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Activity-Wheel Stress and Serotonergic Hypersensitivity in Rats

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MAYEDA, A. R., J. R. SIMON, J. N. HINGTGEN, J. R. HOFSTETTER AND M. H. APRISON. *Activity-wheel stress and serotonergic hypersensitivity in rats.* PHARMACOL BIOCHEM BEHAV 33(2) 349–353, 1989.—Adult male Wistar rats were subjected to activity wheel stress: unlimited access to an activity wheel for up to twelve days and food for 30 to 60 min each day. Each treated rat was paired with a control, the latter being housed in home cages and given sufficient food to maintain a weight similar to the stressed partner. All rats were previously trained on a variable interval schedule for milk reinforcement. When the activity of the stressed rat increased rapidly then decreased suddenly, the pair was decapitated for biochemical analysis. Levels of the serotonin metabolite, 5-hydroxyindoleacetic acid, decreased by 50%, and the B_{max} for ketanserin binding increased by 19% in frontal cortical homogenates from the stressed rats when compared to controls. These data support the concept that stress increases the sensitivity of central serotonin receptors.

Serotonergic hypersensitivity Stress 5-Hydroxyindoleacetic acid Serotonin Ketanserin binding 5-HT_2 receptor

IT has been suggested that chronic stress precipitates depression in certain susceptible individuals (1, 6, 7). Aprison *et al.*, as well as other investigators, have proposed that this interaction is mediated by the serotonin (5-HT) system (6, 8, 36, 38). In this study we were interested in the neurochemical effect of chronic stress on the Aprison *et al.* (3–5, 8) animal model of depression. In this model rats show suppression of food-reinforced operant behavior following administration of the 5-HT precursor 5-hydroxytryptophan (5-HTP).

Aprison and co-workers refined their model of depression in a series of behavioral and neuropharmacological studies (2–8, 16, 19–22) begun in the early 1960's. Rats and pigeons given either 5-HTP or tryptophan showed suppression of operant behavior which paralleled some signs of human depression, i.e., psychomotor retardation with difficulty performing in areas of competence. Data both from this model and from those studies in which 5-hydroxyindoleacetic acid (5-HIAA) concentrations in the CSF of depressed human patients (18) are lower than that of controls led to the hypothesis that certain individuals, predisposed to depression, develop supersensitive postsynaptic 5-HT receptors in response to lower than normal 5-HT release and turnover [for a more complete discussion, see reviews (5, 7, 8)]. Thus, depression in these individuals may result from the impact of a later increase in 5-HT release onto hypersensitive receptors.

Further work using this model tended to support the theory. 5-HTP-induced response suppression correlated with increases in the level of 5-HT in specific areas of the brain. There were no corresponding changes in peripheral tissues. At the same time dopamine and norepinephrine levels were unchanged in the brain and peripheral tissues (4). Response suppression was directly related to the release of 5-HT in the lateral hypothalamus (25). In another study nanogram amounts of 5-HTP administered directly into the lateral hypothalamus in rats precipitated response suppression (22). Response suppression is blocked by pretreatment with methysergide (29), some antidepressants (30) and LY 53857, a selective 5-HT_2 receptor antagonist (21). On the other hand, it is potentiated by acute pretreatment with fluoxetine (29), and chronic treatment with both reserpine (11), and p-chlorophenylalanine (PCPA) (16). PCPA-treated rats also showed a significant decrease in the apparent K_d for 5-HT binding in cerebral cortex (16). These data suggested that potentiated behavioral suppression and hypersensitive 5-HT receptors are associated. The postsynaptic serotonergic theory of depression has been supported by data from other laboratories (28, 36–38).

Recently Aprison and Hingtgen (6) proposed a mechanism by which chronic stress precipitates depression. In some individuals exposure to chronic stress may result in an increase in 5-HT release of sufficient duration to diminish 5-HT release through an effect on the autoreceptor. Increased sensitivity of the postsynaptic 5-HT receptors could follow. These individuals would now be

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predisposed to developing depression when 5-HT interacted with the hypersensitive receptors.

Other investigators have found evidence of hypersensitive 5-HT receptors in chronically-stressed animals. Segawa *et al.* (36) measured a number of neurochemical correlates of 5-HT activity in the brains of rats that had been stressed by forced swimming, long-term isolation or conditioned sedation. These investigators found that the B_{max} for high affinity [^{3}H]-5-HT binding was significantly higher in whole brain crude synaptic membranes of rats exposed to either forced-swimming or conditioned-sedation protocols than in that of controls. 5-HT and 5-HIAA levels in whole brain homogenates from the conditioned-sedation and long-term isolation groups decreased when compared with unstressed rats.

A previous study (6,20) from this laboratory examined the effect of chronic activity-wheel stress (32) on the response suppression induced by 5-HTP. Rats showed a five-fold increase in the period of response suppression following chronic activity-wheel stress compared to the prestress 5-HTP response (6,20). In order to study the neurochemical changes in the serotonin system accompanying these behavioral changes we measured the binding of ketanserin as well as the levels of 5-HT and 5-HIAA in the frontal cortex of rats exposed to chronic activity-wheel stress. We postulated that evidence of increased [^{3}H]-ketanserin binding would occur in the chronically-stressed animals.

METHOD

Behavioral Method

The behavioral protocols used were the same as those described by Aprison and Hingtgen (6,20) and Hellhammer *et al.* (19). Adult male Wistar rats were trained to press a lever for milk reinforcement on a variable-interval 1-min schedule until stable responding was established. The rats then were given intraperitoneal injections of 25 mg/kg of D,L-5-HTP after an initial 15 min of lever-pressing. The 5-HTP-induced period of response suppression was calculated from a comparison of response rates before and after injection (29). This procedure was repeated to establish a stable period of response suppression for each rat.

After 7 days rats were exposed to an activity-wheel stress paradigm. Rats in the treatment group were housed in cages (25 × 15 cm) with free access to an activity wheel (10 × 35 cm dia.), while control rats were housed in home cages but had no access to activity wheels. The body weight in grams and activity level in revolutions of the wheel per hour were recorded daily. During the daily 30- to 60-minute feeding period, the treatment group had free access to food, but the amount of food available to the paired controls was restricted so that their weights remained similar to that of the stressed group. All groups had access to water ad lib. The room in which the rats were housed was maintained on a 12-hr light/dark cycle. Stressed rats were killed between 9:00 a.m. and noon following a peak increase and subsequent decrease in 24-hr activity level; control rats were decapitated the same morning.

Tissue Preparation

After decapitation, the brains were rapidly removed and dissected over ice. Tissues were quickly weighed, frozen in liquid nitrogen and then stored at −80°C. Prior to being assayed, the frozen tissue samples were thawed on ice and homogenized in 5 volumes (w/v) of ice-cold 0.05 M Tris-HCl buffer (pH 7.7) with a Tekmar Ultramax. An aliquot of each homogenate was removed and the levels of 5-HT and 5-HIAA were determined as described below.

In addition, after the brain samples were frozen and stored, the stomach of each rat was removed and examined for ulcers as previously described (19).

Preparation of Membranes for [^{3}H]-Ketanserin Binding Assays

An additional 35 volumes of Tris-HCl buffer was added to the remainder of each tissue homogenate. Homogenates were centrifuged at 40000 × g for 10 min. The pellets were washed by resuspension and centrifugation using 40 volumes of buffer. The washed membrane pellet was resuspended in 400 volumes of buffer and binding assays were performed on 1-ml aliquots (2.5 mg wet weight, 0.1 mg of protein/ml) of this tissue suspension. Proteins of the concentrated homogenate samples were measured by the Lowry method using bovine serum albumin as the standard (26).

Measurement of Ketanserin Binding

Receptor binding assays were performed using the method of Leysen *et al.* (24) with slight modifications. An aliquot of the crude membrane preparation was incubated in 0.05 M Tris-HCl buffer containing 12 concentrations of [^{3}H]-ketanserin (95 mCi/μmol, New England Nuclear, Inc.) from 0.05 nM to 3 nM. Nonspecific binding was determined using 2 μM methysergide (Sandoz Pharmaceuticals). After incubation at 37°C for 15 min, membranes were collected by rapid filtration through Whatman GF/B filters and washed three times with 5 ml of ice-cold buffer. Filters were agitated for 15 min in 10 ml of 3a70b scintillation cocktail (Research Products International) prior to measuring the radioactivity. The radioactivity was measured in a Beckman LS-2800 scintillation counter at 55% efficiency. The ketanserin binding constants were determined using the method of Eadie-Hofstee as adapted by Zivin and Waud (41).

Measurement of 5-HT and 5-HIAA

The 5-HT and 5-HIAA levels were determined using the HPLC assay described by Falkowski and Wei (15). An internal standard of N-methyl 5-HT was added to the frontal cortex homogenates. Protein in each sample was precipitated with 10% trichloroacetic acid and centrifuged at 10000 × g for 10 min. The supernatants were frozen at −20°C. Prior to injection the sample was thawed on ice and adjusted to pH 4.0 with 5 M sodium acetate.

The HPLC stationary phase was a Whatman 3 μm Spherisorb ODS column (4.6 mm × 10 cm) preceded by a Whatman Co:Pell ODS precolumn. The mobile phase was 0.05 M citrate buffer (pH 4.65):acetonitrile:tetrahydrofuran:methanol (97:2.5:0.25:0.25) flowing at 0.8 ml/min. Amperometry (Bioanalytical Systems) on the column effluent was done at the surface of a glassy carbon electrode at 0.55 V. A standard curve was established by standard additions of 5-HT and 5-HIAA to pooled control tissue before the first centrifugation step. The compounds of interest were identified by retention time of standards and quantified by comparison of peak height to the standard curve.

RESULTS

Under the conditions used in this study the time that the rats spent running in the activity wheel increased substantially after 7–10 days, then suddenly decreased. A decrease in the amount of food eaten and a loss in weight accompanied the activity increase. The data in Fig. 1 show the weight and activity changes of a typical rat housed for 9 days in a cage with an attached activity wheel. The glandular portion of the stomachs of the stressed rats contained multiple ulcers. None of the control rats had ulcers.

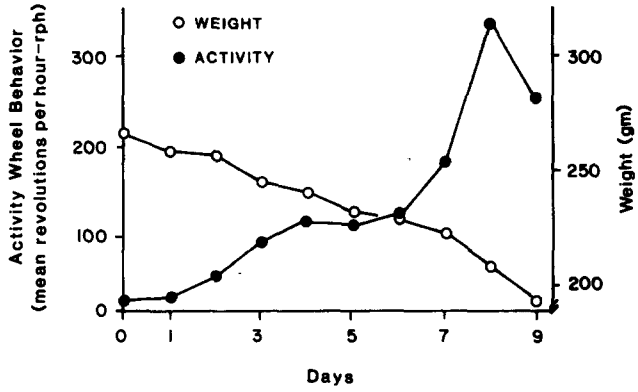

FIG. 1. Body weight and activity of a rat as a function of days housed in a cage with an activity wheel. Activity wheel-stressed rats were allowed 30 to 60 minutes of free feeding per day. Activity was recorded as mean revolutions of the activity wheel per hour.

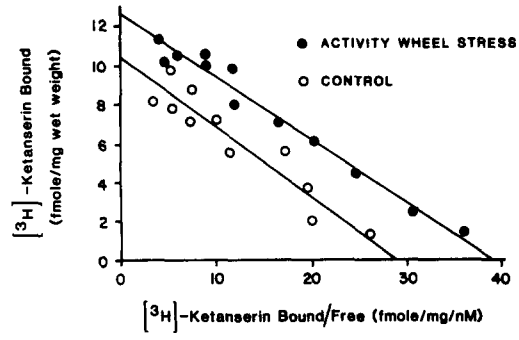

FIG. 2. An Eadie-Hofstee plot of the [^{3}H]-ketanserin binding to frontal cortex homogenates from stress-treated and control rats. The binding constants for the stress-treated rat were B_{max} 12.6 fmole/mg wet weight, K_d 0.32 nM and for the control rat B_{max} 10.5 fmole/mg wet weight, K_d 0.36 nM.

There was a significant 19% increase in the B_{max} of [^{3}H]-ketanserin binding in the cortical homogenates of the stressed rats when compared to controls. There was a nonsignificant increase in the K_d (13%). The average B_{max} and K_d values for the stressed rats and their paired controls are given in Table 1. A representative Eadie-Hofstee plot is shown in Fig. 2.

There was a significant 49% decrease in 5-HIAA levels in the cortical homogenates of stressed rats compared to controls. A small decrease in 5-HT levels (11%) was not significant. The average 5-HT and 5-HIAA levels measured in frontal cortex homogenates of stressed rats and controls are given in Table 2.

DISCUSSION

The results of this study demonstrate that 5-HT_2 receptor hypersensitivity develops in frontal cortices of rats exposed to chronic activity-wheel stress. There was a small but significant increase in B_{max} for ketanserin binding in frontal cortical homogenates of the stressed rats when compared to controls. Ketanserin binding is well accepted (23) as a highly selective assay for 5-HT_2 receptors in the frontal cortex. The increase in B_{max} in our current study suggests that chronically-stressed rats develop an increased number of postsynaptic 5-HT_2 receptors. Previous behavioral studies from this laboratory predicted this outcome. Chronic activity wheel stress produced a five-fold increase in the period of 5-HTP-induced suppression of food-reinforced operant behavior compared to the prestress 5-HTP response (6,20). Given an increase in 5-HT_2 receptor number in the frontal cortex of a rat then equivalent doses of 5-HTP could result in an augmented behavioral response. These data are consistent with the previous finding that 5-HTP response suppression is at least 90% blocked by pretreatment with either a well-established postsynaptic serotonin blocker, methysergide (29), or a specific 5-HT_2 receptor antagonist, LY 53857 (21).

In the current study there was a significant decrease in the 5-HIAA level in frontal cortices of rats exposed to chronic activity-wheel stress. Earlier work from this laboratory (19) showed that activity-wheel stress resulted in decreases in 5-HT and 5-HIAA levels in a number of brain areas including whole cortex; frontal cortex was not examined. Segawa *et al.* (36) found decreased levels of both 5-HT and 5-HIAA in whole brain homogenates of rats exposed to either forced swimming or conditioned sedation stressors when compared to controls. Although other explanations are possible (12), the data suggested to us that certain stressors decrease release of 5-HT in the CNS which is a prelude for the 5-HT_2 receptor hypersensitivity that we observed in this study.

Furthermore, our data are consistent with the results from the following studies on rats treated chronically with PCPA: increased high-affinity ketanserin binding in the striatum (34) and a supersensitivity of 5-HT_2-mediated behaviors such as head twitch (31,40) and the 5-HTP-induced response suppression (16) similar to that observed following stress. In addition there was a 158% increase in B_{max} for spiroperidol binding in the frontal cortex in response to tetrabenazine administration (38) and supersensitivity of 5-HTP-induced response suppression after reserpine administration (11).

Some other investigators (10, 13, 33, 35) found that 5-HT_2 binding sites were unchanged following either PCPA treatment or denervation and suggested that the mechanism for 5-HT_2 behav-

TABLE 1

[^{3}H]-KETANSERIN BINDING CONSTANTS IN CRUDE MEMBRANE PREPARATIONS FROM THE FRONTAL CORTEX OF STRESSED AND CONTROL RATS

Group	n	K_d nM	B_{max} pmol/g Wet Tissue
Stressed	5	0.36 ± 0.03	12.3 ± 0.4*
Controls	5	0.32 ± 0.01	10.3 ± 0.4

The ketanserin binding constants are given as means ± SEM determined using the method of Eadie-Hofstee as adapted by Zivin and Waud (41).

*Significant at $p<0.02$; Student's t-test.

TABLE 2

5-HT AND 5-HIAA LEVELS MEASURED IN FRONTAL CORTEX HOMOGENATES OF STRESSED AND CONTROL RATS

Group	n	5-HT nmol/g Wet Tissue	5-HIAA nmol/g Wet Tissue
Stressed	5	2.03 ± 0.07	2.54 ± 0.30*
Controls	5	2.29 ± 0.17	4.99 ± 0.48

*Significant at $p<0.01$; Student's t-test.

ioral supersensitivity may involve effects distal to the 5-HT_2 receptor-effector complex, i.e., increased sensitivity to the second messenger or down-regulation of another neurochemical system that is inhibitory to serotonergic pathways (13). None of the mechanisms for behavioral supersensitivity proposed above can be discounted at present.

We used frontal cortex in this study because it is a large tissue source that receives serotonergic input from the raphe nucleus but does not contain serotonergic perikarya (17). It also has many connections to the limbic system and is involved in the regulation of mood in humans (14). The findings in the current study closely parallel data on frontal cortex tissue of suicide victims. When compared to controls matched for age and post-mortem delay, suicide victims had as much as 39% increases in the B_{max} of 5-HT_2 receptor binding in frontal cortex (9,27).

We found that chronic activity-wheel stress induced increases in both frontal cortex 5-HT_2 receptor number and behavioral suppression in an animal model of depression. Increased 5-HT_2 receptors may be a useful adaptation to chronic stress and predict the exaggerated response to 5-HTP. One interpretation is that stress precipitates human depression by a mechanism not directly related to these receptors. In that case it is possible that depression would be worse without 5-HT_2 receptor upregulation. However, we interpret these data as supporting the theory that chronic stress produces a predisposition for depression in humans by inducing postsynaptic serotonin receptor upregulation (5–7).

In earlier papers (5, 7, 8) a theoretical rationale for the use of L-tryptophan or L-5-HTP in treating depression was presented. In brief, it is possible that treatment of human depression with either precursor of serotonin would reverse the hypersensitivity of the 5-HT_2 receptor over a period of time. However, in the early stages of treatment with precursors, before the reversal of hypersensitivity, depression may be unchanged or exacerbated. In one study where 5-HTP was found to be an effective antidepressant, Hamilton ratings of depression did not change during the first three days of treatment (39). Hamilton ratings increased during the first three days of treatment in patients given the combination of 5-HTP and clomipramine, although this combined treatment was ultimately superior to treatment with either compound alone. To provide further data on possible interactions between stress, the serotonergic system and depressive states, it would be interesting to see if precursor therapy with chronic L-5-HTP or L-tryptophan would protect animals and humans from the effects of chronic stress before signs of depression develop.

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