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SUPPRESSION OF EVOKED AND SPONTANEOUS RELEASE OF NEUROTRANSMITTERS *IN VIVO* BY MORPHINE

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Abstract—The effects of morphine and naloxone on the release of acetylcholine and amino acids from sensorimotor cortex were studied using an *in vivo* cannula. Morphine (10 mg/kg) reduced the spontaneous release of acetylcholine but was without detectable effect on the spontaneous release of amino acids. It also prevented the release of both acetylcholine and transmitter amino acids (glutamate, aspartate and GABA) induced by application of the depolarizing scorpion venom toxin, tityustoxin (1 μM). These actions of morphine were prevented by naloxone (2 mg/kg).

Morphine and other opiates depress the spontaneous release of acetylcholine *in vitro* [1–5] and *in vivo* [6–8] and depress the release of dopamine [9] and the turnover of biogenic amines [10].

It was recently reported that low concentrations of narcotic analgesics, as well as the endogenous opiates, met-and leu-enkephalin, hyperpolarize a proportion of the neurones in both the guinea pig myenteric plexus [11] and the frog sympathetic ganglion [12]. Such membrane hyperpolarization may reflect the primary action of morphine and indicate the basis for its inhibitory action on the firing of neurones induced by noxious agents (e.g. heat) and by L-glutamate and L-aspartate [13–17]. In addition, this inhibitory action could be a significant component of the analgesic action of morphine.

We have examined the effects of morphine on the spontaneous and evoked release of acetylcholine and amino acid transmitters [18] *in vivo* in an attempt to expose interactions between endogenous opiates and these two systems. The depolarizing peptide tityustoxin, purified from scorpion venom, was employed to evoke neurotransmitter release [19].

MATERIALS AND METHODS

Experiments were carried out with Rowett rats implanted with a special 'swivel' cannula above the exposed sensorimotor cortex. This cannula allows continuous superfusion of the brain surface [20]. All experiments were conducted at least 24 hr after cannula implantation, when rats were awake, unrestrained and behaviourally normal. Superfusate was collected in plastic tubes containing eserine sulphate (1 μg/ml) and sufficient HCl to give a final pH of 3–4. Acetylcholine (ACh) was bioassayed using the guinea pig ileum preparation [5] using Krebs–Henseleit medium containing 10 μg/ml of morphine sulphate. Naloxone does not interfere with the bioassay except when added directly at concentrations (25 μg/ml naloxone) much higher than those present in the samples bioassayed in this study. Amino acids were autoanalysed [21]. Morphine (10 mg/kg) and naloxone (2 mg/kg) were given intraperitoneally. Tityustoxin (1 μM) was administered in saline via the superfusion cannula. The superfusion fluid was normal saline (0.85% w/v plus 1.3 mM Ca^{2+}).

RESULTS

Morphine given i.p. immediately depressed the spontaneous release of ACh, an effect which lasted for at least 3 hr (Fig. 1A), but it did not affect the spontaneous release of glutamate (Fig. 1B) or any of the other amino acids measured (i.e. aspartate, GABA, glutamine, serine, threonine, glycine, alanine, valine methionine, leucine, tyrosine and phenylalanine). In fact, one out of seven animals showed a significantly increased release of glutamate, aspartate and GABA, without change in other amino acids.

In morphine-treated rats, naloxone given i.p. produced a large rebound effect [8], with spontaneous ACh release exceeding its original control levels (Fig. 1A). Again, no effect on amino acid release was detected (Fig. 1B). However, in both of the two experiments performed, naloxone given alone caused some increase (25 per cent) of spontaneous ACh release (Fig. 1A).

We have previously reported [19, 22] that tityustoxin evokes release of ACh and transmitter amino acids from sensorimotor cortex when introduced into the superfusion stream and the data of Fig. 2 clearly show the same effects for glutamate and acetylcholine, the other transmitter candidates aspartate and GABA showing very similar patterns of enhanced release. Tityustoxin has a more consistent and potent action in releasing neurotransmitters both *in vivo*

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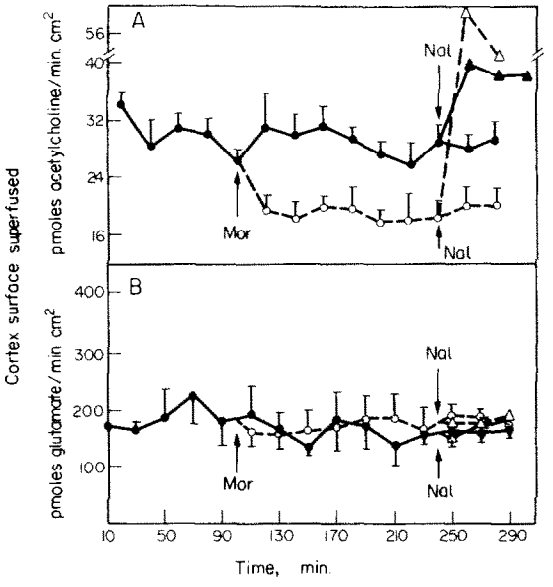

Fig. 1. Effects of morphine and naloxone on spontaneous release of acetylcholine (A) and glutamate (B) from superfused rat sensorimotor cortex. Morphine (Mor; 10 mg/kg) and naloxone (Nal; 2 mg/kg) were administered intraperitoneally at the points indicated. ●, control (N = 7); ○, morphine-treated (N = 7); ▲, naloxone-treated control; △, naloxone given after morphine (N = 2). Data represent the amount of neurotransmitter released to superfusion fluid over a 10 min period ± S.E.M. (flow rate = 6 ml/hr).

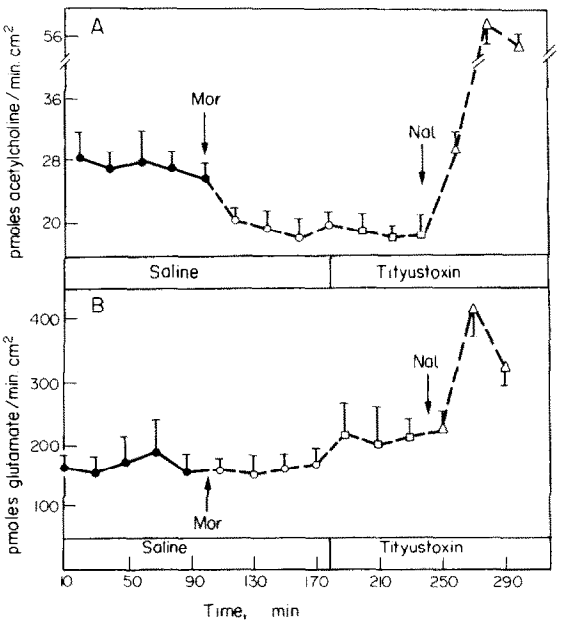

Fig. 3. Effect of morphine and naloxone on tityustoxin-evoked release of acetylcholine (A) and glutamate (B) from superfused rat sensorimotor cortex. Superfusion was started with saline + Ca^{2+} and the same plus tityustoxin (1 μM) was introduced into the superfusion stream where indicated. Morphine (Mor; 10 mg/kg) and naloxone (Nal; 2 mg/kg) were administered intraperitoneally when indicated by the arrows. ●, control (N = 7); ○, morphine-treated (N = 7); □, tityustoxin added (N = 3); △, naloxone (N = 2). Data represent the amount of each transmitter collected during 10 min of superfusion ± S.E.M. (flow rate = 6 ml/hr).

and *in vitro* than other depolarizing agents such as Veratrine or high-K^+; and these effects are both Ca^{2+} and tetrodotoxin-sensitive [19, 22].

The transmitter release observed in these experiments was accompanied by stereotyped grooming movements of the forelimbs, jaw-chatter and myoclonic jerks of the contralateral forelimb which began 10 min after introduction of the toxin and continued during its application. All of these effects were abolished by tetrodotoxin.

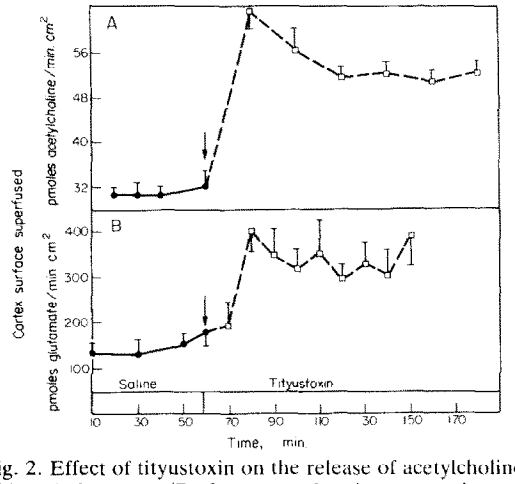

Fig. 2. Effect of tityustoxin on the release of acetylcholine (A) and glutamate (B) from superfused rat sensorimotor cortex. Arrows indicated where tityustoxin (1 μM) in saline (plus Ca^{2+} 1.3 mM) was administered via the cannula. ●, control (N = 4); □, tityustoxin (N = 4). Data represent the amount of each neurotransmitter released and collected during 10 min of superfusion ± S.E.M. (flow rate = 6 ml/hr).

In the present experiments, morphine given i.p. (10 mg/kg) entirely prevented the release to the superfusion stream of ACh and transmitter amino acids (glutamate, aspartate and GABA) induced by tityustoxin (Figs. 3A and B; glutamate is shown as typical for the other transmitter amino acids). These actions of morphine were fully reversed by naloxone given i.p. (2 mg/kg) at various times during tityustoxin infusion (e.g. 140–150 min after morphine; Figs. 3A and B).

The behavioural and physiological responses showed a correlating pattern. Thus, in the presence of morphine, tityustoxin was unable to produce the effects on limb movements and behaviour described above. However, following naloxone administration, all of these characteristic effects reappeared, demonstrating that they had been evoked by the agents (presumably the neurotransmitters) released by the toxin.

DISCUSSION

The anti-convulsant actions of opiates and an enkephalin analogue (FK-33824) on reflex epilepsy in the baboon have recently been reported [23]. In

these experiments the drugs were given intracerebrally or intramuscularly, as their effects were reversed by naloxone. These results would correlate well with our own reported here, but contrast with the many previous reports of a convulsant action of the opiates, usually given in high doses (e.g. up to 50 mg/kg) and causing both behavioural and EEG manifestations of seizures [24–28].

We interpret the ineffectiveness of morphine or naloxone to influence the level of spontaneous release of amino acid neurotransmitters as an indication that these compounds are continuously effluxing from cellular compartments other than nerve terminals, and by non-synaptic mechanisms. The greater part of these compounds would not, in this case, be influenced by agents modulating transmitter release. The much lower level of spontaneous release of ACh appears, on the other hand, to be mostly synaptic in origin (i.e. ACh is present at only 25 per cent of the glutamate level in molecular terms).

In conclusion, our results support an inhibitory action for morphine in the cerebral cortex, and suggest that this is due to a suppression of neurotransmitter release. This could be due to a strong hyperpolarizing effect, powerful enough to overcome, for instance, the potent depolarizing action of tityustoxin [19, 22]. Equally, and perhaps more credibly, morphine could be working by blocking the calcium influx which would normally occur during the depolarization induced by tityustoxin. Such an action of morphine has been demonstrated [29–30] on the calcium current associated with action potentials. However, the naloxone sensitivity of the effect emphasizes the involvement of receptors and limits the appeal of this explanation. The alternative view is that presynaptic opiate receptors are controlling transmitter release and a case for the existence of such receptors in spinal cord and pituitary gland has recently gained considerable momentum [31, 32].

Since naloxone very effectively prevents this suppression, the morphine must be acting via specific receptor-binding sites and these could be sited at any point on the neurones provided interaction leads to reduced firing or to reduced transmitter release (e.g. presynaptically). However, as reduced neuronal firing appears to be a characteristic effect of endogenous opiate peptides [33–36], the former explanation is the more attractive, and in this case both morphine and naloxone could be producing their primary effects at cell bodies located at some distance from the cortical area being superfused, possibly even in different brain regions.

Since we have some evidence that naloxone given alone also enhanced spontaneous ACh release, it seems likely that the suppression of transmitter release by morphine is revealing an influence normally exerted by endogenous opiate peptides in cerebral cortex. Though these agents are not found excessively concentrated in this CNS region, they are present, and morphine-type opiate receptors also abound in sensorimotor cortex [37, 38].

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