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IN VITRO NEUROTRANSMITTER RELEASE IN AN ANIMAL MODEL OF DEPRESSION

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Abstract—Sprague–Dawley rats exposed to uncontrollable shock can be separated by a subsequent shock escape test into two groups: a "helpless" (LH) group which demonstrates a deficit in escape behavior, and a "nonlearned helpless" (NLH) group which shows no escape deficit and acquires the escape response as readily as naive control rats (NC) do. The present studies were designed to examine the correlations between the behavioral differences and the changes of *in vitro* neurotransmitter release seen in these three groups of rats.

The major finding concerned a significant increase in endogenous and K^+-stimulated serotonin (5-HT) release in the hippocampal slices of LH rats. There were no apparent differences in acetylcholine, dopamine and noradrenaline release in the hippocampus of LH rats as compared to NLH and NC rats. These results add further support to previous studies in our laboratory which implicate presynaptic 5-HT mechanisms in the behavioral deficit caused by uncontrollable shock.

Sprague–Dawley rats exposed to uncontrollable footshock develop a behavioral deficit called "learned helplessness" (LH). This paradigm has been proposed as a valid animal model of human depression (Seligman and Maier, 1967; Maier and Seligman, 1976; Willner, 1984, 1990). Indeed, the behavioral outcome of various parameters such as weight loss, sleep, activity, libido and cognitive function are remarkably similar in "learned helplessness or behavioral depression" and in human depression (Anderson *et al.*, 1968; Levine *et al.*, 1973; Weiss *et al.*, 1970; Weiss and Glazer, 1975; Kupfer, 1976; Adrien *et al.*, 1991; Neill *et al.*, 1990, Vogel *et al.*, 1990). Moreover, identical pharmacological treatments are effective in improving depression and LH behavior (Sherman *et al.*, 1979; Petty and Sherman, 1980; Katz and Hersh, 1981; Soubrie *et al.*, 1987).

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; Stahl and Palazidou, 1986), monoaminergic neurochemistry has been extensively investigated with regard to LH. Increased noradrenergic turnover, decreased norepinephrine brain levels, up-regulation of β-adrenergic receptors and increased sensitivity of adenylate cyclase stimulation by norepinephrine have been noted as a result of exposure to uncontrollable shock (Weiss *et al.*, 1975; Anisman and Sklar, 1979; Weiss *et al.*, 1981; Henn *et al.*, 1987; Martin *et al.*, 1990).

Although the original catecholamine hypothesis of affective illness focused largely on norepinephrine, neurochemical and pharmacological evidence also suggests that functional dopaminergic activity is dysregulated in sub-groups of depressed patients (Jimmerson, 1987). Hence, some attention has also been devoted to dopaminergic involvement in LH. Exposure to uncontrollable shock produces dopamine depletion in the caudate nucleus, nucleus accumbens and frontal cortex (Schutz *et al.*, 1979; Thierry *et al.*, 1976). However, in conflict with the above studies, increases in dopamine levels were also reported in the frontal cortex of rats subjected to an avoidance escape test as compared to yoked and no shock controls (Weiss *et al.*, 1981). Additionally, a decrease in dopamine receptor density has been demonstrated in the frontal cortex of rats receiving uncontrollable shock (Cherek *et al.*, 1980) and the debilitating effect of uncontrollable shock exposure on subsequent escape performance was mimicked and exacerbated by DA receptor blockers (Anisman *et al.*, 1979).

Serotonin (5-hydroxytryptamine or 5-HT) mech-

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anisms have also been investigated in the LH paradigm. However, there have been conflicting reports on the involvement of 5-HT in helpless behavior. Increased serotonergic turnover and decreased brain 5-HT levels have been reported in animals exposed to uncontrollable shock (Hellhammer *et al.*, 1984). *In vivo* release of 5-HT from cerebral cortex was lower in LH rats as compared to yoked controls (Petty and Sherman, 1983). However, our laboratory has demonstrated that depletion of 5-HT with *p*-chloro-phenylalanine (PCPA), protected rats from uncontrollable shock effects (Edwards *et al.*, 1986) and that 5-HT levels were elevated in the hippocampus of LH rats as compared to NLH and NC rats (Martin *et al.*, 1990).

Besides dopamine, norepinephrine and 5-HT, acetylcholine has also been implicated in affective illness. The hypothesis that the neurobiology of depression involves an hyperfunction of central muscarinic cholinergic mechanisms has been advanced by Janowsky and colleagues (1972). It has also been demonstrated that chronic uncontrollable stressors activate central muscarinic mechanisms in rats (Janowsky and Risch, 1984).

Despite all the aforementioned studies, there has been no real demonstration of a correlation between the behavioral changes seen in "learned helplessness" and presynaptic activity of norepinephrine, dopamine, acetylcholine and 5-HT.

The current investigations initiate a systematic examination of the *in vitro* release of these four neurotransmitters in the hippocampus of rats showing a behavioral deficit due to uncontrollable stress. We have previously reported that 5-HT levels, 5-HT uptake sites and 5-HT_{1b} receptors were significantly elevated in the hippocampus of LH rats as compared to NLH and NC rats (Edwards *et al.*, 1986, 1991a,b; Martin *et al.*, 1990). We report that the only significant changes in endogenous and K^+-stimulated neurotransmitter release were evident with [^{3}H] 5-HT release in LH rats as compared to NLH and NC rats. No changes in [^{3}H]norepinphrine, [^{3}H]dopamine or [^{3}H]choline release were demonstrated in the LH rats.

EXPERIMENTAL PROCEDURES

Materials

5-[1,2-^{3}H-(N)] hydroxytryptamine creatine sulfate ([^{3}H]5-HT) (sp. act.: 25.2 Ci/mmol), D,L[7-^{3}H-(N)]norepinephrine hydrochloride ([^{3}H]NA: spe. act.: 15.0 Ci/mmol), 3, 4[ring-2,5,6-^{3}H]dihydroxy-phenylethyl-amine hydrochloride ([^{3}H]DA; spe. act.: 45.9 Ci/mmol), choline chloride (methyl-^{3}H; spe. act.: 87.6 Ci/mmol) and Protosol™ Tissue solubilizer were obtained from New England Nuclear (Boston, MA). Hydrofluor™ scintillation cocktail was obtained from National Diagnostics (Palmetto, FL). Fluvoxamine and nomifensine were obtained from Kali Duphar (Weesp, The Netherlands) and Hoechst (Scotchplain, NJ). Neostigmine was purchased from Research Biochemicals (Natick, MA). Other drugs and reagents were purchased from Sigma (St Louis, MO).

Male Sprague–Dawley rats (150–200 g) were obtained from Charles River Breeding Laboratories (Wilmington, MA) and were kept for one week before use, in a temperature/humidity controlled facility. A 12 h 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*. A total of 144 rats were used in 4 separate experiments (one experiment/[^{3}H]ligand release run separately; $n = 6$/rat group).

Behavioral training and shock escape testing

The LH paradigm consists of placing the experimental animals in a Coulbourn chamber with an electrified grid floor as previously described (Edwards *et al.*, 1986). Rats were exposed to uncontrollable shock in a 40 min session where 0.8 mA footshocks are randomly delivered through the electrified grid floor of the Coulbourn chamber with a minimum time of 1.5 s between on and off events. This regimen results in an average schedule of 20 min of footshock.

Twenty-four hours after exposure to uncontrollable shock, each rat was tested in a shock escape paradigm where footshock could be terminated by a single lever press. For the shock escape test, the Coulbourn shock chambers were modified. A lever was mounted 7 cm off the grid floor on one wall of the chamber. A yellow cue light was placed 5 cm above the lever. Shock was delivered at the intensity of 0.8 mA in a pulsating schedule of 35 ms on/35 ms off with the yellow cue light being on during the shock period. Intertrial intervals of 24 s begin with the yellow cue light being out. Fifteen separate trials were given to each rate in a shock escape test. A trial was considered a successful escape to shock if the rat terminates the shock by pressing the lever within 0–20 s. Latencies of 20–60 s were recorded as failures in the shock escape test. Scores were recorded automatically.

As previously described (Edwards *et al.*, 1986), rats scoring 10–15 failures in the 15 trial shock escape test were considered deficient in the escape response (LH group). Rats scoring 0–4 failures in the shock escape test were considered not to be deficient in the shock escape test (nonlearned helpless or NLH group). Naive controls (NC group) received no exposure to uncontrollable shock but were subjected to the shock escape test where they score between 0–4 failures. These NCs were included in the testing paradigm to determine the effects of shock *per se*. Behavioral and biochemical determinations are always carried out on PC rats. From past experiments carried out in our laboratory, these controls do not show any significant changes either behaviorally or biochemically from the NLH rats. Rats scoring 5–9 failures were not included in the release experiments.

All rats were decapitated one day after the shock escape test.

Release experiments

One day after the behavioral testing experiment, LH, NLH and NC male rats ($n = 6$/14 group) were sacrificed by decapitation. The hippocampus was rapidly removed and placed

in ice-cold medium. Slices (0.35 mm thick) were prepared using a McIlwain chopper. In separate experiments, hippocampal slices were incubated with constant shaking under a steady stream of 95% O_2: 5% CO_2 with 0.1 μM [^{3}H]ligands ([^{3}H]5-HT; [^{3}H]DA; [^{3}H]NA; [^{3}H]choline depending on the experiment). Incubation was carried out for 60 min at 37°C in 5 ml of a medium having the following composition (mM): NaCl 118, KCl 4.8, CaCl, 1.25, KH_2PO_4 1.2, $MgSO_4$ 1.2, $NaHCO_3$ 25, glucose 5.5, ascorbic acid 5.7, paragyline 0.05, pH 7.4. After washing with standard medium (3 × 5 ml), 50 μl aliquots of the hippocampal slices were transferred to each of 6 parallel superfusion chambers and were superfused for 110 min at a rate of 100 μl/min. After a 60 min washing period at 37°C to achieve a constant baseline of tritium efflux, 4 min fractions were collected until the end of the experiment. Slices were exposed to one 2 min pulse of a modified depolarizing superfusion medium with elevated K^+ ions (609 mM KCl in superfusion medium substituting an equimolar concentration of NaCl). The following compounds were added to the superfusion medium to prevent the reuptake of neurotransmitter into nerve endings: fluvoxamine (10 μM) in the [^{3}H]5-HT release experiments, nomifensine (30 μM) in the [^{3}H]catecholamine release experiments (Claassen *et al.*, 1977; Potter *et al.*, 1984; Dubocovich and Weiner, 1981; Imperato *et al.*, 1989). After superfusions, tissue slices removed from the superfusion chambers were solubilized in 0.3 ml Protosol and 0.15 ml of acetic acid (2 M). Radioactivity in the solubilized tissue and in aliquots of superfusate was measured by liquid scintillation spectrometry.

Calculations

The efflux of radioactivity in each fraction was calculated as a percentage of the total radioactivity present in the tissue at the start of the fraction collection. The stimulation-evoked overflow was calculated as the difference between the total outflow during and after stimulation and the estimated basal outflow. Data are presented as follows (Farnebo and Hamberger, 1971):

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

$$\% \text{ endogenous release} = 100 \times \frac{(\text{Baseline release})}{(\text{Tissue radioactivity}) + (\text{Baseline release}) + (K^+\text{-induced release})}$$

Means ± SEM are given throughout. Means of behavioral scores for LH, NLH and NC rats were compared by Student's *t*-tests. Release data (% stimulated and % endogenous release) for all three groups were compared by ANOVA followed by *post hoc* contrast test to compare differences between specific means. For ANOVA analysis, the significance was set at 0.05.

RESULTS

The effect of the learned helpless paradigm in rats

Behavioral scores for NC, NLH and LH rats from four separate experiments are shown in Table 1. A total of 144 rats were used in this study. As previously reported (Henn *et al.*, 1987; Edwards *et al.*, 1986) only 20%–30% of all Sprague–Dawley rats trained and tested will exhibit behavioral deficits in the shock escape test scoring 10–15 failures in the 15-trial shock escape test. *In each of four experiments*, 30 rats were exposed to uncontrollable shock and subsequently tested in the shock escape paradigm. Twenty percent of all the rats trained and tested were helpless (n = 24/120; LH group). Seventy-five percent of all the rats trained and tested were non helpless (n = 90/120; NLH group). From that group, we used 24 NLH rats in the release experiments. Rats scoring 5–9 failures (n = 6/120) were not included in the release experiments. We have also used 24 NC rats in these experiments. Control rats (n = 24) are not exposed to uncontrollable shock but were subjected to the shock escape test where they exhibit behavioral scores of 0–4 failures.

Table 1. Shock escape test scores of LH, NLH and NC rats

Groups		Number of failures
Naive controls	NC	2.8 ± 0.12
Non learned helpless	NLH	3.1 ± 0.18
Learned helpless	LH	14.3 ± 0.25†

Data are presented as number of failures ± SEM (n = 24/group).
† t = 3.485, $P < 0.001$ LH vs NC and NLH (Student t-test).

The effect of learned helplessness on [^{3}H]catecholamine and [^{3}H]choline release

Endogenous release and K^+-stimulated release data for [^{3}H]NA, [^{3}H]DA and [^{3}H]acetylcholine ([^{3}H]ACh) are shown in Table 2. No differences either in endogenous release or in K^3-stimulated release of any of the three neurotransmitters were apparent in the LH as compared to the NLH and NC rats.

Table 2. Endogenous and depolarization induced release of radiolabeled catecholamines and acetylcholine from slices of hippocampal tissue of LH, NLH and NC rats

	Efflux rates (% of tissue stores/min)	
	Endogenous	K^L-evoked
[^{3}H]NA release		
LH (6)	0.51 ± 0.11	34.8 ± 1.6
NLH (6)	0.67 ± 0.07	39.3 ± 1.3
NC (6)	0.64 ± 0.05	36.73 ± 1.4
[^{3}H]DA release		
LH (6)	0.56 ± 0.03	11.7 ± 0.52
NLH (6)	0.52 ± 0.04	12.2 ± 0.50
NC (6)	0.63 ± 0.02	11.2 ± 0.43
[^{3}H]ACh release		
LH (6)	0.57 ± 0.02	4.3 ± 0.25
NLH (6)	0.56 ± 0.02	4.8 ± 0.34
NC (6)	0.54 ± 0.06	4.7 ± 0.15

Release expressed as % total tissue content.
Values are means ± SEM with number of determinations (No. rats/group) in parentheses.

The effect of learned helplessness on [^{3}H]serotonin release

By contrast, spontaneous 5-HT release was increased significantly in LH rats as compared to both NLH and NC rats [LH: 1.4 ± 0.16; NLH: 0.86 ± 0.08; NC: 0.89 ± 0.08; data expressed as % basal release $\pm$ SEM, $P \leqslant 0.002$ LH vs NLH and NC rats, Fig. 1(A)].

K^+-induced stimulation of 5-HT release was increased significantly in the LH rats [%stimulated release of [^{3}H]5-HT $\pm$ SEM: 16.75 ± 0.67, LH; 12.3 ± 1.1, NLH; 12.06 ± 0.3, NC; $P \leqslant 0.005$ LH vs NC; $P \leqslant 0.003$ LH vs NLH, Fig. 1(B)].

[K^+]5-HT release was Ca^2 dependent. Basal ^{3}H-efflux was not significantly altered by the omission of Ca^2 from the superfusion medium whereas the K^+-evoked ^{3}H-overflow was mostly abolished by omission of Ca^{2+} [% stimulated release: 1.23 ± 0.17, calcium deficient medium vs 16.75 ± 0.67, Ca^{2+} in superfusion medium; $P \leqslant 0.001$; Fig. 1(B)].

DISCUSSION

The present study of *in vitro* neurotransmitter release was carried out in hippocampal slices from three groups of rats: LH, NLH and NC. The major finding of these experiments involved a significant increase in basal and K^+-stimulated [^{3}H]5-HT release in the LH rats as compared to NLH and NC rats. This increase in presynaptic serotonergic activity contrasts with the finding that LH rats could not be distinguished from NLH and NC rats by comparing [^{3}H]NA, [^{3}H]DA, [^{3}H]ACh release parameters. These data suggest that presynaptic 5-HT regulation may be involved in the modulation of LH behavior.

The behavioral model used in these studies takes advantage of the inherent variability between individual rats in their responsiveness to a regimen of uncontrollable shocks. The release experiments carried out in hippocampal slices can then compare LH rats with unaffected rats receiving the same treatment condition (NLH). The inclusion of the control group (NC) in our comparisons allows for the assessment of the effect of shock *per se* since these control rats are only subjected to the shock escape test. However, in a study of this type, one can only note correlations between the behavioral deficits exhibited by the LH rats and the changes in 5-HT release *in vitro* demonstrated in the same rats. Although no causal inferences can be made, we believe that the changes in 5-HT release demonstrated in these experiments influence the induction of LH behavior. This conclusion is supported by our previous data showing that depletion of 5-HT by PCPA prevents the development of LH (Edwards *et al.*, 1986) and that 5-HT levels are increased in the frontal cortex, hippocampus and hypothalamus of LH rats (Martin *et al.*, 1990). Additionally, manipulations which increase the endogenous levels of 5-HT mimic the effects of an uncontrollable stressor and interfere with the acquisition of an escape response in a shock avoidance paradigm (Brown *et al.*, 1982).

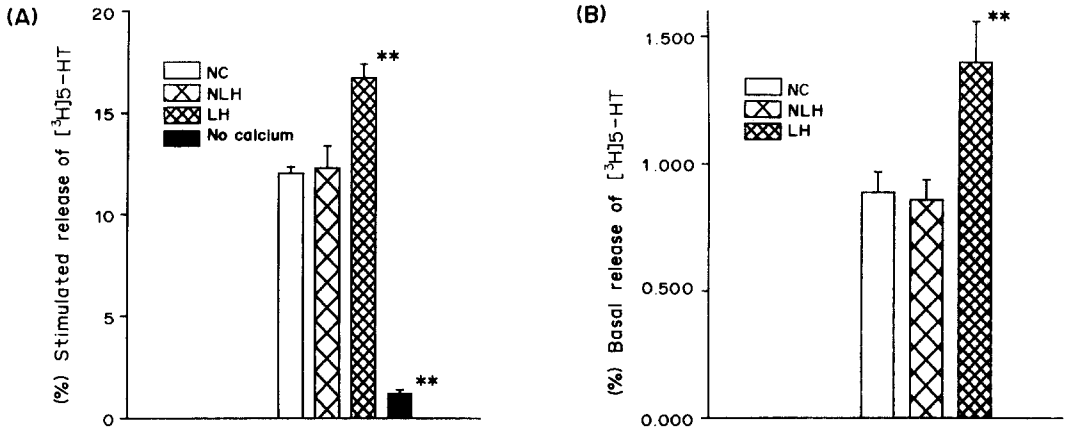

Fig. 1. Basal and K^+-stimulated [^{3}H] 5-HT release in hippocampal slices of LH, NLH and NC rats. Percent endogenous release (A) and percent stimulated release (B) of [^{3}H] 5-HT release were expressed as mean $\pm$ SEM (n = 6/group). Differences in endogenous [^{3}H] 5-HT release and K^+-stimulated [^{3}H] 5-HT release were revealed by ANOVA and *post hoc* contrast test [endogenous release: $F(2, 15) = 6.15$, $P \leqslant 0.01$; LH vs NC, $t = 5.46$, $P \leqslant 0.002$ and LH vs NLH, $P \leqslant 0.002$, *post hoc* contrast test; K^+-stimulated release: $F(2, 15) = 8.2$, $P \leqslant 0.005$; LH vs NC, $t = 4.03$, $P \leqslant 0.005$ and LH vs NLH, $t = 5.02$ $P \leqslant 0.003$, *post hoc* contrast test].

We have recently demonstrated that [^{3}H]5-HT uptake and 5-HT uptake sites labeled with [^{3}H] paroxetine were significantly increased in the hippcampus of LH rats as compared to NLH and NC rats (Edwards *et al.*, 1989, 1991a). In addition, the density of the 5-HT$_{1b}$ receptors in various limbic structures (septum, hippocampus, cortex) was also significantly higher in the LH rats as compared to NLH and NC rats (Edwards *et al.*, 1991b). The consistent evidence for a correlation between behavioral deficits and serotonergic changes in the LH rats suggest that the hippocampal serotonergic pathway may represent a final common output from the various modulatory neuronal systems that have been implicated in the response to stressful stimuli. These changes in serotonergic mechanisms are also evident in rats bred for susceptibility to LH behavior (Fochtmann *et al.*, 1990).

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

The differential regulation of the 5-HT$_{1b}$ receptors in limbic structures of the LH rats (Edwards *et al.*, 1991b) is particularly interesting since these receptors have been identified as the 5-HT autoreceptor in rats and play a role in the modulation of 5-HT release (Middlemiss, 1984). However, we have reported an increase in 5-HT$_{1b}$ receptors in limbic structures and the present study also demonstrates an increase in 5-HT release in hippocampal slices of the LH rats. It is important to recognize that the 5-HT$_{1b}$ receptors are not necessarily restricted to the presynaptic portion of the 5-HT neuron. Combined interaction with pre- and post-synaptic receptors could determine whether the autoreceptor agonist 5-HT will actually inhibit release. We have previously demonstrated with lesion studies that the 5-HT$_{1b}$ sites in the hippocampus occur both pre- and post-synaptically: with approx. one third of these sites occurring on serotonergic terminals and the remainder on neurons or astroglia intrinsic to the hippocampus (Edwards *et al.*, 1987). There is also the possibility that the 5-HT transporter system and the autoreceptor may be linked through functional or molecular events in which case certain conditions or certain agonists will produce anomalous results with regard to autoreceptor function and ultimately the release of 5-HT (Wolf and Kuhn, 1990).

The overall activation of serotenergic systems in limbic structures of an animal model of depression is of particular interest. The terms limbic system and emotion, stress, emotion and disease have been linked for some decades (Selye, 1950). Both clinical and behavioral depression (LH) have been associated with some dysregulation of the hypothalamic–pituitary–adrenal (HPA) axis (Gold *et al.*, 1988; Greenberg *et al.*, 1989; Edwards *et al.*, 1990). The hippocampus has been demonstrated as a site of serotonergic innervation associated with CNS control of the HPA axis and a good correlation exists between the concentrations of cellular receptors for 5-HT and corticosterone (Biegon *et al.*, 1982). The data presented in this article taken together with our findings of HPA axis dysfunction in the LH model (Greenberg *et al.*, 1989); Edwards *et al.*, 1990) suggest that corticosterone may be acting on hippocampal glucocorticoid receptors located post synaptically to the innervating 5-HT neuron whereupon this neuron responds with increased 5-HT syntheses, release and uptake (De Kloet and Reul, 1987). Additional evidences implicating the hippocampus in the pathophysiology of affective disorders can be derived from studies of *post mortem* brain of suicide victims. Increases in serotonin (5-HT$_2$) receptors and β-adrenergic receptors have been demonstrated in cortical and subcortical structures related to the limbic system (Arango *et al.*, 1990).

Our findings contrast with earlier reports of a decrease in calcium-specific 5-HT release from cortical slices in LH rats (Sherman and Petty, 1980). *In vivo* experiments using the push–pull cannula method also demonstrated a decrease in cortical 5-HT in LH rats (Petty and Sherman, 1983). However, the results from the present experiments are in agreement with recent measurements of 5-HT release by *in vivo* microdialysis. Since our data on neurotransmitter release in LH rats are obtained in a hippocampal slice preparation, and the brain slice preparation only represents an artificial simplification of the normal state

of the nervous system, it is important to stress the need for correlation with results from intact preparations and from other techniques. Indeed, recent findings with *in vivo* brain microdialysis suggest that an increase in 5-HT in the extracellular fluid after exposure to uncontrollable stress correlates with the development of LH (Petty *et al.*, 1990). However, no correlation between the degree of LH and K^+ stimulated 5-HT release in the cortex was demonstrated. These findings may contrast with the present data because of differences in the time of sampling for 5-HT (number of days after exposure to inescapable shock). Since we have only examined the release of dopamine norepinephrine, acetylcholine and 5-HT in the hippocampus of LH, NLH and NC rats, it is entirely possible that there might be a differential modulation of these neurotransmitters in other brain regions and that differences might be revealed between the three groups of rats studied. To date, we have only carried out *in vitro* [^{3}H]5-HT release experiments in the hippocampus, cortex, septum and hypothalamus of LH, NLH and NC rats. Increased [^{3}H]5-HT release was evident in all the limbic structures (ongoing studies and unpublished observations).

These changes in 5-HT mechanism in our animal model of depression are of interest since 5-HT has occupied an important place in current theories of the etiology of depression (Meltzer and Lowy, 1987). For the last two decades numerous studies of 5-HT uptake mechanisms and regulation of 5-HT receptors suggest that these mechanisms may be primary etiological factors in the study of depression and may lead to a better understanding of the action of antidepressant drugs and electroconvulsive treatment. We are currently extending the present studies using *in vivo* brain microdialysis and further characterizing the 5-HT_{1b} receptor mechanisms which regulate the release of 5-HT.

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