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Learned Helplessness and In Vivo Hippocampal Norepinephrine Release

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PETTY, F., G. KRAMER, L. WILSON AND Y.-L. CHAE. *Learned helplessness and in vivo hippocampal norepinephrine release.* PHARMACOL BIOCHEM BEHAV 46(1) 231-235, 1993.—Hippocampal norepinephrine release was measured using in vivo microdialysis in rats before and after exposure to inescapable tail shock stress and after testing for learned helplessness. Rats that did not develop learned helplessness after stress had higher basal norepinephrine release after stress than rats developing learned helplessness or than control rats. After the shuttlebox test for learned helplessness, K^+-stimulated norepinephrine release was lower in learned helpless than in nonhelpless or control rats. These results confirm an important role for the hippocampal noradrenergic system in differential behavioral responses to stress.

Hippocampus Norepinephrine Learned helplessness Inescapable stress

LEARNED helplessness is a maladaptive behavioral depression caused by inescapable stress (22). Learned helplessness is often considered to be a model for human depression (29). Norepinephrine has long been implicated in the etiology of human depression (7), and a number of studies on the neurochemistry of learned helplessness have examined the norepinephrine system.

Depletion of norepinephrine with FLA-63 (2) or DSP-4 (20) produces a behavioral deficit similar to that produced by inescapable stress in naive rats. Inescapable stress, but not a comparable escapable stress, decreases levels of norepinephrine in the locus ceruleus (28). Norepinephrine beta receptor blockers prevent the reversal of learned helplessness by antidepressant drugs (12). Generally, this work suggests that a norepinephrine deficit accompanies learned helplessness.

The hippocampus may mediate the effects of norepinephrine in learned helplessness. Bilateral hippocampal norepinephrine lesions with 6-OHDA prevent the reversal of learned helplessness by tricyclic antidepressants (27). Rats demonstrating learned helplessness after inescapable stress have increased density of hippocampal beta receptors (9). Microinjection of norepinephrine had behavioral activity only in hippocampus, of 12 regions studied, and prevented the development of learned helplessness, but did not alter established learned helplessness (25). This suggests elevated extracellular norepinephrine before stress might prevent learned helplessness, and lower extracellular norepinephrine might be a consequence of learned helplessness.

In order to test these hypotheses regarding the relationship of norepinephrine to learned helplessness in vivo, we have used microdialysis in rats to measure norepinephrine release into extracellular space of the hippocampus before and after exposure to inescapable stress and after testing for learned helplessness.

METHOD

Animals

Male Wistar rats weighing 250–275 g (Sasco, Inc.; Omaha, NE) were used for all experiments. Animals were adapted to the laboratory environment for at least a week before study. They were maintained on a 12L : 12D cycle with food and water available ad lib. Prior to experimentation, rats were housed in groups of six, and after surgery they were housed individually.

Microdialysis

All rats were stereotaxically implanted with guide cannulae on day 1. Dialysis probes were constructed with minor modification of the concentric design of Robinson and Whishaw (21) with a cellulose dialysis membrane (cutoff 6000 D). The length of dialysis membrane was 4 mm.

Animals were anesthetized with pentobarbital (45 mg/kg, IP) and guide cannulae were stereotaxically inserted into the ventral hippocampus at the following coordinates relative to bregma and dura: AP, −4.8; ML, ±4.8; DV, −3.5 (15, 23). Animals recovered from surgery and were returned to individual cages where they resumed normal feeding and grooming within 2 to 4 h.

One day after surgery (day 2), the microdialysis probes were inserted into the guide cannulae and connected to a syringe pump and the rats placed in a cylindrical polystyrene

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chamber (30 × 60 cm). Perfusion was maintained at 1 μl/min with Ringer's solution (147 mM NaCl, 2.3 mM $CaCl_2$, 4.0 mM KCl, pH 6.0). Baseline was defined when three consecutive samples with less than 10% variance in concentration were obtained. Aliquots were collected every 20 min and immediately injected onto a HPLC equipped with a coulometric detector. The perfusion consisted of a 1-h washout period followed by 2 h of baseline monitoring. Rats were then disconnected from the perfusion apparatus, placed in the tail shock apparatus, and given inescapable stress or no shock.

On the day after the first perfusion (day 3), rats were again perfused for another 2 h, then tested for learned helplessness.

The day after shuttlebox testing (day 4), rats were again perfused until a stable baseline was established and the perfusing solution was then switched to Ringer's with 100 mM K^+ for 2 additional h (please see Table 1 for experimental design).

At the conclusion of each experiment, crystal violet dye was injected into the probe and animals were sacrificed with an overdose of pentobarbital and decapitated. The brain was removed and dissected under a microscope. Probe placement was visually determined by the location of the probe track, providing rapid verification of anatomical placement. Data from animals in which the probe was not in the ventral hippocampus were discarded from statistical analysis.

Behavioral

Rats were given 100 trials of tail shock in day 2 using the procedure of Maier et al. (11) in Plexiglas chambers with metal tail electrodes. Each trial began with an unsignaled 5-s, 1.05-mA shock, increased 0.3 mA every 20 trials to a final current of 2.0 mA, with a 60-s variable intertrial interval.

Twenty-four hours after being placed in the Plexiglas chambers (day 3), all rats were tested in a shuttlebox using the procedure of Jackson et al. (10). Rats were classified into learned helpless (LH; $n = 7$) and nonhelpless (NH; $n = 5$) groups according to the escape latencies. Previous research (8,18) has shown a mean latency of ≥25 s to reliably differentiate learned helpless rats from NH and from naive control rats whose FR2 mean latency per trial is 12 ± 4 s.

Control groups (CON; $n = 7$) were also studied; they had guide cannulae implanted and were placed in the Plexiglas chambers the next day, where they had electrodes attached and received no tail shock. When controls were tested in the shuttlebox, escape was purposely prevented for the first 15 s, to balance for total shock exposure between learned helplessness and CON groups.

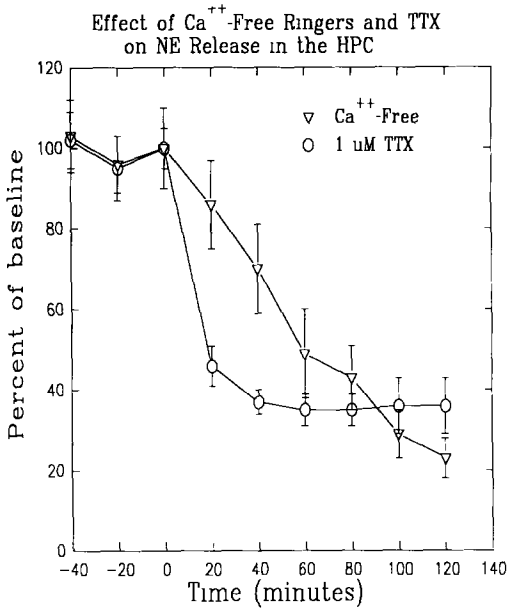
TABLE 1

PROCEDURES OF EXPERIMENT

Day 1:	Guide cannulae implantation
Day 2:	(a) Perfusion (prestress)
	(b) Inescapable stress
Day 3:	(a) Perfusion (pretest)
	(b) Shuttlebox test for learned helplessness
Day 4:	(a) Perfusion (posttest)
	(b) High K^+ perfusion

Norepinephrine Assay

Quantification of norepinephrine in the dialysate was accomplished with a high performance liquid chromatographic system with minor modification of previously described methods (3).



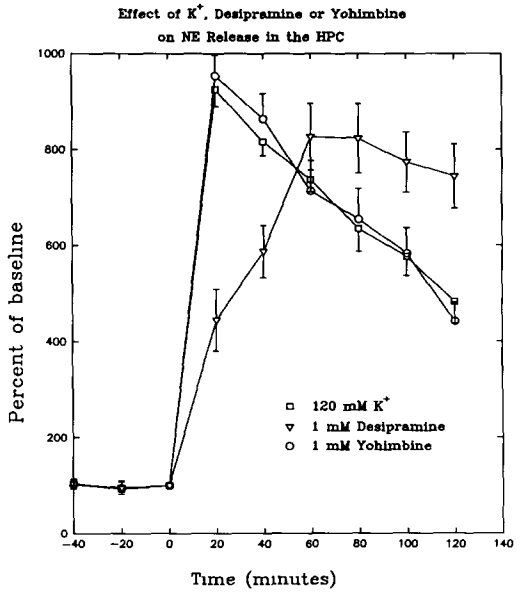


FIG. 1. Characterization of norepinephrine in microdialysis perfusate. (A) Effects on norepinephrine levels in perfusate of hippocampus caused by changing perfusion solution from Ringer's solution to Ca^{++}-free Ringer's solution and to Ringer's solution with 1 mM tetrodotoxin added. Levels expressed as a percent of baseline before solutions were switched at time 0 (arrow). (B) Effects of adding 120 mM K^+, 1 mM desipramine, or 1 mM yohimbine to Ringer's solution. Otherwise as in (A).

The dialysate was injected directly onto the HPLC consisting of a ESA HPLC 5100A and a ODS-Hypersil column (150 × 4.6 mm, 5 μm). The mobile phase contained 75 mM sodium phosphate buffer (pH 3.1), 100 μM EDTA, 1.4 mM sodium octyl-sulfate, and 10% v/v acetonitrile. The flow rate was 1.0 ml/min. The potential of the first (oxidizing) detector cell was set at +0.32 V, and the potential of the second (reducing) detector cell at −0.26 V. The sensitivity of this assay was 0.5–1.0 pg of norepinephrine. Norepinephrine levels were corrected for relative recovery of the probe and expressed in IU.

Characterization of Norepinephrine Release

In a separate series of experiments, groups of six rats were implanted with microdialysis probes in the hippocampus and perfused with Ringer's solution on the following day. After obtaining a stable baseline, perfusing solution was switched to either Ca^{++}-free Ringer's, or Ringer's with added tetrodotoxin (TTX 1 μM), K^+ (120 mM), desipramine (1 mM), or yohimbine (1 mM). Norepinephrine in perfusate was measured every 20 min for an additional 2 h as above, after which animals were sacrificed and probe placement was verified.

RESULTS

Perfusion with Ca^{++}-free Ringer's solution or addition of TTX to the perfusate resulted in a decrease of norepinephrine in perfusate to 40% of baseline. Perfusion with high K^+, yohimbine, or desipramine resulted in an increase of norepinephrine in perfusate to about 800% of baseline (Fig. 1A,B).

The in vitro recovery of norepinephrine was 22 ± 1.4% at 37°C. The prestress basal levels of extracellular norepinephrine in dialysate from LH, NH, and CON were comparable (1.37 ± 0.58 nM, n = 11; 1.24 ± 0.17 nM, n = 6; 1.66 ± 0.5 nM, n = 7).

After stress, the pretest norepinephrine levels in perfusate of the NH group were significantly higher than those of the LH or CON groups, $F(2, 15) = 3.77$, $p < 0.05$ (Fig. 2). Rats that had higher levels of norepinephrine after inescapable stress but before testing had shorter escape latencies and therefore showed less learned helplessness behavior on testing. In other words, the percent increase in norepinephrine levels after inescapable stress inversely correlated with the mean escape latency (Fig. 3: $r = -0.71$, $p < 0.05$). There was no difference in the norepinephrine release measured posttest.

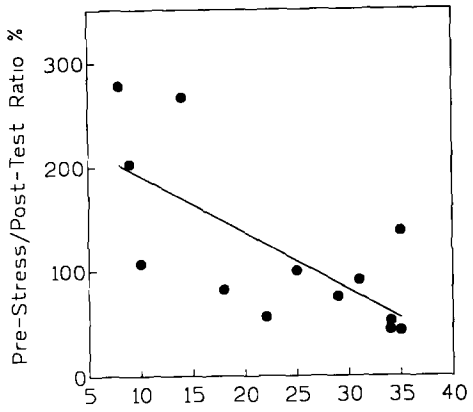


FIG. 3. Correlation of pretest hippocampal norepinephrine release with mean escape latency on FR2 shuttlebox test (n = 13, $r = -0.71$, $p < 0.05$). Data are presented for learned helplessness and NH groups combined.

K^+-stimulated norepinephrine levels in the first hour of perfusion were significantly different between the three experimental groups, $F(2, 15) = 4.10$, $p < 0.05$ (Fig. 4). K^+-stimulated norepinephrine levels of the CON group were increased to 690% over posttest baseline. K^+-stimulated norepinephrine levels of the LH group were only increased to 380% of baseline, and the NH group had intermediate levels (540%) between the CON and learned helplessness groups. There were no differences among groups in K^+-stimulated norepinephrine release in the second hour of perfusion.

DISCUSSION

The experiments characterizing the norepinephrine measured in the perfusate support a neuronal origin for norepinephrine, since the norepinephrine is apparently released by a K^+-stimulated, Ca^{++}-dependent, TTX-sensitive mechanism. Furthermore, the norepinephrine measured in perfusate was increased by desipramine, an inhibitor of synaptic reuptake, and by yohimbine, an alpha$_2$ presynaptic receptor antagonist. The latter results are similar to those of Broderick (6). The present methodology measures extracellular levels (overflow) of norepinephrine and of course cannot distinguish between

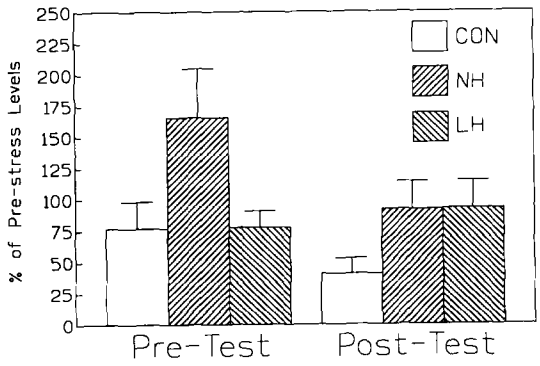


FIG. 2. Hippocampal norepinephrine release before and after shuttlebox test for learned helplessness. *$p < 0.05$ (NH vs. CON and learned helplessness). Values are expressed as percent of prestressed norepinephrine levels.

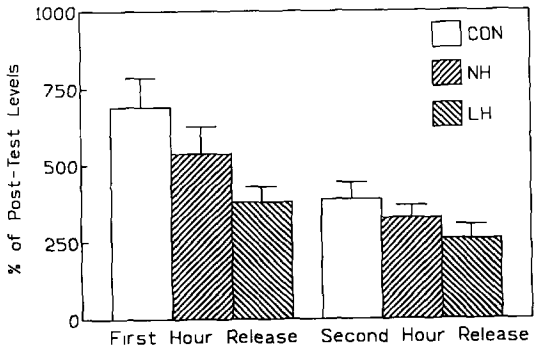


FIG. 4. Potassium-stimulated release of norepinephrine from hippocampus. Groups are significantly different for first hour K^+-stimulated release [ANOVA, $F(2, 15) = 4.1$, $p < 0.05$]. Values are expressed as the percent of posttested baseline levels.

pharmacological manipulations that enhance release vs. those inhibiting reuptake.

The major finding of the present work was that rats that did not become helpless after exposure to inescapable stress (NH) had increased basal hippocampal norepinephrine after stress and before testing, compared to control nonshocked rats and to rats with learned helplessness. There was an inverse correlation between escape latency (degree of learned helplessness) and poststress norepinephrine levels in perfusate.

This suggests that increased extracellular norepinephrine induced by stress has a "protective" effect and prevents development of learned helplessness. Such a result is compatible with our prior finding (24) that direct microinjection of norepinephrine itself into the hippocampus prior to shock exposure prevented the development of learned helplessness. Maintenance of high extracellular norepinephrine in hippocampus may represent a mechanism of action of the tricyclic antidepressants in the prevention of learned helplessness (19).

After testing for learned helplessness, all three experimental groups had comparable levels of hippocampal norepinephrine. This is interesting, since the amount of shock exposure during testing was obviously quite different between the LH and NH groups.

When the posttest perfusion was switched to a high K^+ solution, CON rats had the highest K^+-induced norepinephrine release. Learned helpless rats had the lowest K^+-stimulated norepinephrine release, and rats receiving tail shock stress but not developing learned helplessness (NH) had intermediate levels of K^+-stimulated norepinephrine release.

If one assumes the high K^+ perfusion provides an index of intraneuronal releasable norepinephrine, the data suggest that learned helplessness leads to norepinephrine depletion. Of particular interest is the finding that CON rats, who were exposed to a similar amount of foot shock on testing as learned helplessness rats, had significantly higher K^+-stimulated norepinephrine release than LH rats. Thus, the norepinephrine depletion was not due to the stress of the testing procedure itself. This is confirmed by the fact that the NH rats, with a 15-s foot shock exposure on testing (Fig. 4), had K^+-stimulated norepinephrine release between CON and LH. Therefore, depletion of intraneuronal hippocampal norepinephrine is associated with prior tail shock stress exposure and with subsequently developing learned helplessness as a consequence of stress.

Aversive stimuli are known to increase the spontaneous activity of norepinephrine neurons (26) and to stimulate in vivo norepinephrine release from hippocampus (1,14). However, in the case of noise stress, the hippocampal norepinephrine response is dissociated from the behavioral and neuroendocrine response patterns (5).

Our results are compatible with the idea that the hippocampal norepinephrine system plays a role in the attentional processes involved in interpreting external stress and developing a behavioral response (14).

Other CNS systems have also been implicated in learned helplessness, including GABA (4,16), serotonin (17), and dopamine (29). The hippocampal norepinephrine involvement in learned helplessness is best interpreted in the context of a complex multiregional, multitransmitter phenomenon.

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