EFFECT OF MEPROBAMATE ON CHOLINESTERASE ACTIVITY OF DOG BRAIN

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Centrally acting drugs are capable of affecting cholinergic transmission at a variety of sites and therefore, can play an important role in the investigation of cholinergic machanisms (5, 7, 8, 9 and 12).

In a recent study it has been shown (10) that Meprobamate selectively increases the acetylcholine concentration of the hypothalamus and the hippocampus. These changes were correlated with the fact that Meprobamate seemed to have more selective effect on limbic structures as shown by Kletzkin and Berger (6) that it selectively decreases the after discharge in both the amygdala and hippocampus. Acetylcholine formation, storage and destruction are complex biochemical processes and there are undoubtedly numerous potential points of attack for any drug which may interrupt with the level or release of this transmitter in the central nervous system. Thus, the increase in acetylcholine content of the hypothalamus and the hippocampus after Meprobamate could either be due to increased synthesis of acetylcholine or due to decreased destruction; which could be due to inhibition of cholinesterase. It was, therefore, considered worthwhile to see the effect of Meprobamate on the cholinesterase content of different areas of dog brain both in vivo and in vitro.

MATERIALS AND METHODS

Healthy mongrel dogs of either sex were used in this study. They were divided into two groups. The control group was given 1 ml/kg of saline intravenously whilst the other group received Meprobamate 40 mg/kg intravenously. (The solution was prepared by adding 40 mg of Meprobamate/ml of distilled water, heated on water bath at 50°C; at this stage a few drops of propylene glycol were added. The solution was then cooled to 40°C before administration). After one and a half hour of drug administration, the animals were anaesthetised with ether, the skull was opened and whole brain was removed as quickly as possible. It was immediately transferred to the petri-dish which had been kept in the freezing mixture. The following parts of the brain were removed (*a*) the frontal cortex, (*b*) the hypothalamus, (*c*) the hippocampus and (*d*) the mid-brain.

The acetylcholinesterase activity was measured by the standard Warburg Manometric technique (2). The brain portions were homogenised in calcium free Ringer bicarbonate buffer (0.25 M sodium bicarbonate, 0.15 M sodium chloride and 0.04 M magnesium sulphate were

dissolved in distilled water and saturated with 95% nitrogen and 5% carbon dioxide). The pH of the buffer after saturation was from 7.4 to 7.7. The central compartment of manometer contained 2.7 ml of cold brain homogenate and 0.3 ml of 2 percent acetylcholine chloride in buffer was taken in side arm as substitute. This system contained 50 mg homogenised brain (1:54 dilution). After gassing of the manometer assembly with 95 percent nitrogen and 5 percent carbondioxide for five minutes and equilibrating for ten minutes at 38°C, the acetylcholine chloride from the side arm was tipped into the main compartment. The change in the pressure was recorded at 10 minutes interval for thirty minutes.

For studying the effect of Meprobamate, in vitro, the drug was taken alongwith the homongenate in the main compartment in the concentration of 1×10^{-3}M and 1×10^{-5}M separately and also a control group was kept. The manometers were gassed and equilibrated for 10 minutes at 38°C and tipped. The readings were recorded at 10 minutes interval for 30 minutes.

RESULTS

The acetylcholinesterase enzyme is unevenly distributed in the different regions of brain as illustrated in Table I. The greatest activity is found in the mid-brain and the lowest in the frontal cortex. Our results indicate that Meprobamate in *vivo* has no statistically significant effect on acetylcholinesterase activity in the frontal cortex the hippocampus and the mid-brain. In the case of the hypothalamus though it increased by 13.25 percent, the change was not statistically significant. In *vitro*, the Meprobamate exerted insignificant results in the concentration of $1x10^{-3}$M and $1x10^{-5}$M (Table II). The present studies indicate that the increase in the acetylcholine content of the hypothalamus and the hippocampus are not due to the inhibition of cholinesterase.

TABLE 1

Acetylcholinesterase activity of different areas of dog brain (in vivo) in control and Meprobamate (40 *mg/kg I.V. treated animals. (Results given in ul* co_2 *produced for* 30 *minutes).*

	Frontal cortex	*Hypothalamus*	*Hippocampus*	*Midbrain*
Control dogs (Mean of 10 dogs)	54.22	134.50	105.74	189.62
S.D.	±7.10	±17.29	±18.68	±18.33
Meprobamate treated dogs (Mean of 10 dogs)	57.20	147.80	105.74	167.50
S.D.	±13.27	±11.84	±15.20	±23.68
Probability	>0.5	>0.05	>0.5	>0.1

TABLE II

Acetylcholinesterase activity of different areas of dog brain (in vitro) after Meprobamate (in doses of $1x10^{-3}M$ *and* $1x10^{-5}M$*). Result given in* $ulco_2$ *produced for 30 minutes.*

	Frontal cortex	*Hypothalamus*	*Hippocapus*	*Midbrain*
Meprobamate ($1x10^{-5}M$) (Mean of 10 dogs)	49.60	137.00	102.00	160.00
S.D.	±2.33	±7.81	±11.80	±30.50
Probability	<0.1	<0.5	<0.5	<0.1
Meprobamate ($1x10^{-3}M$) (Mean of 10 dogs)	46.00	129.00	94.00	163.00
S.D.	±14.00	±18.30	±9.8	±30.1
Probability	<0.1	<0.5	<0.1	<0.1

DISCUSSION

There is an ample evidence that the drug induced depression of the central nervous system is associated with the increased cerebral levels of acetylcholine (3, 5 and 8). More recently Malhotra and Mehta (10) showed that this increase in acetylcholine may be selective. After Meprobamate, this increase was only found in the hypothalamus and the hippocampus in the dog. On the other hand the mechanism of the increase produced by the various depressants of the central nervous system is more obscure. The inhibition of the enzyme acetylcholinesterase as the cause of elevated acetylcholine in some areas of the brain after Meprobamate is ruled out by our present studies. The same is true of some other depressants such as methylpentynol and reserpine (5). Again, in the case of phenothiazines, Dasgupta and Mukherjee (1) showed that the rats tranquillized with 15 mg/kg chlorpromazine (CPZ) did not respond with a change in the brain acetylcholinesterase activity 30-45 minutes after intraperitoneal drug injection. However, Fried and Antopol (4) have reported that in *vitro*, very small concentration of CPZ ($5x10^{-7}M$) increases the acetylcholinesterase activity, whereas the ordinarily used concentration ($5x10^{-5}M$) was inhibitory.

The view that the elevated brain acetylcholine after depressants is due to the reduction in the utilization of the neurohormones in the depressed brain though quite attractive, lacks proof. Another possibility is that some or all of these depressants can reduce or prevent the release of acetylcholine form its "bound" from. This is an attractive hypothesis, in the light of the observations of Marley and Paton (11) that methylpentynol—a sedative hypnotic agent inhibits the release of acetylcholine from the perfused superior cervical ganglion of the cat. The same may hold true in case of elevated acetylcholine in the brain after some depressants.

SUMMARY

The effect of Meprobamate on the cholinesterase contents of the frontal lobe, the hypothalamus, the hippocampus and the mid-brain has been studied in dogs under ether anaesthesia both in *vivo* and in *vitro*.

The cholinesterase enzyme was found to be unevenly distributed in different regions of the brain, the greatest activity was found in the mid-brain and the lowest in the frontal cortex. There was no significant effect on acetylcholinesterase activity in different areas in *vivo*. In *vitro* two doses of Meprobamate were studied ($1x10^{-3}$M and $1x10^{-5}$M) but the results were statistically insignificant.

The implications of the results have been discussed.

REFERENCES

1. Dasputa, S.R. and K.L. Mukherjee. *Bul. Calcutta School Trop. Med.* **4**:123, 1956.

2. Dubois, K.P. and G.H. Mangun. Effect of hexaethyl-tetra phosphate on cholinesterase in vitro and in vivo. *Proc. Soc. Exper. Biol. and Med.* **64**:137, 1947.

3. Feldberg, W. Acetylcholine in Metabolism of the Nervous System, Ed. by D. Richter, pp. 493-510, 1957, Pregamon Press, London.

4. Fried, G.H. and W. Antopol. *Appl. Physiol.* **11**:25, 1957.

5. Giarman, N.J. and G. Pepeu. Drug induced changes in brain acetylcholine. *Brit. J. Pharmac. Chemother.* **19**:226, 1962.

6. Kletzkin, M. and F.M. Berger. Effect of Meprobamate on limbic system of the brain. *Proc. Soc. Exp. Biol. Med.* **100**:681, 1959.

7. MacIntosh, F.C. and P.E. Oborin. Release of Acetylcholine from intact cerebral cortex. *Abstr. XIX Int. Physiol. Congr.*, 580-581, 1953.

8. Malhotra, C.L. and P.G. Pundlik. The effect of Reserpine on the acetylcholine content of different areas of the central nervous system of the dog. *Brit. J. Pharmac. Chemother.* **14**:46, 1959.

9. Malhotra, C.L. and P.G. Pundlik. The effect of some anaesthetics on the acetylcholine concentrations of different areas of dog brain. *Brit. J. Pharmac. Chemother.* **24**:348, 1965.

10. Malhotra, C.L. and V.L. Mehta. Effect of Meprobamate on acetylcholine content of different areas of dog brain. *Brit. J. Pharmac. Chemother.* **27**:131, 1966.

11. Marley, E. and W.D.M. Paton. The effect of methylpentynol and methylpentynol carbamate on the perfused superior cervical ganglion of the cat. *Brit. J. Pharmac. Chemother.* **14**:303, 1959.

12. Mathews, E.K. and J.P. Quilliam. Effects of Central depressant drugs upon acetylcholine release. *Brit. J. Pharmac. Chemother.* **22**:415, 1964.