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THE EFFECT OF MEPROBAMATE ON THE ACETYLCHOLINE CONTENT OF CERTAIN AREAS OF DOG BRAIN

BY

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It is well known that the prevailing cerebral activity influences the level of acetylcholine in the brain at any given time. Richter & Crossland (1949) have shown that the amount of acetylcholine increases during anaesthesia but falls during increased cerebral activity. Giarman & Pepeu (1962) showed that a wide variety of depressant drugs increased the cerebral acetylcholine while after certain convulsants the acetylcholine level of the brain falls. Malhotra & Pundlik (1959) showed that after reserpine there was an increase in the acetylcholine content in different areas of the brain except the hippocampus, where there was decrease.

It was considered worth while to see the effect of meprobamate, a tranquillo-sedative belonging to a group where there is overlapping of sedative and tranquillizing properties, on the acetylcholine content of certain areas of dog brain. In this connection it may be mentioned that meprobamate differs from major tranquillizers, viz., rauwolfia alkaloids and phenothiazines, in that it does not produce extrapyramidal effects, does not selectively suppress conditioned avoidance responses and has anticonvulsant properties. At the same time it differs quantitatively from classical sedatives—the barbiturates. Thus, Klerman, Dimascio, Havens & Snell (1960) found that quinalbarbitone in doses of 50 and 100 mg produced more drowsiness than 400 and 800 mg of meprobamate. They also noted that quinalbarbitone interfered with several measures of performance and mental ability while meprobamate had no effect.

METHODS

Twenty mongrel dogs of either sex, weighing 5 to 9 kg, were used. They were divided into two groups of ten each for experimental purposes. The control group was given 1 ml./kg distilled water intravenously to which a few drops of propylene glycol were added. The other group received meprobamate 40 mg/kg intravenously. The solubility of meprobamate in water is low, the solution was prepared by dissolving 40 mg/ml. in distilled water at 50° C. A few drops of propylene glycol were added at this stage when it becomes soluble. The solution was then cooled to 40° C before administration. Both groups were anaesthetized with ether. The animals were bled to death, the skull opened and the following portions of the brain were removed quickly and transferred to weighing bottles which were already kept in a freezing mixture: (*a*) the anterior portion of frontal cortex, (*b*) the hypothalamus, (*c*) the hippocampus, (*d*) the mid-brain with the exception of colliculi, the basis pedunculi and brachium colliculi inferioris, and (*e*) the cerebellar cortex. Acetylcholine

was extracted from these portions of the brain in acidified mammalian Ringer solution at 95 to 100° C, while frog Ringer solution containing 15 μg/ml. physostigmine sulphate was used to assay the acetylcholine on frog rectus abdominis muscle by the method of Nachmansohn described by Anand (1952). The controls were interspersed with drug-treated dogs.

RESULTS

The acetylcholine concentrations of the different areas of dog brain with meprobamate as compared to control dogs under ether anaesthesia are given in Table 1. Dogs treated with meprobamate show a significant increase of acetylcholine in the hypothalamus and the hippocampus; the increase being 45% in the former and 71% in the latter. In other areas of the brain, however, there is a tendency towards diminution but the results are not statistically significant.

TABLE 1

THE ACETYLCHOLINE CONCENTRATIONS OF DIFFERENT AREAS OF THE CENTRAL NERVOUS SYSTEM IN NORMAL AND MEPROBAMATE TREATED DOGS UNDER ETHER ANAESTHESIA

The results are means and standard deviations expressed as μg/g brain tissue. The significance of the differences between means of results under ether and meprobamate and ether alone are calculated by "t" test

		Acetylcholine concentration in μg/g (mean ± S.D.)				
	No. of dogs	Frontal cortex	Hypo-thalamus	Hippo-campus	Cerebellar cortex	Mid-brain
Meprobamate and ether anaesthesia	10	1·6 ±0·305	4·9 ±0·668	5·368 ±1·356	0·425 ±0·291	2·540 ±0·791
Ether anaesthesia	10	2·05 ±0·596	3·5 ±1·035	3·136 ±0·618	0·956 ±0·212	2·770 ±0·413
	P value	>0·05	<0·01	<0·01	>0·05	>0·05
Increase (%)			45%	71%		

DISCUSSION

Our findings indicate that after meprobamate there is a selective increase in the acetylcholine content of the hypothalamus and the hippocampus whilst there is no significant effect on the acetylcholine content of the frontal cortex, the mid-brain and the cerebellar cortex.

In spite of a large amount of work done on it, the exact mechanism and the exact site of action of meprobamate has not been finally determined. Hendley, Lynes & Berger (1954) have shown that the E.E.G. changes indicated that it might produce some thalamic synchronization. Baird, Szekely, Wycis & Spiegal (1957) observed slow-wave activity in the basal ganglia and limbic system in both cats and human beings after meprobamate. Bovet, Longo & Silvestrini (1957) found that meprobamate inhibited a variety of responses to hypothalamic stimulation. A number of investigators (Kletzkin & Berger, 1959; Takagi & Ban, 1960) have shown that meprobamate produces shortening of electrical changes after discharge in the limbic system. Kletzkin & Swan (1959) have further shown that doses of the drug which produce effects on the limbic system were without effect upon the arousal response evoked by stimulation of the reticular formation. They concluded that this may indicate that the limbic system is more sensitive to the action of mepro-

bamate. Berger (1963) compared the effect of meprobamate and the barbiturates on electroencephalographic activity and found that meprobamate rarely, if ever, produces slowing of frequencies lower than 6 cycles/sec even with toxic doses, although this regularly occurs with barbiturates. Thus, it may be concluded that meprobamate acts primarily to block long internuncial neuronal circuits between the cortex and the thalamus. In the light of these observations, it may not be surprising that meprobamate selectively raised the acetylcholine content of the hippocampus and the hypothalamus only in the dose used and has no significant effect on the acetylcholine content of the central core of grey matter in the mid-brain, the frontal cortex and the cerebellar cortex. As already stated Richter & Crossland (1949) have shown that the acetylcholine of the brain bears an inverse relationship to the degree of prevailing activity.

SUMMARY

1. The effect of intravenous meprobamate on the acetylcholine concentration of the frontal lobe, the hypothalamus, the hippocampus, the mid-brain and the cerebellar cortex has been studied in dogs under ether anaesthesia.

2. There was a significant increase in the acetylcholine content of the hypothalamus and the hippocampus after meprobamate, while in other areas of brain studied the changes were insignificant though there was a slight reduction.

3. Attempts have been made to correlate the selective changes in acetylcholine content with the electrical changes in these areas of the brain after meprobamate.

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