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GABAergic Modulation of Learned Helplessness

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PETTY, F. AND A. D. SHERMAN. *GABAergic modulation of learned helplessness*. PHARMAC. BIOCHEM. BEHAV. 15(4) 567–570, 1981.—Previous studies have demonstrated the GABAergic system in the hippocampus to be a major controlling factor in the reversal of learned helplessness by antidepressants. In the present work, animals in which learned helplessness (LeH) was induced by exposure to uncontrollable electric shock demonstrated a decreased depolarization-induced release of GABA relative to controls, and an increased release of hippocampal glutamate. Injection of bicuculline but not glutamate into the hippocampus produced a behavioral state identical to that induced by uncontrollable shock, but had no influence on pain sensitivity. When flux through the "small" hippocampal pool of GABA was determined, chronic treatment with imipramine or iprindole, but not lorazepam or chlorpromazine elevated this measure. These three findings, along with those of prior experiments, suggest a controlling role for GABA in the learned helplessness model of depression.

GABA Learned helplessness Bicuculline

THE learned helplessness (LeH) animal model of depression [19] has demonstrated substantial utility for studies on the mechanism of tricyclic antidepressant drugs. The maladaptive behavior produced by exposure of rats to uncontrollable shock is prevented [18] or reversed [12] by chronic, but not acute, administration of imipramine. This delayed effect is not observed for lorazepam or chlorpromazine, suggesting drug specificity. Depending on the test situation, learned helplessness has also been reversed by desipramine [5], apomorphine or clonidine [1], or electroconvulsive shock [11].

Although clearly not an exact parallel of depression in humans, the learned helplessness model demonstrates sufficient specificity for studies of the possible neuroanatomical and neurochemical loci of antidepressant action. When injected directly into nine cerebral structures of the rat [19], desipramine reversed learned helplessness only in the frontal neocortex. Injections made into the posterior cortex, entorhinal cortex, septum, caudate, amygdala, hippocampus, or lateral geniculate body were ineffective.

Regional injections of serotonin (5-HT), norepinephrine (NE), and 4-aminobutyric acid (GABA) have also been found to be effective in the prevention or reversal of learned helplessness in the rat. Injections of 5-HT into the frontal neocortex or septum reversed the helpless behavior, as did administration of GABA into the hippocampus or lateral geniculate body. Additionally, when bicuculline was injected into the hippocampus, desipramine administration into the frontal cortex was rendered ineffective in reversing LeH. Since some hippocampal afferents arise in the frontal neocortex [20], this pathway might well be involved in the development or reversal of learned helplessness.

Because of the central role of the hippocampal formation in learned helplessness [19], the transmitters involved in this area are of special interest. Intrinsic GABA neurons are known to serve an inhibitory function in the hippocampus [3]. In addition, recent studies suggest that aspartate is the transmitter in the commissural and glutamate in the perforant path inputs to the hippocampus [7, 8, 9, 22]. Thus, both the excitatory (aspartate, glutamate) and inhibitory (GABA) amino acid neurotransmitters may play a role in LeH and may represent the means by which desipramine, injected into the frontal cortex, affects transmission within the hippocampus.

In order to more clearly elucidate the role of these amino acid neurotransmitters, we have studied the spontaneous and calcium-dependent depolarized release of nine amino acids in hippocampi of rats receiving inescapable shock and controls. We have also studied the behavioral effects of intrahippocampally injected glutamate and bicuculline with regard to the development of LeH, and controlled for potential analgesic effects by determining pain sensitivity (vocalization threshold) produced by these two agents. Additionally, we have determined GABA flux in both the "small" (or neuronal and therefore releasable and functionally active) and "large" (or glial and metabolic) hippocampal pools.

GENERAL METHOD

Briefly, learned helplessness training consisted of expo-

sure of the animal (250 g male Sprague-Dawley rats) to 40 minutes of intermittent, random, 0.8 mA scrambled DC shock. The onset and offset of shock was determined on a random basis such that shock averaged 30 seconds off and 30 seconds on. When on, the shock was pulsed on for 360 msec and off for 40 msec.

Testing for learned helplessness involved a lever-press escape task. After a 24 second intertrial interval a shock (0.8 mA, on for 40 of 400 msec) was presented. The shock continued for 70 seconds or until the lever was pressed. A lever-press response within 19 seconds of shock onset was defined as a successful escape. Lever presses with latencies of 20–70 seconds also terminated the shock, but were scored as escape failures.

Control animals routinely performed in the range of 0–5 escape failures (mean and 95% confidence limits). Thus, animals with 6–15 escape failures on 15 trials were defined as being "helpless." These parameters have been described in detail elsewhere [12].

METHOD

Experiment 1

Groups of five animals each were exposed to inescapable footshock as described above, which procedure produces LeH in over 80% of rats. Animals were then returned to their home cages for 24 hours. Twenty-four hours represents the time of maximal behavioral effect of the uncontrollable footshock and a time when the non-specific effects of stress have worn off [14]. After 24 hours hippocampi were removed and chopped into 500 μm slices. The slices were incubated according to the method of Wenthold [21]. This procedure involves washing off slices in buffer containing 3.5 mM KCl and no Ca^{++} to establish baseline release not due to Ca^{++}-induced depolarization. Slices were then incubated in buffer containing 50 mM KCl and 5 mM Ca^{++} in order to assess the calcium-induced release under depolarizing conditions. The supernatant was applied to a cation-exchange column and amino acids were eluted with 0.1 N HCl. After lyophilization of the cation column effluent, amino acids present in each sample were derivatized by sequential reaction with acidified butanol and trifluoracetic anhydride and then were determined by a gas-chromatographic method [23]. Glutamine and asparagine contributions to the glutamate and aspartate peaks were estimated by the method of Collins and Sumner [2].

All determinations were made on an 0.65 weight % ethylene glycol adipate column, 1 m × 2.9 mm, in a Shimadzu GC-7A gas chromatograph equipped with a flame thermionic detector. Peak areas were determined by a Shimadzu C-R1A integrator programmed with molar response ratios for the amino acids investigated.

Statistical analyses were done by Student t-test.

Experiment 2

Groups of seven animals were given intrahippocampal injections of glutamate (1 μg in 0.2 μl of 0.9% saline) or bicuculline (50 ng in 0.2 μl of propylene glycol) or vehicle under ether anesthesia according to previously established coordinates [13]. One hour later, they were placed in the learned helplessness testing paradigm [12] and assessed for the degree to which helplessness was induced by the injections. These animals were not exposed to footshock before testing since the purpose of this section was to determine whether the helpless state could be produced simply by modification of hippocampal transmitter activity.

Another group (n=8) received bicuculline (or vehicle) as above, and their vocalization thresholds to footshock were determined in the testing apparatus to assess for possible effects on pain sensitivity. Each rat was exposed to two series of increasing and two series of decreasing-intensity footshocks varying by 0.05 mA at each step. Shock was presented at 30-second intervals for 40 msec. The shock intensity was assumed to be above threshold when the animal vocalized in response to its presentation and below threshold when no response was elicited. Threshold was defined as the average of the first intensities which produced a response on an ascending series or failed to produce a response on a descending series.

Statistical evaluation of data was by the randomization test for small samples or by Fischer's exact probability test.

Experiment 3

Groups of 24 animals (per drug) were maintained on imipramine, lorazepam, or chlorpromazine (all as the hydrochlorides equivalent to 100 mg of free base per liter) in drinking water for three days. On the fourth day, GABA flux in the hippocampus was determined by the postmortem procedure of Patel, *et al.* [10]. Briefly, this method is used to obtain an estimate of the "small" and "large" pools of GABA. The "small" pool represents the contribution to the total GABA level from neuronal (and releasable, functionally active) plus non-neuronal GABA while the "large" pool consists only of glial GABA and GABA in other metabolic compartments.

Animals were killed by cervical fracture, and the hippocampi removed onto a moist plate maintained at 38° for 30 sec, 1, 2, 3, 5, 10, 15, and 20 min, with three hippocampi for each time point. After the incubation period, tissue was frozen on dry ice, homogenized in trichloracetic acid, and the GABA level of the supernatant was determined following cation-exchange cleanup as previously described. The slope of the curve of concentration vs time for the 0–5 min samples represents contribution to flux from both the "small" and "large" pools, while that for 10–20 min is flux from the "large" pool only. By subtraction it is then possible to obtain the flux from the "small" neuronal pool.

Statistical comparisons were made with Student's t-test.

RESULTS

Experiment 1

In animals exposed to inescapable shock 24 hours before sacrifice (Table 1), the spontaneous release of glutamate was elevated compared to nonshocked controls. Depolarization in the absence of calcium produced an increased level of glutamate and aspartate compared to controls. Depolarization in the presence of calcium resulted in increased levels of glutamate plus decreased levels of GABA present in the medium. Calcium-induced depolarization release of alanine, glycine, aspartate, glutamate, and GABA (Table 2) could be demonstrated, but only for GABA and glutamate were these different from non-shocked controls. The ratio of glutamate/GABA was elevated in all three media in the helpless animals.

Experiment 2

Intrahippocampal injection of glutamate was without effect on behavior in the test situation. Injection of bicuculline (Table 3) produced animals which behaved no differently than animals which received helplessness training, but sub-

TABLE 1

LEVELS OF SELECTED AMINO ACIDS IN MEDIA

	3.5 mM KCl, no $CaCl_2$		50 mM KCl, no $CaCl_2$		50 mM KCl, 5 mM $CaCl_2$	
	Control	Helpless	Control	Helpless	Control	Helpless
ALA	0.45 ± 0.22	0.64 ± 0.19	0.86 ± 0.13	0.98 ± 0.13	1.35 ± 0.21	1.48 ± 0.21
VAL	0.43 ± 0.17	0.29 ± 0.08	0.47 ± 0.12	0.36 ± 0.11	0.51 ± 0.10	0.34 ± 0.13
GLY	0.73 ± 0.20	0.90 ± 0.18	2.20 ± 0.33	2.34 ± 0.39	2.97 ± 0.30	3.08 ± 0.33
THR	0.11 ± 0.09	0.10 ± 0.08	0.27 ± 0.09	0.30 ± 0.10	0.26 ± 0.07	0.26 ± 0.09
GABA	0.94 ± 0.30	1.11 ± 0.26	1.29 ± 0.17	1.40 ± 0.21	2.13 ± 0.19	1.73 ± 0.21↓
PHE	0.04 ± 0.05	0.06 ± 0.07	0.06 ± 0.05	0.05 ± 0.04	0.04 ± 0.05	0.07 ± 0.04
ASP	0.72 ± 0.18	0.94 ± 0.17	0.97 ± 0.13	1.21 ± 0.15↑	1.24 ± 0.14	1.41 ± 0.20
GLU	1.71 ± 0.42	2.55 ± 0.50↑	2.70 ± 0.36	3.61 ± 0.49↑	5.71 ± 0.53	7.73 ± 0.49↑
LYS	0.23 ± 0.11	0.20 ± 0.07	0.35 ± 0.09	0.39 ± 0.08	0.44 ± 0.10	0.30 ± 0.12

Data represent mean ± S.D. ng present in medium of two two-min samples per mg wet tissue weight, n=5. Arrows represent significant increase (↑) or decrease (↓) compared to controls ($p<0.01$, Student t-test).

TABLE 2

SUMMARY TABLE OF DEPOLARIZATION RELEASE DATA

	Ca-Induced	
	Control	Helpless
ALA	0.49	0.50
VAL	0.04	−0.02
GLY	0.77	0.74
THR	−0.01	−0.04
GABA	0.84	0.33↓
PHE	−0.02	0.02
ASP	0.27	0.20
GLU	3.01	4.12↑
LYS	0.09	−0.09

Data as in Table 1. Calcium-induced release was determined by subtracting the levels of amino acid present in 50 mM KCl medium (without Calcium) from the level present in 50 mM KCl medium containing Calcium. Arrows represent significant increase (↑) or decrease (↓) compared to control (n=5, $p<0.01$, Student t-test).

TABLE 3

BEHAVIORAL EFFECTS OF INTRAHIPPOCAMPAL INJECTIONS OF BICUCULLINE OR VEHICLE

	Escape Failures 1–5	Helplessness 6–15
Bicuculline	1	6
Vehicle	7	0

	Pain Sensitivity
Bicuculline	0.84 ± 0.19 mA
Vehicle	0.81 ± 0.16 mA

Data for the upper table represent the frequency of animals exhibiting 1–5 (normal) or 6–15 (helpless) escape failures in 15 trials ($p=0.002$ by Fischer exact probability test).

Data for the lower table represents mean ± S.D. mA required to produce vocalization in the test apparatus (n=8).

TABLE 4

SUMMARY OF POST-MORTEM GABA FLUX PARAMETERS

	"Large" Pool	"Small" Pool
Control	43 ± 4	72 ± 6
Imipramine	50 ± 5	103 ± 8
Iprindole	48 ± 6	105 ± 10
Lorazepam	45 ± 11	72 ± 15
Chlorpromazine	58 ± 3	76 ± 4

Flux parameters were calculated by the method of Patel, *et al.* [10].

Data represent mean nmoles/g ± S.E.

stantially different from controls. No difference in vocalization threshold could be determined by this method following administration of bicuculline or vehicle (Table 3).

Experiment 3

The intercepts of the 0–4 minute curves or the 10–20 minute curves were not different with regard to experimental treatment. The estimated flux of the "small" pool was significantly elevated in the hippocampi of animals receiving imipramine or iprindole compared to animals receiving no drug, lorazepam, or chlorpromazine (Table 4).

GENERAL DISCUSSION

These data may be summarized as follows: (1) Calcium-dependent release of GABA was decreased, and release of glutamate was increased in the hippocampi of helpless animals. (2) The helpless state could be produced by intrahippocampal administration of bicuculline, but not glutamate. (3) This helpless state was not due to differences in pain (vocalization) threshold. (4) Chronic administratin of two antidepressants which had previously been shown to reverse LeH [12] (but not other psychoactive drugs) resulted in an increased flux of the "small" GABA pool in hippocampus.

These data together with a previous study demonstrating

reversal of LeH by intrahippocampal GABA injection [19] suggest a central role for the hippocampal GABAergic system in the learned helplessness phenomenon.

Some alternative explanations also should be considered. For example, since the method used for estimating amino acid release is a batch system which does not remove released material from potential reuptake into the slices, it cannot be determined whether the presence of decreased amounts of GABA and increased amounts of glutamate in the medium bathing hippocampi of helpless animals was due to increased efflux from the tissue or to decreased high affinity uptake. The failure to demonstrate Ca^{++}-induced release of several amino acids not thought to be associated with neurotransmission (e.g. alanine, valine, lysine) suggests that this procedure does represent a reasonable approximation of condition *in vitro*.

The behavioral measure also must be considered carefully. For example, it was observed that the behavior of animals given intrahippocampal doses of bicuculline was the same in helplessness testing as animals exposed to unavoidable shock. Although it is tempting to assume that the same mechanism is involved, this may not necessarily be the case. The behavioral test of learned helplessness involves the acquisition of an escape response. The poor performance of animals exposed either to bicuculline, or to uncontrollable shock, could be due to deficits in learning ability, motivation, attentional variables, or memory. Thus, it is possible that one treatment might affect the motivation of animals, while another might interfere with the ability to learn the escape response. These two different mechanisms could not be distinguished on the basis of the present testing system.

The data on hippocampal release suggested the possibility that input to the hippocampus from the entorhinal cortex was excessive in helpless animals since glutamate release was increased. This would suggest that learned helplessness might be reversed by the injection of a glutamate antagonist into the entorhinal cortex. In previous studies, injections of GABA, NE, or 5-HT into this area [19] were not effective in reversing learned helplessness. Although competition between GABAergic and glutamatergic systems has been noted in some systems [15], glutamate did not produce the helpless state when injected intrahippocampally, while the GABA blocker bicuculline did. This fails to support the hypothesis of direct involvement of glutamate in learned helplessness.

The data involving GABAergic involvement, although subject to some important limitations, are much stronger, since some behavioral confirmation was obtained. The finding that systemic administration of two antidepressants elevated the flux of the "small" GABA pool suggests that these agents might reverse the helpless state by increasing functional neuronal GABA activity in the hippocampus. To the extent that the hippocampal GABAergic system (which appears to be central to both development and reversal of LeH) might also be involved with the depressive state in humans, a possible mechanism is therefore suggested by which antidepressants are clinically effective.

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REFERENCES

1. Anisman, H., A. Suissa and L. S. Sklar. Escape deficits induced by uncontrollable stress: Antagonism by dopamine and norepinephrine agonists. *Behav. Neural Biol.* **28:** 34–51, 1980.
2. Collins, F. S. and G. K. Sumner. Determination of glutamine and glutamic acid in biological fluids by gas chromatography. *J. Chromat.* **145:** 456–463, 1978.
3. Curtiss, D. R., A. W. Duggan, G. A. R. Johnston and H. McLennan. Antagonism between bicuculline and GABA in the cat brain. *Brain Res.* **33:** 57–73, 1971.
4. Henn, F. A. and A. Hamberger. Glial cell function: Uptake of transmitter substances. *Proc. natn. Acad. Sci. U.S.A.* **68:** 2686–2693, 1971.
5. Leshner, A. I., R. Hofstein and D. Samuel. Intraventricular injection of antivasopressin serum blocks learned helplessness in rat. *Pharmac. Biochem. Behav.* **9:** 889–892, 1978.
6. Minchin, M. C. W. and L. L. Iverson. Release of H gamma-aminobutyric acid from glial cells in rat dorsal root ganglia. *J. Neurochem.* **23:** 533–540, 1974.
7. Nadler, J. V., W. F. White, K. W. Vaca, D. A. Redburn and C. W. Cotman. Aspartate and glutamate as possible transmitters of excitatory hippocampal afferents. *Nature, Lond.* **260:** 538–540, 1976.
8. Nadler, J. V., W. F. White, K. W. Vaca, D. A. Redburn and C. W. Cotman. Characterization of putative amino acid transmitter release from slices of rat dentate gyrus. *J. Neurochem.* **29:** 279–290, 1977.
9. Nadler, J. V., W. F. White, K. W. Vaca, B. W. Perry and C. W. Cotman. Biochemical correlation of transmission mediated by glutamate and aspartate. *J. Neurochem.* **31:** 147–155, 1978.
10. Patel, A. J., A. L. Johnson and R. Balazs. Metabolic compartmentation of glutamate associated with the formation of γ-aminobutyrate. *J. Neurochem.* **23:** 1271–1279, 1974.
11. Petty, F., J. L. Campbell and A. D. Sherman. Learned helplessness and ECS. American Psychiatric Association Annual Convention, New Research, San Francisco, May 1980.
12. Petty, F. and A. D. Sherman. Reversal of learned helplessness by imipramine. *Commun Psychopharmac.* **3:** 371–375, 1980.
13. Petty, F. and A. D. Sherman. Regional aspects of the prevention of learned helplessness by desipramine. *Life Sci.* **26:** 1447, 1980.
14. Petty, F. and A. D. Sherman. A neurochemical differentiation between exposure to stress and the development of learned helplessness. *Drug Dev. Res.*, in press.
15. Price, M. T., J. W. Olney, J. V. Mithell, T. Fuller and T. J. Cicero. Luteinizing hormone releasing action of Nmethyl aspartate is blocked by GABA or taurene but not by dopamine antagonists. *Brain Res.* **158:** 461–465, 1978.
16. Roberts, P. J. Amino acid release from isolated rat dorsal root ganglia. *Brain Res.* **74:** 327–332, 1974.
17. Seligman, M. E. P. The learned helplessness animal model of depression. In: *Research in Neurosis*, edited by H. M. van Praag. New York: Academic Press, 1978, pp. 154–171.
18. Sherman, A. D., G. L. Allers, F. Petty and F. A. Henn. A neuropharmacologically relevant animal model of depression. *Neuropharmacology* **18:** 891, 1979.
19. Sherman, A. D. and F. Petty. Neurochemical basis of the action of antidepressants on learned helplessness. *Behav. Neural Biol.* **30:** 119–134, 1980.
20. Van Hoesen, G. W., D. N. Pandya and H. Butters. Some connections of the entorhinal (Area 28) and perirhinal (Area 35) cortices of the rhesus monkey. II. Frontal lobe afferents. *Brain Res.* **95:** 23–38, 1975.
21. Wenthold, R. J. Release of endogenous glutamic acid, aspartic acid, and GABA from cochlear nucleus slices. *Brain Res.* **162:** 338–343, 1979.
22. White, W. F., J. V. Nadler, A. Hamberger, C. W. Cotman and J. T. Cummins. Glutamate as transmitter of hippocampal perforant path. *Nature, Lond.* **270:** 356–357, 1977.
23. Zumwalt, R. W., K. Kuo and C. W. Gehrke. A nanogram and picogram method for amino acid analysis by gas liquid chromatography. *J. Chromat.* **57:** 193–208, 1971.