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PRESYNAPTIC SEROTONIN MECHANISMS IN RATS SUBJECTED TO INESCAPABLE SHOCK

EMMELINE EDWARDS,[1]* W. KORNRICH,[2] PHYLLIS V. HOUTTEN[1] and F. A. HENN[1]

[1]Department of Psychiatry and Behavioral Science, and [2]Department of Medicine, State University of New York at Stony Brook, Stony Brook, NY 11794-8101, U.S.A.

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Summary— After exposure to uncontrollable shock training, two distinct groups of rats can be defined in terms of their performance in learning to escape from a controllable stress. Learned helpless rats do not learn to terminate the controllable stress, whereas non-learned helpless rats learn this response as readily as naive control rats do. The present studies were designed to examine the correlations between the behavioral differences and the changes of presynaptic serotonergic activity, seen in these groups of rats. The major findings concerned presynaptic serotonergic effects in the hippocampus and hypothalamus of learned helpless rats. In the hippocampus, these included a statistically significant increase in three presynaptic 5-hydroxytryptamine (5-HT) mechanisms: K^+-induced release of [^{3}H]serotonin, high affinity uptake of [^{3}H]serotonin and maximum density of binding sites for uptake of 5-HT, measured with [^{3}H]paroxetine. In the hypothalamus, there was a differential modulation of all three presynaptic 5-HT mechanisms. A significant decrease in: K^+-induced release of [^{3}H]serotonin, in high affinity uptake of [^{3}H]serotonin and the maximum binding site density of [^{3}H]paroxetine binding was observed. No changes in uptake site binding was seen in other regions of the brain examined. These results implicate presynaptic serotonin mechanisms in the behavioral deficit caused by uncontrollable shock. In addition, a limbic–hypothalamic pathway may serve as a control center for the behavioral response to stress.

Key words—learned helplessness, stress, K^+-induced serotonin release, serotonin uptake, paroxetine, depression.

Serotonin has occupied a central place in the current theories of the etiology of depression for nearly two decades. It has been proposed that a decrease in serotonergic activity may increase vulnerability to affective disorders or may in fact produce depression (Murphy, Campbell and Costa, 1978). The alternative view that an excessive serotonergic activity causes depression has also been put forward (Aprison, Takahashi and Tachiki, 1977; Ogren, Fuxe, Agnati, Gustafsson and Holm, 1979).

Early research has concentrated on the possibility of a decrease in the output of 5-HT as the cause of depression or as a factor increasing the vulnerability to this disorder (Coppen, Prange, Hill, Whybrow and Noguera, 1980). In the last decade, the improved understanding of the reuptake process for 5-HT and the identification of multiple subtypes of pre- and post-synaptic 5-HT receptors have suggested that these mechanisms may be primary etiological factors of depression and may also be critical to a better understanding of the action of antidepressant drugs and electroconvulsive treatment.

Animal models of depression have been extensively used to test the hypotheses of the neurobiology of depression, the mechanism of action of antidepressant drugs and to screen for new potential antidepressant therapies. The Learned Helplessness model is one of the most recognized and valid animal models of human depression (Maier and Seligman, 1976; Willner, 1984, 1990). After exposure to an inescapable stress, Sprague–Dawley rats show a deficit in learning to escape a controllable stress. This maladaptive behavior is called "learned helplessness" (Seligman and Maier, 1967). There is a behavioral correlation between learned helplessness and the vegetative symptoms of clinically depressed patients (Anderson, Cole and McVaugh, 1968; Levine, Madden, Conner, Moskal and Anderson, 1973; Weiss and Glazer, 1975). Moreover, the behavioral deficit, induced in rats by exposure to inescapable shock, is specifically reversed by pharmacological treatment with antidepressants (Petty and Sherman, 1980; Sherman, Sacquitne and Petty, 1982; Martin, Soubrie and Simon, 1986).

Since human depression has been hypothesized to be related to some dysfunction in monoaminergic neurotransmission (Schildkraut, 1965; Van Praag and Korf, 1971; Jesberger and Richardson, 1985), monoaminergic neurochemistry has been extensively investigated with regard to learned helplessness. Changes in noradrenergic mechanisms, including an increase in turnover of noradrenalin and a decrease in levels of norepinephrine in brain, an up-regulation of beta-adrenergic receptors and an increase in the sensitivity of the stimulation of adenylate cyclase by norepinephrine (Weiss, Glazer, Pohorecky, Brick and Miller,

*To whom correspondence and reprint requests should be addressed.

1975; Anisman and Sklar, 1979; Weiss, Goodman, Losito, Corrigan, Charry and Bailey, 1981; Henn, Edwards and Johnson, 1987; Martin, Edwards, Johnson and Henn, 1990) have been noted as a result of exposure to uncontrollable shock.

The metabolism of serotonin (5-hydroxytryptamine) has also been investigated in the learned helplessness paradigm. However, there have been some conflicting reports on the involvement of serotonin in learned helplessness. Animals exposed to uncontrollable shock had lower levels of 5-HT and higher levels of 5-hydroxyindoleacetic acid (5-HIAA) in brain than did yoked control animals (Hellhammer, Rea, Bell, Belkien and Ludwig, 1984). The *in vivo* release of 5-HT from the cerebral cortex was less in uncontrollably shocked rats, which developed a behavioral deficit, than in those which did not (Petty and Sherman, 1983). These data concur with recent animal data indicating that blockers of the uptake of 5-HT (which increase 5-HT transmission) are effective in the reversal of learned helplessness (Martin, Soubrie and Pueuch, 1990). However, it was previously reported that depletion of 5-HT by treatment with *p*-chlorophenylalanine protected from uncontrollable shock (Edwards, Johnson, Anderson, Turano and Henn, 1986). Manipulations which increase endogenous levels of 5-HT were found to interfere with the acquisition of an escape response, mimicking the effects of an uncontrollable stress (Brown, Rosellini, Samuels and Riley 1982).

The present investigations initiated a systematic examination of serotonin presynaptic mechanisms in rats showing a behavioral deficit due to uncontrollable stress. It was previously demonstrated that levels of 5-HT were significantly elevated in the hippocampus of learned helpless rats, as compared to nonlearned helpless and control rats (Edwards *et al.*, 1986; Martin *et al.*, 1990). Significant changes in K^+-induced release of [^{3}H]serotonin, high affinity uptake of [^{3}H]serotonin and binding of uptake sites for 5-HT to [^{3}H]paroxetine are reported in learned helpless, nonlearned helpless and naive control rats.

METHODS

Materials

5-[1,2-^{3}H-(*N*)]Hydroxytryptamine creatinine sulphate ([^{3}H]-5-HT) (sp. act.: 25.2 Ci/mmol), (alpha(−)-*trans*-4-(*p*-fluorophenyl)-3-3,4-methyl-enedioxyphenoxy-methyl-piperidine) ([^{3}H]paroxetine) (sp. act.: 26.5 Ci/mmol) and Protosol(TM) tissue solubilizer was obtained from New England Nuclear (Boston, Massachusetts). Hydrofluor(TM) scintillation cocktail was obtained from National Diagnostics (Palmetto, Florida). Fluoxetine was a gift from Eli Lilly (Indianapolis, Indiana). Other drugs and reagents were purchased from Sigma (St Louis, Missouri).

Male Sprague–Dawley rats (150–200 g) were obtained from Charles River Breeding Laboratories (Wilmington, Massachusetts) and were kept for 1 week before use, in a temperature/humidity controlled facility. A 12 hr light–dark cycle was maintained (lights on from 8:00 a.m. to 8:00 p.m.). Behavioral training and testing experiments were performed between 8:00 a.m. and 2:00 p.m. Food and water were available *ad libitum*.

Behavioral training and shock escape testing

All experimental animals were placed in a Coulbourn experimental chamber with an electrified grid floor, as previously described (Edwards *et al.*, 1986). Briefly, an initial training session consisted of the delivery of a series of 0.8 mA footshocks, through the electrified grid floor of the Coulbourn chamber, for 40 min. The onset and offset of these shocks were randomly established by a probability generator. Hence, this schedule resulted in a cumulative total of 20 min of shock, with a minimum time of 1.5 sec between on and off events.

Twenty-four hours after the training session, each rat was tested in a shock escape paradigm, where a footshock could be terminated with a single bar press. The 0.8 mA shock was delivered on a pulsating off and on schedule with a 35 msec period and was accompanied by a yellow cue light. Fifteen trials were given to each rat. If a rat failed to respond by the end of 60 sec of shock, the trial was terminated. A 24-sec intertrial interval began without the presence of the yellow cue light. Latencies of 0–20 sec from initiation of shock to the bar press response, were recorded as successful escapes. Latencies between 20–60 sec from initiation of shock were registered as escape failures.

On the basis of previous work (Edwards *et al.*, 1986), rats scoring 10–15 failures in the shock escape test were considered deficient in the escape response (learned helpless group). Rats having 0–4 failures in the testing session were considered not to be deficient in the shock escape test (non-learned helpless). Naive controls received no shock training but were subjected to the shock escape test, where they scored between 0–4 failures. These rats were only subjected to the shock test, enabling the effects of shock *per se* to be determined. Ninety to ninety-five percent of all naive rats tested scored 0–4 failures. Rats scoring 5–9 failures to escape were not included in the experiments. Rats were decapitated 1 day after the shock escape test.

Release of [^{3}H]5-HT from slices of hippocampus and hypothalamus

General methods to study the release of compounds from tissue (Chase, Katz and Kopin, 1969; Farnebo and Hamberger, 1970; Becker, Casteneda, Robinson and Beer, 1984) were modified to study the release of labeled neurotransmitter from slices of hippocampus and hypothalamus. Immediately after dissection of the hippocampus and hypothalamus, 350 × 350 μm sections were prepared, using a McIlwain tissue chopper (Brinkman, Westbury, New York). Slices were washed three times in ice-cold Krebs'–Ringer

buffer with the following composition (mM): 118 NaCl, 4.8 KCl, 1.25 $CaCl_2$, 1.2 KH_2PO_4, 1.2 $MgSO_4$, 25 $NaHCO_3$, 5.5 Glucose, 5.7 ascorbic acid. The buffer had been equilibrated earlier with 95% O_2:5% CO_2 for 30 min, bringing the pH to 7.4. After washing, the slices were incubated with constant shaking under a stream of 95% O_2:5% CO_2 at 37°C for 60 min in Krebs'–Ringer buffer, with 50 μM pargyline and 0.1 μM [^{3}H]5-HT. After the incubation, the slices were washed three times with Krebs'–Ringer buffer and 50 μl aliquots of the slices of tissue were transferred to superfusion chambers (Becker *et al.*, 1984). Inlets to these chambers were connected to a masterflex microprocessor pump drive (Cole Palmer Inst., Chicago, Illinois), which delivered Krebs'–Ringer buffer at 37°C to the slices of brain in the superfusion chambers, at a rate of 100 μl/min for a 1-hr period of stabilization. At this time, the slices were exposed to a 2-min pulse of a modified depolarizing, Krebs'–Ringer solution, in which the concentration of KCl was increased to 60 mM and the concentration of NaCl was accordingly decreased from 118 to 58 mM. The superfusion medium contained 1 μM fluoxetine or 10 μM fluvoxamine, which blocked neuronal uptake of serotonin. Four minute fractions of the superfusate were collected just before the depolarizing pulse (baseline release) and just after the depolarizing superfusion (K^+-induced release), for a total of 50 min. Radioactivity in aliquots of these fractions was determined by liquid scintillation spectrometry. The slices of tissue were removed from the superfusion chamber, incubated in 0.3 ml of Protosol tissue solubilizer for 12 hr, acidified in 0.15 ml of 2 M acetic acid and counted for tritium (tissue radioactivity). Data based on release of total tritium are presented as follows (Farnebo and Hamberger, 1970):

$$\% \text{ stimulated release} = 100 \times \frac{(K^+\text{-induced release}) - (\text{baseline release})}{(\text{tissue radioactivity}) + (K^+\text{-induced release})}$$

$$\% \text{ spontaneous release} = 100 \times \frac{(\text{baseline release})}{(\text{tissue radioactivity}) + (\text{baseline release}) + (K^+\text{-induced release})}$$

Uptake of [^{3}H]5-HT from synaptosomal preparations of hippocampus and hypothalamus

A microassay for the specific synaptosomal high affinity uptake of [^{3}H]5-HT was performed on the hippocampus and hypothalamus, as described by Azmitia, Brennan and Quaterman (1983). Briefly, the hippocampi and hypothalami from learned helpless, nonlearned helpless and control rats (n = 8/group) were homogenized in ice-cold 0.32 M sucrose (50 mg wet wt/ml sucrose solution), in a glass homogenizer fitted with a Teflon pestle. The homogenates were centrifuged at 500 g for 10 min. The supernatant was collected and subsequently centrifuged at 12,000 g for 10 min at 4°C to sediment the synaptosomes in a pellet. After the supernatant was discarded, the crude synaptosomal pellet was resuspended in ice cold Krebs'–Ringer buffer (10 vol of original tissue wt).

Uptake assays for [^{3}H]5-HT were performed in triplicate in 96 well plates, with a total reaction volume of 300 μl, containing 15 μl (1–3 mg protein/ml) of the synaptosomal preparation. Using an 8 channel repetitive dispenser pipet, the reaction was begun by adding 20 μl of [^{3}H]5-HT (final concentration: 5×10^{-8} M) to the reaction mixture at 37°C. After 5 min, the synaptosomes were collected through GF-B Whatman filters, by means of an automatic cell harvester (Brandel, Gaithersburg, Maryland). The trapped synaptosomes were washed with cold Krebs'–Ringer buffer (3 × 5 ml), dried and counted in a Beckman LS 8100 scintillation counter to determine the amount of radioactivity retained. Uptake of [^{3}H]5-HT was expressed as fmol/mg/protein, after correction for uptake in the presence of 1 μM fluoxetine. Protein levels were determined by the method of Lowry, Rosebrough, Farr and Randall (1951).

Binding of [^{3}H]paroxetine: uptake sites for 5-HT

The binding of [^{3}H]paroxetine was determined in various regions of the brain: cortex, septum, hippocampus, hypothalamus and striatum, as previously described (Marcusson, Bergstrom, Erikson and Ross, 1988). Briefly, samples of brain from learned helpless, nonlearned helpless and control groups of rats were homogenized in 15 volumes of ice-cold buffer (50 mM Tris–HCl, 120 mM NaCl, 5 mM KCl, pH 7.4), using a polytron homogenizer (Brinkman, New York) at setting 4 for 5 sec. After centrifugation (48,000 g, 10 min, 4°C), the pellet was homogenized in the same buffer. After another centrifugation (48,000 g, 10 min, 4°C), the final pellet was resuspended in Tris buffer (pH 7.4) to a final tissue concentration of 40–60 μg of protein/ml in the binding assay. The homogenates were incubated for 60 min at 22°C with [^{3}H]paroxetine (0.005–2 nM) in a total volume of 1.6 ml. Incubation was terminated by addition of 5 ml of ice-cold Tris buffer (pH 7.4) and rapid vacuum filtration through Whatman GF/B filters, using a 24-channel cell harvester (Brandel, Gaithersburg, Maryland). The filters were washed with three 5 ml rinses of ice-cold buffer. The radioactivity on the filters was determined by liquid spectrometry. Non-specific binding was estimated in the presence of 100 μM 5-HT. Protein was determined by the method of Lowry *et al.* (1951). The data from the equilibrium saturation experiments were analyzed by a computerized program, using a weighted non-linear least squares curve fitting (McPherson, 1985).

Data analysis

Means of behavioral scores, measured in the shock escape test for learned helpless, nonlearned helpless and control rats were compared by independent t-test. The uptake data [^{3}H]5-HT for learned helpless, nonlearned helpless and control rats ($n = 8$) were expressed as fmol/mg protein/5 min and compared by one-way analysis of variance (ANOVA), followed by a *post hoc* contrast test, which compared differences between specific means. Differences in densities of binding sites for [^{3}H]paroxetine (B_{max}) for learned helpless, nonlearned helpless and control rats ($n = 8$/group), in various regions of the brain, were analyzed by one-way ANOVA followed by a *post hoc* contrast test. When multiple t-tests were performed, a α value was reduced to 0.025 to compensate for the experiment error rate. For ANOVA analysis, the significance was set at 0.05.

RESULTS

The behavioral scores of control, nonlearned helpless and learned helpless rats are expressed as the number of failures ± SEM ($n = 24$/group). A total of 149 rats was used in this study. Naive control rats were not exposed to uncontrollable shock (no training) but were tested in the shock escape test, scoring 3.2 ± 0.17 failures. Nonhelpless rats did not exhibit any performance deficits in the shock escape test and scored an average of 3.8 ± 0.31 failures. By contrast, helpless rats scored 13.8 ± 0.60 failures in the shock escape test. As previously reported (Henn *et al.*, 1987; Edwards *et al.*, 1986), only 20–30% of all Sprague–Dawley rats trained and tested exhibited behavioral deficits in the shock escape test, scoring 11–15 failures in the 15-trial test. In these experiments, 120 Sprague–Dawley rats were trained and tested in the learned helplessness paradigm. Twenty percent of the rats trained and tested ($n = 24$) were helpless. These rats were divided in three groups of 8 and were used in the release, uptake and binding studies. Seventy-five percent ($n = 90$) of the rats trained and tested were non-helpless. Twenty-four nonhelpless rats were used, divided in three groups of 8 for the release, uptake and binding assays. Rats scoring 5–9 failures in the shock escape test were not used in the biochemical assays. Twenty-four naive control rats were also used in these experiments. These rats were not trained but were subjected to the shock escape test.

In the hippocampus, the spontaneous release of 5-HT was increased significantly in learned helpless rats as compared to both nonlearned helpless and control rats (learned helpless: 1.4 ± 0.16; nonlearned helpless: 0.86 ± 0.08; control: 0.89 ± 0.08; data expressed as percentage release ± SEM, $n = 8$, $P < 0.002$ learned helpless vs nonlearned helpless and control rats, data not shown). The K^+-stimulated release of serotonin was increased significantly in the learned helpless rats (percentage stimulated release of [^{3}H]serotonin ± SEM: 17.5 ± 0.7, learned helpless vs 11.94 ± 0.8, control and 12.3 ± 1.7, nonlearned helpless; $P < 0.002$ learned helpless vs control, $P < 0.01$ learned helpless vs nonlearned helpless, Fig. 1).

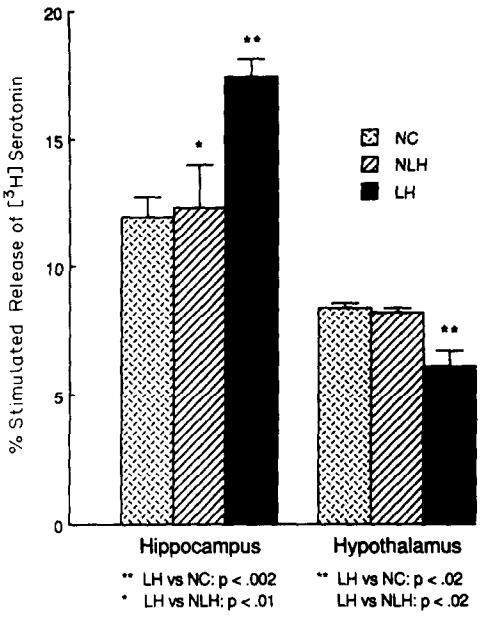

Fig. 1. K^+-Stimulated release of [^{3}H]serotonin in slices of hippocampus and hypothalamus of learned helpless (LH), nonlearned helpless (NLH) and control (NC) rats. The percentage stimulated release of [^{3}H]serotonin is expressed as mean ± SEM ($n = 8$/group). Differences in K^+-stimulated release of [^{3}H]serotonin were revealed by one-way ANOVA and followed by a *post hoc* contrast test (Hippocampus: $F(2,21) = 11.5$, $P < 0.005$; LH vs NC, $P < 0.002$, LH vs NLH $P < 0.01$, *post hoc* contrast test; Hypothalamus: $F(2,21) = 4.53$, $P < 0.025$; LH vs NC, $P < 0.02$, LH vs NLH, $P < 0.02$, *post hoc* contrast test).

In the hypothalamus there was no difference in the spontaneous release of serotonin in all three groups examined (learned helpless: 0.84 ± 0.04; nonlearned helpless: 1.02 ± 0.2; control: 0.83 ± 0.2; data expressed as percentage release ± SEM, $n = 8$/group; data not shown). However K^+-stimulated release of serotonin was decreased in the learned helpless rats (percentage stimulated release of [^{3}H]serotonin ± SEM: 6.1 ± 0.6, learned helpless vs 8.4 ± 0.2, control and 8.2 ± 0.2, nonlearned helpless; $P < 0.02$ learned helpless vs nonlearned helpless and control, Fig. 1).

Figure 2 represents the high affinity uptake of [^{3}H]5-HT in synaptosomes from hippocampus and hypothalamus of learned helpless, nonlearned helpless and control rats. Data is expressed as fmol/mg protein/5 min ± SEM. There was a significant increase in uptake of [^{3}H]5-HT in the hippocampus of learned helpless rats, as compared to nonlearned helpless and control rats (2975 ± 461, learned helpless vs 1545 ± 228, control and 1594 ± 151, nonlearned helpless; $P < 0.02$ learned helpless vs nonlearned helpless and control rats. By contrast, the high affinity uptake of [^{3}H]5-HT in hypothalamic synaptosomes was reduced in the learned helpless rats (3004 ± 341, learned helpless

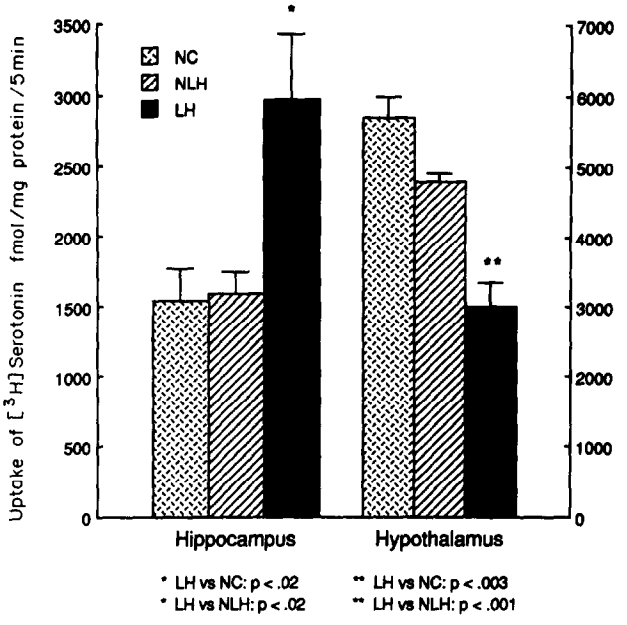

Fig. 2. High affinity uptake of [^{3}H]serotonin in synaptosomes from hippocampus and hypothalamus of learned helpless (LH), nonlearned helpless (NLH) and control (NC) rats. Data expressed as fmol/mg protein/5 min are means ± SEM of individual determinations (n = 8/group). Differences in uptake of [^{3}H]-serotonin were revealed by one-way ANOVA and a *post hoc* contrast test (Hippocampus: F (2,21) = 5.75, $P < 0.01$; LH vs NC and NLH, $P < 0.02$, *post hoc* contrast test; Hypothalamus: F (2,21) = 8.62, $P < 0.005$; LH vs NC, $P < 0.003$, LH vs NLH, $P < 0.001$, *post hoc* contrast test).

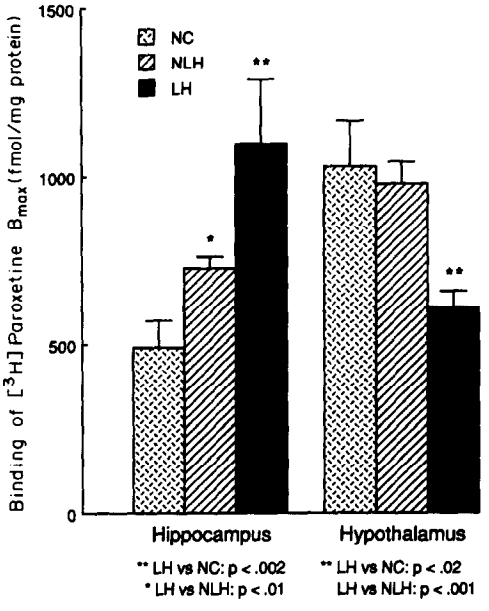

Fig. 3. Binding sites for [^{3}H]paroxetine in the hippocampus and the hypothalamus of learned helpless (LH), nonlearned helpless (NLH) and control (NC) rats. Data are expressed as maximum number of uptake sites for 5-HT (B_{max} ± SEM). Hippocampi and hypothalami were not pooled but assayed individually for all saturation experiments, run in duplicate (n = 8/group). Differences in binding of [^{3}H]paroxetine in the three groups studied control, nonlearned helpless and learned helpless were revealed by one-way ANOVA and a *post hoc* contrast test (Hippocampus: F (2,21) = 7.26, $P < 0.005$; LH vs NC $P < 0.002$, LH vs NLH $P < 0.01$, *post hoc* contrast test. Hypothalamus: F (2,21) = 6.46, $P < 0.01$; LH vs NC $P < 0.02$, LH vs NLH, $P < 0.001$, *post hoc* contrast test).

vs 5714 ± 290, control and 4783 ± 134, nonlearned helpless rats; $P < 0.001$ learned helpless vs nonlearned helpless; $P < 0.003$ learned helpless vs control rats).

The binding of [^{3}H]paroxetine was measured in membranes from the hippocampus and hypothalamus of learned helpless, nonlearned helpless and control rats. Data is expressed as fmol/mg protein (B_{max}) (Fig. 3). There were no differences in the K_d values (ranging from 0.08 to 0.11 nM) for learned helpless, nonlearned helpless and control rats. Changes in the binding of [^{3}H]paroxetine were evident in the learned helpless rats in the hippocampus and hypothalamus but not in the other regions of the brain examined: cortex, septum, striatum (data not shown). A significant increase in uptake sites was seen in the hippocampus of the learned helpless rats (fmol/mg protein ± SEM: 1096 ± 188, learned helpless vs 491 ± 79, control and 724 ± 35, nonlearned helpless, $P < 0.002$ vs control and $P < 0.01$ learned helpless vs nonlearned helpless). There was an apparent effect of shock on binding of [^{3}H]paroxetine. Nonlearned helpless rats displayed an increase in binding of [^{3}H]paroxetine, as compared to control rats (fmol/mg protein: 724 ± 35 nonlearned helpless vs 491 ± 79, control). However, the learned helpless rats were significantly different from the nonlearned helpless rats (119% above naive controls vs 42%, $P < 0.01$).

In the hypothalamus, there was a significant reduction of uptake sites for 5-HT in the learned helpless rats (fmol/mg protein ± SEM: 608 ± 49, learned helpless vs 1029 ± 135, control and 977 ± 63, nonlearned helpless, $P < 0.02$ learned helpless vs control and $P < 0.001$ learned helpless vs nonlearned helpless).

The K_d values, ranging from 0.08 to 0.1 nM, were similar in the three groups studied.

DISCUSSION

The present study was carried out with tissue from the brain of three groups of rats: learned helpless, nonlearned helpless and control.

The major finding of these experiments concerned the differential modulation of serotonin mechanisms in the hippocampus and hypothalamus of the learned helpless rats. In the hippocampus of the learned helpless rats, an overall hyperactivity of serotonergic neurotransmission was demonstrated by significant increases in the spontaneous release of 5-HT, K^+-induced release of 5-HT, high affinity uptake and maximum number (B_{max}) of uptake sites, measured with binding of [^{3}H]paroxetine. By contrast, there was a consistent reduction in serotonin mechanisms in the hypothalamus, as demonstrated by the significant decreases in release of [^{3}H]5-HT, uptake of [^{3}H]5-HT and maximum number (B_{max}) of uptake binding sites, measured with [^{3}H]paroxetine. The present data thus suggest that presynaptic serotonin mechanisms in the hippocampus and hypothalamus may be involved in the modulation of learned helpless behavior.

In the hippocampus of learned helpless rats, spontaneous and K^+-stimulated release of 5-HT were increased by 50% as compared to nonlearned helpless and control rats. With the increase in spontaneous release seen in the hippocampus of the learned helpless rats an apparent decrease in uptake might be expected, possibly due to the isotopic dilution of the tracer from the endogenous 5-HT. However, in the learned helpless rats, the uptake of [^{3}H]5-HT was increased by 90%. It appeared that the presence of an uptake inhibitor in the superfusion medium of the release studies, prevented the displacement of [^{3}H]5-HT from its intraneuronal binding sites by any unlabeled 5-HT which was also transported into the 5-HT neuron by the same uptake mechanism. In the learned helpless rats, the magnitude of the increase in uptake was larger than the increase in release. It is possible that the total increase in the 5-HT uptake system did not result in a single releasable pool of 5-HT. An alternative explanation may also be that in the hippocampus of learned helpless rats, there is a feedback mechanism, mediated by the 5-HT$_{1b}$ receptors, identified as the presynaptic autoreceptor (Middlemiss, 1984).

The density of binding sites for [^{3}H]paroxetine was increased by 120% in the hippocampus of learned helpless rats. This change in binding of [^{3}H]paroxetine did not fully parallel the increase in actual uptake, suggesting that the nature and function between uptake parameters and the measurement of uptake binding sites is still unclear. Although [^{3}H]paroxetine binds to a high-affinity site, probably located on serotonin nerve terminals, this site may be closely related but not identical with the uptake mechanism for 5-HT (Mellerup and Plenge, 1986).

Studies investigating the mechanisms of the reuptake process for 5-HT and the identification of subtypes of pre- and postsynaptic 5-HT receptors, suggest that these mechanisms may be primary etiological factors in the study of affective disorders and may be critical in reaching an understanding of the action of antidepressant drugs and electroconvulsive treatment (Meltzer and Lowy, 1987). The present experiments indicate significant changes of serotonergic neurotransmission in the learned helpless rats. This further attests to the validity of the "learned helpless" paradigm as a model of depression. A differential regulation of 5-HT$_{1b}$ receptors in limbic structures and hypothalamus of the learned helpless rats has also recently been demonstrated (Edwards, Harkins, Wright and Henn, 1991). These changes are particularly interesting since the 5-HT$_{1b}$ receptors have been identified as the presynaptic 5-HT autoreceptor in rats (Middlemiss, 1984).

The present study, along with previous findings from this laboratory, suggest that there may be a neuroanatomical circuit which provides a link between the limbic system, mediating affect and the hypothalamus mediating responses to stress. It has demonstrated that there is a dysregulation of the hypothalamic–pituitary axis in the learned helpless rats (Haracz, Minor, Wilkins and Zimmerman, 1988; Greenberg, Edwards and Henn, 1989). In addition, bilateral adrenalectomy facilitates the induction of "helpless behavior" (Edwards, Harkins, Wright and Henn, 1990). Recent data in the literature agree with the theory that the ascending serotonergic system, that originates in the raphe nuclei and innervates a variety of hypothalamic and limbic areas (Steinbusch, 1981), plays an important role in the control of the hypothalamic–pituitary axis by the CNS (Ganong, 1980; Tuomisto and Mannisto, 1985). The hippocampus is a major site of serotonergic innervation, associated with the control of the hypothalamic–pituitary axis by the CNS and also contains large concentrations of cellular receptors for serotonin and corticosterone (Biegon, Rainbow and McEwen, 1982). The data presented in this paper, taken together with the findings of neuroendocrine disturbances in the model of depression used here (Greenberg *et al.*, 1989; Edwards *et al.*, 1990), support the hypothesis that in the learned helpless rats, corticosterone may be activating hippocampal neurons, located postsynaptically to the innervating 5-HT neuron, whereupon this neuron responds with increased synthesis, release and uptake of 5-HT. Preliminary experiments in this laboratory are assessing endogenous levels of corticosterone in Sprague–Dawley rats, that are subsequently subjected to the training and testing paradigm, in an attempt to correlate the induction of helpless behavior to endogenous levels of corticosterone. Interaction mechanisms between the biogenic amines, specifically serotonin and glucocorticoids, may provide the

functional link mediating stress and "helpless behavior" in this model.

The present studies were designed to determine some neurochemical correlates of behavioral differences between individual rats, after identical treatment with uncontrollable shock. The major finding was an increase in presynaptic serotonin mechanisms, in the hippocampus of learned helpless rats. A statistically significant decrease in the uptake, release and binding of 5-HT to uptake sites for serotonin was also noted in the hypothalamus of learned helpless rats. Thus, changes in serotonergic mechanisms correlated with the occurrence of the behavioral deficit, caused by uncontrollable shock.

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