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Pressure suppresses serotonin release by guinea pig striatal synaptosomes

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Gilman SC, Colton JS, Hsu SC, Dutka AJ. Pressure suppresses serotonin release by guinea pig striatal synaptosomes. Undersea Biomed Res 1988; 15(2):69–77—Exposure to high pressure produces neurologic changes in humans which manifest as tremor, EEG changes, and convulsions. Since previous studies have implicated the involvement of the serotoninergic system in these symptoms, it was of interest to study serotonin release at high pressure. Synaptosomes isolated from guinea pig striatum were used to follow serotonin efflux at 68 ATA. The major observation was a decrease in [^{3}H]serotonin release from depolarized striatal synaptosomes at 68 ATA. In view of the role of serotonin as an inhibitory neurotransmitter in this area, the observed decrease in synaptic release leads us to conclude that decreased serotoninergic activity in striatal neurons probably is contributing to the hyperexcitability associated with HPNS.

serotonin efflux
high pressure nervous syndrome
synaptosome
striatum

INTRODUCTION

Exposure to pressures greater than 27 atmospheres absolute (ATA) produces a disturbance of neurologic function referred to as the high pressure neurologic syndrome (HPNS) (1). Neurologic changes of HPNS manifest as tremor, myoclonic episodes, EEG changes, and convulsions (2, 3).

Although a number of pharmacologic agents have been tested to protect against the adverse effects of pressure (4, 5), little information is available about the direct action of high pressure on synaptic transmission in the CNS. Gillard et al. (6) recently used a differential pulse voltammetry technique to study the generation of serotonin metabolites in the rat striatum during exposures to high pressure. They found that exposure to 61–121 ATA produced a significant increase in the striatal concentration of 5-HIAA, the major metabolite of serotonin.

This finding suggests that pressure accelerates the turnover of serotonin in presynaptic nerve terminals in the striatum. Such an alteration in receptor function would be expected to affect the release kinetics of the striatal serotoninergic system. There-

fore, we used an in vitro presynaptic nerve terminal preparation to determine the extent to which the synaptic release of [^{3}H]serotonin by striatal neurons is affected by high pressure exposure. The synaptic vesicle (synaptosome) preparation was chosen because it has been shown to retain the transport properties of the nerve ending in situ (7–9) and to exhibit a calcium-dependent release of neurotransmitter substances (10). Also, synaptosomes offered an advantage as an experimental model in that multineuronal or neuroglial interactions could be eliminated.

MATERIAL AND METHODS

Animals

Adult male Hartley short-haired guinea pigs (300 and 400 g) were obtained from a commercial source.

Materials

Radioactive 5-[1,2-^{3}H(N)]hydroxytryptamine binoxalate (serotonin; 15–30 Ci/mmol) was purchased from New England Nuclear Corporation, Boston, MA. All other chemicals were purchased from Sigma Chemical Company, St. Louis, MO.

Synaptosomal isolation procedure

Each guinea pig was killed by decapitation, and the striatum dissected into 20 vol of 0.32 *M* sucrose buffered with 5 m*M* HEPES. Synaptosomes were prepared by density gradient centrifugation as previously described (11). Briefly, a homogenizer was used to disperse the tissue. Centrifugations were carried out at 3°C in an ultracentrifuge using a Sorvall digital integrator to obtain consistent centrifugation. The initial homogenate was centrifuged at 1100 × *g* for 5 min to yield a nuclear pellet and low speed supernatant. The supernatant was centrifuged at 10,000 × *g* for 10 min to yield a mitochondrial pellet which was resuspended in sucrose and homogenized. This homogenate was layered over a Ficoll gradient and centrifuged at 26,000 × *g* for 30 min. After centrifugation, the purified synaptosomes were removed, diluted with sucrose, and pelleted.

Radioisotope uptake

The method used for [^{3}H]serotonin loading and efflux measurement of synaptosomes was developed by modification of the methods of Gilman (12) and Schlicker et al. (13). The final synaptosome pellet was suspended in 20 vol of buffer. [^{3}H]Serotonin was added and the mixture was incubated at 37°C for 30 min to allow uptake by the tissue. After uptake, a 1.0-ml aliquot of the synaptosome suspension was placed on a filter unit consisting of a 0.45-μm nylon membrane filter positioned on the plastic multiperforated support of a 10-ml perfusion chamber. The perfusion chamber was then connected to a polystaltic pump, and the filters immediately washed with 25 ml of a high sodium, calcium-free buffer medium at 37°C, using the highest pump speed.

Compression studies

After the [^{3}H]serotonin-loaded synaptosomes were washed, the filter unit was attached to a superfusion apparatus that was mounted in a hyperbaric chamber. The hyperbaric chamber was sealed and pressurized to 1.3 ATA with oxygen, and to a final pressure of 68 ATA with helium. The rate of compression was 4 ATA/min. Oxygen partial pressure, as measured with a paramagnetic O_2 analyzer, was maintained at 0.49 ± 0.01 ATA throughout pressurization. A recirculation atmosphere control system was used to regulate CO_2 at < 0.0005 ATA, as measured by an infrared analyzer. Temperature within the hyperbaric chamber was maintained at 37° ± 2°C during compression, as previously described (13). During pressurization and the release studies the temperatures of the buffer media and perfusion chamber were constantly monitored by microthermistors, and maintained at 37° ± 0.5°C using YSI model 73A temperature control units (Yellow Springs Instrument Co., Yellow Springs, OH).

Upon reaching 68 ATA, the filters were perfused at a rate of 500 μl/min with 5 ml of buffer medium and 3 perfusate "wash" fractions collected directly into scintillation vials. At 3 min after the beginning of superfusion, the buffer medium was quickly replaced with 10 ml of a 5 m*M* K^+, nondepolarizing or 10 ml of a depolarizing, 70 m*M* K^+ "efflux" medium containing 1.2 ml of Ca^{2+}. The composition was (in millimolars): NaCl, 145 or 80; KCl, 5 or 70; $CaCl_2$, 2.5; $MgCl_2$, 1.2; KH_2PO_4, 1.2; HEPES, 20; glucose, 10; pH 7.4. Nine fractions containing 500 μl each of perfusate were collected every minute directly into scintillation vials. The hyperbaric chamber was then decompressed to 1 ATA at the rate of 4.03 ATA/min. In all experiments, aliquots from the same synaptosome preparation were used for obtaining the 1 and 68 ATA release values. Control aliquots from the same preparation were treated identically to the experimental group, except that pressure exposures were sham (1 ATA).

Assay of samples

Biofluor (15 ml) was added to the scintillation vial and the radioactivity of each perfusate was determined. The radioactivity remaining on the filters at the end of the superfusion was also counted. Each filter was placed in a scintillation vial containing 500 μl of 1% sodium dodecyl sulfate. After agitation, 15 μl of Biofluor was added and the radioactivity of the filter was measured.

Expression of results

Fractional efflux was expressed as percentage released per minute of total radioactivity, i.e.,

$$\text{efflux} = \frac{\text{cpm in 500 μl filtrate}}{\text{total radioactivity}}$$

where total radioactivity was the sum of all 12 fractional filtrate cpm's and cpm remaining on the filter. Statistical significances at each time point were determined using *t* tests.

RESULTS

[^{3}H]Serotonin release at 1 ATA

Figure 1 shows the time course of depolarization-induced efflux of [^{3}H]serotonin from synaptosome fractions isolated from the striatum. Application of the high K^+ medium containing Ca^{2+} initially induced a more than fourfold increase in the release of [^{3}H]serotonin by the synaptosome preparation. After the first 3 min, the evoked release declined rapidly, returning within 8 min to levels not significantly greater than the resting efflux levels.

Synaptosomes were perfused with a depolarizing, 70 m*M* K^+ calcium-free medium, with $MgCl_2$ replacing the omitted $CaCl_2$ to determine the extent to which the release observed with depolarization was calcium-dependent. Figure 1 shows the effect of calcium removal on depolarization-induced [^{3}H]serotonin release. As can be seen, the removal of calcium from the depolarizing medium reduced initial release evoked by high K^+ by over 80%.

Compression effects on [^{3}H]serotonin

Figure 2 shows the effect of compression to 68 ATA on the nondepolarized release of [^{3}H]serotonin. Pressure was not found to cause any significant changes in the spontaneous efflux of serotonin by these st iatal synaptosomes ($P > 0.05$).

As shown in Fig. 3 *A*, the evoked release of [^{3}H]serotonin from synaptosomes in the presence of 1.2 m*M* Ca^{+2} was substantially reduced (i.e., 65%) by compression

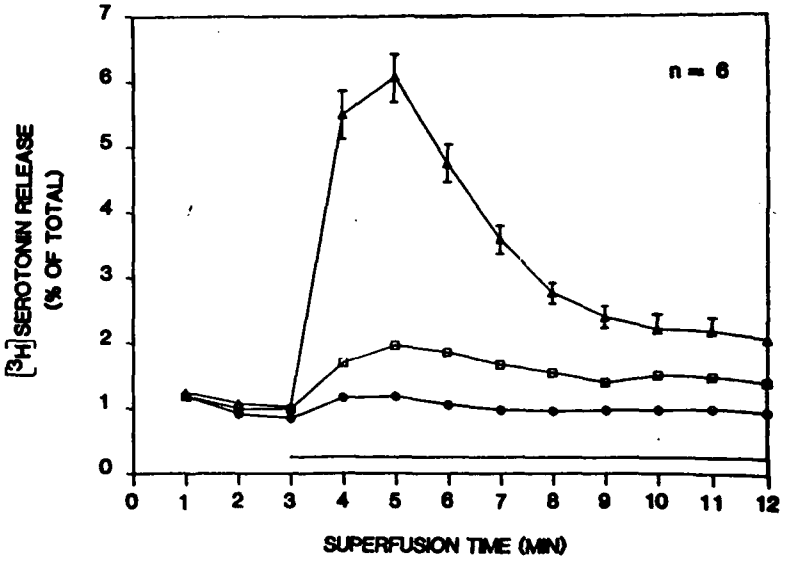

Fig. 1. Release of [^{3}H]serotonin from guinea pig striatal synaptosome fractions. *Triangle*, depolarized, Ca^{2+} present; *circle*, depolarized, Ca^{2+} absent; *square*, nondepolarized, Ca^{2+} present. After preloading with [^{3}H]serotonin, synaptosome aliquots were superfused with a high sodium, calcium-free buffer medium for 3 min, then 70-m*M* K^+-containing medium with or without Ca^{2+} or 5-m*M* K^+-containing medium with Ca^{2+} was similarly superfused for the 9 min marked by *horizontal bar* (i.e., 3–12 min).

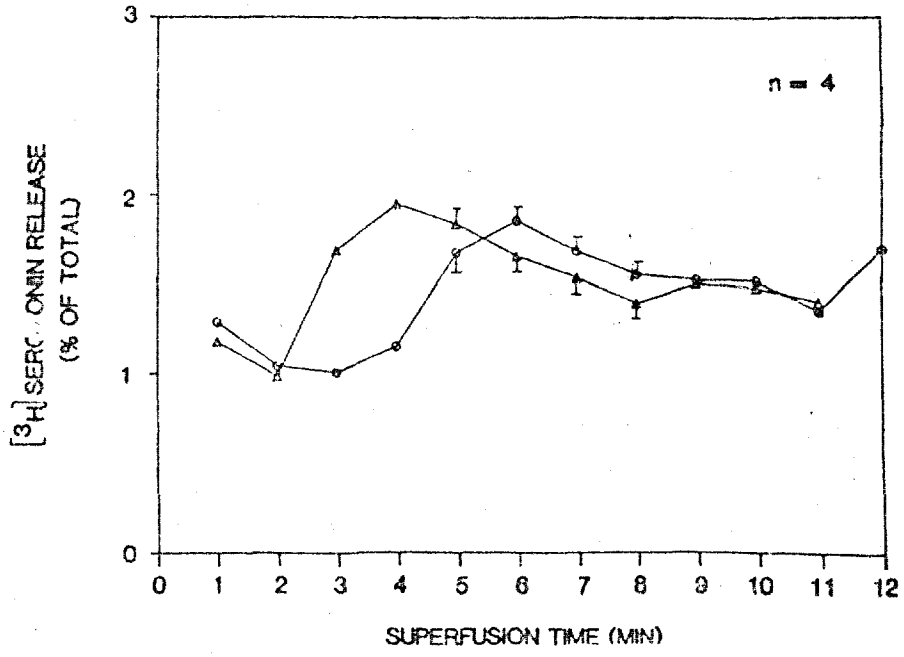

Fig. 2. Effect of compression to 68 ATA on the spontaneous (5-m*M* K^+-evoked) release of [^{3}H]serotonin. *Triangle*, 1 ATA; *circle*, 68 ATA.

to 68 ATA. Analysis of the data showed that the depression in serotonin release by these synaptic terminals was significant at the 4-min superfusion time ($P < 0.05$). On the other hand, the release in the absence of Ca^{2+} was little affected by compression ($P > 0.05$; Fig. 3 *B*). Figure 3 *C* shows the difference in [^{3}H]serotonin release between the Ca^{2+}-containing and Ca^{2+}-free media, which represents the calcium-dependent component of [^{3}H]serotonin release. Statistical comparison between the 1 ATA control curve and the 68 ATA calcium-dependent curve showed that [^{3}H]serotonin release at the 4-min superfusion time was significantly lower in the presence of pressure than in the corresponding control condition ($P < 0.05$). This indicates that pressure exposure depressed the initial depolarization-induced, calcium-dependent release of [^{3}H]serotonin by striatal synaptosomes isolated from the striatum.

DISCUSSION

Electron microscopic examination and the measurement of the cytoplasmic markers showed a high purity synaptosome preparation which retains an intact plasma membrane, with no apparent nonselective changes in permeability, which would be reflective of membrane damage. Also important to data interpretation was that high pressure exposure has not been found to have any disruptive effect on the synaptosomal plasma membrane (13).

High K^+ medium containing 1.2 m*M* Ca^{2+} initially produced a rapid increase in the release of [^{3}H]serotonin by the synaptosome preparation, followed by a decline in release approaching resting efflux levels. The removal of calcium from the depolarizing medium reduced [^{3}H]serotonin release evoked by high potassium by over 80%. This indicates that the evoked release of serotonin by striatal synaptosomes is highly dependent on the influx of calcium.

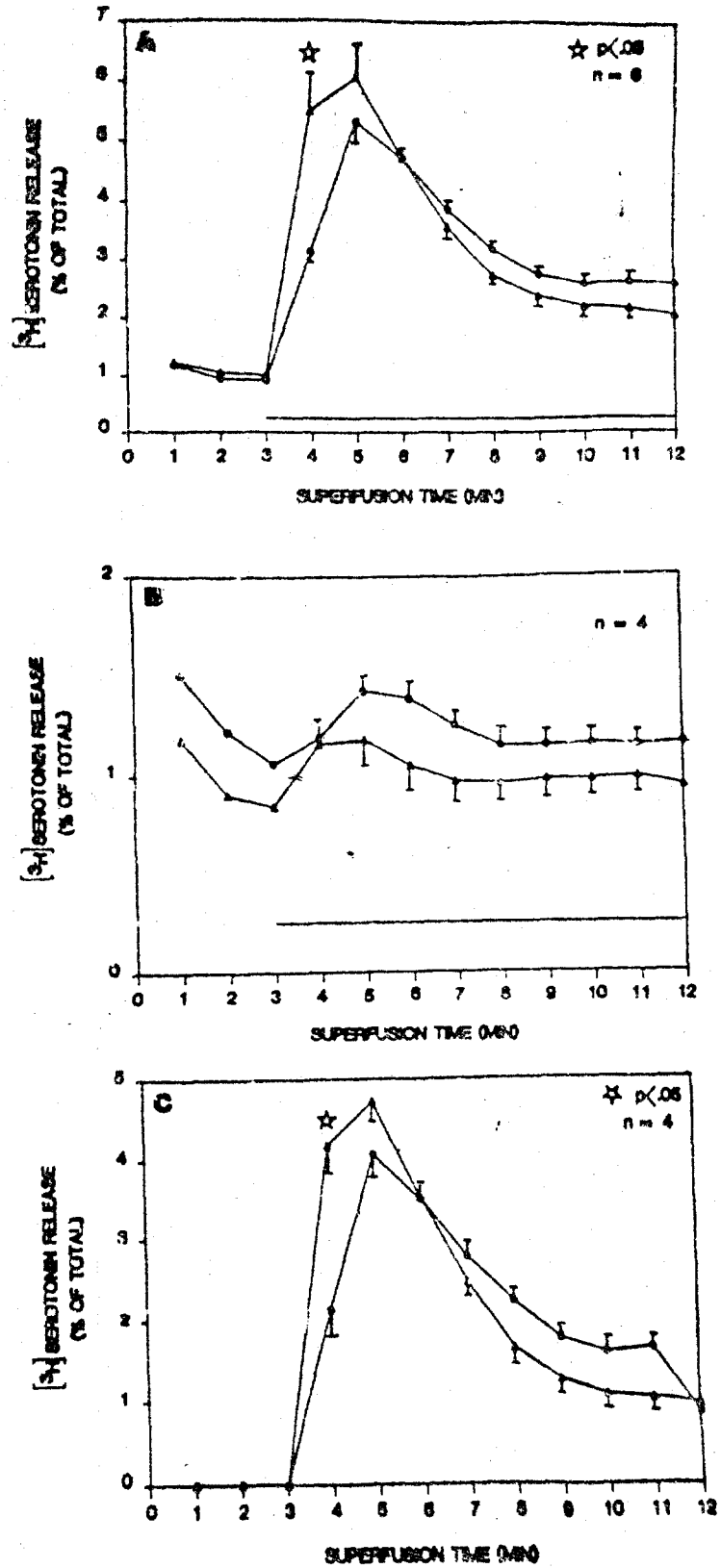

Fig. 3. Effect of compression to 68 ATA on the high K^+-evoked release of [^{3}H]serotonin from striatal synaptosomes. *A*, in the presence of Ca^{2+}; *B*, in the absence of Ca^{2+}; *C*, Ca^{2+}-dependent release, i.e., the difference between *A* and *B*. *Triangle*, 1 ATA; *circle*, 67.7 ATA.

The major observation in the present study was a 65% decrease in the initial efflux of [^{3}H]serotonin from depolarized striatal synaptosomes after compression to 68 ATA. This suppression of serotonin release parallels decreases noted previously in the calcium-dependent release of other neurotransmitters with pressure (11, 14, 15).

The pressure-induced decrease in the initial rate (viz in the first 1–2 min) of transmitter efflux seen in this and other studies (11, 14, 15) suggests that a concomitant decrease in voltage-sensitive calcium influx might be occurring at high pressure. However, in another study (13) we have observed that although $^{45}Ca^{2+}$ movement into synaptosomes is significantly depressed at 68 ATA, the initial (i.e., the first minute) amount of Ca^{2+} influx, although depressed, was not significantly different from control values (1 ATA). This relationship between the initial amount of $^{45}Ca^{2+}$ uptake and the significant depression of initial transmitter efflux leads us to propose that high pressure helium may alter transmitter release in more than one way.

First, high pressure most likely does interfere with voltage-sensitive calcium channel gating. Second, high pressure also probably interrupts some aspect of the intraterminal cascade for the calcium-dependent release of transmitter (e.g., calcium-calmodulin interaction, kinase II activity, membrane phosphorylation, or the interaction of the vesicle membrane with the terminal membrane) (16–18). Further support for this notion is provided by a preliminary study in our laboratory with the calcium ionophore, A23187, applied to synaptosomes subjected to high pressure. This study indicates that this ionophore does not alter the decrease in transmitter release at 68 ATA. A23187 circumvents the membrane-bound Ca^{2+} channel by forming a Ca^{2+}-H+ exchanger and therefore should facilitate the rise in intraterminal Ca^{2+} (19, 20). Since the effect of high pressure appears to be universal on calcium-dependent transmitter release, alteration of any or all of the above-mentioned sites could affect transmitter release at most synapses.

The increase in the serotonin metabolite, 5-HIAA, found by Gillard et al. (6) suggests an increase in metabolic activity or a decrease in the removal of this compound. Removal of 5-HIAA from the brain requires a membrane-bound protein carrier. Inhibition of this carrier by the drug Probenecid has been shown to produce an accumulation of 5-HIAA in brain tissue (21, 22). Since removal requires a membrane-bound protein carrier, it is reasonable to suspect that high pressure helium could exert its effect on the membrane to depress carrier activity, causing 5-HIAA levels to rise. It is unlikely that this inhibition of metabolite removal suppresses serotonin production, because serotonin synthesis does not appear to be feedback regulated (23). Therefore, the depression of transmitter release found in our study is not adequately explained by changes in serotonin metabolism or metabolite removal.

Whether this pressure-induced phenomenon is a contributory factor in the actual development of the symptoms of HPNS is currently unknown. However, the decrease in the initial rate of release of serotonin observed in synaptosomes may reflect in vivo events occurring in the synaptic terminal.

Since serotonin is believed to function as a modulator to presynaptically inhibit transmitter release (24,25), a small change in the timing of release of serotonin could result in a marked diminution of its inhibitory function. This could result in a significantly enhanced excitatory response. Coupled with similar events occurring in other inhibitory systems (viz GABA and glycine), symptomatic neurologic changes characteristic of HPNS could result.

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Gilman SC, Colton JS, Hsu SC, Dutka AJ. La pression supprime la libération de sérotonine par les synaptosomes striataires de cobaye.—Undersea Biomed Res 1988; 15(2):69–77. L'exposition à la pression élevée produit des changements neurologiques chez les humains, lesquels se manifestent par le tremblement, des changements dans l'EEG et des convulsions. Puisque des études antérieures ont impliqué le système nerveux sérotoninergique dans ces symptomes, il est d'intérêt à étudier la libération de sérotonine sous haute pression. Des synaptosomes isolés du corps strié de cobaye furent utilisés pour suivre l'efflux de sérotonine à 68 ATA. L'observation principale fut une diminution dans la libération de [^{3}H]sérotonine de synaptosomes striataires dépolarisés à 68 ATA. Vu le rôle de la sérotonine comme neurotransmetteur inhibiteur dans cette zone, la diminution observée dans la libération synaptique mène à conclure que la réduction de l'activité sérotoninergique dans les neurones striataires contribue probablement à la surexcitabilité associée avec le syndrome nerveux de la haute pression.

Gilman SC, Colton JS, Hsu SC, Dutka AJ. Supresión por presión de la liberación de serotonina en sinaptosomas del cuerpo estriado de conejillos de Indias.—Undersea Biomed Res 1988; 15(2):69–77. La exposición a presiones altas produce cambios neurológicos en humanos, que se manifiestan como temblor, cambios electroencefalográficos y convulsiones. Debido a que trabajos previos han implicado la participación del sistema serotoninérgico en estos síntomas, nuestro interés residió en el estudio de la liberación de serotonina a presiones altas. Se empleó sinaptosomas del cuerpo estriado de conejillos de indias para estudiar el eflujo de serotonina a 68 ATA. El hállazgo más sobresaliente fue la disminución en la liberación de [^{3}H]serotonina en sinaptosomas despolarizados de cuerpo estriado a 68 ATA. Debido a que la función de la serotonina a este nivel es de un neurotransmisor inhibitorio, la disminución observada en la liberación sináptica nos lleva a concluir que la reducción en la actividad serotoninérgica en las neuronas del cuerpo estriado probablemente contribuye a la hiperexitabilidad asociada al Síndrome Neurológico de Presión Alta (SNPA).

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