDA-stimulated release was significantly reduced but not eliminated by TTX in both tissues. This reduction of release indicates that activation of the NMDA receptor-channel complex effects release of [^{3}H]DA directly at the terminal as well as causing release by events distal to the terminal in both the STR and NACC.

Stimulation of neurotransmitter release by elevation of the extracellular K concentration has been used by many groups in studies of the mechanism of

transmitter release and its modulation by regulatory agents. Increasing the external concentration of K in the superfusion buffer from 5 mM to 20 mM with a concomitant decrease in Na concentration stimulates release of neurotransmitter. Release is stimulated either by direct activation of voltage-sensitive Ca conductances or by depolarizing the neuron sufficiently to activate neuronal firing, which indirectly activates Ca influx (Illes,1986). At concentrations below 30 mM, the latter effect is presumed to predominate as transmitter release evoked under these conditions is usually readily inhibited by tetrodotoxin. L-Glutamate and the glutamate analogue NMDA act on tissues by action at specific receptors. In contrast, elevated K indiscriminately depolarizes all neurons present.

Release of [^{3}H]DA stimulated by K was enhanced in both STR and NACC by the removal of Mg (compare Table 3 and Table 4). This is consistent with the presence of excitatory inputs that can be activated by glutamate released as a result of depolarization by elevated K and that synapse on local NMDA-type receptors . However, as in the case of spontaneous overflow, K-stimulated release of [^{3}H]DA is not significantly reduced in STR or NACC by either MK-801 (data not shown) or CNQX (Fig. 5). These results suggest that K-stimulated [^{3}H]DA release does not depend substantially on the release of L-glutamate or the action of AMPA/kainate- or NMDA-type glutamate receptors in the rat STR or NACC. Indeed, in the STR, Bowyer et al. (1992) have presented a detailed case against the facilitation of release of [^{3}H]DA by L-glutamate which is released by elevated K. Potentiation of K-stimulated release in the absence of Mg was not blocked by the glutamate receptor antagonists MK-801, 6,7-dinotroquinoxalinedione (DNQX), or kynurenate. Release of endogenous L-glutamate by K depolarization may not produce additional DA release through stimulation of NMDA receptors because the concentration of K necessary to

evoke significant L-glutamate release also blocks NMDA receptor-mediated DA release. These data suggest no significant role for activation of glutamate receptors in K-stimulated DA release.

TTX greatly reduced K-stimulated release in the NACC (Table 3). This indicates that much of the K-stimulated release from the NACC is dependent on excitatory input from sites distal to the terminal. In the presence or absence of Mg, TTX slightly enhanced K-stimulated release in the STR (Table 3 and 4). These results are consistent with a relatively greater depolarization-activated inhibitory contribution by neurons regulating the dopaminergic terminals in the STR and a significant excitatory contribution in the NACC. In order to further elucidate the mechanism of the enhanced release of [^{3}H]DA in the presence of TTX, and since inhibitory GABAergic inputs to the STR and NACC have been demonstrated (Brownstein and Palkovits, 1984), K-stimulated release was measured in the presence of an antagonist (picrotoxin) and an agonist (muscimol) selective for receptors for the inhibitory neurotransmitter GABA (Table 8). Although the density of $GABA_A$ and $GABA_B$ receptors is similar in STR and NACC (Bowery et al., 1987), only compounds selective for the $GABA_A$ receptor subtype were tested due to the projected expense of $GABA_B$ reagents if used in this experimental design. In the NACC, as predicted from the net excitatory input revealed by TTX treatment, neither picrotoxin (50 μM) nor muscimol (1 μM) had a significant effect on K-stimulated release. Furthermore, neither compound had any effect on the TTX-induced reduction of release. TTX enhances release in STR (Tables 3 and 8). This enhancement of release by TTX might indicate attenuation of the inhibitory effect of GABA. Blockade of the $GABA_A$ receptors by picrotoxin (50 μM) did not significantly increase DA release (Table 8). It seems likely that the failure of $GABA_A$ agents to clarify the mechanism of TTX-induced facilitation of K-stimulated release is due to the

difficulty of measuring subtle changes in the small change in release induced by TTX in the STR. Alternatively, $GABA_B$ receptors may be more important in the regulation of DA release in the STR. Recently, $GABA_B$ receptors have been shown to modulate glutamate release in the neostriatum (Uchimura and North, 1991; Nisenbaum et al., 1992).

In the STR, release of [^{3}H]DA stimulated by K is not affected by selective blockade of the DA transporter with GBR 12909 (Fig 11) and is slightly enhanced by non-selective blockade of the monoamine transporters by the highest concentrations tested of FLX (Fig 12), NOM (Fig 13), or cocaine (Fig 14). The enhancement observed is probably due to increased overflow of released [^{3}H]DA evident upon blockade of reuptake into dopaminergic and serotonergic nerve terminals (Nissbrandt et al., 1991). In the NACC however, K-stimulated release was inhibited by GBR 12909 in a concentration-dependent manner (Fig 11). Since NOM and cocaine had only a modest inhibitory effect on K-stimulated release in NACC at high concentrations, the inhibition by GBR may be via a mechanism unrelated to transporter blockade. GBR 12909 has been shown to bind to σ sites (Contreras et al., 1990); therefore, the effect of GBR 12909 on K-stimulated release in NACC may be due to action at these sites, raising the possibility that modulation of DA release by σ ligands might be more important in NACC than in STR. The mechanism for the apparent inhibition of K-stimulated release by GBR 12909 in the NACC will be discussed further in the section on endogenous DA release. In contrast to K-stimulated release, release stimulated by NMDA in the STR was greatly enhanced (5x) by selective blockade of the DA transporter with GBR 12909 (Fig 11) as well as by non-selective blockade of the monoamine transporters by NOM (Fig 13) and cocaine (Fig 14). These agents showed no significant effect or only modest

enhancement of NMDA-stimulated release in the NACC (Figs 11, 13, and 14) and FLX had little effect in either tissue (Fig. 12).

The actions of selective (GBR 12909 and FLX) and non-selective (NOM and cocaine) transporter blockers further demonstrate that inhibition of monoamine transporters does not reduce the release elicited by K (except for GBR in the NACC, where a different mechanism may be implicated, but see below) or NMDA. Indeed, the most common result was an enhancement (modest for K, profound for NMDA) of release. It seems likely that there is a high density of DA re-uptake sites in close proximity to the locations from which NMDA activates [^{3}H]DA release in STR and a somewhat lower density at the sites from which K elicits release. In contrast, for NMDA- and K-stimulated release in NACC, immediate re-uptake by closely aligned sites may be less significant in determining the amount of released [^{3}H]DA recovered in the superfusate. Serotonergic terminals may also play a role in accumulating released DA in NACC (Feuerstein et al., 1986). These results might indicate that for NMDA receptor-mediated excitatory amino acid pathways, volume conduction mechanism without tight synaptic junctions may be more significant in NACC than in STR.

b. Release of endogenous dopamine

In contrast to release of [^{3}H]DA, potassium stimulated significantly greater release ($p < 0.05$) of endogenous DA from STR than from NACC (Table 5) under control conditions. K-stimulated release of endogenous DA, as expected of an exocytotic/vesicular process, was significantly reduced by removal of external Ca in both STR and NACC. In agreement with release of

[^{3}H]DA, K-stimulated release of endogenous DA appeared to be enhanced by Mg-free buffer.

L-Glutamate induces release in the presence of Mg (Fig. 7). This release is strongly Ca-dependent. NMDA (25 μM), in Mg-free buffer, also releases DA (Table 6). These data are consistent with the proposed mechanism of action of the NMDA receptor-channel complex. Thus, in both STR and NACC, release can be activated by either glutamate or NMDA.

The presence of GBR 12909 would be expected to enhance the amount of DA overflow by inhibiting re-accumulation of released DA. GBR 12909, which greatly enhanced fractional release of [^{3}H]DA by NMDA in STR but not NACC, showed the same preferential enhancement of release of endogenous DA in STR. Release was increased eleven-fold in STR and less than six-fold in NACC. These data support the above hypothesis suggesting a relatively high density of DA re-uptake sites in close proximity to the locations from which NMDA activates DA release in STR, a somewhat lower density at the sites from which K elicits release, and a less significant role for re-uptake in release in NACC.

3. COMPARISON OF MEASUREMENT OF ENDOGENOUS DOPAMINE WITH MEASUREMENT OF [^{3}H]DOPAMINE

Release of [^{3}H]DA (Tables 3 and 4) was expressed as a percentage above an arbitrary baseline determined by spontaneous overflow under various conditions (Table 1). The radiolabeled compound represents an unknown fraction of the total DA pool in the neuron. Endogenous overflow represents an absolute amount of neurotransmitter collected per mg of protein. This makes detailed quantitative comparisons between overflow of endogenous DA and overflow of [^{3}H]DA very difficult. The patterns which emerge however, are a

useful indication of the general validity of the two experimental paradigms. Spontaneous overflow of both DA and [^{3}H]DA is greater in STR than in NACC. GBR 12909 enhances spontaneous overflow in both tissues. The enhancement is greater in STR than in NACC for overflow of both DA and [^{3}H]DA.

Difficulties arise when comparing K-stimulated release. For [^{3}H]DA, K stimulates a greater percentage of release in NACC than in STR. Addition of GBR 12909 does not change the relative amount of [^{3}H]DA released in STR but decreases the relative amount released in NACC. Endogenous DA release differs in that K-stimulated release is greater in STR than NACC. Addition of GBR 12909 enhances release in both tissues but to a greater extent in STR, further amplifying the difference relative to NACC. The similarity between STR and NACC is reduced to the statement that, for both DA and [^{3}H]DA, there is a greater relative increase of K-stimulated release in the STR. The most obvious confounding variable is the radiolabel. The explanation I favor for the different sensitivities of STR or NACC to treatment with GBR 12909 is that the pre-loaded radiolabel is distributed to the proposed pools of DA (Fig. 1) differently in the STR and NACC, at least under these experimental conditions. Although the spontaneous overflow was stable throughout the collection period, the pool subject to K-stimulated release may not have reached a steady state or may have a different relationship with the pool(s) responsible for spontaneous overflow. Alternatively, the proposed effect of GBR 12909 on σ-receptors, as mentioned above, may be the mechanism by which the potentiating effect of GBR is attenuated in the NACC. The mechanism for the relative inhibition of K-stimulated release in the NACC requires further study.

The detailed mechanism for the release of DA stimulated by K, L-glutamate, or NMDA is obviously complex. A confounding factor may be the differential effects of re-uptake blockade in STR and NACC. Also, the release of

DA may depend on the interaction of intermediate stimulatory and/or inhibitory substances, the analysis of which is beyond the scope of this study.

4. TRANSPORTER-MEDIATED RELEASE

a. D-Amphetamine-stimulated release

It is likely that there is an equilibrium between DA in vesicles and DA free in the cytoplasm. Since DA is largely ionized at physiological pH and cannot pass through the membrane by diffusion, it can be released either from vesicles by an exocytotic mechanism or from the cytoplasm via the DA transporter operating in reverse. Transporter-mediated uptake of DA into DA terminals strictly depends on the existence of an inward Na gradient (Holz and Coyle, 1974). Assuming a symmetrical transport process, inversion of the Na gradient allows efflux of cytoplasmic DA via the transporter (Raiteri, 1978; Ficher and Cho, 1979; Raiteri, 1979). D-amphetamine has been shown to stimulate [^{3}H]DA release by exchange diffusion, a mechanism which requires the DA transporter (Liang and Rutledge, 1982; Fischer and Cho, 1978; Parker and Cubeddu, 1986a). Efflux of pre-loaded [^{3}H]DA is also caused by other transporter substrates, such as dopamine, fenfluramine, and tyramine, being transported into the tissue (Heikkila et al., 1975; Liang and Rutledge, 1982; Parker and Cubeddu, 1986b; Fisher and Cho,1979). Although D-amphetamine can reach equilibrium concentrations within the neuron by passive diffusion through the membrane and effect release of DA from vesicular stores, the release of neurotransmitter from the neuron is dependent upon the DA transporter. In this model, the release of DA would be a function of the monoamine gradient, the Na gradient, and the availability of the transporter on the inside of the

membrane (Rutledge, 1978). Exchange diffusion is not related to Ca-dependent exocytotic events (Fischer and Cho, 1979; Raiteri et al., 1979) and succeptibility to inhibition by DA transporter blockers is considered as evidence in favor of this mechanism. Transporter-dependent release has been defined as efflux which persists in reduced Ca and which is blocked by NOM (Arbuthnott et al., 1990a,b).

In contrast to release stimulated by K or L-glutamate, D-amphetamine-stimulated release was not strongly Ca-dependent in either the STR or NACC (Table 3). Increased intraneuronal Ca increases intraneuronal Na (Rutledge, 1978); therefore, decreased intraneuronal Ca could presumably result in reduced intraneuronal Na, reducing availability of co-substrate for transport. This may explain the effect of removing Ca on release stimulated by D-amphetamine (and 5-HT). The presence of TTX or the absence of Mg (compare Table 3 with Table 4) had no significant effect on release stimulated by D-amphetamine in either tissue.

The release stimulated by D-amphetamine in STR and NACC was inhibited in a concentration-dependent manner by selective blockade of the DA transporter by GBR 12909 (Fig. 11), non-selective blockade of monoamine transporters by NOM (Fig. 13) and cocaine (Fig. 14), as well as by concentrations of *FLX greater than or equal to 1 μM (Fig. 12). These results are* consistent with a release mechanism for amphetamine which is transporter-dependent (exchange diffusion) and not dependent on exocytotic release.

b. Serotonin-stimulated release

(i) Release of [^{3}H]dopamine

5-HT has been shown to cause a variety of effects by activation of receptors associated with pharmacologically distinct classes of binding sites (Schmidt and Peroutka, 1989). However, I found no direct evidence to indicate that 5-HT-stimulated release of [^{3}H]DA is mediated by 5-HT receptors in STR or NACC. Antagonists at 5-HT_2 or 5-HT_3 receptors did not reduce the release of [^{3}H]DA induced by 5-HT (Figs 8 and 9). Under conditions employed in this study, 5-HT-stimulated release of [^{3}H]DA is not Ca-dependent in either STR or NACC (Table 3). Inhibition of DA transport consistently reduces 5-HT-stimulated release in a concentration dependent manner in both STR and NACC, consistent with the effects described by Nurse, et al. (1988). In the STR, D-amphetamine- and 5-HT-stimulated release is inhibited almost identically by selective blockade of the DA transporter by GBR 12909 (Fig. 11) or non-selective blockade of the transporters for DA and other monoamines by NOM (Fig. 13). This indicates that both D-amphetamine and 5-HT have the same requirement for the DA transporter to effect release of pre-loaded [^{3}H]DA. Cocaine, the other non-selective transporter blocker tested, also inhibits [^{3}H]DA release stimulated by both both D-amphetamine and 5-HT (Fig. 14). Blockade of the 5-HT transporter with concentrations of FLX below 1 μM (Fig. 12) has no significant effect on release. Only when concentrations of FLX are high enough to block DA transport (>1 μM) (Izenwasser et al., 1990a) does it reduce D-amphetamine- or 5-HT-stimulated release. In the NACC, DA release is reduced in a manner similar to that in the STR, although at certain concentrations of uptake inhibitor in the NACC (10 nM NOM, 100 nM NOM and 1 μM cocaine) statistically significant differences between 5-HT- and D-amphetamine-

stimulated release are observed. In both tissues however, 5-HT exhibits similar characteristics to D-amphetamine with respect to blockade of monoamine transport and stimulation of release. Thus, 5-HT releases DA in a manner consistent with exchange diffusion via the DA transporter.

Although there are similarities between 5-HT- and D-amphetamine-stimulated release, there are also differences. Release stimulated by D-amphetamine is not reduced in the absence of Mg. Release stimulated by 10 μM 5-HT is markedly reduced in the absence of Mg though release stimulated by 100 μM 5-HT is unaffected. This argues for a saturable process in which 100 μM 5-HT produced a supramaximal stimulus. TTX has no effect on release stimulated by 5-HT in the presence of Mg. However, in the absence of Mg, the presence of TTX partially restores the release stimulated by 10 μM 5-HT in the NACC. 10 μM 5-HT may cause receptor-mediated effects (Westfall and Tittermary, 1982) opposing exchange diffusion; the inhibitory action of reduced Mg might be partially offset in the presence of TTX or overcome by increased exchange diffusion elicited by 100 μM 5-HT.

The discrepancy in Mg sensitivity between high and low concentrations of 5-HT may reflect an overall metabolic change (e.g. reduction in efficiency of transport caused by low Mg/reduced ATP utilization) or Mg might be required for maximal binding of 5-HT to the transporter. If the release process activated by 5-HT is in any way energy dependent, it could be inhibited by lack of Mg. However, D-amphetamine-induced release of monoamine from STR has been shown to be potentiated by metabolic inhibitors affecting Na/K ATPase activity (Rutledge, 1978). Direct inhibition of Na/K ATPase (e.g. with ouabain) elevates Na within the nerve without reducing ATP content. Elevation of the

concentration of Na within the nerve provides increased levels of co-substrate for transport. Assuming that low intraneuronal Na concentration is the rate limiting factor for efflux by exchange diffusion, increasing intraneuronal Na by interfering with ATPase activity would increase efflux. Alternatively, uptake itself has been reported to be ATP-dependent (Cao et al., 1990a,b) and Mg may be required for ATP utilization in uptake. Reduced transporter efficiency because of a reduced ability to utilize ATP for uptake, due to lack of the necessary co-factor Mg, might be overcome by increasing the concentration of 5-HT from 10 μM to 100 μM. D-Amphetamine-stimulated release may be less dependent upon transporter efficiency due to the greater lipophilicity of D-amphetamine, allowing its inward movement by diffusion across the cell membrane. In any event, the response to removal of Mg argues against a similar mechanism to that of K or L-glutamate. These data are consistent with a release mechanism which is transporter dependent (exchange diffusion) and only marginally dependent on exocytotic release.

(ii) Release of endogenous dopamine

Release of endogenous DA by 10 μM 5-HT is similar in all important respects to release of radiolabeled DA (Table 7) in both STR and NACC. Removal of Ca does not decrease release in either tissue. The presence of GBR 12909 (1 μM) virtually abolishes 5-HT-stimulated release. The absence of Mg appeared to diminish 5-HT-stimulated release although this effect did not attain statistical significance. These data support the hypothesis above which suggests that release of endogenous DA is a transport-mediated process.

5. SIGNIFICANCE

The physiological and pharmacological significance of the ability of 5-HT to release DA in NACC and STR via a transporter-dependent exchange process requires comment. The STR and NACC are innervated by serotonergic neurons from more than one raphe nucleus (Steinbush, 1984). The dorsal raphe nucleus primarily innervates the striatum (Olpe and Koella, 1977) while the NACC receives a relatively dense input of serotonergic fibers from the medial raphe nuclei (Vertes and Martin, 1988). The 5-HT fiber distribution in NACC is non-homogeneous, with regions of dense innervation interspersed with regions of sparser innervation. It is possible that 5-HT, released as a result of activity in either or both of the 5-HT containing raphe nuclei, might reach sufficient concentration in the extracellular space surrounding the DA terminal to evoke DA release through a transporter dependent mechanism, especially in regions of NACC or STR with dense serotonergic innervation. This effect would be facilitated in the presence of inhibitors of monoamine uptake into 5-HT neurons (e.g. FLX). The effects of non-selective inhibitors of monoamine re-uptake (e.g. cocaine) on local extracellular DA concentrations would be very dependent on the relative densities of dopaminergic and serotonergic terminals in the immediate vicinity.

Studies conducted here also provide evidence for a substrate for local control of DA release in STR and NACC by glutamate or other excitatory amino acids, activity both at the level of the DA nerve terminal and through connecting interneurons. Under my experimental conditions, the local release of glutamate, such as might result from firing of cortex-STR or cortex-NACC neurons, may directly promote DA release or may facilitate release induced by firing of the dopaminergic neurons.

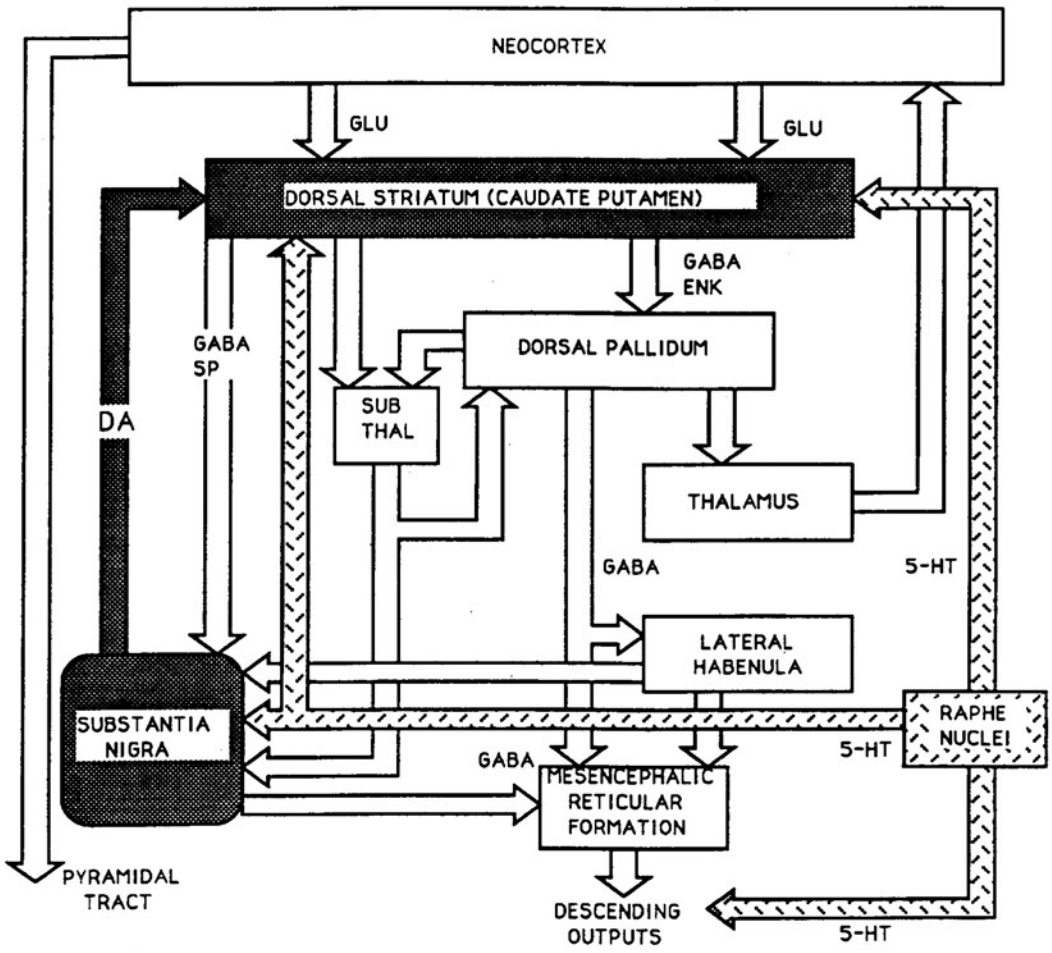
I have shown that various stimulators of [^{3}H]DA release are influenced by altered ionic conditions, receptor antagonists, or uptake inhibition. Specifically, I have demonstrated that 5-HT can stimulate the release of [^{3}H]DA in a manner which closely resembles release stimulated by D-amphetamine. However, there are some intriguing differences. For example, D-amphetamine-stimulated [^{3}H]DA release is not sensitive to removal of Mg, but 5-HT-stimulated release is inhibited by removal of Mg from the medium. As 5-HT can influence the release of [^{3}H]DA, the factors which influence the release of [^{3}H]5-HT are of interest. The evidence presented above indicates that released 5-HT may well be a factor increasing the synaptic concentration of DA. Preliminary data indicates that some of the same factors which influence the release of [^{3}H]DA can affect the release of [^{3}H]5-HT in a similar manner (Appendix 3). Specifically, DA stimulates the release of [^{3}H]5-HT in a Ca-insensitive, Mg-sensitive manner which can be inhibited by transport blockade.

I have used some of the same superfusion paradigms to release endogenous DA. This is to demonstrate that the release effects previously demonstrated are not merely artifacts of using tissue which has been pre-loaded with radiolabeled monoamine. Although the interpretation of these data is not always unequivocal and the reconciliation of endogenous data with radiolabeled data may not be a trivial endeavor, in general, the endogenous data support the major assertions made on the basis of release of [^{3}H]DA. It has been proposed that several pools of monoamine exist and that monoamine which is imported into the neuron via the transporter is destined for a readily-releasable pool which may be different from transmitter synthesized within the neuron.

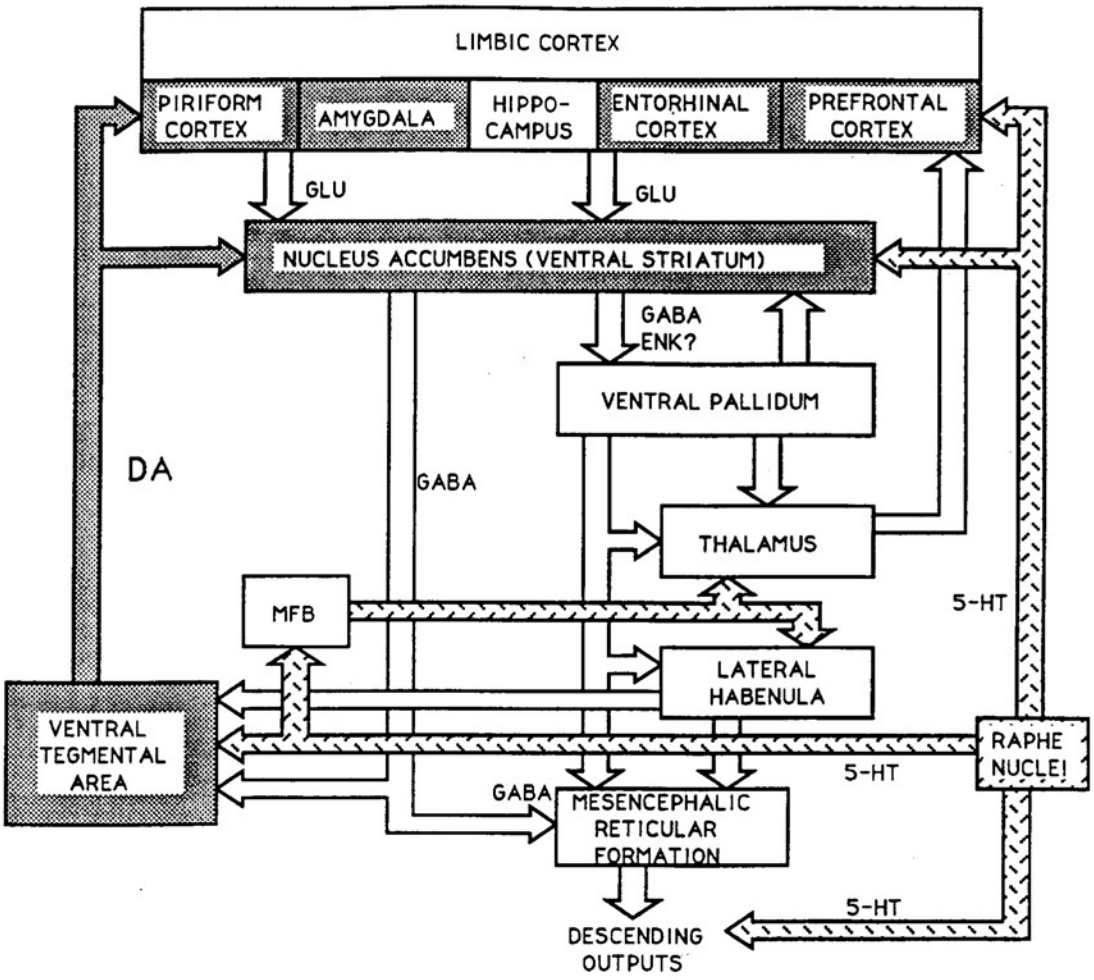
CONCLUSIONS

I have demonstrated differences between the STR and NACC with respect to DA release stimulated by a variety of compounds. For example, more [^{3}H]DA is released in NACC than in STR by elevated K, L-glutamate, and 10 μM 5-HT. Also, D-amphetamine releases more [^{3}H]DA in STR than in NACC. In contrast, more endogenous DA is released in STR by K, NMDA (in Mg-free buffer), and 5-HT. NMDA-stimulated overflow of [^{3}H]DA is greatly potentiated by blockade of monoamine re-uptake in STR but only marginally increased in NACC. K-stimulated release is potentiated by TTX in STR but inhibited in NACC. In general however, these differences are more quantitative than qualitative and indicate that release of DA is regulated in a similar manner in STR and NACC with respect to the experimental conditions employed in this study.

I have also demonstrated that 5-HT can induce release of DA by a mechanism which is insensitive to 5-HT receptor blockade and TTX, does not require external Ca, and requires the plasma membrane DA transporter. This mechanism may be an important consideration in studies of the role of 5-HT in the modulation of DA release.

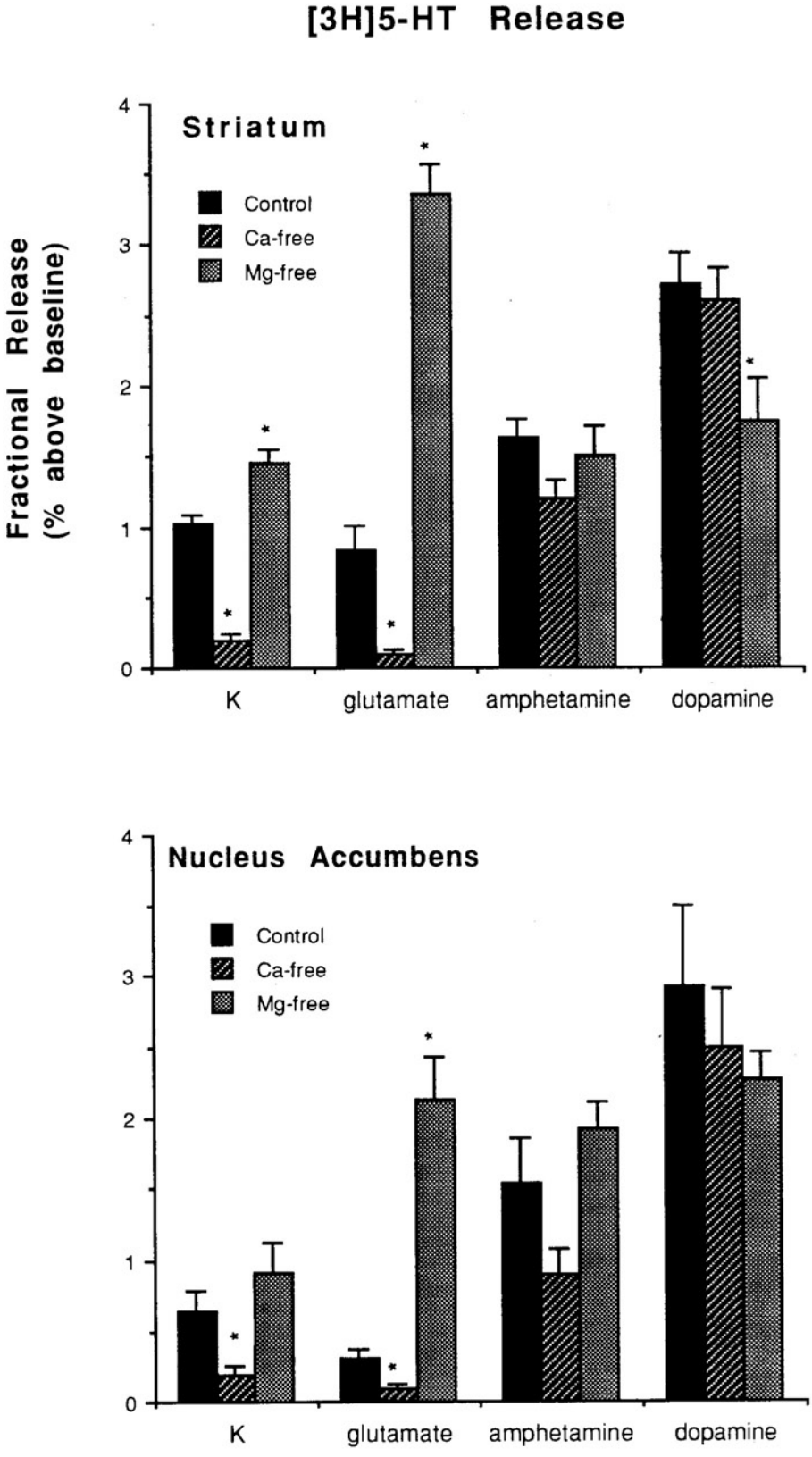


Appendix 1. Pathways related to DA projections to STR. Some major pathways related to the DA projection from the substantia nigra to the striatum (caudate/putamen). Some known or probable transmitters in the individual connections are indicated. ENK, enkephalin; SP, substance P. Adapted from Bjorklund and Lindvall 1984.



Appendix 2. Pathways related to DA projections to NACC. Some major pathways related to the DA projection from the ventral tegmental area to the nucleus accumbens. Some known or probable transmitters in the individual connections are indicated. ENK, enkephalin; SP, substance P. Adapted from Bjorklund and Lindvall 1984.

Appendix 3. Stimulation of [^{3}H]5-HT release from slices of STR or NACC of rat brain in various buffers. Data are expressed as percent of radioactivity released by the stimulating agent relative to that contained in the tissue at the beginning of the stimulation interval (Fractional Release, % above baseline). Concentrations of releasers were: 20 mM K, 1 mM glutamate, 10 μM amphetamine and 10 μM dopamine. Reported values of fractional release are means from 5 independent experiments in STR and 4 independent experiments in NACC, each of which was performed in triplicate, ± s.e.m. "Control" buffer indicates 2.5 mM CaCl and 1.2 mM $MgSO_4$. "Ca-free" indicates buffer with 1 mM EGTA and no $CaCl_2$. "Mg-free" indicates buffer with no $MgSO_4$. Experiments were performed as for release of [^{3}H]DA as described in "Methods".


[3H]5-HT Release
Striatum
Control
Ca-free
Mg-free
Fractional Release
(% above baseline)
4
3
2
1
0
K
glutamate
amphetamine
dopamine
Nucleus Accumbens
Control
Ca-free
Mg-free
4
3
2
1
0
K
glutamate
amphetamine
dopamine

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