Pharmacology Biochemistry & Behavior, Vol. 22, pp. 531–533, 1985. © Ankho International Inc. Printed in the U.S.A. 0091-3057/84 $3.00 + .00

Intracortical Glutamate Injection Produces Helpless-Like Behavior in the Rat

FREDERICK PETTY,*† CHERYL McCHESNEY*†[1] AND GERALD KRAMER*

**Veterans Administration Medical Center, Iowa City, IA 52240*
and †Department of Psychiatry, University of Iowa College of Medicine
500 Newton Road, Iowa City, IA 52242

Received 5 May 1984

PETTY, F., C. McCHESNEY AND G. KRAMER. *Intracortical glutamate injection produces helpless-like behavior in the rat.* PHARMACOL BIOCHEM BEHAV **22**(4) 531–533, 1985.—Acute injection of glutamate into frontal neocortex of naive rats produced a subsequent deficit in escape performance behavior that was similar to that produced by exposure to uncontrollable shock. The behavioral deficit was dose-related. The behavioral deficit was similar in time-course to that produced by 15 min (but not 40 min) of exposure to learned helplessness induction. Unlike learned helplessness produced by exposure to inescapable shock, the behavioral deficit produced by intracortical glutamate injection was not prevented by chronic intraperitoneal administration of imipramine.

Cortex Glutamate Helplessness Stress

EXPOSURE to uncontrollable, unavoidable, uncued aversive stimuli leads to subsequent deficits in escape/avoidance behavior, a phenomenon termed learned helplessness [6]. Learned helplessness has been demonstrated in a variety of animal species including human [3] and has recently been demonstrated in the rat to fulfill established criteria for an animal model of depression [5]. That is to say, it has some behavioral similarity with depression, and it is reversed in a pharmacologically specific manner by agents useful in the treatments of depression: tricyclic antidepressants, atypical antidepressants, electroconvulsive shock, and monoamine oxidase inhibitors [10]. Most importantly, chronic treatment by these agents is required to effect a behavioral response, in a manner analogous with the clinical situation.

If the antidepressant drugs are administered by intracranial injection, however, a behavioral reversal of helplessness occurred acutely, after recovery from anesthesia. However, of nine brain regions studied, antidepressant drugs reversed learned helplessness only when injected into frontal neocortex [8]. Based on a series of experiments with intracranial injections with antidepressant drugs and with putative neurotransmitters, we have proposed two different neurochemical pathways for learned helplessness prevention and reversal [8]. Briefly, for reversal, antidepressants appear to stimulate a serotonergic nucleus in the cortex which in turn activates a GABAergic locus in the hippocampus which finally activates a septal serotonergic locus. Pathways for prevention are considerably more complex. Antidepressants will prevent the development of helplessness when injected into either frontal neocortex, hippocampus, or lateral geniculate body. GABA will prevent the development of helplessness in frontal cortex, hippocampus, and lateral geniculate body, suggesting that antidepressant drugs may work through GABA in helplessness prevention. This idea is confirmed by the fact that benzodiazepines, which are generally thought to exert their action partly through GABAergic mechanisms, prevent helplessness when administered in an acute manner [7]. Additionally, we demonstrated that helpless behavior can be induced in naive animals by the injection of bicuculline into the hippocampus, an area in which injection of GABA both prevents and reverses learned helplessness [8].

Whether a naturally occurring neurotransmitter could produce helpless behavior remained to be demonstrated. Because the frontal neocortex is the only brain structure we have found in which both prevention and cure can be effected by antidepressant drugs, and because GABA and glutamate have mutually antagonistic roles in the central nervous system, we have studied the behavioral effects in naive animals of intracortical glutamate injection.

METHOD

General

Sprague-Dawley male 200–250 g rats were used in all experiments. Animals were group-housed with free access to food and water and maintained on a 12 hour on/off light-dark cycle. To minimize potential circadian effects, all experiments were performed between 0700 and 1200. Stereotaxic

[1]Requests for reprints should be addressed to C. McChesney, University of Iowa College of Medicine, Department of Psychiatry, 500 Newton Road, Iowa City, IA 52242.

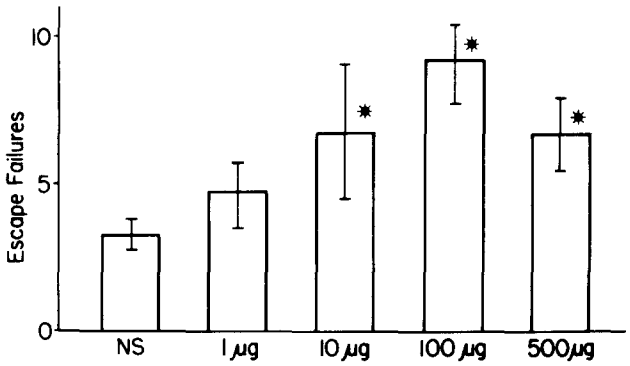

FIG. 1. Escape performance after injection of various doses of glutamic acid hydrochloride into frontal neocortex. Data are presented as mean±S.E.M. for 15 trials. *10 μg, 100 μg, 500 μg were significantly increased at $p<0.05$ (t-test) as compared to normal saline.

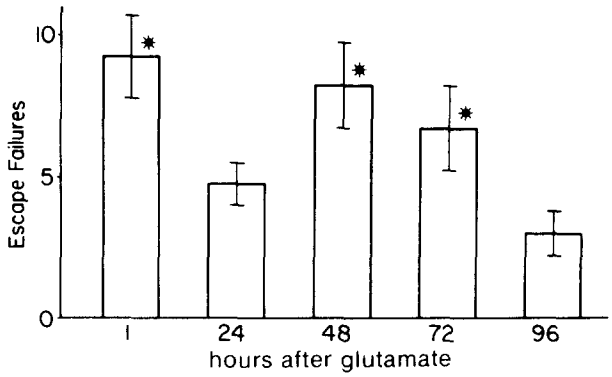

FIG. 2. Escape performance after intracortical injection of glutamic acid hydrochloride (100 μg) with varying intervals between injection and testing. Animals tested at 24 hours were not significantly different from control (t-test). Data are presented as mean±S.E.M. *Animals at 1, 48, and 72 hours after injection were significantly different from animals tested at 24 and 96 hours after injection $p<0.05$.

injections were made bilaterally under ether anesthesia using the coordinates of Pellegrino *et al.* [4] (3.0 mm anterior, 2.0 mm lateral, 1.0 mm vertical, from bregma). In all cases an injection volume of 1.0 μl was administered over a period of 1.0 min.

Behavioral testing was performed using our standard protocol [8]. Animals were placed in an aluminum Colbourn modular test cage equipped with a stainless steel grid floor and a lever 3 cm above the floor. After a 1 min familiarization, 15 trials of escape testing were administered. Each trial began with a 24 sec intertrial interval after which a 0.7 mA shock was pulsed on for 40 msec every 400 msec. Shock remained on for 60 sec or until a bar-press was performed. Escapes with latencies greater than 15 sec were scored as escape failures while escapes with less than 15 sec were scored as successes. Using these parameters, naive animals routinely scored 5 or less escape failures in 15 trials while animals subjected to our standard learned helplessness induction (40 sec of pulsed random footshock) routinely scored 6–15 escape failures in 15 trials (mean and 95% confidence limits).

Experiment 1

Thirty rats were used for this experiment. Groups of 6 were injected intracortically with either saline, or 1, 10, 100 or 500 μg of glutamic acid hydrochloride (dose calculated as the free base) dissolved in saline. Animals were tested with the bar-press escape task 1 hour after injection to allow complete recovery from ether anesthesia. An additional control group of 6 rats received an injection of 1 μl pH 2.0, 0.05 M HCl/KCl buffer intracranially to frontal neocortex to control for possible pH effects.

Experiment 2

Thirty animals were divided into groups of 6, except for the 24 hour group which included 13 rats, and were injected intracortically with 100 μg of glutamic acid hydrochloride in saline under ether anesthesia. Animals were then tested on the bar-press escape task at either 1 hour, or 1, 2, 3, or 4 days after injection.

Experiment 3

Four groups of six rats were injected intraperitoneally once a day for 5 days with either saline or imipramine (10 mg/kg) a dose found to prevent learned helplessness when given chronically [7]. On the 6th day both imipramine- and saline-injected animals were both randomly assigned to receive either saline or 100 μg of glutamic acid hydrochloride intracranially into the frontal neocortex under ether anesthesia. All animals were then tested 1 hour after injection.

RESULTS

Experiment 1

A curvilinear dose response curve was obtained for interference with escape behavior by intracortical glutamate (Fig. 1), with 500 μg demonstrating less effect than 100 μg. In no case did animals exhibit any evidence of seizures or other gross motor abnormalities. The control group that received pH 2.0 HCl/KCl buffer received 335±395 shocks and had 2.16±2.99 escape failures which is not significantly different from normal saline.

Experiment 2

When tested 24 hours after intracortical injection of glutamate, escape performance was essentially in the normal range. Again, animals tested 1 hour after injection of glutamate demonstrated significant performance deficits, as did those tested at 48 and 72 hours after injection. By 96 hours, behavior had returned to normal (Fig. 2).

Experiment 3

Chronic intraperitoneal injection of imipramine did not prevent the development of escape performance deficits caused by intracortical glutamate injection (Fig. 3).

DISCUSSION

Injection of glutamate into frontal neocortex produced escape performance deficits similar to those caused by behavioral induction of learned helplessness with uncontrolla-

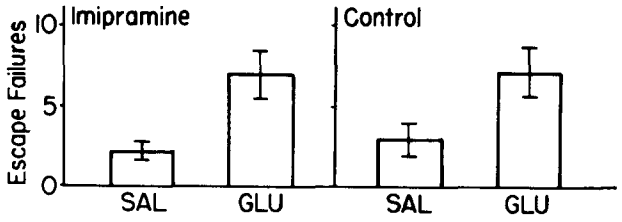

FIG. 3. Effect of chronic injection of imipramine or saline on behavioral deficits in escape performance induced by intracortical injection of glutamic acid hydrochloride (100 μg). Data are presented as mean±S.E.M. For both imipramine and saline control, differences between saline and glutamate were significant ($p<0.05$, n=6, t-test).

ble footshock when animals were tested 1 hour after injection. However, the time-course of glutamate-induced behavioral deficits was not that seen following behavioral induction of learned helplessness with our standard 40 min protocol [8]. Specifically, behaviorally-induced learned helplessness led to profound performance deficits which were not only still seen 24 hours after induction, but also persisted for more than 4 days. On the other hand, cortical glutamate injection produced behavioral deficits which were dissipated by 4 days. Of particular interest is the fact that the behavioral deficit produced by intracortical glutamate injection was not seen at 24 hours, but then reappeared. This time-course is similar to that seen when animals were exposed to only 15 min of helplessness induction [9]. With 15 min of behavioral helplessness induction, animals' escape performance, when tested at 24 hours, was in the normal range, but escape failures were significantly higher than normal at 2 and 3 days.

Another difference between the presently-reported phenomenon and learned helplessness involved prevention by chronic administration of imipramine. Behavioral helplessness was prevented by this treatment, but the behavioral deficit caused by intracortical glutamate was not.

It is possible, of course, that the behavioral effects caused by intracortical glutamate injection were caused by a nonspecific neurotoxic or lesion effect. This seems unlikely for several reasons. First, direct visual examination under the dissecting microscope of the injection site (in Area 10 of the prefrontal cortex) revealed no obvious tissue damage. Second, comparable concentrations of glutamate have been used with intracranial injections to selectively stimulate cell bodies in highly localized regions of the CNS without demonstrable neurotoxic effects [2]. Third, we (unpublished data) and others [1] have not found cortical lesions to have significant behavioral effects in the learned helplessness paradigm. This suggests that the results described are probably due to direct effects of glutamate as an excitatory neurotransmitter.

We have previously hypothesized that different neurochemical pathways are responsible for the prevention and for the cure of learned helplessness [8]. Thus it may be that the behavioral deficit caused by intracortical glutamate injection are related to yet another pathway, since chronic treatment with imipramine activated both the reversal pathway and the prevention pathway in behavioral learned helplessness. Weiss *et al.* [11] have commented at some length as to the differences in short- and long-term responses to stress. In this context, it is tempting to speculate that the pathway involving "glutamate helplessness" involves a tonic inhibition by GABA along with a tonic stimulation by glutamate, and that alterations of this pathway caused by the intracortical glutamate injection result in a short-term behavioral phenomenon characterized by a dissipation within 24 hours. On the other hand, the classic reversal pathway for learned helplessness involving cortical serotonin may reflect a more profound state of helplessness, in that a greater time is required for its induction, and in that it is sensitive to chronic treatment with tricyclic antidepressants. Additional research looking at other brain structures, other putative neurotransmitters and a wide spectrum of pharmacologic agents should clarify these possibilities.

In summary, these preliminary data again confirm the utility of the learned helplessness animal model of depression as a tool for the study of the complex interaction between stress, anxiety and depression.

ACKNOWLEDGEMENT

This work was supported in part by the Veterans Administration.

REFERENCES

1. Elmes, D. G., L. E. Jauard and P. D. Swart. Helplessness in hippocampectomized rats: response perseveration? *Physiol Psychol* **3:** 51, 1975.
2. Goodchild, A. K., R. A. L. Dampney and R. Bandler. A method for evoking physiological responses by stimulation of cell bodies but not axons of passage, within localized regions of the central nervous system. *J Neurosci Methods* **6:** 351, 1982.
3. Hiroto, D. S. and M. E. P. Seligman. Generality of learned helplessness in man. *J Pers Soc Psychol* **31:** 311, 1975.
4. Pellegrino, L. J., A. S. Pellegrino and A. J. Cushman. *A Stereotaxic Atlas of the Rat Brain*. New York: Plenum Press, 1979.
5. Petty, F. and A. D. Sherman. Animal models of psychiatric illness: Pharmacological aspects. In: *Psychopharmacology*, edited by D. G. Grahame-Smith, H. Hippius and G. Winokur. Amsterdam: Excerpta Medica, 1983, p. 444.
6. Seligman, M. E. P. and S. F. Maier. Failure to escape traumatic shock. *J Exp Psychol* **74:** 1, 1967.
7. Sherman, A. D., G. L. Allers, F. Petty and F. A. Henn. A neuropharmacologically-relevant animal model of depression. *Neuropharmacology* **18:** 891, 1979.
8. Sherman, A. D. and F. Petty. Neurochemical basis of the action of antidepressants on learned helplessness. *Behav Neural Biol* **30:** 119, 1980.
9. Sherman, A. D. and F. Petty. Additivity of neurochemical changes produced by learned helplessness and imipramine. *Behav Neural Biol* **35:** 344, 1982.
10. Sherman, A. D., J. L. Sacquitne and F. Petty. Specificity of the learned helplessness model of depression. *Pharmacol Biochem Behav* **16:** 449, 1982.
11. Weiss, J. M., H. I. Glazer, L. A. Pohorecky, J. Brick and N. E. Miller. Effects of chronic exposure to stressors on avoidance-escape behavior and on brain norepinephrine. *Psychosom Med* **37:** 522, 1975.