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Effects of Elevated Calcium on Learned Helplessness and Brain Serotonin Metabolism in Rats

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TRULSON, M E, K ARASTEH AND D W RAY *Effects of elevated calcium on learned helplessness and brain serotonin metabolism in rats* PHARMACOL BIOCHEM BEHAV **24**(3) 445–448, 1986 —The effects of elevated calcium on learned helplessness in rats was tested by maintaining animals on either distilled water or water containing 2 5% calcium Animals that were maintained on drinking water containing high calcium showed elevated levels of brain and serum calcium Rats that were maintained on high calcium drinking water showed significantly longer escape latencies than their non-calcium counterparts after they were pretreated with inescapable electric shocks Lower levels of 5-hydroxyindoleacetic acid (5HIAA) were found in the forebrain and brainstem of animals maintained on high calcium drinking water There was no significant correlation between blood or brain calcium or 5HIAA levels and latency of escape We conclude that elevated levels of calcium enhance learned helplessness and decrease brain serotonin turnover The relationship between depressive states and calcium homeostasis is worthy of further investigation

Serotonin Learned helplessness Depression Calcium Raphe neurons

SEROTONIN has been extensively investigated for its potential role in depression Some antidepressants result in increased synaptic serotonin [5, 7, 15] Petty and Sherman [11] reported that a decreased release of serotonin was associated with learned helplessness, a model for depression in animals Furthermore, postmortem studies in humans have reported a decreased level of serotonin and its metabolites in the brains of suicide victims [2,19] Thus, a variety of studies has suggested that serotonin may play a role in depressive states

Mineral metabolism in depressive illness has also been the subject of investigation for many decades Calcium metabolism in mood disorders has been studied since the 1940's Changes in calcium levels associated with depressive illness have been reported [3, 4, 6, 9] However, since these studies are purely correlational in nature, they have not determined whether the altered calcium levels is a cause or a consequence of the disease state Moreover, most of these studies have measured serum calcium levels, which do not necessarily reflect brain calcium concentrations In addition to patients with the primary diagnosis of depression, patients with hyperparathyroidism also suffer from mood disturbances The etiology of at least some of these disorders has been hypothesized to be related to abnormal calcium metabolism [13] Increased levels of calcium in the cerebral spinal fluid have been reported during the manifestation of depression in these patients, while the reverse occurs during recovery from such symptoms [9]

Recent data from our laboratory revealed that small elevations of calcium ions (10–15%) significantly suppressed the activity of serotonin-containing dorsal raphe neurons by approximately 35% *in vitro* [23] Elevated calcium has been found to suppress the activity of serotonergic neurons *in vivo* as well (unpublished data) Therefore, the abnormal levels of serotonin frequently found in depression may constitute a secondary effect rather than a primary one

Based on these findings, we hypothesized that increased calcium levels within the physiological range have depressogenic effects. Testing this hypothesis could best be accomplished using an animal model of depression. Seligman has suggested four criteria for the usefulness of an animal model of a human disease state These criteria are that the following conditions should be similar (1) physiological and behavioral symptoms, (2) etiology, (3) cure, and (4) prevention If the animal model and human condition are similar on one or more of these criteria they can be tested in predicting a similarity in the remaining criteria [17,18]

The learned helplessness model of depression is a relevant model since it has been shown to correspond with many of the behavioral symptoms of human depression The model has also been reported to be specific in that the induced helplessness can be reversed by antidepressants but not other drugs [21] Seligman and his colleagues [10,16] found that, following administration of inescapable electric shock, dogs failed to escape even when they were presented with an

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escapable situation. That the effect was due to the inescapability rather than the shock itself was demonstrated by the fact that equal amounts of escapable shock failed to produce helplessness. The effect has been replicated with several species of animals, including humans and rats [8].

The following experiments were conducted to determine the effect of elevated calcium on the acquisition of learned helplessness and on brain serotonin metabolism in rats.

METHOD

Behavioral Testing

Sixteen male Sprague-Dawley rats weighing between 330–380 g were housed in individual cages and maintained on ad lib food and water. The test apparatus consisted of a 26.7×30.5×24.1 cm Lehigh Valley test chamber and a sound-attenuated cubicle. The front, top, and back panels of the apparatus were made of Plexiglas while the side panels were made of stainless steel. The animals were observed through a one-way mirror, and a fan provided a masking noise and ventilated the test chamber. Electric shock was delivered through an electromechanical device consisting of a 110 V power supply, a remote hand switch and a recorder. The lever was mounted 3.8 cm from the floor and had to be pressed 3 consecutive times in order to terminate the shock.

The rats were randomly assigned to 2 groups (N=8/group). Group I was maintained on distilled water containing 2.5% calcium (as the chloride salt) and 0.25% saccharine sodium. The saccharine was added to compensate for the somewhat bitter taste of the calcium solution. Group II was maintained on distilled water containing 0.25% saccharine sodium. All rats were maintained on water and food ad lib for two weeks prior to the test procedure. During training and testing, no food or water was available to the animals. Each rat received one session of 75 trials of inescapable shock with the bar present, but inoperative, in the chamber. The shocks were random and unsignaled. The duration of each trial was 12 seconds with the intensity of 1 mA. The intertrial interval was variable. It averaged 60 seconds and ranged from 10 to 110 seconds. Each session lasted an average of 90 minutes. Forty-eight hours after this pretreatment session, each rat was tested for the acquisition of learned helplessness. The test session consisted of 20 trials of escapable shock with 3 bar presses as an escape response. If the rat failed to escape, the shock lasted for 60 seconds. Intensity of the shock and the intertrial interval were identical to those of the pretreatment.

Serotonin Metabolism

Ten days after the last test session the animals described above were decapitated and their brains were removed. This 10-day interval was allowed to permit the animals to return to baseline levels, i.e., to allow for any effects due to the training and testing procedures to dissipate. During this period the subjects were maintained on the drinking water described above. Thus, the animals were maintained on high calcium or saccharine drinking water for a total of 26 days prior to neurochemical analyses. The brain was dissected into forebrain and brainstem and the samples were weighed and frozen on dry ice, then stored in a freezer at −40°C until the time of assay. Ten ml of homogenizing solution (10 ml of 1.0 M sodium metabisulfite, 10 ml of 1.0 M disodium EDTA, 270 ml of 0.05 M perchloric acid, and 10 ml of a 5 μg/ml solution of N-methyldopamine (MeDA, as the internal standard) were added to the frozen brain samples in an Eppendorf tube. The sample was then sonicated for 1 minute with a microtip Fisher sonic dismembraner (Model 300) and then centrifuged for 5 minutes at 12,000 rpm. The supernatant was decanted onto a bioanalytical systems microfilter, and the filtrates centrifuged for 10 minutes at 3,000 rpm. The filtrate was transferred to an Eppendorf tube and frozen until assayed. The samples were thawed and 250 μl were pipetted into conical autosampler vials, and the vials were capped and loaded onto the autosampler.

The high pressure liquid chromotagraph system consisted of a Beckman Altex Model 334, controlled by a Model 421 computerized controller. The column was an Ultrasphere ODS 5 μ column. Three model 110A pumps were connected to a Model 500 autosampler equipped with a 50 μl loop and a CrLB integrator. The detector was a Bioanalytical Systems LC-3 amphomeric detector with a glass carbon electrode. The following parameters were used: Detector, E=0.62 V, Sensitivity=1.20 nA/V, Flow rate=1.4 ml/min, Gradient, The buffer was 0.15 M chloroacetic acid containing 0.8 mM sodium octyl sulfate, pH 2.8. 100% buffer was followed by 92% 8% buffer acetonitrile over the first 6 minutes of the 40-minute run, and then remained constant at 92% 8% for the remainder of the run. The system then recycled for 10 minutes back to 100% buffer. Suitable standards were run concurrently with the samples. For calculation of the unknown concentration the following formula was used. For example, for serotonin (5HT)

$$\frac{\dfrac{\text{(Peak area 5HT)}}{\text{(Peak area NMeDA) sample}}}{\dfrac{\text{(Peak area 5HT)}}{\text{(Peak area NMeDA) standard}}} \times \text{concentration of standard} = \text{concentration of 5HT}$$

Concentration was then expressed as ng/g. This system is capable of producing reliable data for serotonin and 5-hydroxyindoleacetic acid (5HIAA) in the same sample.

Calcium Assays

Twenty male Sprague-Dawley rats weighing between 330–380 g were used in this experiment. The animals (N=10/group) were maintained on distilled water containing 0.25% saccharine or 2.5% calcium in distilled water containing 0.25% saccharine for 14 days and then the training procedures were replicated (with virtually identical outcome). Ten days later the rats were decapitated, blood was collected in heparinized tubes from the cervical wound, and the brains were removed and dissected into forebrain and brainstem. The tissues were then weighed and frozen at −40°C until assayed. Serum and brain tissues were assayed for calcium concentrations using a modification of the spectrophotometric method of Baginski *et al.* [1]. Briefly, this method uses the metalochrome dye, O-creosolphthyalein complexone, which forms an intensely colored complex of calcium and can be quantitated by measuring the absorbance at 575 nm. The values were corrected for reagent blanks, and measured against calcium standards.

RESULTS

The rats maintained on high calcium drinking water had a significantly longer mean latency of escape response than

TABLE 1

BRAIN SEROTONIN AND 5-HYDROXYINDOLEACETIC ACID (5HIAA) IN CONTROL AND ELEVATED CALCIUM RATS

Group	Forebrain Serotonin	Forebrain 5HIAA	Brainstem Serotonin	Brainstem 5HIAA
Control	2 31 ± 0 10	2 40 ± 0 12	1 36 ± 0 15	1 82 ± 0 08
Calcium	2 64 ± 0 09*	1 79 ± 0 13*	1 70 ± 0 09*	1 28 ± 0 10*
(% change)	(+14 3%)	(−26 3%)	(+25 0%)	(−29 7%)

Rats were maintained on calcium drinking water (2 5%) containing saccharine (0 25%) or saccharine alone (0 25%) for 10 days following the test procedure Data are presented as Means ± S E M s for 8 rats/group

*Significantly different from control group, $p<0\ 05$, two-tailed t-test

TABLE 2

EFFECTS OF CALCIUM IN THE DRINKING WATER ON BRAIN AND SERUM CALCIUM LEVELS

Group	Serum	Brain Forebrain	Brain Brainstem
Control	8 14 ± 0 04	2 43 ± 0 02	2 42 ± 0 02
Calcium	10 10 ± 0 46*	2 88 ± 0 02*	2 88 ± 0 02*
(% change)	(+24 1%)	(+18 5%)	(+19 0%)

Rats were maintained on calcium drinking water (2 5%) containing saccharine (0 25%) or saccharine alone (control) for 14 days prior to assay Data are presented as means (mM) for 10 rats/group ± S E M

*Significantly different from control, $p<0\ 001$, two-tailed t-test

their non-calcium counterparts ($t(12)=2\ 27$, $p<0\ 025$) The group mean latencies were 33 0 seconds for the calcium group and 18 3 seconds for the non-calcium group

The group of animals maintained on high calcium drinking water had significantly higher levels of serotonin in the forebrain than the non-calcium group ($t(12)=2\ 75$, $p<0\ 02$) The amount of serotonin in the brainstem, although somewhat elevated, was not significantly different from controls The amount of the metabolite of serotonin, 5-HIAA in both forebrain and brainstem areas of the calcium group were significantly lower than that of the non-calcium group ($t(12)=4\ 0$, $p<0\ 01$) (Table 1)

Calcium assays revealed a significant increase (18–24%) in the amount of serum, brainstem and the forebrain calcium of the rats maintained on the calcium drinking water in comparison to the control group ($t(18)=14\ 67$, $p<0\ 0005$) (Table 2)

There was no significant correlation between serum or brain calcium levels and latency of escape, nor was brain 5HIAA levels significantly correlated with latency of escape However, there was a trend in this direction for both variables, and the lack of significance may be due to the low variability within each measure

An identical experimental protocol using Mg^{++} rather than Ca^{++} produced no significant differences between control and experimental groups

DISCUSSION

The present data suggest that elevated calcium may play a critical role in certain depressive states Animals maintained on drinking water containing elevated calcium concentrations, sufficient to increase plasma and brain calcium levels by approximately 20%, greatly enhanced the acquisition of the learned helplessness response This paradigm is generally considered to be a valid animal model of depression [25] The fact that serotonin metabolism was decreased in the animals on the elevated-calcium drinking water is consistent with previous studies by Sherman and Petty [18], who showed that there is a decrease in serotonin metabolism during training on the learned helplessness paradigm These latter investigators, however, have not examined calcium during training on the learned helplessness task Thus, it is not known whether calcium homeostasis is altered during learned helplessness training in normal rats, however, our data clearly demonstrate that elevated calcium greatly enhances acquisition of the behavior in animals maintained on drinking water containing elevated calcium levels That the effect is not due to the excess chloride in the water (calcium was administered as the chloride salt) is demonstrated by the fact that elevated magnesium chloride (2 5%) in the drinking water had no significant effect on the acquisition of the learned helplessness task Since elevated calcium alters the activity of certain other neurotransmitter systems, we cannot be certain that the observed behavioral effects are due to altered serotonin metabolism

Currently, the most popular hypothesis concerning the biological basis of depressive states is that they result from a decreased metabolism of serotonin and/or norepinephrine. Recent studies from our laboratory have demonstrated that elevated calcium, within the physiological range (i e , 10–15%), produces a significant depression of the activity of serotonin-containing dorsal raphe neurons both *in vivo* and *in vitro* [23] Elevated calcium has recently been found to decrease the activity of norepinephrine-containing locus coeruleus neurons *in vitro* as well (unpublished data) That the effect of elevated calcium on monoamine transmitter turnover is not simply a general phenomenon is indicated by the fact that even very high levels of calcium (i e 7 2 mM) do not alter the activity of dopamine-containing substantia nigra neurons under identical *in vitro* recording conditions (unpublished observations) Interestingly, elevation of magnesium ions, which does not alter the acquisition of the learned helplessness task, as described above, produces no significant change in the activity of either serotonin- or norepinephrine-containing neurons recorded *in vitro* [23]

While a considerable amount of clinical data suggests that there is altered calcium homeostasis in various mood disorders, there exist conflicting data in the literature. Much of this conflicting data may be attributable to the fact that previous studies have not categorized depressed patients according to different forms of depression [3, 6, 9, 24]. That is, one form of depression may result from elevated calcium levels while another form of depression may have a different etiology. This issue is currently under study in our laboratory using human depressed patients.

The present data have implications for the treatment of depressed patients. If elevated levels of calcium is, in fact, the primary cause of certain types of depressive states then treatment strategies should be aimed at restoring calcium homeostasis to normal.

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