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CIRCADIAN RHYTHMS AND THE TOXICITY OF CENTRAL NERVOUS SYSTEM DR--ETC(U)
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CIRCADIAN RHYTHMS AND THE TOXICITY OF CENTRAL NERVOUS SYSTEM DRUGS,

FINAL REPORT.

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OBJECTIVES

(a) To determine the circadian toxicity rhythms for specific central nervous stimulants (amphetamines) and depressants (ethanol) in rodents, (b) to study the circadian pharmacological and physiological responses of the indicated drugs in therapeutic and/or sublethal doses, (c) to relate the influence of circadian behavioral and physiological drug-induced changes to endogenous biogenic amines and body chemistry circadian rhythms.

ABSTRACT

Twenty-four hour LD50 rhythms were determined for dl-amphetamine in isolated and aggregated mice. In both cases peak toxicity occurred between the 4th and 7th hour of the twelve hour dark period. The twenty-four hour toxicity circadian pattern for both isolated and aggregated groups were similar but dl-amphetamine was approximately six times more toxic in aggregated than in isolated groups. The greatest percent change in amphetamine toxicity for aggregated mice was approximately four times that noted for isolated groups. Peak toxicity for dl-amphetamine in isolated groups was significantly greater during the month of January when compared to the month of June of the same year. In additional studies on the effects of amphetamine in sublethal doses, on twenty-four hour rhythms of whole brain amines and blood glucose levels in mice, the blood glucose levels for control animals showed a diurnal rhythm. Amphetamine treatment (doses 5 mg/kg) decreased blood glucose levels by approximately 50% near the end of the dark phase but the decrease was not significant following treatment near the end of the light phase. In the same investigation, brain norepinephrine

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showed a twenty-four hour rhythm for control animals but amphetamine treatment had no influence on the regular diurnal norepinephrine pattern. Control serotonin circadian rhythms were not shown for the mouse whole brain and amphetamine treatment was ineffective in phase-shifting the serotonin control responses.

In additional studies involving specific brain parts, norepinephrine and serotonin rhythms occurred in both the mouse midbrain and caudate nucleus. Amphetamine treatment (5 mg/kg) caused characteristic biphasic responses for both blood glucose and brain norepinephrine. Amphetamine treatment at 1300 hours (serotonin troughs) caused a sustained elevation in serotonin for both brain tissues. The elevation lasted the duration of the light period or approximatley some 15 hours following treatment. These findings suggest that both toxicity and the therapeutic effectiveness of the amphetamines are influenced by the time of the day the drug is administered and that probably this circadian toxicity is associated with alterations in brain amines and the environmental arrangement of the animals (acute isolation versus aggregation). Individual animal variations in drug efficacy, potency and the expression of many dangerous side effects might be related to the daily and/or seasonal environmental parameters. It is possible that the implications of this research might require that in studies dealing with the abuse of these compounds in human patients, one might need to examine and be cognizant of the customs and habits under which the drugs are taken.

Diurnal rhythms for dopamine, norepinephrine and acetylcholine have been observed for the rat whole brain, midbrain, cortex, caudate nucleus and cerebellum. The acrophases for norepinephrine and dopamine at the indicated times occurred at identical periods during the day (dark phase) and exhibited similar circadian patterns. The possibility of a neurohumoral role for dopamine versus norepinephrine in the mechanism of action of CNS stimulant (amphetamine) and depressant (alcohol) is indicated.

In another experiment, male Sprague Dawley rats injected with 2 mg/kg of ethyl alcohol and analyzed one hour later at eight different times of the day showed diurnal rhythms for the urine and specific tissues. Brain and liver rhythmic patterns were similar, peaking at D-6 and troughing at L-9. Urine and blood levels were greatest at D-3, D-6 and least at L-3 and L-9 respectively. All animals were circadinized for three weeks on 12 hour dark, 12 hour light cycles. The increased concentrations of blood alcohol might indicate a decrease in enzymatic metabolism during the dark phase and/or greater enzymatic competition with the alcoholic degradative products at a time when the animal is most active. Tissue alcohol levels were determined by the enzymatic method of Bonnichsen (1965).

Concentrations of biogenic amines in brain tissues of rats and mice have been shown to vary with the time of day. Several investigations have shown the toxicity of certain CNS depressants and stimulants to the circadian rhythm. This investigation was designed to evaluate the toxicity of ethanol and to correlate this with endogenous and possibly modulator amine levels in the brain. Ethanol LD_{50} (Litchfield and Wilcoxan, 1949) values were determined in the mouse and found to be circadian. All animals were

adapted for a minimum period of four weeks to a 12 hour light, 12 hour dark programmed illumination cycle. Specific endogenous brain amines were analyzed by the fluorometric method of Welch and Welch (1966) at different times of the day before and following drug treatment in an effort to directly associate ethanol toxicity with body chemistry and physiological function.

The midbrain, cortex, caudate nucleus, cerebellum and whole brain of male Sprague Dawley rats were analyzed for dopamine and norephinephrine levels in identical tissues at different times of the day. The acrophases for both compounds occurred during the dark phase of the 12-hour dark-light photoperiod. Dopamine concentrations were greater than norephinephrine concentrations in specific brain areas. The acrophases for norepinephrine were more sustained and followed dopamine peaks for most periods examined on the cycle as would probably be expected since dopamine is the precursor for norepinephrine synthesis. Norepinephrine and dopamine showed similar patterns during the light phase of the photoperiod with troughs occurring during this phase for both compounds. All animals were adapted to a laboratory condition with automatically timed light-dark photoperiods for a minimum period of three weeks at ambient temperature of 23 ± 1°C before experimentation. The catecholamine levels in whole brain and in brain parts were extracted and anlyzed according to the fluorometric procedure of Welch and Welch (1966). The sensitivity of other analytical methods for catecholamines were studied and compared. It is possible that different neurohumoral roles for dopamine and norepinephrine and the importance of dopamine in chronotherapy is indicated.

PARTIALLY SUPPORTED RESEARCH

1. INVOLVEMENT OF BRAIN BIOGENIC AMINES IN THE SEXUAL MATURITY OF THE RAT.

Serotonin (5-hydroxytryptamine, 5-HT), norephinephrine and dopamine levels were measured in different parts of the rat brain in order to determine the role of biogenic amines in sexual maturity. Pregnant mare serum gonadotropin (PMS) was injected into 25 day old immature rats for the induction of ovulation. Animals were sacrificed at 6 hour intervals for 72 hours and the cortex, cerebellum, caudate nucleus and hypothalamus were isolated and analyzed for their biogenic amine content using the method of Welch and Welch. Levels of serotonin reached a maximum peak after 12 hours in all brain parts except for the hypothalamus which reached its peak at 54 hours after PMS injection. Norepinephrine was found to reach its maximum value between 60 and 66 hours after PMS injection except for the cerebral cortex. Dopamine determinations revealed that its maximum levels occur 12 hours after pretreatment in all parts except for the cerebellum. It was concluded from this work that PMS has a definite effect on the biogenic amine levels of the brain and also that these biogenic amines might be involved in sexual maturity and ovulation.

2. EFFECTS OF CNS STIMULANTS ON THE SEXUAL MATURITY INDUCTION OF THE IMMATURE RAT.

The effects of pentylentetrazol, picrotoxin and theophylline were studied in pregnant mare serum gonadotropin (PMS) and testosterone induced sexual maturity in the immature rats. In animals treated with PMS, pentylentetrazol injection at a level of 5 mg/kg and 10 mg/kg caused a significant increase in ovarian and uterine weights. Higher doses of pentylentetrazol (20 and 40 mg/kg) depressed ovarian and uterine weights in comparison to the 10 mg/kg group. Testosterone injection alone induced vaginal opening but ovulation did not take place. PMS induced ovulation was stimulated significantly by simultaneous injections of testosterone. Picrotoxin or theophylline were without effect on the ovarian weights or ovulation. When PMS, testosterone and pentylentetrazol were injected simultaneously, ovarian weights were 100% more than PMS treated rats. However, the uterine weights did not change significantly from the PMS treated group. The results of this experiment indicated that pentylentetrazol has shown specific activity by stimulating the release of gonadotropin.

CURRENT REPORTS AND PUBLICATIONS

Walker, C.A. and Charlton, C.G., "The effects of amphetamines on twenty-four hour rhythms of brain amines and blood glucose in mice," (submitted for publication, Int. J. of Chronobiology).

Walker, C.A. and Whitworth, U.G., "Diurnal changes of dopamine and norepinephrine in different areas of the rat brain and phase-shifting by ethyl alcohol," (presented at the Neuroscience Meeting in St. Louis, Mo., October, 1974).

Walker, C.A. and Ekpoudia, I.K., "Circadian rhythms of cholinacetylase and cholinesterase from identical tissues in different parts of the rat brain," (presented at the Neuroscience Meeting in St. Louis, Mo., October, 1974).

Walker, C.A. and Charlton, C.G., "Diurnal rhythms of blood glucose and its relationship to the diurnal rhythms of norepinephrine and serotonin in the mouse cortex and midbrain," (presented at the Fall Meeting of the American Society for Pharmacology, Montreal, Canada, August, 1974).

Parker, V.D., Soliman, K.F.A. and Walker, C.A., "Involvement of brain biogenic amines in the sexual maturity of the rat," Fed. Proc., 1975.

Howell, J., Soliman, K.F.A. and Walker, C.A., "Effects of CNS stimulants on the sexual maturity induction of the immature rat," Proc. Ann. Xavier Bio. Med. Symp. 3, 55, 1975.

Soliman, K.F.A. and Walker, C.A., "Effect of estrogen and progesterone on the circadian periodicity of blood cells," XII Int. Chronobiology, 1975.

Soliman, K.F.A. and Walker, C.A., "Diurnal periodicity for ethanol absorption tissue levels and metabolism in the rat," Int. Soc. Chronogiol., 1975.