MECHANISM OF CHOLINERGIC POTENTIATION OF TRANQUILLIZER ACTIVITY

By

P.N. Saxena

Department of Pharmacology, J. L. N. Medical College, Aligarh Muslim University, Aligarh.

A number of chemical substances have been identified in the brain which may function in the transmission of nerve impulses within the central nervous system. These substances include epinephrine, norepinephrine, 5-hydroxytryptamine and acetylcholine. It has often been suggested, on circumstantial evidence, that drugs which modify behaviour elicit marked change in the concentration of these chemical substances in specific areas of the central nervous system. One such concept has been put forward by Proctor *et al* (8) who consider that alteration of the CNS ratio of cholinergic to adrenergic and / or serotonergic effects favours tranquillizer activity. The present study was undertaken to test this hypothesis.

Extension of hexobarbital narcosis by promazine was selected as a criterion for assessment of tranquillizer activity. The CNS ratio of cholinergic / adrenergic / serotonergic activity was altered by use of drugs and the effect of this alteration of ratio on the extension of hexobarbital narcosis was observed. The data were analysed by means of the "t" test.

MATERIALS AND METHODS

Male albino mice weighing 22 to 32 Gm were divided in groups of ten. Access to food and water was allowed till the beginning of the experiment. Each group was used only once in a week. The room temperature varied between 19-22° C during the period of the experiment.

Hexobarbitone sodium was prepared as a 5 *mg./ml.* solution in distilled water. It was injected into the tail vein by means of a continuous slow injector at the rate of 0.005 *ml/gm* bodyweight/second. This dose produced loss of righting reflex in 100 percent of the animals and was determined by a preliminary experiment. The mouse was then taken out of the wire-gauze holder and placed on its back and left undisturbed till it resumed its upright posutre. The duration of the loss of righting reflex was measured from the time of starting the injection to the time when the animal resumed its upright posture.

The animals were premedicated 30--40 minutes before injection of hexobarbital with one or more of the following drugs (dose in *mg./kg.*): promazine hydrochloride (4.0), prostigmine bromide (0.04), atropine sulphate (0.2), hydergine (0.1), tetraethylammonium chloride (TEAC) (2.0), lysergic acid diethylamide (LSD 25) (0.1), and normal saline. 5-Hydroxytryptophane (5-HTP) (100.0) and pargyline hydrochloride (10.0) were injected 4 hours before hexobarbital. The dose of each drug or combination of drugs was contained in 10 *ml./kg.* bodyweight of the final solution and was injected intraperitoneally.

In a subsequent experiment, the dose-response curve for intraperitoneal hexobarbital was established in the promazine pretreated mice, using groups of 10 mice for each dose level

of hexobarbital. The narcotic dose in 50 percent of the animals (ND50) was calculated by the method of probit analysis. The effects of adding (*i*) prostigmine, (*ii*) prostigmine+atropine and (*iii*) prostigmine+pargyline to the promazine pretreatment were observed. Only the above combinations were employed for quantitative study as they showed significant effect in the preceding study.

RESULTS

Reasonably large doses of promazine and prostigmine, when administered alone or together, failed to produce loss of righting reflex. Each diminished motor activity and prolonged hexobarbital narcosis. Atropine and pargyline, on the other hand, shortened hexobarbital narcosis. These results are not shown in the accompanying Table. In this study, each of the above drugs was administered at a dose level which by itself was ineffective in significantly altering hexobarbital narcosis.

Table I. summarizes the observations of the experiment and the results of statistical analysis.

TABLE I

Effect of Drugs on Promazine Potentiation of Hexobarbital Narcosis

Pretreatment with		*Mean duration in minutes of hexobarbital narcosis*	*Standard error of the means*
Saline	(n* 40)	8.4	1.03
Promazine	(n 20)	10.0	2.04
Prostigmine		11.0	1.20
Atropine		6.8	1.45
Pargyline		7.2	1.68
5-HTP		12.5	2.10
LSD		14.0	2.81
Hydergine		12.5	3.86
TEAC		9.6	2.31
Promazine+Prostigmine	(n 25)	19.0	1.96
Promazine+Prostigmine+Atropine		11.0	1.50
Promazine+Prostigmine+Pargyline		8.0	2.85
Promazine+Prostigmine+5-HTP		14.8	3.00
Promazine+Prostigmine+LSD		15.0	2.43
Promazine+Prostigmine+5-HTP+LSD		18.0	4.70
Promazine+Prostigmine+Hydergine		16.5	2.50
Promazine+Prostigmine+TEAC		17.0	2.47

*n, the number of animals tested with each combination was ten except where indicated.

The mean duration of hexobarbital narcosis in the control saline group was 8.4 minutes with an standard error of 1.03. Pretreatment with a small dose of promazine produced no alteration in the mean duration (10.0±2.04 minutes). When prostigmine was combined with promazine, the duration of narcosis was significantly (p=0.05) prolonged (19.0±1.20 minutes). Addition of atropine or pargyline effectively (p=0.05) reversed this prolongation.

The effects of 5-HTP and LSD were identical; each increased hexobarbital narcosis and reversed the promazine-prostigmine induced prolongation. Neither effect was statistically significant. The reversal of prolongation was, however, entirely eliminated when 5-HTP and LSD were added together to the promazine-prostigmine combination.

Hydergine tended to enhance hexobarbital narcosis but reversed the prolongation of narcosis produced by promazine prostigmine combnaion. The effects were statisticaly not significant. TEAC did not affect either of them.

The following figure presents the log-dose probit-response relationship of intraperitoneal hexobarbital in mice pretreated with promazine (*a*), promazine+prostigmine, (*b*), promazine prostigmine+atropine, (*c*) and promazine+prostigmine+pargyline, (*d*). The ND50 with its standard error is indicated for each combination.

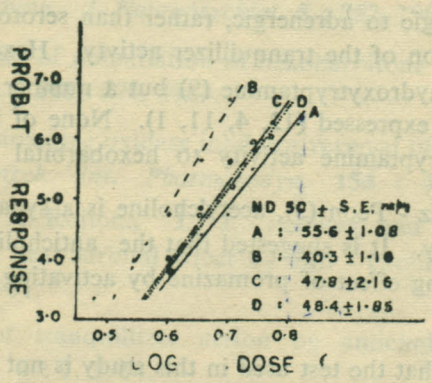

Fig. I

The dose-response relationship of hexobarbital and the ND50 with Standard Error.

The mice were pretreated with

A : promazine
B : promazine+prostigmine
C : promazine+prostigmine+atropine
D : promazine+prostimgne+pargyline

It is inferred from the figure that addition of prostigmine to the pretreatement reduces the ND50 of hexobarbital by about 25 percent. Atropine or pargyline effectively antagonises this potentiating action of prostigmine.

DISCUSSION

This study confirms the earlier findings that hexobarbital narcosis is prolonged by phenothiazines as well as anticholinesterases (7, 2, 5) and shortened by monoamine oxidase inhibitor (10).

The present finding of a significant prolongation of narcosis and reduction of ND50 of hexobarbital in the promazine pretreated mice by prostigmine supports the hypothesis of Proctor *et al* (8) that raising the cholinergic activity in the brain increases tranquillizer action of a drug. Reversal of the prostigmine enhancement of tranquillizer activity by atropine which reduces cholinergic activity, is in favour of this concept.

Further support is lent to the hypothesis by the observation that administration of pargyline which raises the adrenergic-serotonergic activity and consequently relatively lowers the cholinerigc activity in the brain, reverses the enhancing effect of prostigmine on promazine-potentiation of hexobarbital narcosis. A nearly similar finding is that of Proctor (6) who obtained reversal of parathione-extension of hexobarbital narcosis by monoamine oxidase inhibitor.

Modification of brain serotonergic activity by administration of 5-HTP or LSD does not influence the prostigmine enhancement of tranquillizer activity of promazine. This suggests that it is the ratio of cholinergic to adrenergic, rather than serotonergic, activity in the brain which subserves the potentiation of the tranquillizer activty. Hexobarbital narcosis has been shown to be prolonged by 5-hydroxytryptamine (9) but a number of different views regarding its mode of action have been expressed (12, 4, 11, 1). None of these views supports a relationship of brain 5-hydroxytryptamine activity to hexobarbital narcosis.

According to Hernandez - Peon (3), acetylcholine is a synaptic excitatory transmitter within the hypnogenic pathway. It is suggested that the anticholinesterase, prostigmine, enhances the narcosis potentiating effect of promazine by activating this cholinergic hypnogenic system.

It may be pointed out that the test used in this study is not a highly specific test for tranquillizer activity and the design of the experiment provides only a whole animal unitary evidence rather than evidence for the specific parts of the central nervous system involved.

SUMMARY

The effect of alteration of the ratio of cholinergic to adrenergic and / or serotonergic activity in the brain on the tranquillizer action has been studied using promazine induced prolongation of hexobarbital narcosis in mice as a test for tranquillizer activity. An increase in the cholinergic activity produced by an anticholinesterase, prostigmine, favours tranquillizer activity. A decrease in cholinergic activity produced by a cholinolytic, atropine or an increase in adrenergic activity produced by a monoamine oxidase inhibitor, pargyline, reverse this

effect. Modification of the brain serotonergic activity by 5-HTP and LSD or peripheral autonomic blockade by hydergine and tetraethylammonium have no significant effect on the prostigmine potentiation of tranquillizer activity. It is suggested that the anticholinesterase enhances the narcosis potentiating effect of the tranquillizer by activating the cholinergic hypnogenic pathway.

Thanks are due to Messrs. May and Baker, Dagenham, England, for their generous supply of Hexobarbitone Sodium.

REFERENCES

1. Aprison, M. H. and J. N. Hingtgen,. Neurochemical correlates of behaviour. *J. Neurochem.* **12** : 959, 1965.
2. Fink, G. B. and E. A. Swinyard,. Comparison of anticonvulsant and psychopharmacologic drugs. *J. Pharmaceutical sciences.* **51** : 548, 1962.
3. Hernandez Peon, R. Central neurohumoral transmission in sleep and wakefulness. *Progress in Brain Research.* Vol. 18 : 1965. Editors - K. Akert, C. Bally and J. P. Schade. Elsevier Publishing Company.
4. Mahler, D. J. and F. L. Humoller. The potentiation of hexobarbital hypnosis by 5 - hydroxytryptamine. *J. Neuropsychiat.* **5** : 252, 1964.
5. Mc Cance, I. Studies of potentiation of hexobarbitone infused intravenously. *Arch. int. Pharmacodyn.* **148** : 270, 1964.
6. Proctor, C. D. Monoamine oxidase inhibitor reversal of parathion extension of hexobarbital action. *Arch. int. Pharmacodyn.* **154** : 468, 1965.
7. Proctor, C. D. , S. A. Ridlon., J. J. Fudema, and V. S. Prabhu. Factors affecting extension of hexobarbital effect by anti - cholinesterase. *Fed. Proc.*, **20** (1) Part I, 395, 1961.
8. *Idem.* Extension of tranquillizer action by anticholinesterases. *Toxicol. and Appl. Pharmacol.* **6** : 1, 1964.
9. Rumke, Chr. L. Die Verlangerung der Hexobarbitalnarcosa dusch kurz vorher intraperitoneal oder subcutan verabreichtes Serotonin. *Arch. fur Experimentelle Pathologic und Pharmakologic.* **243:** 298, 1962.
10. Stock, K. and E. Westermann. Untersuchungen uber den Mechanismusder narkoseverkurzenden Wirkung von Monoaminoxydase - Hammistoffen. *Ibid.* **243,** 44, 1962.
11. Wada, J. A., D. Hill, and J. Wrinch. Reversal by atropine of 5-hydroxytryptophane induced behavioral changes in cats and monkeys. *J. Neuropsychiat.* **4** : 413. 1963
12. Wulfsohn, N. L. and W. M. Politzer. 5 - Hydroxytryptamine in anaesthesia. Anaesthesia. **17** : 64, 1963.