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THE EFFECT OF RESERPINE ON THE ACETYLCHOLINE RELEASE OF THE CEREBRAL CORTEX OF DOG UNDER DIFFERENT ANAESTHETICS

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It is well known that the amount of acetylcholine present in the brain is influenced by the level of cerebral activity. Richter & Crossland (1949) showed that the acetylcholine 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). Giarman & Pepeu (1962) showed that a wide variety of depressant drugs increase the cerebral acetylcholine, while there is reduction in the level of total acetylcholine in the brain after administration of certain convulsants. In a series of papers Malhotra and his co-workers (Malhotra & Pundlik, 1959 ; Malhotra & Das, 1962 ; Malhotra & Prasad, 1963 ; and Malhotra & Pundlik, 1965) have shown that reserpine increases the acetylcholine content of various areas of the dog brain. MackIntosh & Oborin (1953) found that the output of acetylcholine from the surface of the cortex decreases when the depth of anaesthesia is increased. Mitchell & Silver (1963) found that the acetylcholine released when collected by the cup method varies with the anaesthetic used. It was more with sodium pentobarbitone than with ether. Mathews & Quilliam (1964) have estimated the release of acetylcholine after central depressant drugs. They have not used brain tissue but cat perfused superior cervical ganglion preparation and rat phrenic nerve diaphragm preparation. They have shown a close relationship between the release of acetylcholine and ganglionic and neuromuscular transmission.

The present paper deals with the effect of reserpine on the acetylcholine release under different anaesthetics.

METHODS

Forty-two mongrel dogs weighing between 6 and 9 kg were divided into six groups of seven each.

In the first group intravenous saline 0.5 ml./kg was given and one hr later the dogs were anaesthetized with ether. The parietal areas of cerebral cortex were exposed and a push-pull cannula was inserted to the depth of 4 mm in the right parietal area. It was perfused with mammalian Ringer containing physostigmine 15 μg/ml. at the rate of ten drops/min. Perfusate was collected for twenty min. Immediately after perfusion both the parietal areas were removed and the acetylcholine content of the perfusate and the two areas was estimated.

In the second group reserpine 0.5 mg/kg was given intravenously (volume 0.5 ml./kg). After 1 hr the dogs were anaesthetized with ether and the same procedure was adopted as in the first

group. In the third group 35 mg/kg pentobarbitone sodium was given intraperitoneally. After one hr twenty min right and left parietal areas were removed and the acetylcholine content was estimated.

In the fourth group pentobarbitone was given intraperitoneally and this was followed immediately by 0.5 mg/kg of reserpine intravenously. After one hr and twenty min the right and left parietal areas were removed for estimation of acetylcholine.

In the fifth group the right and left parietal areas, one centimetre away from mid line (one centimetre in diameter and four millimetres deep), were removed under pentobarbitone anaesthesia. After one hr another piece of right parietal region about 1 cm posterior to the removed one was perfused with eserinized Ringer and perfusate collected for twenty min. At the end of perfusion this area and the corresponding area on the left side was removed. The acetylcholine content of all the four areas and the perfusate was estimated. In the sixth group the dogs were anaesthetized with sodium pentobarbitone and both parietal areas were removed as in the fifth group. Reserpine 0.5 mg/kg was then injected intravenously and one hr later the right parietal area posterior to the removed area was perfused for twenty min as in the fifth group and subsequently excised. Acetylcholine in all cases was estimated on frog rectus abdominis muscle by the method of Nachmansohn, as described by Anand (1952).

RESULTS

Table 1 shows that after reserpine under ether anaesthesia there is increase in acetylcholine content in the brain and in the perfusate. It is interesting to note that the acetylcholine content is higher on the right side than on the left.

After sodium pentobarbitone the values for acetylcholine are higher than with ether alone, but did not cause a significant increase in acetylcholine (Table 2).

TABLE 1

ACETYLCHOLINE CONTENT OF RIGHT AND LEFT PARIETAL AREAS AND PERFUSATE UNDER ETHER ANAESTHESIA

The results are mean and standard deviations expressed as ng/g brain tissue and for released acetylcholine as ng/min. Perfusion was done with Ringer solution containing physostigmine. Significance of difference between means of treated and untreated or right and left side calculated by Students " t " test.

Control dog			Reserpinised dog		
Rt. parietal	Lt. parietal	Perfusate	Rt. parietal	Lt. parietal	Perfusate
1564±318	1305*±232	4·85±0·9	2418±513	2066±384	**12·3±1·25

* $0{\cdot}001 < P < 0{\cdot}5$ (difference between left and right side of control dog's brain).
** $P < 0{\cdot}001$ (difference between treated and control dog).

TABLE 2

ACETYLCHOLINE CONTENT (ng/gm OF BRAIN) OF RIGHT AND LEFT PARIETAL AREAS UNDER SODIUM PENTOBARBITONE ANAESTHESIA

The differences between reserpine-treated dogs and control dogs were not significant.

Control dogs		Reserpinised dog	
Rt. parietal	Lt. parietal	Rt. parietal	Lt. parietal
1685±265	1574±235	1712±245	1596±132

Table 3 shows that under pentobarbitone sodium alone there is no significant difference between the acetylcholine content of adjacent parietal areas immediately and one hr twenty min after anaesthesia. Perfusion of the right parietal area for twenty min with eserinized Ringer also did not make any difference in that area. With reserpine there was no significant increase in the acetylcholine content of the parietal areas. However, the perfusate showed a significant increase in acetylcholine release.

TABLE 3

ACETYLCHOLINE CONTENT OF RIGHT (A_1 A_2) AND LEFT (B_1 B_2) PARIETAL AREAS AND RELEASE OF ACETYLCHOLINE UNDER SODIUM PENTOBARBITONE ANAESTHESIA IN NORMAL AND RESERPINE TREATED DOGS

Acetylcholine concentrations are expressed as ng/g brain tissue and ng/min. for released acetylcholine.
* Value significantly different from control.

Control dogs					Reserpinised dogs				
Before perfusate		After perfusate		Perfusate	Before perfusate		After perfusate		Perfusate
A_1 (Rt. parietal)	B_1 (Lt. parietal)	A_2 (Rt. parietal)	B_2 (Lt. parietal)		A_1 (Rt. parietal)	B_1 (Lt. parietal)	A_2 (Rt. parietal)	B_2 (Lt. parietal)	
1660 ±385	1443 ±184	1559 ±224	1418 ±272	7·2±1·8	1642 ±287	1403 ±164	1649 ±326	1472 ±259	*17·17±3·02

DISCUSSION

Malhotra & Pundlik (1965) showed that the acetylcholine content of different areas of the dog brain varies according to the anaesthesia used, the values being higher with sodium pentobarbitone than with ether. The present studies are in conformity with the previous findings as far as acetylcholine content of parietal areas is concerned. It is further observed that reserpine causes a significant increase in acetylcholine content under ether anaesthesia but not under sodium pentobarbitone. In this connection it is interesting to note that Giarman & Pepeu (1962), while studying the effects of drug-induced changes in brain acetylcholine, reported that the most striking aspect of increase in the levels of acetylcholine after certain central depressants is the relative efficiency of acetylcholine metabolism in maintaining the level of neurohormone within relatively narrow limits. They further postulated that this imposition of a ceiling on the level of acetylcholine achievable in the brain may be related to a depression of synthetic processes by acetylcholine itself. It appears that the sodium pentobarbitone does not allow the brain content of acetylcholine to go beyond a certain level, so that excessive acetylcholine formed under this anaesthetic alone as well as under reserpine is released (Table 3). As far as release of acetylcholine with the push-pull cannula device is concerned, our results indicate that acetylcholine release is faster with sodium pentobarbitone anaesthesia than with ether ; probably acetylcholine is being removed as soon as synthesized. Mitchell & Silver (1963) have also found that the acetylcholine release in ng/min/cm^2 cortex is larger with sodium pentobarbitone than with ether. The proportion of acetylcholine released by reserpine, however, is not greater under pentobarbitone than with ether.

Feldberg (1957) has reported that under deep anaesthesia, when the electrical activity is decreased, release of acetylcholine is reduced and the content is high, while it falls in the waking state, when activity and output are decreased. In our study, however, we have found that release of acetylcholine after reserpine is less under pentobarbitone anaesthesia than under ether anaesthesia. It may be due to the fact that reserpine and sodium pentobarbitone combination is more depressant than the reserpine and ether one.

Recently (Quastel & Birks, 1962 ; and Birks, 1963) have suggested that the normal influx of sodium ion at nerve terminals may play a role in mobilizing cellular acetylcholine for release and in triggering the synthesis of acetylcholine. It has been suggested by Thesleff (1956) that, since central depressant drugs may act by inhibition of the neuronal membrane " sodium carrying mechanism " responsible for the production of a normal

nerve action potential, this may be of importance for the release of acetylcholine from the prejunctional region. Our finding that pentobarbitone and reserpine combination as compared to ether and reserpine combination is in accord with this view, although it does not exclude the possibility suggested by Matthews & Quilliam (1964) that centrally acting drugs might more directly interfere with acetylcholine synthesis and thus would lead ultimately to a reduced release of acetylcholine.

SUMMARY

1. The effect of intravenous administration of reserpine on the acetylcholine concentration of right and left parietal areas and acetylcholine release from perfused right parietal area under ether and sodium pentobarbitone anaesthesia was studied in six groups of seven dogs each.

2. The acetylcholine content of parietal areas is higher after sodium pentobarbitone than after ether anaesthesia.

3. After reserpine under pentobarbitone sodium there is no significant increase in the brain acetylcholine, but there is under ether anaesthesia.

4. Release of acetylcholine from a push-pull cannula is greater with pentobarbitone than with ether, both under control conditions and after the administration of reserpine. However, the percentage increase in acetylcholine released by reserpine is not greater under pentobarbitone.

REFERENCES

ANAND, B. K. (1952). Influence of temperature on vagal inhibition and liberation of acetylcholine in frog hearts. *Am. J. Physiol. (Lond.)*, **168**, 218–225.

BIRKS, R. T. (1963). Effects of sodium and potassium ions on acetylcholine release. *Abstr. 6th Meet Canad. Fed. Biol. Sci.*, P.10.

CROSSLAND, J. & MERRICK, A. J. (1954). The effect of anaesthesia on the acetylcholine content of brain. *J. Physiol. (Lond.)*, **125**, 56–66.

FELDBERG, W. (1957). Acetylcholine in metabolism of the nervous system. Ed. by D. Richter, pp. 493–510. London: Pergamon Press.

GIARMAN, N. J. & PEPEU, G. (1962). Drug-induced changes in brain acetylcholine. *Br. J. Pharmac. Chemother.*, **19**, 226–234.

MACKINTOSH, F. C. & OBORIN, P. E. (1953). Release of acetylcholine from intact cerebral cortex. *Abstr.* **19**, *Int. Physiol. Congr., Montreal*, 580.

MALHOTRA, C. L. & DAS P. K. (1962) Effect of reserpine on the acetylcholine content of the heart, the ileum and the hypothalamus of the dog. *Br. J. Pharmac. Chemother.*, **18**, 190–193.

MALHOTRA, C. L. & PRASAD, K. (1963). The effect of intra (cerebro) ventricular reserpine on the acetylcholine content of the heart, ileum and hypothalamus of the dog. *Br. J. Pharmac. Chemother.*, **21**, 355–360.

MALHOTRA, C. L. & PUNDLIK, P. G. (1959). The effect of reserpine on the acetylcholine content of different areas of the central nervous system of the dog. *Br. J. Pharmac. Chemother.*, **14**, 46–47.

MALHOTRA, C. L. & PUNDLIK, P. G. (1965). The effect of some anaesthetics on acetylcholine concentrations of different areas of dog brain. *Br. J. Pharmac. Chemother.*, **24**, 348–351.

MATHHEWS, E. K. & QUILLIAM, J. P. (1964). Effects of central depressant drugs upon acetylcholine release. *Br. J. Pharmac. Chemother.*, **22**, 415–440.

MITCHELL, J. F. & SILVER, A. (1963). The spontaneous release of acetylcholine from the denervated hemidiaphragm of the rat. *J. Physiol. (Lond.)*, **165**, 117–129.

QUASTEL, D. M. J. & BIRKS, R. T. (1962). Effects of sodium ion on acetylcholine metabolism in a sympathetic ganglion. *Abstr. 5th meet. Canad. Fed. Biol. Sci.*, P.64.

RICHTER, D. & CROSSLAND, J. (1949). Variation in acetylcholine content of the brain with physiological state. *Am. J. Physiol.*, **159**, 247–255.

THESLEFF, S. (1956). The effect of anaesthetic agents on skeletal muscle membrane. *Acta physiol. Scand.*, **37**, 335–349.