Brit. J. Pharmacol. (1965), **24**, 348–351.

THE EFFECT OF SOME ANAESTHETICS ON THE ACETYLCHOLINE CONCENTRATIONS OF DIFFERENT AREAS OF DOG BRAIN

BY

C. L. MALHOTRA AND P. G. PUNDLIK

From the Department of Pharmacology and Therapeutics, Lady Hardinge Medical College, New Delhi, India

(*Received May* 25, 1964)

The amount of acetylcholine in the brain during different levels of cerebral activity has been estimated by various workers. Richter & Crossland (1949) have shown that acetylcholine content of the brain bears an inverse relationship to the degree of prevailing cerebral activity. Thus, the amount of acetylcholine increases during anaesthesia, but falls during conditions of increased cerebral activity. Crossland & Merrick (1954) found that the amount of acetylcholine which could be extracted from the brains of rabbits, mice and rats increased as a result of anaesthesia, no increase being observed during induction. Further, they showed that the increase in the acetylcholine concentration depended, within limits, on the depth of anaesthesia attained but was independent of its duration. The importance of drug-induced changes in brain acetylcholine is being recognized (Tobias, Lipton & Lepinat, 1946; Malhotra & Pundlik, 1959; Giarman & Pepeu, 1962). Most of the work on brain acetylcholine after administration of drugs has been done on whole brains of small animals like mice and rats in which no anaesthetic is used and the animals are killed either by decapitation or by immersion into liquid air. In our laboratory, we have been using bigger animals like dog and monkey and in such experiments an anaesthetic has to be administered before taking out different regions of the brain. It was thus considered worth while to see the influence of some commonly used anaesthetics, namely ether, chloroform and sodium pentobarbitone, on the acetylcholine level of certain regions of the dog brain.

METHODS

Three groups of ten mongrel dogs of either sex weighing between 5.5 and 10.5 kg were used. Group 1 was given ether by inhalation, group 2 was anaesthetized with chloroform by inhalation and group 3 received 35 mg/kg of sodium pentobarbitone, intraperitoneally as a 2.5% solution. When the animals were completely anaesthetized as judged by the disappearance of certain reflexes, they were bled to death. The skull was opened and the following areas of the brain were transferred quickly to weighing bottles which had already been kept in freezing mixtures; part of the hippocampus, the entire hypothalamus, the anterior portion of the frontal cortex and part of the cerebellar cortex.

Acetylcholine was extracted from these portions of the brain in acidified frog-Ringer solution containing physostigmine at 95 to 100° C, and was assayed on frog rectus abdominis muscle by the method of Nachmansohn described by Anand (1952). This uses standard solutions made up in tissue extracts in which the acetylcholine has previously been destroyed. The experiments with different anaesthetics were randomized.

RESULTS

The acetylcholine concentrations of the different areas of brain of dogs anaesthetized with chloroform, ether or sodium pentobarbitone are given in Table 1. There is a wide variation in the acetylcholine content of these areas, the maximum level being in the hippocampus and the minimum in cerebellar cortex, irrespective of the anaesthetic used.

TABLE 1

THE ACETYLCHOLINE CONCENTRATIONS OF DIFFERENT AREAS OF THE CENTRAL NERVOUS SYSTEM OF THE DOG WITH DIFFERENT ANAESTHETICS

The results are means and standard deviations, expressed as μg/g of fresh tissue. Differences between means of ether and chloroform (decrease) and pentobarbitone and chloroform (increase) are expressed as percentages of the chloroform means. Significances between means of results with chloroform and ether (P_1) and between means of results with chloroform and sodium pentobarbitone (P_2) are calculated by the *t*-test

			Acetylcholine concentration in			
Anaesthetic	No. of dogs		Hippo-campus	Hypo-thalamus	Frontal cortex	Cerebellar cortex
Chloroform	10	Mean	5·9±1·2	4·3±0·8	2·5±0·8	0·6±0·2
Ether	10	Mean	3·1±0·6	3·0±0·6	1·9±0·6	0·8±0·2
		Decrease	47·1%	29·8%	24·1%	—
		P_1	<0·001	<0·01	<0·05	>0·08
Sodium pentobarbitone	10	Mean	7·4±0·6	5·4±0·4	3·3±0·6	0·6±0·04
		Increase	24·6%	25·9%	30·8%	—
		P_2	<0·005	<0·005	<0·05	<0·6

Dogs anaesthetized with ether showed decreased acetylcholine concentrations in the different areas of brain compared to those of the chloroform-treated group. The extent of the decrease was about the same in hypothalamus and frontal cortex, namely 29.8 and 24.1% respectively of the values for chloroform anaesthesia. In the hippocampus the decrease was much more, namely 47.1%. The change in cerebellar cortex was statistically not significant. In contrast, dogs anaesthetized with sodium pentobarbitone showed significantly higher acetylcholine levels in all the areas except cerebellar cortex. They exceeded the levels for chloroform anaesthesia by, respectively, 24.6, 25.9 and 30.8% in hippocampus, hypothalamus and frontal cortex.

The results show that the acetylcholine concentration was lowest for ether and highest for sodium pentobarbitone anaesthesia.

DISCUSSION

Our findings indicate that the concentrations of acetylcholine in certain regions of dog brain varied considerably depending upon the anaesthetic used in spite of the fact that the depth of anaesthesia, as judged by reflexes, was almost the same with the three anaesthetics. However, the degree of struggling during the induction stage was different in each instance. The dogs struggled a great deal during the induction period with ether; struggling was less with chloroform and least with sodium pentobarbitone anaesthesia, when the dogs seemed to go to sleep gradually. Hence the nervous activity was greatest with ether, less with chloroform and least with sodium pentobarbitone. The quantitative variations in the acetylcholine concentrations of different areas of brain of dogs subjected to these anaesthetics may be the result of differences in the degree of nervous activity during the induction stage. As already pointed out, Richter & Crossland (1949) have shown that the acetylcholine content

of the brain bears an inverse relationship to the degree of nervous activity. Giarman & Pepeu (1962) have shown that, in rats, drug-induced depression of the central nervous system is associated with an elevation in level of total acetylcholine with a wide variety of depressant drugs with one notable exception: the subacute administration of reserpine, which increased cerebral acetylcholine after the first dose but allowed return to control levels after subsequent doses, despite continued sedation of the animals.

The effect of anaesthetics on the acetylcholine level is important for the study of small, well-defined areas in the brain of large animals. Such areas may differ in their metabolisms and responses to drugs. Thus, a mild degree of hypoxia may induce changes in some parts of the brain and not in others (Himwich, 1951), or, as we have shown (Malhotra & Pundlik, 1959), reserpine raises the acetylcholine content of the hypothalamus whereas it lowers that of the hippocampus.

The mechanism of the increase produced by various anaesthetics is obscure. It is unlikely to be due to inhibition of acetylcholinesterase, since Bernheim & Bernheim (1936) have shown that the anaesthetics have no such action. It may be that the utilization of acetylcholine is reduced in the depressed brain, though this explanation lacks experimental proof. Another possibility is that there may be decreased liberation of acetylcholine from central cholinergic neurones as a result of depressed cerebral activity accompanying anaesthesia. MacIntosh & Oborin (1953) have shown that, during anaesthesia, the release of acetylcholine by the brain into a Ringer-Locke solution containing physostigmine and in contact with the brain is reduced. Marley & Paton (1959) have also shown that methylpentynol, a sedative-hypnotic agent, inhibits the release of acetylcholine from the perfused superior cervical ganglion of the cat.

SUMMARY

1. The effect of ether, chloroform and sodium pentobarbitone on the acetylcholine concentrations of the hippocampus, hypothalamus, frontal cortex and cerebellar cortex was studied in dogs.

2. Portions of brain were removed when the animals were completely anaesthetized. Extracts were made and assayed for acetylcholine content on the frog rectus abdominis preparation.

3. The acetylcholine concentration of the hippocampus, hypothalamus and frontal cortex was greatest with sodium pentobarbitone, less with chloroform and least with ether anaesthesia. There were no significant differences in the cerebellar cortex.

4. These differences may be related to the differences in behaviour of the animals during induction of anaesthesia, in which struggling was greatest with ether, less with chloroform and least with sodium pentobarbitone.

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