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Effects of Morphine, Nalorphine and Naloxone on Neocortical Release of Acetylcholine in the Rat

John D. Matthews*, Gaston Labrecque**, and Edward F. Domino

Michigan Neuropsychopharmacology Research Program, Department of Pharmacology, University of Michigan, Ann Arbor, Michigan 48104

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Abstract. The effects of morphine (10 mg/kg), nalorphine (1 and 10 mg/kg), and naloxone (1 mg/kg) were studied on the neocortical release of acetylcholine (ACh) in midpontine pretrigeminal transected rats. Morphine and, to a lesser extent, nalorphine decreased ACh release. Naloxone was ineffective alone but antagonized the action of morphine.

Key words: Acetylcholine — Morphine — Nalorphine — Naloxone — Neocortex.

Introduction

There is good evidence that morphine and related narcotic agonists in large doses elevate total brain acetylcholine (ACh) in rats (Herken *et al.*, 1957; Giarman and Pepeu, 1964; Agarwal and Bhargava, 1964; Maynert, 1967) and mice (Hano *et al.*, 1964; Howes *et al.*, 1969). The increase in total brain ACh could be caused by inhibition of acetylcholinesterase (AChE). Although high concentrations of narcotic agonists and antagonists inhibit AChE *in vitro* (Berheim and Berheim, 1937; Young *et al.*, 1955; Foldes *et al.*, 1959; Hein and Powell, 1967) such a mechanism is probably not responsible for their *in vivo* effect on total brain ACh. The concentration of morphine required to produce AChE inhibition *in vivo* is achieved only after parenteral administration of nearly lethal doses (Johannesson, 1962). Enhanced choline acetyltransferase (ChAc) activity is also unlikely to explain the increase in total brain ACh found after narcotics. Inhibition of ChAc has been reported *in vitro* (Torda and Wolff, 1946; de la Lande and Bentley, 1955; Morris, 1961) as well as

* Present address: Loma Linda Medical School, Loma Linda, California.

** Predoctoral fellow with scholarships from Laval University and Province of Quebec, Canada. Present address: Department of Pharmacology, Laval University, Quebec City, Canada.

in vivo (Thal and Wajda, 1968; Datta *et al.*, 1971). Inhibition of ChAc would lower not elevate brain ACh.

Another possible mechanism by which morphine and related narcotic agonists elevate total brain ACh could be inhibition of ACh release from central cholinergic neurons. An ACh antirelease effect of morphine has been reported in cats (Mitchell, 1963) and rabbits (Beleslin and Polak, 1965; Beleslin *et al.*, 1965). Jhamandas *et al.* (1971) showed that this antirelease effect was shared by all narcotic agonists tested as well as by nalorphine, a well known mixed agonist-antagonist. The pure antagonist, naloxone, was devoid of any ACh antirelease action but antagonized the effect of the narcotic agonists.

Very recently Lundholm *et al.* (1972) and Pepeu *et al.* (1972) reported that ACh is released from the neocortex of the rat. There is no study of the effect of morphine and related narcotic agonists and antagonists on the release of ACh from rat neocortex. This paper presents the characteristics of ACh release from the neocortex of midpontine pretrigeminal transected rats as well as on the effect of morphine, nalorphine and naloxone on the neocortical release of ACh in analgesic and behaviorally effective doses.

Methods

Twenty-four adult male Holtzman rats weighing between 400 and 500 grams were used in these experiments. They were initially anesthetized with a diethyl ether-air mixture. When surgical anesthesia was achieved, a tracheotomy was performed and a small polyethylene tube inserted into the trachea. It was thus possible to administer additional amounts of anesthetic as required during surgery and to assist respiration. Respiratory assistance was necessary in only one case throughout all the experiments. The rats were then placed in a Stoelting rat stereotaxic apparatus. A midpontine pretrigeminal transection was performed by a special spatula followed by removal of the bone and the dura over the cerebrum in preparation for placement of the collecting cup. When surgery was complete, the rats were given about one hour to recover from the trauma.

The collecting cup was constructed of brass and was coated with the electrode insulating agent, compound 741. The cup covered an area of 0.38 cm^2, which included the following Broadman areas: 1, 2, 3, 4, 5, 6, 17, 18, 23, 29C (Krieg, 1946). The cup was filled with a 0.4 ml aliquot of mammalian Locke's solution containing (concentration in g/1): NaCl, 9.0; KCl, 0.42; $CaCl_2$, 0.24; $NaHCO_3$, 0.5; glucose, 1.0; and physostigmine sulfate, 0.1. Every 10 min the cup content was siphoned and immediately bioassayed for ACh using the dorsal muscle of the leech, according to the method of Murnaghan (1958).

The muscle was suspended in a 2 ml bath. The upper end of the muscle was connected to a small mirror which reflected a beam of light to a graduated scale approximately 5 feet away, allowing a 200 fold magnification.

The samples collected from the cups were diluted with distilled water (1 volume diluted to 1.4 volume) for the purpose of achieving inotonicity with leech Locke's

solution. The samples were then compared with standard solutions of ACh · Cl containing the same concentration of physostigmine. In order to assure that maximal cholinesterase inhibition had occurred in the area of the cortex beneath the cup, samples for the first 10 min interval were not assayed. Evidence that the substance released into the cup was either ACh or a very similar choline ester has been given previously (Mitchell, 1963; Szerb, 1963; Bartolini and Pepeu, 1967).

In all experiments, the narcotic agonists and antagonists were injected i.p. The doses (calculated as base) were chosen as in the analgesic and behaviorally effective range. They are known to affect rat brain ACh utilization (Domino and Wilson, 1972).

Results

Effects of Narcotic Agonists and Antagonists on the Release of ACh from the Neocortex

a) Characteristics of Spontaneous Release of ACh. It has previously been shown in midpontine pretrigeminal transected cats that ACh is spontaneously released from the neocortex into a cup filled with mammalian Locke's solution. Our results show that ACh is also released from the neocortex of rats when using the same preparation as with cats. Although the release of ACh varied from animal to animal, it increased linearly during collection periods of 5, 10 and 15 min, as shown in Fig. 1. The mean rates of ACh release $\pm$ S.E. during collections of 5, 10 and 15 were 2.60 $\pm$ 0.16 ng/min/cm^2 from 6 cups in 4 rats, 2.24 $\pm$ 0.09 ng/min/cm^2 from 59 cups in 22 rats, 2.30 $\pm$ 0.11 ng/min/cm^2 from 9 cups in 5 rats. A group comparison t test showed that there was no significant difference between any of the means ($P > 0.1$).

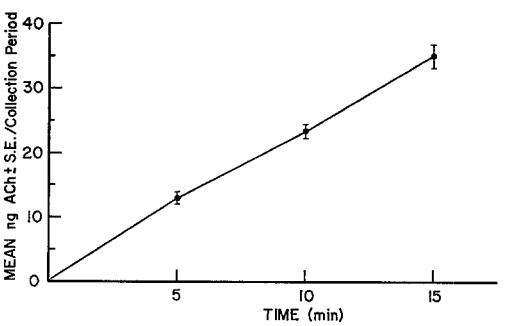

Fig. 1. Evidence of linear release of ACh from the rat neocortex. Each point represents the mean of at least 4 animals. The amount of ACh is expressed as total ACh release in ng/collection period/cm^2 of cortex. For convenience, a 10 min interval was used in the remaining study

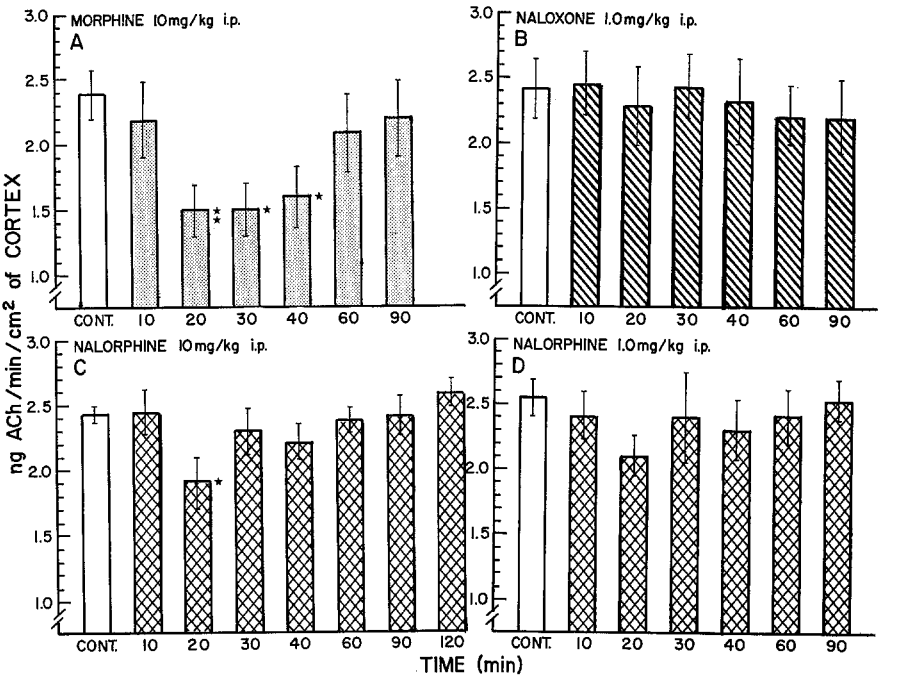

Fig. 2. Effects of morphine, nalorphine and naloxone on neocortical ACh release in the rat. The four bar graphs (A—D) indicate the time course of action of the various narcotics on mean ACh release. A minimum of 4 animals per collection period was used. The small vertical lines indicate $\pm$ S.E. A group comparison "t" test when significant from control is indicated by $^{*}P < 0.05$, $^{**}P < 0.01$. Symbols used also apply to Fig. 3

Since ACh release was linear over the first 15 min, a collection period of 10 min was chosen for convenience. The effect of narcotic agonists and antagonists on neocortical ACh release was determined only when the control level of ACh output was stable.

b) Effects of Narcotic Agonists and Antagonists. Intraperitoneal injection of morphine in a dose of 10 mg/kg produced a decrease in the release of ACh from neocortex. The time course of the anti-release action of morphine is shown in Fig. 2A. Twenty minutes after injection a nadir was reached. ACh release remained low for 40 min after injection. The mean $\pm$ S.E. of the amount of ACh released at 20 min was 1.46 ± 0.16 ng/min/cm², which represents a 35% decrease from the mean of the control values of 2.36 ± 0.19 ng/min/cm². Sixty minutes

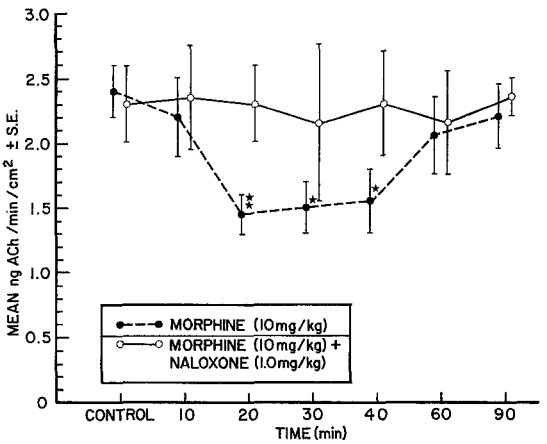

Fig. 3. Antagonism of morphine ACh antirelease by naloxone in the rat. Naloxone completely blocked the ACh antirelease effect of morphine as noted

after injection of morphine, ACh release was not significantly different from control ($0.3 > p > 0.2$).

The pure antagonist, naloxone, in a dose of 1 mg/kg did not significantly change the release of ACh through the 90 min sampling period. Fig. 2B shows the time course of events. The difference between the mean of the controls and the mean of any one of the collection periods after injections of the drug was not statistically significant ($P > 0.05$).

Nalorphine, a mixed antagonist-agonist, produced a slight decrease in the release of ACh from the neocortex when injected intraperitoneally in doses of either 1 or 10 mg/kg. Figs. 2C and 2D show that ACh release reached a maximum by 20 min after injection. This onset of action was the same as for morphine. The 1 mg/kg of nalorphine caused a 17.4% decrease in ACh release during the 10—20 min collection period, as compared with controls ($P < 0.1$), whereas the 10 mg/kg dose showed a 21.5% decrease ($P < 0.05$). Throughout the following 70 min, ACh release was not significantly different from controls.

c) Effect of Naloxone on the Reduction of ACh Release by Morphine. When naloxone (1 mg/kg) and morphine (10 mg/kg) were simultaneously injected intraperitoneally, the reduction in ACh release produced by morphine was essentially reversed. Fig. 3 shows the time course of morphine alone and with naloxone. The difference between the mean

for controls and the mean for any one of the collecting periods for naloxone-morphine was not statistically significant ($P > 0.7$).

Discussion

This preliminary study demonstrates conclusively that the acute pretrigeminal midpontine transected rat preparation is suitable for measuring the release of ACh from the neocortex. ACh release is linear with time. The total amount of ACh released is slightly higher than that obtained in the cat.

Evidence that morphine in reasonable analgesic doses reduces rat cortical release of ACh for about one hour, confirms similar results in the cat (Jhamandas *et al.*, 1971; Labrecque and Domino, unpublished observation, 1972). The data are also consistent with the observation that this dose of morphine is very effective in preventing brain ACh depletion induced by HC-3 (Domino and Wilson, 1972). An especially important observation is that in the rat, nalorphine also reduces ACh release, but the effect is transient with recovery in 30 min. This explains the failure of Domino and Wilson (1972) to show that nalorphine has an agonistic effect on brain ACh depletion in the rat. In contrast to the very short duration of action of nalorphine in the rat, in the cat it has a longer ACh antirelease effect than morphine (Labrecque and Domino, unpublished observation, 1972). As would be expected, naloxone, a pure antagonist, in the dose used did not alter ACh release from the rat neocortex. However, it did prevent the effect of morphine when given simultaneously.

A major criticism of the present study is the failure to do complete dose-effect curves. Such data is already available for the anti-ACh depleting effects of narcotic analgesics in the rat (Domino and Wilson, 1973). The present study further supports the hypothesis that morphine and related narcotic agonists reduce brain ACh release in the rat as well as the cat (Jhamandas *et al.*, 1971) and the rabbit (Beleslin *et al.*, 1965).

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Dr. E. F. Domino
Department of Pharmacology
University of Michigan
Ann Arbor, Michigan 48104, U.S.A.