EFFECT OF SOME DRUGS ON THE ACETYLCHOLINE RELEASE FROM THE CEREBRAL CORTEX OF CAT

By

C.L.MALHOTRA AND S.PRASAD

Department of Pharmacology, Lady Hardinge Medical College, New Delhi

Feldberg (1) reported that there is a close relation between acetylcholine content of the brain, its electrical activity and the release of acetylcholine from the cortex. In deep anaesthesia when activity is decreased and the output of acetylcholine is reduced the content is high; it falls in the waking state when activity and output are increased. The central release of acetylcholine from the surface of the intact cortex has been estimated under different conditions. MacIntosh and Oborin(2) showed that the output of acetylcholine from the surface of the cortex decreased when the depth of anaesthesia is increased. Mitchell (3) found that the acetylcholine released when collected by the cup method varied with the anaesthetic used. It was more with sodium pentobarbitone than with ether, and the release of acetylcholine was increased when the animal was atropinised. Mathews and Quilliam (4) have estimated the release of acetylcholine after central depressant drugs. They have, however, not used brain tissue but cat perfused superior cervical ganglion preparation and rat phrenic nerve diaphragm preparation and have shown a close relationship between the release of acetylcholine and ganglionic and neuromuscular transmission. Malhotra and Mehta (5) have reported increased release of acetylcholine from the cortex after reserpine under different anaesthetics, by using pushpull cannula in dogs. It was further shown that increased release was more under ether than under sodium pentobarbitone. The present paper deals with the effect of certain drugs on the acetylcholine release under different anaesthetics in cats.

A technique similar to that of Mitchell (3) has been used in the present study.

MATERIALS AND METHODS

Twenty four cats weighing between 2 to 5 *kg* were divided into four groups of six each. Cats were anaesthetised with sodium pentobarbitone (35 *mg /kg*) given intraperitoneially, or ether. In all the experiments the trachea was cannulated and femoral vien was exposed for intravenous injection. The head was clamped in a rigid frame and the large part of the cerebral cortex was exposed on both sides of midline. The dura was opened and circular glass cups open at both ends were then lowered gently on the surface of the exposed parietal cortex on both sides and adjusted so that there was no obvious disturbance of the blood supply. The junction between the brain and cups was sealed by applying warm paraffin. The cups were each filled with 1.0 ml. of escrinised (2 *mg* /100 c.c.) mammalian Ringer solution (NaCl 9.00 KCl. 0.42, $CaCl_2$ 0.24, $NaHCo_3$ 0.2, Glucose 2.0 *gm/litre*).

Thirty minutes before begining the collection of the samples, the cups were filled with eserinised Ringer solution and at the end of this period the solutions were removed by suction and discarded. The chambers were again filled with 1.0 ml. of Ringer solution which was

left in contact with cortex for 30 minutes and then removed by suction into a graduated tube for assay of acetylcholine on the eserinsied frog rectus abdominis muscle preparation in a small bath (2.5 ml). Before assay the cup solutions were diluted with distilled water so as to make them isotonic with frog's Ringer solution. They were compared with standard solution of acetylcholine containing eserine. Samples from both sides of cortex were pooled together for assay purposes. First sample before giving any drug served as control, the drug was then injected intravenously and three samples of 30 minutes duration each (total 90 minutes) were collected.

Cats in group one and two were anaesthetised with pentobarbitone sodium. Group one was given atropine sulphate 2 *mg/kg.* and group two was given atropine methyl nitrate 4 *mg./kg.* intravenously. Group three and four were anaesthetised with pentobarbitone sodium and ether respectively and reserpine 0.5 *mg/kg* was given intravenosuly.

Acetylcholine in all cases was estimated on frog rectus abdominis muscle by the method of Nachmansohn, as described by Anand (6).

RESULTS

It is seen that after atropine sulphate, there is significant increase in spontaneous release of acetylcholine from the parietal cortex of cats, but there is no significant difference in the level of acetylcholine collected at different time intervals of atropine sulphate (1-30 minutes, 30-60 minutes, and 60-90 minutes). After atropine methyl nitrate there is no significant change in the level of acetylcholine in all the samples as compared to controls (Table I).

TABLE I

Effect of atropine sulphate and atropine methylnitrate on spontaneous release of acetylcholine from the parietal cortex of cats under sodium pentobarbitone anaesthesia. Results are expressed as acetylcholine release, ng/min/cm² Cortex ±S.D.

Drug	Control	After Drugs		
		1—30 mts.	30—60 mts	60—90 mts.
Atropine So_4 2 *mg/kg.* I.V.	0.9413±0.087	1.6789±0.283 P<0.001	1.811±0.172 P<0.001	1.9166±0.168 P<0.001
Atropine Methylnitrate 4 *mg/kg* I.V	1.0883±0.362	1.0430±0.310 P>0.8	1.0583±0.374 P<0.8	1.0361±0.338 P>0.8

Our studies also show that the release of acetylcholine from the parietal cortex is less under ether than under sodium pentobarbitone anaesthesia though the difference is not significant (Table II). It is further seen that after reserpine there is significant increase in the spontaneous release of acetylcholine from parietal cortex in the first sample. The release is more under ether than under sodium pentobarbitone anaesthesia. The values of acetylcholine in the second and third samples, after reserpine, show no significant change as compared to control.

TABLE II

Effect of reserpine on the spontaneous release of aceylcholine from the parietal cortex of cats under sodium pentobartiotone and ether anaesthesia. Results are expressed as acetylcholine release. ng/min/cm,² cortex±S.D.

Drug	Anaesthesia	Control	After Drug		
			1—30 mts.	30—60 mts	60—90 mts.
Reserpine 0.5 *mg/kg* I.V.	Sodium pentobarbitone	0.9144±0.227	1.4583±0.291 P<0.01	1.1569±0.454 P>0.3	0.9819±0.287 P>0.8
Reserpine 0.5 *mg/kg* I.V.	Ether	0.7163±0.235	1.7833±0.432 P<0.001	1.0433±0.509 P>0.1	0.8100±0.364 P>0.6

DISCUSSION

MacIntosh and Oborin(2) showed that undercutting the cortex abolished the spontaneous release of acetylcholine and that cholinergic vasodilator nerves made no significant contribution to the liberated acetylcholine. It, therefore, seemed likely that the acetylcholine was cortical in origin. Mitchell (3) has supported this view since after removing the cortex and collecting from underlying white matter no acetylcholine could be recovered. The possibility that acetylcholine collected from the cortex was being liberated from some distant site in the brain or from blood, has been ruled out by Mitchell(3). More direct evidence to suggest the release of acetylcholine from within the cortex was obtained by using pushpull cannula (Malhotra and Mehta(5).

The present study shows that after atropine sulphate there is significant increase in the release of acetyecholine under sodium pentobarbitone anaesthesia. These results are in conformity with the findings of MacIntosh and Oborin(2) and Mitchell (3). Giarman and Pepeu(7) have reported that atropine and hyoscine sharply lower brain acetylcholine. These workers have ruled out the possibility that these drugs enhance the activity of acetylcholinesterase. They have put forward the view that atropine and hyoscine might produce blockade of central acetylcholine receptors as they do to peripheral acetylcholine receptors and thus lead to the movement of the physiologically liberated acetylcholine to acetylcholinesterase and destruction, as well as points distant from the site of storage and release. This will account for the diminished acetylcholine content of brain areas, and an increased spontaneous output of acetylcholine from the parietal cortex of the cats.

In the present study, it has also been observed that with atropine methyl nitrate, there is no effect on the release of acetylcholine from the cortex. Atropine methyl nitrate does not cross the blood brain barrier, and therefore, acts only on the peripheral acetylcholine receptors.

The release of acetylcholine is more under sodium pentobarbitone than with ether. Similar findings have been reported by Mitchell (3) and Malhotra and Metha(5). It has been found that after reserpine, the release of acetylcholine is less under sodium pentobarbitone than

under ether anaesthesia in the first sample taken after 30 minutes. These findings are in conformity with those of Malhotra and Mehta(5). It may be due to the fact that reserpine and sodium pentobarbitone combination is more depressant than the resperine and ether one. The samples collected 60 minutes and 90 minutes after reserpine showed no significant difference in the release of acetylcholine as compared to the control values. The exact mechanism of release of acetylchoine is not known. Influx of sodium ion at nerve terminals may play a role in mobilising cellular acetylcholine for release and in triggering the synthesis of acetylcholine (Quastel and Birks(8) and Birks(9). Thesleff (7) has seuggested that since central depressant drugs may act by inhibition of the neuronal membrane "sodium carrying mechanisms" responsible for the production of a normal nerve action potential, this may be of importance for the release of acetylcholine from the prejunctional region. In our experiments, pentobarbitone and reserpine combination seems to be more depressant as compared to ether and reserpine one and thus support this view, although it does not exclude the possibility suggested by Mathews and Quilliam(4) that centrally acting drugs might more directly interfere with acetylcholine synthesis and thus would lead ultimately to a reduced release of acetylcholine.

REFERENCES

1. Feldberg, W. Acetylcholine in Metabolism of the Nervous System. Ed. by D Richter, 493, Pergamon Press, London, 1957.

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

3. Mitchell, J.F. The spontaneous and evoked release of acetylcholine from the cerebral cortex. *J. Physiol.* **165**:98, 1963.

4. Mathews, E.K. and J.P. Quilliam. Effect of central depressant drugs upon Acetylcholine Release. *Br. J. Pharmac. Chemother.*, **22**:415, 1964.

5. Malhotra C.L. and V.L. Mehta. The effect of Reserpine on the Acetylcholine Rlease of the cerebral cortex of dog under different anaesthetics. *Br. J. Pharmac.* and *Chemother.* **27**:131, 1966.

6. Anand B.K. Influence of temperature on vagal inhibition and liberation of Acetylcholine in frog hearts. *Am. J. Physiol.* **168**:218, 1952.

7. Giarman, N.J. and G. Pepeu. Drug-induced changes in brain Acetylcholine. *Br. J. Pharmac. Chemother.* **19**:226, 1962.

8. Qastel D.M. and R.T. Birks. Effects of Sodium ion on Acetylcholine metabolism in a sympathetic ganglion. *Abstr. 5th Meet. Canad. Fed. Biol. Sci.* : p. 64, 1962.

9. Birks, R.T. Effects of Sodium and Potassium Ions on Acetylcholine Release. *Abstr. 6th Meet. Canad. Fed. Biol. Sci.* : p. 10, 1963.

10. Thesleff, S. The effect of anaesthetic agents on skeletal muscle membrane. *Acta. Physiol. Scand.* **37**:335, 1956.