The Effect of Catnip, an Hallucinogen, on the Neurotransmitters Serotonin, Norepinephrine and Dopamine

by

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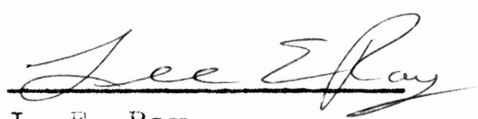

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ABSTRACT

The concentration of Norepinephrine in rat brain was compared to the levels in mouse and rat brains which had previously been studied. Whole brains of white rats where extracted and the concentration of Norepinephrine was determined fluorometrically. Present studies indicated that the concentration of this neurotransmitter was the same in the rat brain as it was in the mouse although there was a large variance in the sample. In other experiments, the brain was divided into four regions, 1. the medulla and pons, 2. the cerebral (right) hemisphere, 3. the cerebral (left) hemisphere, and 4. the cerebellum, prior to extraction and assay. The concentration of norepinephrine in these areas varied, with the concentration in the medulla and pons higher and the cerebellum lower than previous reports in mouse and rat brains.

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INTRODUCTION

The concentration and location of neurotransmitters in the brain has a definite effect on the behavior of an animal. It has been reported that the medullary and midbrain raphe system and its serotonin-containing neurons may be a major sleep-inducing structure (Szara, 1970; Albers et. al., 1972; McGeer, 1971) and it has been suggested that behavioral effects are dependent upon the availability of serotonin in the brain. It has been reported that increasing catecholamine concentrations, or decreasing serotonin concentrations seem to enhance sexual activity (McGeer, 1971). The injection of the neurotransmitter norepinephrine decreases spontaneous motor activity (Myers, 1974) while a close correlation between the physiological development of the brain and the concentration of norepinephrine (Cooper et. al., 1974) has been found.

Certain drugs are capable of modifying the concentration of neurotransmitters in the brain. Lysergic acid diethylamide (LSD), in very low concentrations, reversibly blocks the spontaneous activity of raphe neurons. The primary site of action of this hallucinogenic drug is in the medulla rather than the midbrain (Szara, 1970; Quarton et. al., 1967). The drug resperpine apparently blocks the synthesis of serotonin, causing tranquilizing effects and depression.

Iproniazid is a monamine oxidase inhibitor which causes an increase in the levels of serotonin in the brain resulting in excitation and delusions (Quarton et. al., 1967).

Drastic changes in the levels of dopamine in an organism become manifest in Parkinson's disease. The major cause of Parkinson's disease is a failure to convert tyrosine into L-DOPA, the result of a non-functioning tyrosine hydroxylase system. Physical damage can cause this disease, and in victims who had recieved damage to the brain, the levels of tyrosine hydroxylase are one-sixth the normal value in the corpus striatum (McGeer, 1971).

Concentrations of the neurotransmitters affect the internal biochemistry of the neurons. The depolarization of the membrane of the axon initiates the release of neurotransmitters at the pre-synaptic vesicles, allowing the propagation of the impulse to the post-synaptic receptor site of another neuron (Katz, 1966). It has been shown that a large increase in c-AMP content occurs in neurons exposed to solutions of norepinephrine or histamine, indicating that biochemical changes are being triggered by the external changes in the norepinephrine levels. Further studies indicate that the overall effect of neurotransmitters on the organism is a result of their effects on internal cellular components (Nathanson et. al. , 1977; Russell et. al., 1975).

MATERIALS AND METHODS

Male and female white rats (250-350 g) were maintained on LAB-BLOX rat chow diet (Wayne Corporation) and water ab libitum. The rats were stunned and decapitated with a pair of large scissors.

DISSECTION

The brain was quickly and carefully removed and placed in physiological saline while the meningies were removed. The brains were divided into four parts; 1. the left cerebrum, 2. the right cerebrum, 3. the cerebellum, and 4.the medulla oblongata and pons. The dissection of the brain was performed as follows: first, the cerebellum, medulla oblongata, and pons were separated from the cerebrum with one cut. The second cut was made between the cerebellum and the medulla oblongata. The three sections could be taken through the extraction immediately or frozen for use at a latter date. (Glowinski et. al., 1966).

EXTRACTION PROCEDURES

The following procedures were performed on each individual sample. Using a razor blade, a sample was cut into small pieces and transferred to an homogenizer where it was homogenized with 2.8 ml of cold .01 N HCl and

0.2 ml of 10% EDTA. The homogenizer was rinsed with 3.0 ml of .01N HCl and transferred to a 50 ml bottle which contained 25 ml of n-butanol and 4.0 gm of NaCl. The vial was shaken for 10 minutes at 250 excursions / minute and the sample was centrifuged (3000X g, 8 min.). The pellet was discarded and the n-butanol was transferred to a 150 ml bottle which contained 40 ml of n-heptane and 3.0 ml of phosphate buffer (pH= 7.3). The samples were shaken for 10 minutes and then centrifuged (2000 x g, 8 min.). The n-heptane was discarded and the phosphate buffer was acidified to pH 3.5-4.0 with 3N HCl. A 20 ml aliquot of peroxide-free ether was added to the sample and shaken for 10 minutes. The ether phase was discarded and 0.5 ml aliquots of the aqueous layer were used for the fluorometric assays (Welch et. al., 1969).

Extraction blanks were prepared by replacing the brain homogenate with an equivalent amount of .01 N HCl. This was added to the butanol-NaCl mixture and the extraction procedures described above were carried out.

Recovery was calculated by adding a known amount of a standard amine solution (See under Fluorometric assays) to a known volume of homogenate (spiked solution) and performing the extraction and fluorometric assays. The difference in fluorometric readings between the spiked solution and a comperable unspiked solution gives the amount of nore-

pinephrine recovered. The ratio of the recovered norepinephrine to that of the known amount of norepinephrine added will give the percent recovery.

FLUOROMETRIC ASSAYS

The norepinephrine was assayed by adding 0.5 ml of 2M acetate buffer (pH=6.8) to 0.5 ml sample; followed by the sequential addition of .1 ml iodine solution,.15 ml of .1 N sodium thiosulfate, and .2 ml of alkaline ascorbic acid/ ethylene diamine. A five minute period elapsed between the addition of each of the above compounds to allow them to react fully. (An exception is the acetate buffer. The iodine solution can be added immediately after the acetate buffer has been added.) The iodine solution was prepared by dissolving 1.269 gm of Iodine and 5 gm of NaI in distilled water and diluting to 100 ml. The solution was stored in a dark bottle in the cold. The ethylene diamine solution should be prepared before the start of each set of assays and is made by dissolving 50 mg of ascorbic acid in 0.63 ml of .01 N HCl, and adding 5.6 ml of 10 N NaOH and 0.1 ml of ethylene diamine solution. After the addition of all solutions, the norepinephrine sample is placed within 10 cm of a fluorescent light and allowed to develop for 35 minutes. The fluorescence is then read at 510 nm after excitation at 400 nm.

Dopamine concentration is determined by adding 0.5 ml of 2 M acetate buffer (pH=6.8) to a 0.5 ml sample, followed by the addition of 0.1 ml of 0.1 N iodine solution. The following solutions are added in sequence: 0.2 ml alkaline sodium sulfite/EDTA, and .25 ml 1:1 glacial acetic acid/HCl conc. A five minute wait is required between the addition of each compound. The sample is developed by placing it in boiling water for 45 minutes, allowing it to cool to room temperature, and reading the fluorescence at 380 nm after excitation at 335 nm.

Serotonin can be measured by acidifying an aliquot (0.5 ml) with 0.5 ml of 6 N HCl and reading the fluorescence immediately at 535 nm after excitation at 295 nm.

A standard curve was prepared from the known "standard amine solution." The standard was prepared by diluting stock solutions containing 1 mg/ml of norepinephrine, serotonin, or dopamine, with .01 N HCl, to working solutions of 100 ng/ml. Then, 10 ml of the serotonin and dopamine working standards and 5 ml of the norepinephrine working standard were diluted together to 50 ml with .01 N HCl to form a standard amine solution. Different volumes of this were used to obtain the point for the standard curve.

RESULTS

Ten samples of whole brain where extracted and analyzed for norepinephrine. The average of all ten samples was 376 ng $\pm$ 141 ng/g tissue(See table 1.). This is in agreement with previous reports (Cooper, et. al, 1974).

Recovery factors were determined in five experiments (See Table 2.). The recovery values varied from 11.0% to 58.5%; the 58.5% was statistically invalid but, using the other four values, the average recovery value was 20.3%.

Four samples were used to determine the contribution of the organic solvent to the fluorescent measurements. The average value was .005 and was subtracted from all the sample readings in the experiments.

A standard curve was prepared by plotting fifteen points and drawing the most accurate straight line through them using least squares analysis (See Figure 1.).

One experiment was performed in which the brain was divided into the medulla, right and left cerebral hemispheres, and cerebellum. The concentration of norepinephrine in the medulla was 272 ng/ g tissue, which is lower than concentrations reported for mice (Welch et. al., 1969) and rats (Cooper, 1974.). The concentration in the cerebellum was 420 ng/g tissue which is higherthan reported for the rat (Cooper, 1974.). The norepinephrine concentration in the

right and left cerebral hemispheres is lower than those concentrations previously reported in the mouse and higher than the values reported in the rat (Cooper et.al., 1974) (See Table 3 and 4).

DISCUSSION

Previous experiments have indicated that the normal level of norepinephrine in rat brains is 490 ng/g tissue (Cooper et. al., 1974) while an experiment on mice produced results which had a range of 350-450 ng/g tissue (Welch et. al., 1969).

The norepinephrine levels in the whole rat brain support earlier readings in mice (Welch et. al., 1969). The range of concentrations of norepinephrine seem to be greater in the rat brains than in man or mouse brains (Gardner,et.al., 1967, Welch et.al., 1969,Cooper et. al, 1970).

The similarities between the mouse and rat is not shared by other animals. In fact, there appears to be a steady decrease in the levels of norepinephrine as the animal increases in size (Cooper, et. al.,1966; Gardner, et. al.,1967). For example, in man the values are 240ng/g tissue for the cerebral hemispheres, 720 ng/g tissue for the medulla oblongata, and 170 ng/g tissue for the cerebellum(See Table 4.)

The experiment in which the brain was dissected into the cerebrum, medulla, and cerebral hemispheres, the results did not agree with earlier reports in these areas of the brain(See Table 4.)(Cooper et. al, 1970).

It is possible that certain factors could have caused the levels of the transmitters to change. The rats were transported and could have been nervous at the time the experiment was preformed. If any of the rats saw the dissection or stunning process, the levels of the neurotransmitters could also change. Due to lack of time, proper filters, and proper fluorescent lamps, the concentrations of dopamine and serotonin were not determined.

CONCLUSIONS

The concentration of norepinephrine in rat brain has been determined and compared to values reported for mice, rats, and men. The concentrations of norepinephrine in rat brain is in agreement with those reported in mice and rats (Cooper et.al., 1970; Welch et.al., 1969). However, different sections of the rat brain did not agree with earlier reports in either the mouse or rat (Cooper et.al, 1970; Welch et.al, 1969). The rat appears to have a wider range of these concentrations than a mouse. As mentioned in the discussion, this could be due to arousal of the animal.

Figure 1.

Standard Curve

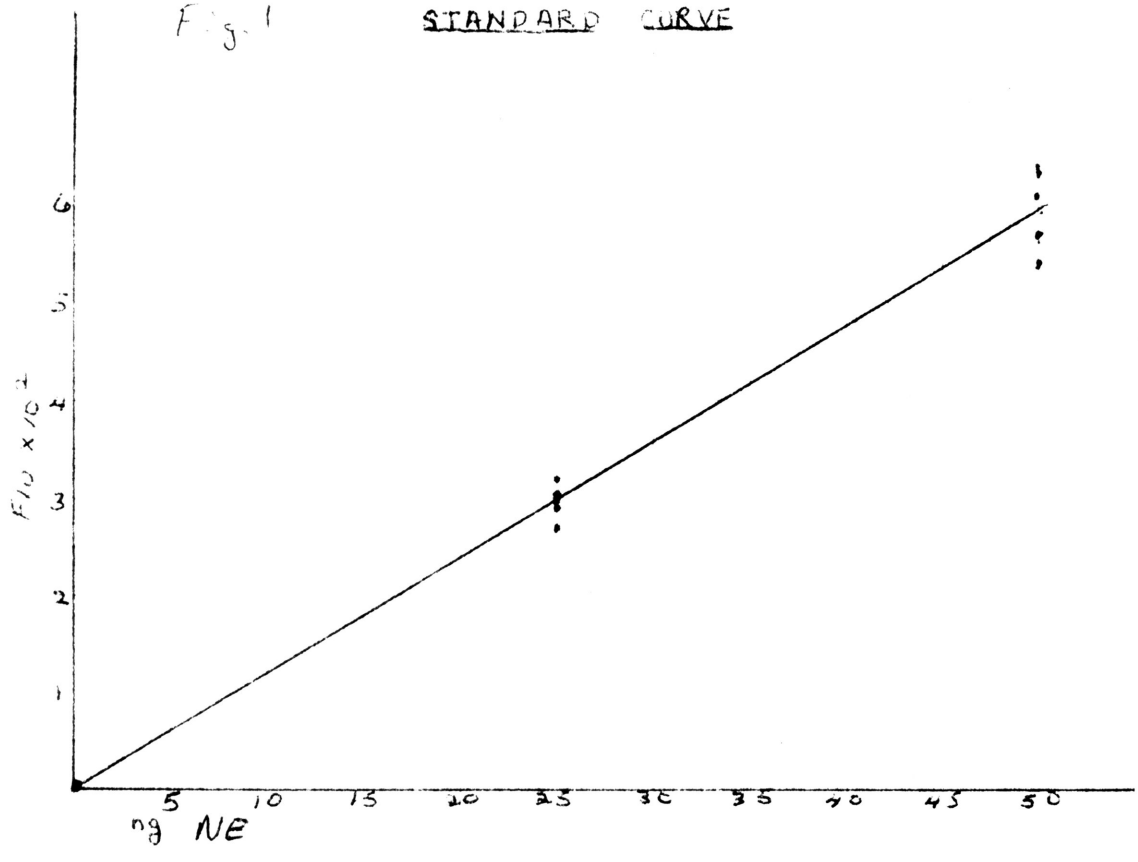

TABLE 1

Concentration of Norepinephrine in Whole Rat Brains

Experiment	Sample	Weight of Tissue Assayed	Fluorescence	Net Fluorescence*	NG/G Tissue
12	A	1.170 g	.1260	.1210	437
	B	1.350 g	.1245	.1195	362
19	A	.230 g	.0405	.0355	644
24	B	.290 g	.0240	.0190	276
	C	.280 g	.0211	.0160	226
	D	.270 g	.0240	.0190	303
28	A	.310 g	.0222	.0172	462
	B	.300 g	.0285	.0235	667
30	A	.086 g	.0120	.0070	330
	B	.086 g	.0060	.0010	57

Average: 376ng $\pm$ 141 ng

The net readings have been corrected for reaction (F=.0150-.0210) and extraction (F= 0.0050) blanks.

TABLE 2

Recoveries of Norepinephrine from Extraction of Whole Brain

Experiment	Sample	Amount of Norepinephrine Added	% Recovery
19	B	100 ng	58.5
28	AS	150 ng	11.0
	BS	150 ng	19.2
30	AS	500 ng	25.1
	BS	1000 ng	25.8

TABLE 3

Concentration of Norepinephrine in Sections of Rat Brain

Experiment	Sample	Weight of Tissue Assayed	Fluorescence	Net Fluorescence*	NG/G Tissue
15	L.Hem.	.30 g	.0300	.0250	353
	R.Hem.	.38 g	.0285	.0235	263
	Med.	.30 g	.0240	.0190	272
	Cere.	.15 g	.0195	.0145	420

* The net readings havebeen corrected for reaction(F=0.0) and extraction (F=0.005) blank.

TABLE 4

Concentration of Norepinephrine in Various Species

Brain Region	Species	Norepinephrine(ng/g tissue)
Whole Brain	Man	X
Whole Brain	Mouse	350-450
Whole Brain	Rat	490
Medulla	Man	370-720
Medulla	Mouse	500-800
Medulla	Rat	720
Cerebral Hemisphere	Man	30-240
Cerebral Hemisphere	Rat	180
Cerebral Hemisphere	Mouse	350-500
Cerebellum	Man	60-170
Cerebellum	Rat	170
Cerebellum	Mouse	X

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