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DEPRESSION BY MORPHINE AND CHLORALOSE OF ACETYLCHOLINE RELEASE FROM THE CAT'S BRAIN

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Both Paton (1957) and Schaumann (1957) have shown that morphine inhibits the release of acetylcholine from the isolated guinea-pig ileum suspended in eserinized physiological salt solution. The resting output of acetylcholine as well as the output during stimulation of the nerve endings in the intestinal wall is reduced when morphine is present in the bath fluid. According to these authors the inhibition of acetylcholine release is responsible for the paralysing action of morphine on the guinea-pig intestine. The present experiments were designed to find out whether morphine similarly depresses the release of acetylcholine from the central nervous system.

When the cerebral ventricles of the cat are perfused with physiological salt solution containing an anticholinesterase acetylcholine appears in the effluent and it has recently been shown (Beleslin, Carmichael & Feldberg, 1964) that the greatest amount originates from structures lining the anterior horns of the lateral ventricles. In the present experiments therefore the method of perfusing the anterior horn of a lateral ventricle with an anticholinesterase was used to study the effect of morphine on the output of acetylcholine. In a similar way, the effect of chloralose was examined.

METHODS

Cats of both sexes weighing between 1·8 and 3·6 kg were anaesthetized with chloralose (40–70 mg/kg) injected into the cannulated right femoral vein. To allow cannulation of the femoral vein, anaesthesia was induced with ethyl chloride and ether. The trachea was cannulated. With the cat lying on its belly the head was fixed to the ear bars and the mouthpiece of a head holder similar to that of a Horsley–Clark stereotaxic instrument. The surface of the skull was widely exposed and the muscles covering the atlanto-occipital membrane were dissected away.

The anterior horn of the left lateral ventricle was perfused with neostigmine methyl sulphate in a concentration of 1/50,000. The method used was that of multiple cannulation of the ventricular system which is based on the principle that drugs perfused through one part of the ventricular system are denied access to those parts which are cannulated and perfused with artificial c.s.f.

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Three cannulae were inserted into the left lateral ventricle, one into the anterior, one into the inferior horn and one into its body. Another cannula was inserted into the body of the right lateral ventricle and a fifth into the aqueduct. The neostigmine (as well as the morphine and chloralose) was delivered through the cannula in the anterior horn, whereas artificial c.s.f. was delivered through the cannulae in the inferior horn and in the body of the right lateral ventricle. The rate of flow through each of the three cannulae was 0·1 ml./min. The cannula in the body of the left lateral ventricle served as outflow, and the cannula in the aqueduct was kept closed throughout the experiment. The details of the method have been described elsewhere (Carmichael, Feldberg & Fleischhauer, 1964).

The effluent was collected in successive half-hour samples which were assayed against acetylcholine chloride on the eserinized dorsal muscle of the leech suspended in a 2 ml. bath. The acetylcholine content of the samples is expressed as the mean output in ng/min of acetylcholine chloride. The acetylcholine solutions used in the assays were made up so as to contain the same concentrations of neostigmine, morphine and chloralose as those present in the dilutions of effluent tested on the leech muscle.

The artificial c.s.f. used for perfusion as well as for dilution of neostigmine, morphine, and chloralose, was a modification of that described by Merlis (1940). Its composition was (g/l.): NaCl, 8·1; KCl, 0·25; $CaCl_2$, 0·14; $MgCl_2$, 0·11; $NaHCO_3$, 1·76; Na_2HPO_4, 0·07; $CO(NH_2)_2$, 0·13; and glucose, 0·61.

At the end of each experiment, bromophenol blue was perfused through the anterior horn cannula for 15 min in order to find out which parts of the ventricular walls were stained and had thus been reached by the drugs. In all experiments there was deep staining of the walls of the anterior horn and of the olfactory recess of the left lateral ventricle. There was no staining of the left inferior horn or of the right lateral ventricle indicating that their perfusion with artificial c.s.f. had prevented the neostigmine reaching these parts of the ventricular system. In several experiments there was some staining of the walls of the third ventricle indicating that the closure of the aqueduct had not always prevented the neostigmine delivered through the anterior horn cannula from entering the third ventricle. However, since there was no inflow from the aqueductal cannula acetylcholine derived from structures lining the third ventricle could at most have contributed to a small extent to the total acetylcholine content of the effluent.

RESULTS

On prolonged perfusion of the anterior horn of a lateral ventricle with an anticholinesterase the acetylcholine content of the effluent increases. This is shown in Table 1, which gives for seven experiments the acetylcholine output in successive ½ hr samples collected during perfusion of the anterior horn with neostigmine. As shown in the last column the mean acetylcholine output rises from 0·8 ng/min in the first, to 1·9 in the fifth, and then to 5 ng/min in the tenth sample. If neostigmine is not added to the fluid perfusing the anterior horn, no acetylcholine is detected in the effluent.

Morphine. Perfusion of the anterior horn with morphine, 1/400,000, produces no overt symptoms in the anaesthetized cat, whether or not neostigmine is included in the perfusion fluid.

Table 2 gives the results of seven experiments in which, after 2½ hr perfusion with neostigmine alone, morphine 1/400,000 is included in the perfusion fluid as well. It is seen that there is no further rise in the acetyl-

choline output. In five of the experiments the output actually decreases, but this depression is not maintained.

It is not possible to suppress completely the appearance of acetylcholine in the effluent from the anterior horn if the procedure is reversed and a perfusion with morphine 1/400,000 precedes that of morphine with neostigmine, although in this condition the morphine would be able to

TABLE 1. Acetylcholine output from the anterior horn of a lateral ventricle during 5 hr perfusion with neostigmine methyl sulphate 1/50,000

Min	ACh output in ng/min in successive samples							Mean
30	0·5	0·5	1·0	0·8	0·6	0·9	1·3	0·8
60	1·0	0·6	1·2	1·3	1·5	1·0	1·3	1·1
90	1·2	0·9	1·5	1·2	1·8	1·1	1·7	1·3
120	1·8	1·1	1·4	1·5	2·5	1·3	2·2	1·4
150	2·3	1·2	1·4	1·8	3·0	1·8	2·3	1·9
180	2·5	2·0	2·0	2·0	3·4	2·2	2·7	2·3
210	3·1	2·0	2·8	3·0	2·4	2·3	3·3	2·6
240	3·6	2·5	2·0	4·1	2·4	3·6	3·9	3·1
270	—	3·6	6·1	7·0	2·9	3·7	3·8	4·4
300	—	5·2	6·1	7·8	3·2	3·7	4·2	5·0

TABLE 2. Acetylcholine output from the anterior horn of a lateral ventricle during 5 hr perfusion with neostigmine methyl sulphate 1/50,000. During the last 2½ hr the perfusing fluid contained morphine sulphate 1/400,000 as well

Min	ACh output in ng/min in successive samples							Mean
30	0·7	0·7	0·6	0·8	0·7	0·3	0·3	0·6
60	0·6	0·6	0·8	1·4	0·8	1·0	0·6	0·8
90	1·1	1·0	1·1	1·2	1·0	1·1	0·5	1·0
120	1·0	1·3	1·0	1·2	1·1	1·3	0·6	1·1
150	1·4	1·2	1·3	2·1	1·4	1·6	1·0	1·5
	Morphine 1/400,000 included in perfusion fluid							
180	1·3	1·3	1·7	1·7	1·1	1·3	0·6	1·3
210	1·2	1·5	1·7	1·3	1·0	1·3	0·8	1·3
240	1·3	1·8	1·8	1·2	0·8	1·3	0·8	1·3
270	1·2	1·8	1·2	1·2	1·2	1·5	0·9	1·3
300	1·8	2·4	1·2	1·6	1·5	1·7	1·1	1·6

penetrate the brain tissue before the neostigmine. The results of two such experiments are given in Table 3. The anterior horn is first perfused for 1 hr with morphine 1/400,000 before the neostigmine 1/50,000 is added to the perfusing fluid as well; the perfusion is then continued for 5 hr. The table gives the acetylcholine output for the ten successive ½ hr samples collected during this 5 hr period. Some acetylcholine appears in the first sample and the amounts increase as perfusion continues. The mean output rises from 0·3 ng/min in the first, to 1·4 ng/min in the last sample. The depression produced by morphine in the acetylcholine output is thus not appreciably greater than in the experiments of Table 2.

It is also not possible to suppress completely the appearance of acetylcholine in the effluent by increasing the morphine concentration in the perfusion fluid. The result of such an experiment in which the concentration was increased eight times, to 1/50,000, is included in Table 5. In this experiment the perfusion with morphine was preceded by perfusion with chloralose 1/25,000.

TABLE 3. Acetylcholine output from the anterior horn of a lateral ventricle during 5 hr perfusion with both neostigmine methyl sulphate 1/50,000 and morphine sulphate 1/400,000, following a preliminary perfusion for 1 hr with morphine sulphate alone

Min	ACh output in ng/min in successive samples		Mean
30	0·3	0·3	0·3
60	0·5	0·6	0·6
90	1·1	0·7	0·9
120	1·2	0·6	0·9
150	1·3	0·7	1·0
180	1·3	1·0	1·2
210	1·5	0·8	1·2
240	1·4	1·0	1·2
270	1·5	1·0	1·3
300	1·4	1·3	1·4

TABLE 4. Acetylcholine output from the anterior horn of a lateral ventricle during 5 hr perfusion with neostigmine methyl sulphate 1/50,000. Intravenous injection of morphine sulphate 2 mg/kg after the first 2½ hr perfusion

Min	ACh output in ng/min in successive samples					Mean
30	0·6	1·0	0·9	0·7	0·8	0·8
60	0·8	0·7	0·9	0·6	1·3	0·8
90	1·0	1·0	0·9	1·0	1·2	1·0
120	0·9	0·8	1·5	1·5	1·6	1·3
150	1·6	0·7	—	2·7	1·9	1·7
	Intravenous injection of morphine 2 mg/kg					
180	1·4	0·7	0·8	1·8	1·4	1·2
210	0·8	0·8	0·8	2·1	1·4	1·2
240	0·8	0·8	1·9	2·3	1·6	1·5
270	0·9	1·2	1·8	3·0	1·7	1·7
300	0·9	1·2	1·9	3·0	1·8	1·8

Morphine inhibits the acetylcholine output also when given intravenously. Table 4 gives the results of four experiments in which the anterior horn is perfused for 5 hr with neostigmine and the morphine (2 mg/kg) is injected after the first 2½ hr perfusion. The injections of morphine depressed the acetylcholine output to about the same extent as in the experiments in which morphine was added to the perfusion fluid. One effect of the injections was pronounced slowing of respiration which necessitated artificial ventilation in two experiments; there were no signs of excitation.

Chloralose. Perfused through the anterior horn, chloralose has an effect like morphine on the acetylcholine output, but only when perfused in strong concentrations. Such perfusions did not produce changes in respiration nor any other overt symptoms in the anaesthetized cat.

TABLE 5. Acetylcholine output from the anterior horn of a lateral ventricle during 5 and 6½ hr perfusion with neostigmine methyl sulphate 1/50,000. During the second 2½ hr the perfusing fluid contained chloralose 1/25,000 as well, which in the one experiment was substituted by morphine sulphate 1/50,000 for the last 1½ hr perfusion

Min	ACh output in ng/min in successive samples		Mean
30	1·3	0·7	1·0
60	1·5	0·7	1·1
90	1·6	0·8	1·2
120	1·5	1·3	1·4
150	2·2	1·8	2·0
	Chloralose 1/25,000 included in perfusion fluid		
180	2·1	1·8	2·0
210	2·3	2·0	2·2
240	2·9	2·0	2·5
270	3·9	2·4	3·2
300	4·3	3·0	3·7
	Morphine 1/50,000 included in perfusion fluid		
330	—	1·8	—
360	—	1·8	—
390	—	2·1	—

TABLE 6. Acetylcholine output from the anterior horn of a lateral ventricle during 5 hr perfusion with neostigmine methyl sulphate 1/50,000. During the last 2½ hr the perfusing fluid contained chloralose 1/10,000 as well

Min	ACh output in ng/min in successive samples				Mean
30	1·3	0·7	0·6	1·3	0·9
60	1·7	1·0	0·7	1·2	1·1
90	2·7	1·0	0·7	2·2	1·6
120	3·3	1·0	0·8	2·2	1·8
150	4·0	1·1	1·0	2·8	2·2
	Chloralose 1/10,000 included in perfusion fluid				
180	3·3	1·3	1·1	2·5	2·1
210	2·9	1·1	1·2	2·1	1·8
240	2·7	1·0	1·3	1·8	1·7
270	2·9	1·3	1·5	—	1·9
300	2·8	1·6	1·7	—	2·0

On perfusion with chloralose in a concentration of 1/25,000, the acetylcholine output is scarcely affected, if at all. Two such experiments are given in Table 5. On the other hand, perfusion with chloralose 1/10,000

inhibits the output to about the same extent as morphine 1/400,000, as shown by comparison of the results in Table 6 with those in Table 2. In the four experiments of Table 6, the mean acetylcholine output rises from 0·9 to 2·2 ng/min during the first 2½ hr period of perfusion with neostigmine alone, but there is no further rise, in fact the mean output decreases slightly, during the following 2½ hr when the perfusing fluid contained chloralose 1/10,000 as well. Chloralose has a depressant effect on the acetylcholine output also when given intravenously to deepen anaesthesia (Beleslin, Carmichael & Feldberg, 1964).

DISCUSSION

The present experiments were performed to find out if morphine, which inhibits the release of acetylcholine from the guinea-pig small intestine, has a similar action on the central nervous system. The results show that morphine reduces the output of acetylcholine from the perfused anterior horn of a lateral ventricle when it is either added to the perfusion fluid, or injected intravenously and so reaches the brain from the blood stream. As the acetylcholine which enters the anterior horn is mainly derived from the caudate nucleus (Beleslin, Carmichael & Feldberg, 1964) the effect of morphine must be mainly on this structure. However, in experiments carried out with Dr D. H. Sproull (Beleslin, Polak & Sproull, 1965) we have found that morphine apparently has a similar effect on the cerebral cortex since it reduces the acetylcholine output into the perfused subarachnoid space. So the effect appears to be a more general one and not confined to a particular part of the brain.

The effect of morphine on the acetylcholine output is not specific. Chloralose has the same effect, but only when perfused through the anterior horn in much stronger concentrations. This difference may reflect the fact that morphine is a more potent substance than chloralose, and produces central effects in much smaller doses. The effect of chloralose is not entirely new. It was known that deepening the anaesthesia by intravenous chloralose reduced the acetylcholine output from the cerebral cortex (MacIntosh & Oborin, 1953; Mitchell, 1963) as well as into the perfused anterior horn (Beleslin, Carmichael & Feldberg, 1964).

The effect of morphine could be the result of increased destruction, of diminished formation or of diminished release of acetylcholine. An increased destruction could only be assumed if morphine were able to reactivate cholinesterase that has been inhibited by neostigmine. There is no evidence that morphine has such an action. Concerning the effect of morphine on synthesis of acetylcholine the results are equivocal. Torda & Wolff (1946, 1947) found depression of choline acetylase activity but

only with extremely strong concentrations of morphine. On the other hand, neither Kumagi, Ebashi & Takeda (1954), nor Schaumann (1957), obtained evidence for such an effect of morphine. It is therefore more likely that the reduction produced by morphine in the output of acetylcholine from the perfused cerebral ventricles, or from the perfused subarachnoid space, results from the same mechanism which is effective on the guinea-pig intestine, i.e. inhibition of the release of acetylcholine, and the same conclusion applies to the effect of chloralose. This view is supported by the changes in the acetylcholine content of the brain produced by morphine and anaesthetics, if we assume that inhibition of the release results in a rise of the acetylcholine content. It has been known for some time that in anaesthesia the acetylcholine content of the brain increases (Tobias, Lipton & Lepinat, 1946; Richter & Crossland, 1949; Elliott, Swank & Henderson, 1950; Wajda, 1951) and the same has recently been shown to occur after morphine (Giarman & Pepeu, 1962; Hano, Kaneto, Kakunaga & Moribayashi, 1964).

The present experiments were carried out under anaesthesia and, as in this condition perfusion of the cerebral ventricles with morphine did not produce overt symptoms in the cat, the results do not enable us to make definite statements concerning the significance of the inhibition of acetylcholine release for the specific central actions of morphine.

One explanation, however, of the inhibitory effect of morphine as well as of anaesthetics on the release of acetylcholine would be depression of activity in cholinergic internuncials. Interneurones appear to be particularly susceptible to the action of anaesthetics as pointed out by various authors (Bremer, 1937; Bárány, 1947; Funderburk, King & Unna, 1951; French, Verzeano & Magoun, 1953; King, 1956) and according to Wikler (1944) the ultimate locus of the depressant action of morphine may also be on internuncials.

SUMMARY

1. In cats anaesthetized with chloralose the anterior horn of the left lateral ventricle was perfused with neostigmine 1/50,000 and the acetylcholine in the effluent was determined in successive ½ hr samples.

2. Acetylcholine appears in the effluent and the amounts increase on prolonged perfusion. During 5 hr perfusion the mean acetylcholine output per minute rises from 0·8 ng in the first, to 1·9 ng in the fifth, and to 5 ng in the tenth sample.

3. If after 2½ hr perfusion with neostigmine alone morphine 1/400,000 is added to the perfusing fluid as well, or 2 mg/kg morphine are injected intravenously, the output of acetylcholine does not continue to rise.

4. It is not possible to suppress completely the acetylcholine output by

either perfusing the anterior horn first with morphine alone and then with morphine and neostigmine, or by increasing the morphine concentration in the perfusing fluid.

5. Chloralose has an action similar to that of morphine on the acetylcholine output but has to be added to the perfusing fluid in much stronger concentration, i.e. 1/10,000, to produce an effect like that of morphine 1/400,000.

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