

PILOCARPINE MODULATION OF AN EFFECT OF CHLORPROMAZINE ON MOUSE BEHAVIOR

CHARLES D. PROCTOR
AND

ANDREW MIDELKA, JR.

*Gillette Memorial Laboratory
Departments of Psychiatry and Pharmacology
School of Medicine, Meharry Medical College*

ABSTRACT.—Pilocarpine, given subcutaneously after prior administration of atropine methyl bromide, enhances the induction of loss of righting reflex without true general anesthesia caused by chlorpromazine in mice. The same dose of pilocarpine failed to exert this effect when atropine sulfate was substituted analogously for the atropine methyl bromide.

Chlorpromazine has been shown to produce a depression in mice which can cause loss of the righting reflex (Burton, Sodd and Goldin, 1957). Utilizing three different test systems, Proctor and his co-workers (1964) have demonstrated extension of the action of the phenothiazine tranquilizer, perphenazine, caused by low dosages of organophosphorous anticholinesterase agents in mice. The action of the anticholinesterase agents in producing this enhancement of tranquilizer action was accomplished under conditions which best support a concept that their effect was exerted by a cholinergic action on the central nervous system (CNS). These reports have engendered interest on our part in the question as to whether or not a receptor-active, CNS-active cholinergic agent such as pilocarpine (Pfeiffer and Jenny, 1957) could enhance the ability of chlorpromazine to abolish the righting reflex of mice, and, if so, whether or not such enhancement

could be associated with a probable CNS site of action. The present communication presents the results obtained when the pilocarpine effect on chlorpromazine induction of the loss of the righting reflex in mice was observed under the influence of the anticholinergic agents, atropine methyl bromide and atropine sulfate. Atropine methyl bromide was given under conditions so as to expect it to exert only peripheral anticholinergic action, while atropine sulfate was given in a way whereby it could be expected to yield both CNS and peripheral action (Pfeiffer and Jenny, 1957; Proctor et al. 1964; Goodman and Gilman, 1965; Stein, 1963). These atropine derivatives were employed with a view toward influencing any pilocarpine effect ascertained by the respective distribution patterns of their anticholinergic action.

MATERIALS AND METHODS

The studies were carried out in adult albino mice weighing 20-30 g. Drugs were utilized in the following forms: chlorpromazine hydrochloride, pilocarpine nitrate, atropine sulfate, and atropine methyl bromide. All dosages have been expressed in terms of the drug salt used. Distilled water solutions of the drugs used for injection were made up fresh on the day of use. Pilocarpine was administered subcutaneously. All the other drugs were given intraperitoneally. Subcutaneous injections were made in a volume of 0.005

ml/g; intraperitoneal injections in a volume of 0.01 ml/g. Each animal, treated or control, received the same number and volume of injections on a similar time schedule and by analogous routes, using isotonic saline as a "sham" injection where necessary. Time schedule for injection of drugs, indicated in minutes prior to making the first test for loss of righting reflex, was as follows: chlorpromazine, 30 min; atropine methyl bromide or atropine sulfate, 10 min; pilocarpine, 0 min (i. e., immediately before the first test of the reflex). The animals were tested for loss of the righting reflex at ten minute intervals over a period of two hours. Loss of the reflex was assumed when present for one or more minutes. Any animal failing to spontaneously exhibit return of the reflex was left undisturbed and was included in the total of animals having loss of righting reflex for the given interval of time being assessed. Each experimental group consisted of 10 mice. Statistical significance of the data was assessed for each 10 min time interval by X^2 determination. Significance of differences was set at or below the 5 per cent probability level.

RESULTS

In running preliminary experiments designed to locate dosages of chlorpromazine which would be useful for testing the pilocarpine enhancing effect an interesting phenomenon was observed. Beginning with the 25 mg/kg chlorpromazine dose (used originally by Burton, Sodd and Goldin, 1957) and testing several lower dosages of chlorpromazine, it was found that the tranquilizer could cause loss of the righting reflex without inducing true anesthesia. Animals responding in this way tried to escape when righted and prodded, and attempted to bite objects brought near their heads. Such behavior differs from that of animals which are in the surgical state of anesthesia and presents the possibility that the mice were merely "in-

different" to being placed on their backs. This kind of behavior, in contrast to a state more nearly approximating true surgical anesthesia, was most marked at 10 mg/kg dose of chlorpromazine, dosages of 5 mg/kg, 10 mg/kg, 15 mg/kg, 20 mg/kg and 25 mg/kg of the tranquilizer having been tried. At the 10 mg/kg dose every animal in the group of 10 animals tested elicited the kind of behavior in question at some time during the two hour test period and none of them was ever truly anesthetized. Higher dosage effects were less readily distinguishable from general anesthesia. The 5 mg/kg chlorpromazine dosage failed to produce loss of righting reflex in any animal over the same period of observation in the trial run.

Figure 1 presents the results obtained when the 5 mg/kg chlorpromazine dosage in mice was subjected to the influence of 11 mg/kg pilocarpine administered subsequently to either atropine methyl bromide or atropine sulfate. It allows comparison of these findings with those obtained from groups of animals receiving 10 mg/kg chlorpromazine; 5 mg/kg chlorpromazine; 5 mg/kg chlorpromazine plus 20 mg/kg atropine MeBr; and saline-injected controls, respectively.

It is apparent that 11 mg/kg pilocarpine, given subsequently to 20 mg/kg atropine methyl bromide and 5 mg/kg chlorpromazine (curve II), produced considerable enhancement of the capacity of 5 mg/kg chlorpromazine to induce loss of the righting reflex (curve III). Loss of the reflex did not represent the surgical stage of anesthesia in these animals. This enhancement converted a vir-

tually subthreshold 5 mg/kg dosage of the chlorpromazine to about the same level of effect elicited from 10 mg/kg chlorpromazine given alone

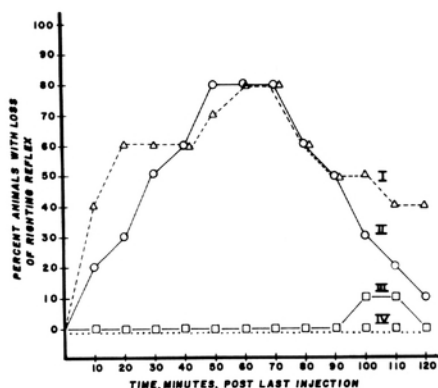


FIGURE 1.—Time course of the effect of 11 mg/kg pilocarpine (pilocarp.) on loss of righting reflex in groups of 10 mice pretreated with 5 mg/kg chlorpromazine (CPZ) and either 20 mg/kg atropine methyl bromide (AtMeBr) or 5 mg/kg atropine sulfate (AtSO₄). Experimental curves obtained from animal groups (n=10) receiving other dosage regimens are presented for comparison.

Key:

Curve I:

10 mg/kg CPZ

Curve II:

5 mg/kg CPZ + 20 mg/kg AtMeBr + 11 mg/kg pilocarp.

Curve III:

5 mg/kg CPZ

Curve IV:

Curve obtained for each of the following:

(a) 5 mg/kg CPZ + 5 mg/kg AtSO₄ + 11 mg/kg pilocarp.

(b) 5 mg/kg CPZ + 20 mg/kg AtMeBr

(c) Saline controls

p values, X²: In comparing per centage values from either Curve I or II with either Curve III or IV at a given time interval: 50 and 60% response, $p < 0.05$; 70 and 80% response, $p < 0.01$; 40% response and lower, $p > 0.05$. In comparing Curve I with Curve II, $p > 0.1$ at all time intervals.

(curve I). It can also be seen that when 5 mg/kg atropine sulfate replaced the 20 mg/kg atropine methyl bromide as the anticholinergic agent given prior to the 11 mg/kg pilocarpine, the latter was no longer able to enhance the 5 mg/kg chlorpromazine effect on the reflex (curve IV (a)). A similar inability was exhibited (a) by 20 mg/kg atropine methyl bromide given with the chlorpromazine but without the pilocarpine (curve IV (b)), and (b) by the saline controls (curve IV (c)).

DISCUSSION

The experiments performed appear to demonstrate that under certain conditions pilocarpine can enhance the effect of chlorpromazine in causing loss of the righting reflex without inducing true anesthesia in mice. The pilocarpine effect is apparently exerted when it is given subsequent to prior administration of atropine methyl bromide but not when it is given following prior administration of atropine sulfate. The pattern of influence of these two anticholinergic agents on this pilocarpine modulation of chlorpromazine effect would make it appear that the response to pilocarpine is more related to a CNS action than to a peripheral action of pilocarpine.

Reversal of the modulation was effected by atropine sulfate but not by atropine methyl bromide when each was given under conditions so fixed as to expect CNS and peripheral anticholinergic action from the former but only peripheral action from the latter (Goodman and Gilman, 1965; Pfeiffer and Jenny, 1957; Proctor et al., 1964; Stein, 1963). The compara-

tive effect of atropine sulfate and atropine methyl bromide on the pilocarpine extension of chlorpromazine effect which has been observed exhibits a degree of similarity to the effect of pilocarpine on the pole climbing conditioned response in the rat first observed by Pfeiffer and Jenny (1957). These investigators found that pilocarpine inhibited this conditioned response when given after atropine methyl bromide but not when given after atropine sulfate.

The present findings are also compatible with a working hypothesis which has been advanced earlier (Proctor, et al., 1964). This hypothesis states that tranquilizer activity may be due in part to a depression of CNS adrenergic activity, caused either by blockade (Marrazi, 1953, 1957) or by reduction in CNS neurohumor level (Brodie, et al., 1960; Costa, 1960), with a resultant relative ascendancy of cholinergic activity which contributes to tranquilizing action.

The present data provides evidence that pilocarpine, a CNS-active cholinergic agent (Pfeiffer and Jenny, 1957; Pfeiffer, 1959), can enhance a gross tranquilizer response due to chlorpromazine, a tranquilizer known to block the action of adrenergic amines on CNS synapses (Marrazi, 1957), under conditions which best support interpretation of a CNS site of action for the pilocarpine. It is believed that such data yield support for the working hypothesis which has been advanced previously (Proctor, et al., 1964), even though it is understood that the data provide whole animal unitary evidence in support of this speculation rather than analytical evidence

for the specific parts of the CNS that may be involved in the behavioral effect observed.

ACKNOWLEDGMENTS

This work was supported in part by a grant from the Ernest T. Collins fund by research grants 5-SO1-5422-05 and 1-SO-1-FR-05341 from the National Institutes of Health, U.S. Public Health Service.

Dr. Midelka's present address is the University of Indiana Medical Center, Indianapolis, Indiana.

LITERATURE CITED

- BRODIE, B. B., K. F. FINGER, F. B. ORLANDS, G. P. QUINN, and F. SULSER. 1960. Evidence that tranquilizing action of reserpine is associated with changes in brain serotonin and not in brain norepinephrine. *J. Pharmacol. exp. Ther.*, 129: 250-256.
- BURTON, R. M., M. A. SODD, and GOLDIN. 1957. The analeptic action of lysergic acid diethylamide and d-amphetamine on reserpine-sedated mice. *Arch. int. Pharmacodyn.*, 112: 188-198.
- COSTA, E. 1960. The role of serotonin in neurobiology. *Intern. Rev. Neurobiol.*, 2: 175-227.
- GOODMAN, L. S., and A. GILMAN. 1965. The pharmacologic basis of therapeutics. New York. Macmillan.
- MARRAZI, A. S. 1953. Some indications of cerebral humoral mechanisms. *Science*, 118: 367-370.
- MARRAZI, A. S. 1957. The effects of certain drugs on cerebral synapses. *Ann. N. Y. Acad. Sci.*, 66: 496-507.
- PFEIFFER, C. C., and E. H. JENNY. 1957. The inhibition of the conditioned response and the counteraction of schizophrenia by muscarinic stimulation of the brain. *Ann. N. Y. Acad. Sci.*, 66: 753-764.
- PFEIFFER, C. C. 1959. Parasympathetic neurohumors; possible precursors and effect on behavior. *Intern. Rev. Neurobiol.*, 1: 195-244.
- PROCTOR, C. D., S. A. RIDLON, J. J. FUDEMA, and V. G. PRABHU. 1964. Extension of tranquilizer action by anticholinesterases. *Toxicol. appl. Pharmacol.*, 6: 1-8.
- STEIN, L. 1963. Anticholinergic drugs and the central control of thirst. *Science*, 139: 46-48.

Manuscript received Oct. 27, 1967.