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Effects of narcotic analgesics and antagonists on the *in vivo* release of acetylcholine from the cerebral cortex of the cat

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Summary

1. In cats under light allobarbitone anaesthesia, the effects of intravenous injections of narcotic and non-narcotic analgesics, of a general depressant, and of narcotic antagonists were investigated on the spontaneous release of acetylcholine (ACh) from the surface of the sensorimotor cortex.

2. The narcotic analgesics morphine (0·1, 1·0 and 5 mg/kg), meperidine (1·0 and 2·0 mg/kg), methadone (1·0 mg/kg) and codeine (5·0 and 10·0 mg/kg) greatly reduced ACh release.

3. The non-narcotic analgesics pentazocine (1·0 and 2·0 mg/kg) and propoxyphene (5·0 and 10·0 mg/kg) as well as the depressant chlorpromazine (0·25, 0·5 and 1·0 mg/kg) also greatly reduced ACh release.

4. Two of the three narcotic antagonists examined, levallorphan (0·1, 1·0 and 5 mg/kg) and nalorphine (1·0 mg/kg) had the property of reducing ACh release. They were thus partial agonists. With levallorphan the greatest reduction occurred with the smallest dose injected and the effect was regularly obtained, whereas with nalorphine a reduction was obtained in some experiments only. The third, naloxone, was a specific narcotic antagonist and did not reduce the ACh release in any dose (0·01, 0·1, 0·5 and 1·0 mg/kg) examined. In a dose of 1·0 mg/kg it actually produced a small increase in Ach release.

5. Naloxone (0·1–1·0 mg/kg) restored the reduction in ACh release produced by the narcotic analgesics and by the partial agonist levallorphan. It partially restored the reduction produced by the non-narcotic analgesics and by nalorphine, but had no effect on the reduction produced by chlorpromazine.

6. The relevance of these results with regard to analgesia and to the narcotic abstinence syndrome is discussed.

Introduction

Evaluation of the action of narcotic analgesics such as morphine on cholinergic neurones has been the subject of several studies aimed at elucidating the mechanisms of action of narcotic agents. Some of these studies on peripheral cholinergic

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junctions have shown that morphine inhibits the release of acetylcholine (ACh). Both Schaumann (1957) and Paton (1957) showed that morphine impairs the release of ACh from the electrically stimulated guinea-pig ileum. Morphine also inhibits release of ACh from the cat superior cervical ganglion (Pelikan, 1960) and more recently it has been found to produce a similar effect at the neuromuscular junction in frog sartorius and rat diaphragm muscles (Frederickson & Pinsky, 1971 and unpublished observations). Other narcotic analgesic drugs related structurally to morphine decrease the contractor response of the guinea-pig ileum to electrical stimulation (Cox & Weinstock, 1966 ; Gyang & Kosterlitz, 1966). The kinetic parameters of various narcotic analgesics were investigated in some detail by Kosterlitz & Watt (1968) who classified these agents in terms of their apparent effect on morphine receptors in the ileum. According to this classification analgesic drugs such as morphine, codeine, meperidine, and methadone are defined as agonists ; nalorphine and levallorphan as partial agonists (and partial antagonists), and a drug such as naloxone is classified as a true antagonist.

It has been suggested that the brain contains an ascending system of cholinergic neurones (Shute & Lewis, 1967) which releases ACh from the cerebral cortex (MacIntosh & Oborin, 1953 ; Mitchell, 1963 ; Phillis, 1968), and it is conceivable that morphine modifies the release of this substance at the level of the cortex. Although considerable attention has been focussed on the diverse actions of morphine in the CNS, its effect on the release of putative transmitter substances, such as ACh, has received relatively little study. Beleslin & Polak (1965) perfused the cerebral ventricles in cats with artificial cerebrospinal fluid (c.s.f.) and found that the addition of morphine to the perfusion fluid led to a modest decrease in the ACh output of the effluent. Subsequently, Sharkawi & Schulman (1969) have shown that morphine inhibits the potassium-induced release of radioactive labelled ACh from rat brain cortex slices. Recent experiments conducted in this laboratory indicate that the spontaneous release of ACh from the cat cerebral cortex *in vivo* is impaired by morphine, either injected intravenously or applied topically to the cortical surface. A similar inhibitory action on ACh release was produced by a partial agonist such as levallorphan, but not by a true morphine antagonist such as naloxone (Jhamandas, Pinsky & Phillis, 1970). The investigation described here is an extension of this work and compares the action of morphine on release of cortical ACh with that of other agonists such as codeine, meperidine, methadone, and the effects of narcotic antagonists such as levallorphan, nalorphine and naloxone. It also includes information about the impairment of ACh release by the analgesics propoxyphene and pentazocine, which are classified as non-narcotic substances on the basis of their low addiction potential (W.H.O. Expert Committee on Drug-Dependence, 1964, 1966). The effect of chlorpromazine on cortical release of ACh has been examined as well. This drug produces analgesia in animal tests (Cahn & Herold, 1967 ; Silvestrini & Quadri, 1970).

The initial aim of this study was to evaluate the effect of narcotic analgesics on ACh release from the cerebral cortex and to ascertain whether alterations in the activity of the ascending cholinergic system in the brain could be implicated in analgesia. It became apparent that the specific morphine antagonists caused a rapid and complete reversal of the morphine effect on ACh release. These effects may be implicated in the withdrawal syndrome precipitated by such antagonists in morphine addicted animals.

Methods

Experiments were carried out on fifty-five cats of either sex weighing 2·5–4·0 kg. The animals were anaesthetized initially with intravenous sodium thiopentone and this anaesthetic was continued until the termination of surgery, after which they were maintained for the duration of experiment under light allobarbitone anaesthesia (Dial Compound, CIBA Ltd., 0·4 ml/kg).

A tracheal cannula was placed in all animals. The cephalic vein in one of the forelimbs was exposed, and a polyethylene catheter inserted for drug administration. The animal was placed in a stereotaxic frame, the muscle and skull base overlying the sensorimotor cortex removed bilaterally. The dura mater was resected to expose the sensorimotor cortices. Perspex cylinders, of inside diameter 10 mm, were placed bilaterally on the sensorimotor cortices and all exposed areas were covered with a 2–3 mm thick layer of 4% agar in physiological saline. A non-leaking seal between the cups and the cortical surface was made by coating the lower rims with Dow Corning Silicone adhesive which, after curing, formed a soft flexible rim on the cylinders. The cups were filled with artificial c.s.f. (Na^+, 152·8; K^+, 2·65; Ca^{++}, 1·05; Cl^-, 157·6 mEq/l. and glucose 763 mg/l.) containing 5×10^{-5} g/ml neostigmine bromide. This solution was left in contact with the cortex for 30 minutes. After this the cortical surface was irrigated several times with the same solution and the cups were filled with 0·4 ml of artificial c.s.f. This solution was collected after 15 min and discarded. Subsequent collections were made at 15 min intervals and assayed biologically for acetylcholine. All drugs were administered intravenously and a 5 min wash period was allowed after each drug injection.

Assay procedure

The solutions collected from the sensorimotor cortex were assayed on hearts of the bivalve mollusc, *Mercenaria mercenaria.* The identification of the inhibitory factor as acetylcholine has been described in earlier papers (Phillis, 1968; Jhamandas *et al.*, 1970). The molluscs were obtained from Pacific Biomarine Supply Co. (Venice, California) and kept in a refrigerated aquarium (14–15° C) filled with recirculating artificial sea water (Instant Ocean, supplied by Aquarium Systems, Ohio). The hearts performed best when molluscs had been kept 2–3 weeks before use. If used during the period immediately after shipment the hearts were usually sensitive to mechanical disturbances.

Threads were attached to both atrio-ventricular junctions and the hearts suspended in 1 ml baths. Cardiac contractions were recorded on an ink writing recorder (Grass, 5B) via a strain gauge transducer (Grass FT-03). A short helical coil of fine wire, inserted between the heart and transducer, allowed the tissue to contract isotonically. Control solutions of acetylcholine, and unknown solutions collected from the cortical surface, were injected into the bath through a fine hypodermic needle. Two or three hearts were mounted side-by-side and each solution was tested simultaneously on all hearts. Threshold sensitivity of the hearts to acetylcholine ranged from 2×10^{-10} to 10^{-9} g/ml.

Drugs

The drugs used were morphine sulphate (B.D.H.), meperidine hydrochloride (Demerol, Winthrop Laboratories), methadone hydrochloride (Methadone, Pitman-

Moore Ltd.), codeine phosphate (B.D.H.), levallorphan tartrate (Lorfan, Hoffman-La Roche Ltd.), nalorphine (Nalline, Charles E. Frosst & Co.), naloxone hydrochloride (Endo Drugs Ltd.), pentazocine lactate (Talwin, Winthrop Laboratories), propoxyphene hydrochloride (Darvon, Eli Lilly & Co.), chlorpromazine hydrochloride (Largactil, Poulenc Ltd.).

Results

Spontaneous release of ACh

In cats maintained under light allobarbitone anaesthesia there was a spontaneous release of ACh into the cups overlying the sensorimotor cortex. The rate of this resting release from the cortical surface varied from animal to animal and also fluctuated in a single animal but in the main only with regard to the first three or four periods of collection. The mean rates of release of ACh recorded during three successive 15 min collection periods from twenty-six cortical hemispheres were $(2{\cdot}66 \pm 0{\cdot}40$ ng/min)/cm^2, $(3{\cdot}50 \pm 0{\cdot}43$ ng/min)/cm^2 and $(3{\cdot}90 \pm 0{\cdot}38$ ng/min)/cm^2. The effects of drugs were examined only when the level of resting ACh release was stable and not less than (2·0 ng/min)/cm^2.

Morphine, meperidine, methadone and codeine

The four analgesic agonists with narcotic action decreased the resting output of ACh from the sensorimotor cortex. Table 1 shows the mean decrease in the first and second 15 min period of collection after the injection of 1 mg/kg of either morphine, meperidine, or methadone, and of 5 mg/kg codeine. With morphine, a consistent effect was obtained with as little as 0·1 mg/kg, whereas

TABLE 1. *Effect of morphine and morphine-like drugs on the release of acetylcholine from the sensorimotor cortex in the cat*

Drug (mg/kg)	Control*	ACh release ((ng/min)/cm^2) In first 15 min after drug	P	In second 15 min after drug	P	Degree of reversal by naloxone†
Morphine sulphate (1·0) (*n*=12)‡	3·41±0·74	1·30±0·25	<0·02	0·76±0·16	<0·005	++
Meperidine hydrochloride (1·0) (*n*=8)	3·24±0·60	1·37±0·33	<0·02	0·71±0·11	<0·005	++
Methadone hydrochloride (1·0) (*n*=6)	3·84±0·47	1·42±0·31	<0·005	1·05±0·15	<0·001	++
Codeine phosphate (5·0) (*n*=4)	4·28±0·14	3·06±0·71	<0·20	1·12±0·40	<0·001	++
Pentazocine lactate (2·0) (*n*=6)	4·20±0·30	1·61±0·35	<0·001	1·46±0·17	<0·001	+
Propoxyphene hydrochloride (5·0) (*n*=4)	4·23±0·66	2·87±0·73	>0·20	2·25±0·63	<0·05	+
Chlorpromazine hydrochloride (1·0) (*n*=6)	5·44±0·84	2·99±0·21	<0·02	1·94±0·18	<0·005	−
Levallorphan tartarate (0·1) (*n*=6)	2·94±0·87	0·74±0·27	<0·05	0·91±0·38	<0·05	++
Nalorphine hydrochloride (1·0) (*n*=10)	2·38±0·42	3·16±0·99	>0·4	3·40±0·99	>0·3	
Naloxone hydrochloride (1·0) (*n*=6)	3·40±0·40	4·27±0·56	>0·2	4·53±0·47	>0·10	

* Control release represents average of release during two 15 min periods immediately preceding drug administration. Post-drug collections commenced 5 min after injection. Mean values ± S.E.
† Naloxone injected 30–45 min after administration of the agonist, (++) indicates a complete restoration of ACh to predrug values, (+) indicates a partial reversal, (−) indicates non-reversal.
‡ *n*=Number of cortical hemispheres.

codeine was the least potent of all and had to be injected in doses of 5–10 mg/kg. Its effect also differed from that of the other three substances in that it was more delayed; the decrease in the ACh release was only small during the first 15 min after injection. Typical experiments are shown for each of the four drugs in the histograms of Figs. 1–4.

In the experiment of Fig. 1, the output of ACh from the right cortex decreased from 2·0 to (0·4 ng/min)/cm² in the first 15 min after the injection of 1 mg/kg morphine and then remained at this level during the subsequent two collections. Essentially the same effect was obtained in the left cortex. In the experiment of Fig. 2, an injection of 1 mg/kg meperidine reduced the ACh release in the right sensorimotor cortex from 4·0 to 1·3 (ng/min)/cm² in the first 15 min and a second injection of the same dose of meperidine reduced the output to (0·3 ng/min)/cm². Again, an essentially similar result was obtained in the left cortex. In the experiment of Fig. 3, the injection of 1 mg/kg methadone decreased the resting ACh release in the right sensorimotor cortex from 5·5 to 2·0 (ng/min)/cm² in the first, and to (0·8 ng/min)/cm² in the fourth collection period; in the left sensorimotor cortex the control value of 6·0 was reduced to 2·8 and (1·0 ng/min)/cm² in the corresponding two collection periods after the injection. As shown in Fig. 4, the release of ACh from both cortices was hardly affected in the first 15 min after the injection of 5 mg/kg codeine but remained near the control value of (4·5 ng/min)/cm². However, in the following 30 min, the release fell in the right cortex to 2·0 and in the left, to (1·0 ng/min)/cm².

Pentazocine, propoxyphene and chlorpromazine

These three 'non-narcotic' analgesics also depressed the ACh release, and in Table 1, their effects are compared with those of the four analgesic agonists with

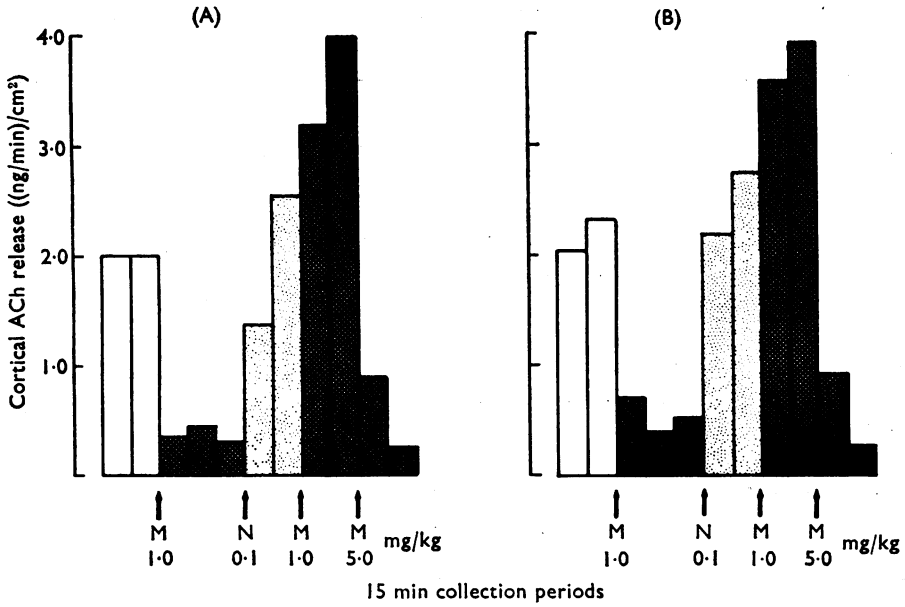

FIG. 1. Rates of ACh release from right (A) and left (B) sensorimotor cortices before and after intravenous morphine (M) and naloxone (N), in the doses indicated below the histogram. Five minutes' break between collections after each drug injection.

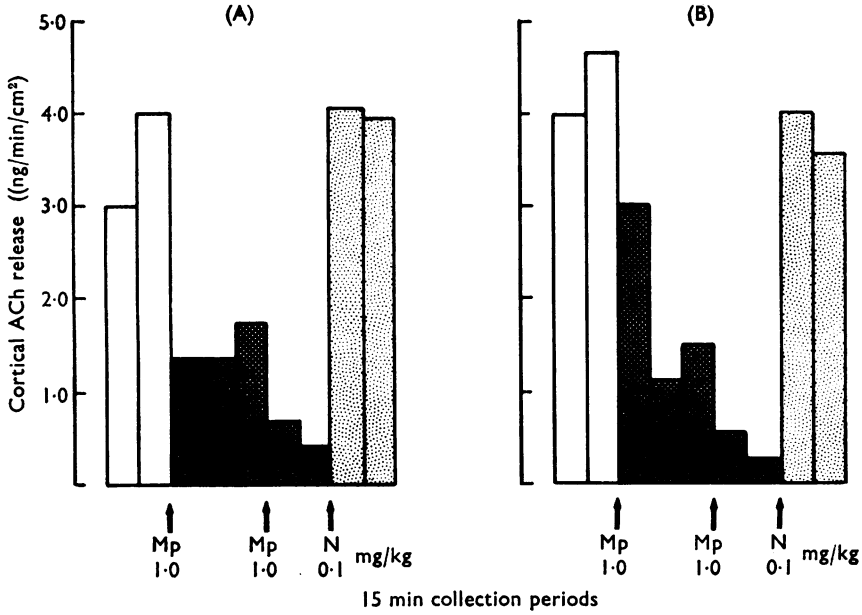

FIG. 2. Rates of ACh release from right (A) and left (B) sensorimotor cortices before and after intravenous meperidine (Mp) and naloxone (N), in the doses indicated below the histogram. Five minutes' break between collections after each drug injection.

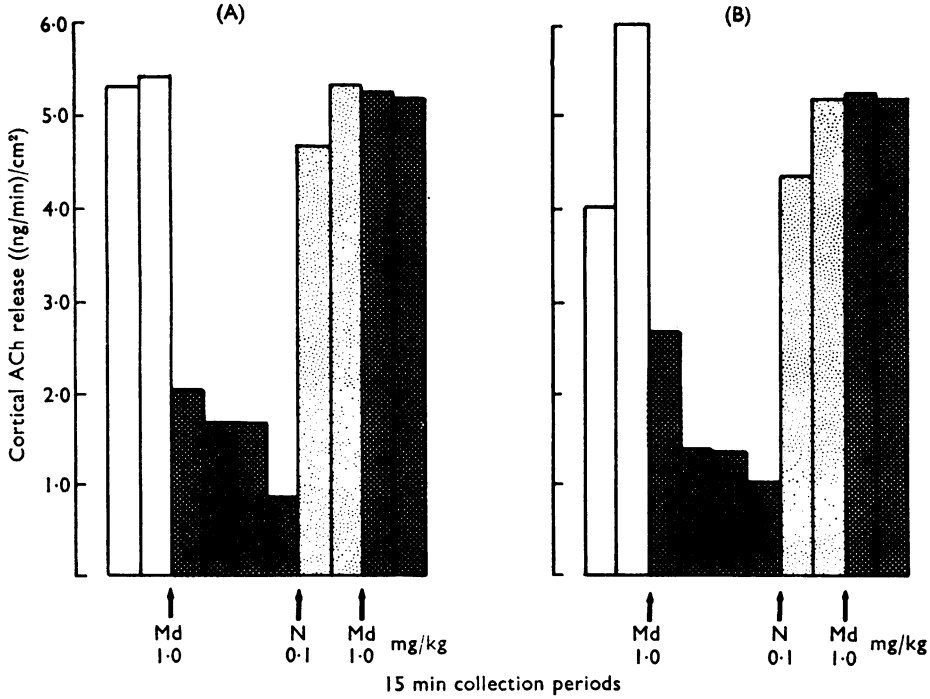

FIG. 3. Rates of ACh release from right (A) and left (B) sensorimotor cortices before and after intravenous methadone (Md) and naloxone (N), in the doses indicated below the histogram. Five minutes' break between collections after each drug injection.

narcotic action. With 1 mg/kg pentazocine the depression was not long lasting. This is shown in the experiment Fig. 5 in which the resting release of ACh was reduced in the first 15 min from 5·4 to 1·3 (ng/min)/cm² in the right, and from 4·8 to 1·8 (ng/min)/cm² in the left cortex, but had risen beyond the preinjection level in the third collection period. A second injection, this time of 2 mg/kg pentazocine, produced a greater depression of the ACh release which fell to 0·5 in the right, and to (0·9 ng/min)/cm² in the left cortex.

To obtain a decrease in the release of cortical ACh, with propoxyphene, it had to be injected in doses of 5 or 10 mg/kg. Then, like codeine, it had no great effect in the first 15 min, but caused a reduction in the subsequent collection periods.

Chlorpromazine decreased the ACh release when given in doses of 0·25, 0·5 and 1 mg/kg. In the experiment of Fig. 6, the injection of 1 mg/kg greatly reduced the ACh output from both cortices. The output fell during the three collection periods in the right cortex from 4·0 to 2·3, 2·0 and (0·9 ng/min)/cm², and in the left cortex from 5·0 to 3·0, 1·5 and (0·9 ng/min)/cm².

Naloxone, nalorphine and levallorphan

Of these three narcotic antagonists only levallorphan caused a great reduction in the release of ACh and only when given in a small dose (0·1 mg/kg). Naloxone and nalorphine on the other hand, caused a small increase in the output of ACh as shown for the mean values given in Table 1 for six experiments with 1 mg/kg

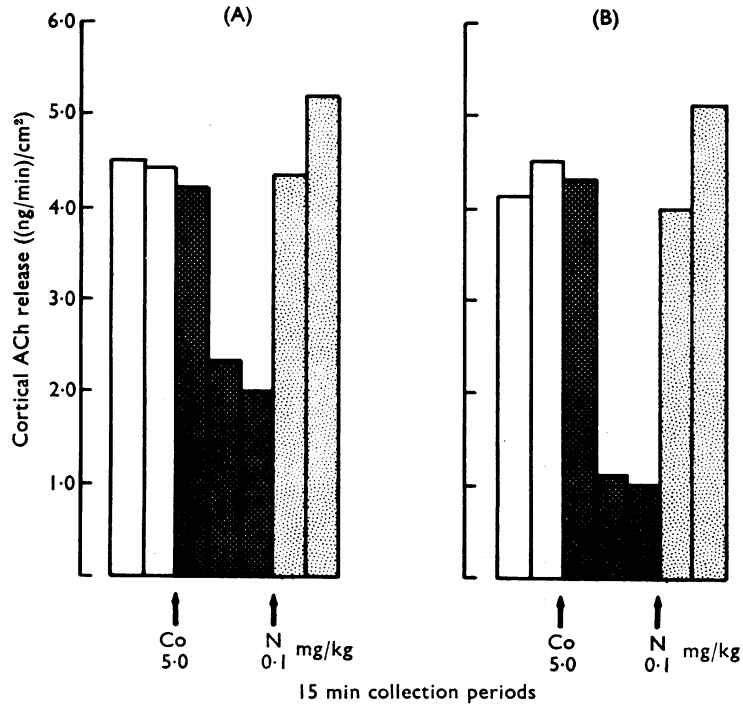

FIG. 4. Rates of ACh release from right (A) and left (B) sensorimotor cortices before and after intravenous codeine (Co) and naloxone (N), in doses indicated below the histogram. Five minutes' break between collections after each drug injection.

of naloxone and for ten with 1 mg/kg of nalorphine. The increases, however, were not significantly greater than those which occurred spontaneously, and in two experiments with nalorphine the resting output of ACh actually decreased somewhat. No decrease, however, was obtained in any of the experiments with naloxone, even when given in smaller doses.

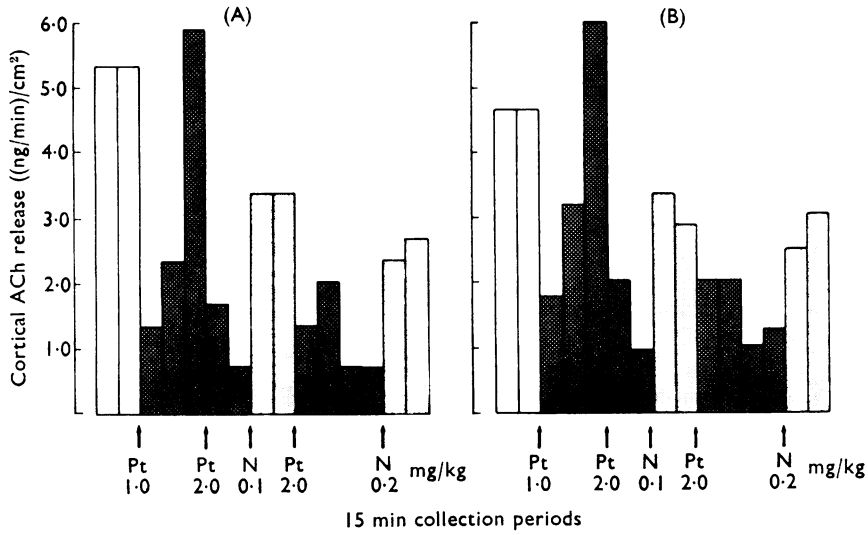

FIG. 5. Rates of ACh release from right (A) and left (B) sensorimotor cortices before and after intravenous pentazocine (Pt) and naloxone (N), in doses indicated below the histogram. Five minutes' break between collections after each drug injection.

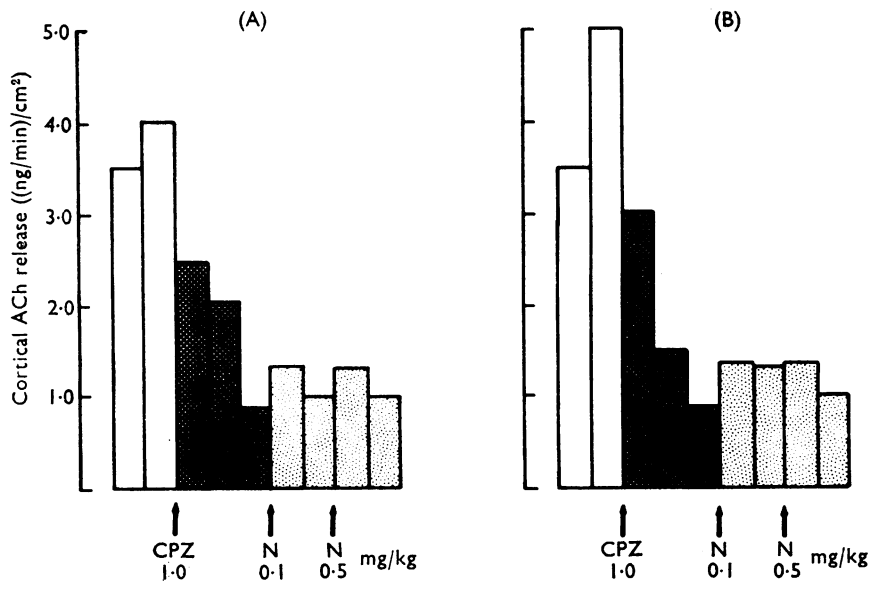

FIG. 6. Rates of ACh release from right (A) and left (B) sensorimotor cortices before and after intravenous chlorpromazine hydrochloride (CPZ) and naloxone (N), in doses indicated below the histogram. Five minutes' break between collections after each drug injection.

Figure 7 illustrates the results of an experiment in which 0·01, 0·1 and 1 mg/kg naloxone were injected intravenously. The release of ACh in the right cortex was hardly affected by these injections and that from the left was slightly increased after the first injection. Naloxone was thus ideally suited for testing against the agonists with and without narcotic action, and most of the tests were carried out with this antagonist. Naloxone was also tested against the two antagonists levallorphan and nalorphine as they reduced the resting ACh release from the cortex and were thus partial agonists as well.

Table 1 gives the mean values from six experiments with levallorphan. They show that the reduction in ACh after an injection of 0·1 mg/kg levallorphan was of the same order as that produced by 1 mg/kg morphine, meperidine, or methadone. This is also evident from the experiment of Fig. 8 in which the ACh release fell in the first 15 min collection after 0·1 mg/kg levallorphan from 5·8 to (1·9 ng/min)/cm² in the left, and from (5·7 to 1·4 ng/min)/cm² in the right cortex. The figure further illustrates that this reduction was fully restored by a subsequent injection of 1 mg/kg naloxone. The ACh output rose to the control values in the first 15 min collection after this injection of naloxone, and continued to rise in the subsequent collections. In experiments in which levallorphan was injected in larger doses than 0·1 mg/kg, for instance, in doses of 1 or 5 mg/kg, the reduction in ACh output was only small, but it was again abolished by naloxone. Thus naloxone acted as an antagonist to levallorphan. The reduction observed in the two experiments with 1 mg/kg nalorphine was counteracted only partly by 1 mg/kg naloxone.

Effect of naloxone, nalorphine and levallorphan on the reduction in ACh release produced by the analgesic agonists with and without narcotic action

Agonists with narcotic action

The reductions in ACh output produced by morphine, methadone and codeine, were restored and prevented by the antagonists as shown for naloxone in the following figures.

Figure 1 illustrates the morphine-naloxone antagonism in an experiment in which the injection of 1 mg/kg of morphine had reduced the ACh release in the right sensorimotor cortex from 2·0 to (0·4 ng/min)/cm² in the first 15 min and the release had remained at this low level until 0·1 mg/kg naloxone was injected. The ACh release then rose to 1·4 in the first, and to (2·5 ng/min)/cm² in the second collection period. At this stage, a second injection of 1 mg/kg morphine failed to depress the ACh release; instead the release continued to rise to (4·0 ng/min)/cm². The injection of a larger dose of morphine (5 mg/kg), however, was effective and produced a great depression: the release of ACh fell to (0·3 ng/min)/cm². Essentially the same result was obtained in the left sensorimotor cortex. Figure 7 shows, in another experiment, that the injection of 2 mg/kg morphine hardly reduced the ACh release in the sensorimotor cortices when given after three previous injections of 0·01, 0·1 and 1 mg/kg naloxone. Both nalorphine (2·5 mg/kg) and levallorphan (5 mg/kg) had the same effect as naloxone (0·1 mg/kg) on the depression of the ACh release produced by morphine. They quickly restored the ACh release to premorphine levels.

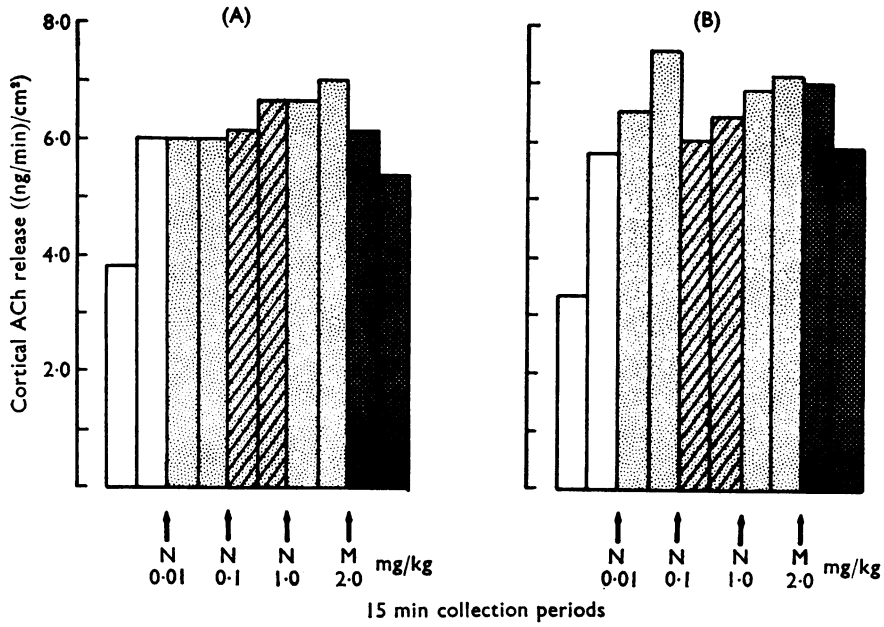

FIG. 7. Rates of ACh release from right (A) and left (B) sensorimotor cortices before and after intravenous naloxone (N) and morphine (M), in doses indicated below the histogram. Five minutes' break between collections after each drug injection.

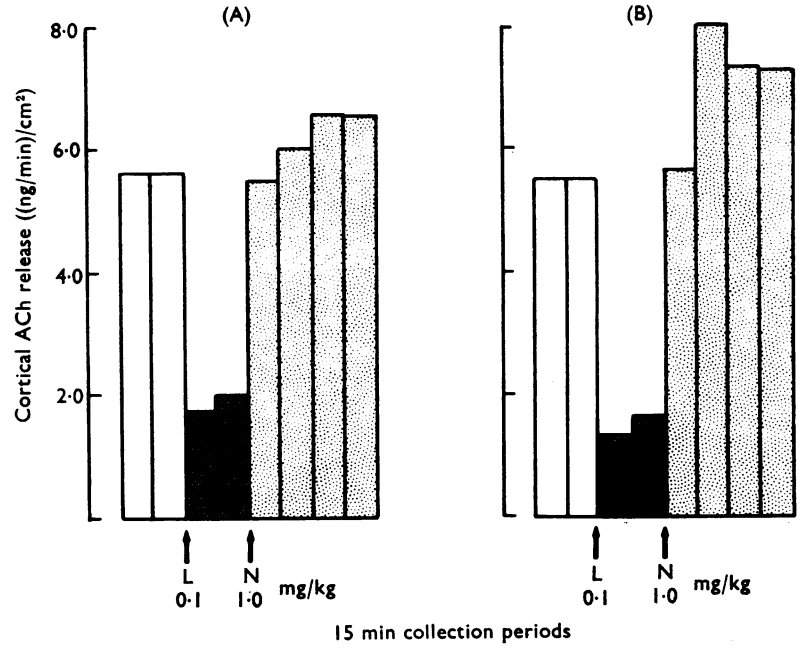

FIG. 8. Rates of ACh release right (A) and left (B) sensorimotor cortices before and after intravenous levallorphan (L) and naloxone (N), in doses indicated below the histogram. Five minutes' break between collections after each drug injection.

Figure 2 illustrates the meperidine-naloxone antagonism in an experiment in which two injections of 1 mg/kg meperidine had reduced the ACh release in the two sensorimotor cortices to 0·3 and (0·2 ng/min)/cm². The injection of 0·1 mg/kg naloxone resulted in immediate restoration of ACh to the premeperidine level of about (4 ng/min)/cm². In this experiment a subsequent injection of 1 mg/kg meperidine (not shown in the figure) no longer reduced the ACh release. To obtain a reversal of the depressant action of meperidine on the ACh release with nalorphine, this antagonist had to be injected in a dose of 2·5 mg/kg.

Figure 3 illustrates the methadone-naloxone antagonism in an experiment in which the injection of 1 mg/kg methadone had reduced the release of ACh in the sensorimotor cortices to 0·8 and (1·0 ng/min)/cm². Administration of 0·1 mg/kg naloxone caused an immediate reversal of the methadone-induced depression ; the ACh release rose to about (5 ng/min)/cm². A subsequent injection of 1 mg/kg methadone was ineffective in reducing the ACh release.

Figure 4 finally illustrates the codeine-naloxone antagonism in an experiment in which the injection of 5 mg/kg of codeine had reduced the ACh release of about (4·5 ng/min)/cm² to 2·0 in the right, and to (1·0 ng/min)/cm² in the left sensorimotor cortex. After the injection of 0·1 mg/kg naloxone the ACh release in both cortices was restored to the control levels in the first collection period, and in the second collection period the ACh release rose beyond the control levels.

Agonists without narcotic action

As indicated in the last column of Table 1, naloxone restored the depression of ACh release produced by pentazocine and propoxyphene to some extent only and had scarcely any effect on the depression of ACh release produced by chlorpromazine.

The partial pentazocine-naloxone antagonism is illustrated by the experiment of Fig. 5. Two injections of pentazocine, the first of 1 mg/kg, the second of 2 mg/kg, had reduced the ACh release in the right cortex from 5·4 to (0·6 ng/min)/cm², and in the left cortex from 4·7 to (0·9 ng/min)/cm². After the injection of 0·1 mg/kg naloxone the ACh release was restored to approximately (3·5 ng/min)/cm² in both cortices. The partial restoration was reversed by a further injection of 2 mg/kg pentazocine and again restored by a subsequent injection of naloxone, this time of 0·2 mg/kg. However, restoration in both cortices was only to about (3 ng/min)/cm². The depressant action of propoxyphene on ACh release was also partially reversed by naloxone (0·1 mg/kg) (Table 1).

Figure 6 illustrates an experiment in which naloxone was given after 1 mg/kg chlorpromazine had reduced the ACh release to less than (1 ng/min)/cm² in both cortices. Neither an initial injection of 0·1 mg/kg naloxone, nor later of 0·5 mg/kg, was able to reverse the action of chlorpromazine.

Discussion

The results of our investigation show that morphine, as well as morphine-like drugs such as meperidine, methadone, and codeine, depress the spontaneous release of acetylcholine from the cerebral cortex of a lightly anaesthetized cat. All four analgesics have been pharmacologically classified as narcotic agonists (Gyang & Kosterlitz, 1966). The relatively high dose of codeine required to produce a

depression of ACh release and the delay in onset of its action suggest that the action of codeine depends on its conversion to an active metabolite, or that its penetration into the brain occurs more slowly.

Antagonism of the depressant action on ACh release by naloxone, a true morphine antagonist (Kosterlitz, Lees & Watt, 1969) suggests a common site of action for the depressant action of morphine and the other three narcotic agents, but as suggested by Jhamandas, *et al.* (1970), more than one site of action is possible.

Levallorphan and nalorphine are classified according to Kosterlitz & Watt (1968) as partial agonists as well as antagonists of morphine. This dual action was also revealed in our experiments with regard to their action on the ACh release. In small doses levallorphan depressed release as much as morphine, and nalorphine exhibited a similar depressant action, but to a lesser extent, in a few experiments only. Yet both drugs antagonized the reduction in ACh release produced by morphine. On the other hand, the true antagonist, naloxone, had only this action without itself depressing the ACh release, whatever dose was injected. In large doses it increased the ACh release. This increase was superimposed on the resting release. The same effect was produced in some experiments with nalorphine. This increase is hard to assess on the basis of the present evidence; it is also not possible to say whether the same or different receptor sites are involved in the depressant and stimulating action of nalorphine on the ACh release. In this connexion it is interesting to note that nalorphine has a biphasic effect also on glucose utilization in mouse brain slices; first it inhibits and later it stimulates the glucose utilization (Howes, Harris & Dewey, 1970).

Pentazocine and propoxyphene have been classified as 'non-narcotic' agents on the basis of their low addicting potential, but there are reports of abuse liability with both drugs (Jaffe, 1970). In our experiments, both depressed the release of ACh to the same extent as morphine, but their depressant effect was reversed only partially by naloxone. It is interesting to note in this respect that nalorphine and levallorphan have been used clinically to counteract the toxic effects of propoxyphene but with limited success (Chapman & Walsek, 1962; Fraser, 1968; Gary, Mahler, De Myttenaere, Liggero, Scott, Mattusiak & Schreimer, 1968), and that naloxone has been successful in reversing the respiratory depression obtained with pentazocine by 60% or more (Kallos & Smith, 1968).

The third 'non-narcotic' analgesic, chlorpromazine, differed from pentazocine and propoxyphene in that the depression it produced on the ACh release was not reversed by naloxone. This strongly suggests that the site of action of chlorpromazine is not the same as that affected by morphine and the other narcotic and non-narcotic analgesics.

An increase in the ACh concentrations of the brain is an acute effect of morphine administration and has been observed by various authors (Hano, Kaneto, Kakunaga & Moribayashi, 1964; Crossland & Slater, 1968; Large & Milton, 1970). Crossland & Slater (1968) also found that morphine increased the bound ACh of brain tissue and decreased the free ACh. Increased synthesis of ACh in the brain can hardly be responsible for the rise in brain ACh since morphine has the opposite effect and decreases synthesis in the brain (Howes, *et al.* 1970). The rise is therefore most likely to be due to the effect shown in this investigation, that is, depression of the release of ACh, which probably occurs from the sites which bind ACh in nervous tissue.

Several investigations have been carried out to study the relationship between changes in the brain acetylcholine and the analgesic response produced by morphine (Maynert, 1967; Howes, Harris, Dewey & Voyda, 1969). Maynert (1967) has rejected the role of brain ACh in analgesia, chiefly on the ground that morphine-induced increase of ACh is not blocked by simultaneous administration of nalorphine, and that in rats tolerance does not develop to this action of morphine on repeated administration for 9 days. However, Crossland & Slater (1968) showed that nalorphine blocks the action of morphine on 'free' and 'bound' acetylcholine in the rat brain. Furthermore, Large & Milton (1970) found that tolerance develops to the morphine-induced increase in brain ACh of rats if the animals have been maintained on morphine injections for 6–10 weeks. In incubated mouse brain slices, Howes *et al.* (1970) found that not only morphine but also its antagonists, nalorphine and naloxone, decreased the ACh synthesis and the potassium-induced release of ACh. Since the effects were produced by morphine as well as by its antagonists they concluded that it was not related to their analgesic activity. The results of our investigation do not support this conclusion. They demonstrate a clear separation between drugs which have analgesic properties and those which have not. Those with analgesic properties, for example morphine, other agonists and partial agonists such as nalorphine and levallorphan, decreased the release of acetylcholine, whilst naloxone, which is practically devoid of analgesic activity (Blumberg, Dayton, George & Rapaport, 1961) did not have this action. It is thus tempting to suggest that a decrease in the release of ACh at some cholinergic synapses in the brain may be a component of the central actions produced by morphine.

The effect of an antagonist which reverses the depression in ACh release produced by morphine presumably involves removal or displacement of morphine from the tissue sites which it normally occupies, and this removal in its turn leads to the increase in ACh release. This increased ACh release in the brain may partly be responsible for the hyperactive state which follows acute withdrawal of morphine in animals and man. In view of this it is interesting to note that the use of ACh antagonists has been recommended to ameliorate the abstinence syndrome (Ramkhen, 1968) and that chlorpromazine, shown in the present experiments to suppress ACh release, has been used and recommended for the treatment of the narcotic withdrawal syndrome (Friedgood & Ripstein, 1955; Zelson, 1970).

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