Biochemical Pharmacology, Vol 31, No. 6, pp 1019–1023, 1982
Printed in Great Britain.

0006-2952/82/061019-05 $03 00/0

MORPHINE SUPPRESSION OF NEUROTRANSMITTER RELEASE EVOKED BY SENSORY STIMULATION *IN VIVO*

JOAQUIM COUTINHO-NETTO, ABDUL-SALEM ABDUL-GHANI and HENRY F. BRADFORD*
Department of Biochemistry, Imperial College, London SW7 7AZ, U.K.

(*Received* 20 *June* 1981; *accepted* 9 *October* 1981)

Abstract—The effects of morphine and naloxone on the release of acetylcholine and amino acid neurotransmitters from sensorimotor cortex were studied employing an *in vivo* superfusion cannula. Morphine (20 mg/kg) reduced the spontaneous release of acetylcholine but had no detectable effect on the spontaneous release of amino acids. It also suppressed the release of acetylcholine and amino acid neurotransmitters evoked by sensory stimulation of the contralateral sensorimotor cortex via the brachial plexus. Naloxone (5 mg/kg) prevented all of these inhibitory actions of morphine. Naloxone also caused a significant increase in spontaneous acetylcholine release.

Several studies have shown that morphine reduces the release of neurotransmitters from both *in vitro* and *in vivo* preparations [1–9], and will depress their turnover [10]. We recently reported that morphine administered systemically depressed the resting release *in vivo* to cortical superfusion cannulae of acetylcholine and completely prevented its stimulated release evoked by topically applied depolarizing toxin. The amino acid neurotransmitters glutamate, aspartate and GABA were similarly effected [11, 12]. These results implied control of neurotransmitter release processes in the cerebral cortex by endogenous opiates, since the effects were entirely prevented by naloxone. In order to further test the physiological significance of these phenomena we have investigated the effects of morphine and naloxone on neurotransmitter release evoked by stimulation of the cerebral cortex through neural pathways, which contrasts with the topically applied chemical depolarizing agent employed in the previous study [12].

MATERIALS AND METHODS

Experiments were carried out with Rowett rats implanted with a special 'swivel' cannula above the exposed sensorimotor cortex under halothane/nitrous oxide anaesthesia. This cannula allows continuous superfusion of the brain surface [13]. For sensory cortical stimulation, the brachial plexus contralateral to the cannula was exposed under anaesthesia and a small electrode of the kind developed by Dobkin was placed around all the nerves of the plexus [14] and the animals were allowed to recover. Several days later after wound healing, electrical stimulation of the brachial plexus was performed by square wave pulses of the following specifications: amplitude 2–7 V; current 1–3 mA; pulse duration 1 msec; frequency 50 Hz. Periods of 8–8.5 min of stimulation were applied and always evoked clearly visible muscular jerking in the forelimb. All experiments were conducted at least 12 hr after cannula and electrode implantation, when the rats were awake, unrestrained and behaviourally normal. Superfusate was collected during 10 min periods at a flow rate of 6 ml/hr into plastic tubes containing eserine sulphate (1 μg/ml) and sufficient HCl to give a final pH of 3–4.

The procedure was to collect one control sample and then to collect during 10 min of brachial plexus stimulation. This was followed by collection of a third sample to examine whether release patterns had returned to normal. This sequence was repeated three times on each animal. After this morphine (20 mg/kg) was injected i.p. and a further 9 samples were collected as previously with or without stimulation. Naloxone (5 mg/kg) was then injected i.p. and another sequence of 9 samples were collected as before. The superfusion fluid employed was normal saline (0.85% w/v) plus 1.3 mM Ca^{2+}. The collected samples were divided for the determination of amino acids and acetylcholine. Acetylcholine (ACh) was bioassayed using the guinea pig ileum preparation [15] 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) much higher than those present in the samples bioassayed in this study. Amino acids were autoanalysed [16].

RESULTS

Delivering the relatively small currents (5–15 μA average current) involved to the brachial plexus of awake animals for 8–9 min caused continuous vibratory movements together with myoclonic jerks of that limb, whilst the animals tended to remain motionless, and did not show any signs of pain or distress during the procedure.

We have previously shown that brachial plexus stimulation causes release of neurotransmitters only from the contralateral sensorimotor cortex area [11, 12] and Fig. 1A shows the clear-cut release of glutamate which was evoked by this procedure in

* To whom correspondence should be addressed.

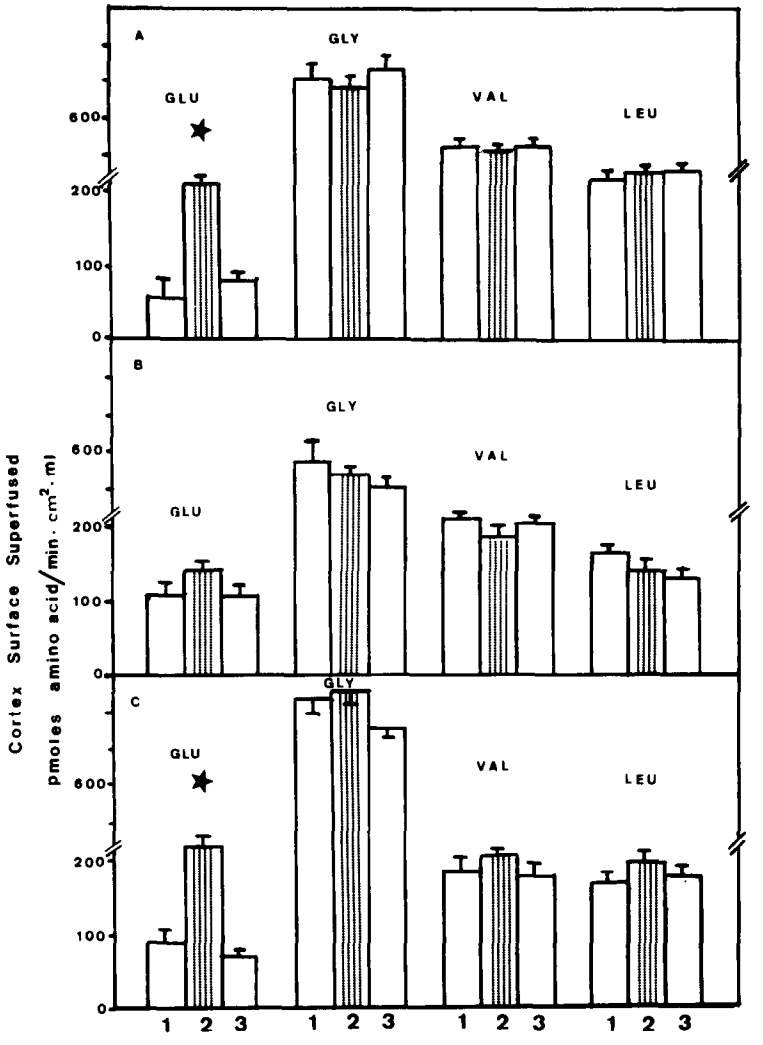

Fig. 1. *In vivo* release of amino acids from superfused sensorimotor cortex during control periods and during periods of stimulation of the contralateral brachial plexus. (A) drug-free, (B) after morphine, and (C) after naloxone. Histograms are as follows: (1) 10 min samples before stimulation, (2) 10 min sample during stimulation, and (3) 10 min sample after stimulation. Morphine (20 mg/kg) was injected intra-peritoneally after collection of control samples, and naloxone (5 mg/kg) was injected after morphine administration (see Materials and Methods). Histobars represent the mean ± S.E.M. of 12 observations in 4 animals. Glu, glutamate; gly, glycine; val, valine; leu, leucine. The star indicates glutamate release in A and C is significantly different from glutamate release in B with $P > 0.001$.

the present experiments. Typical chromatograms of superfusate showing the extent of these effects are shown in Fig. 2. No changes were produced in levels of non transmitter amino acids measured in superfusate (e.g. glycine, valine and leucine, Fig. 1), though aspartate and GABA were released as previously reported.

Similarly, brachial plexus stimulation evoked release of ACh from contralateral sensorimotor cortex (Fig. 3A). However, following i.p. morphine injection (20 mg/kg), the level of spontaneous ACh was immediately depressed (Fig. 3B), though spontaneous levels of release of glutamate and other amino acids were unaffected (Fig. 1B) as previously reported [12]. This dose of morphine did, however, effect the *evoked* release of both ACh and glutamate, causing complete suppression (Figs. 1B and 3B). Administration of naloxone (5 mg/kg) to these morphine treated animals prevented these inhibitory actions of morphine, and the levels of evoked release of glutamate (Fig. 1C) and ACh (Fig. 3C) returned to normal when brachial plexus stimulation was again applied. Naloxone alone (5 mg/kg) caused a significant increase in ACh release (Fig. 3C) indicating a prevailing inhibition of ACh release by endogenous opioids.

When brachial plexus stimulation was carried out with animals under deep or light anaesthesia (hal-

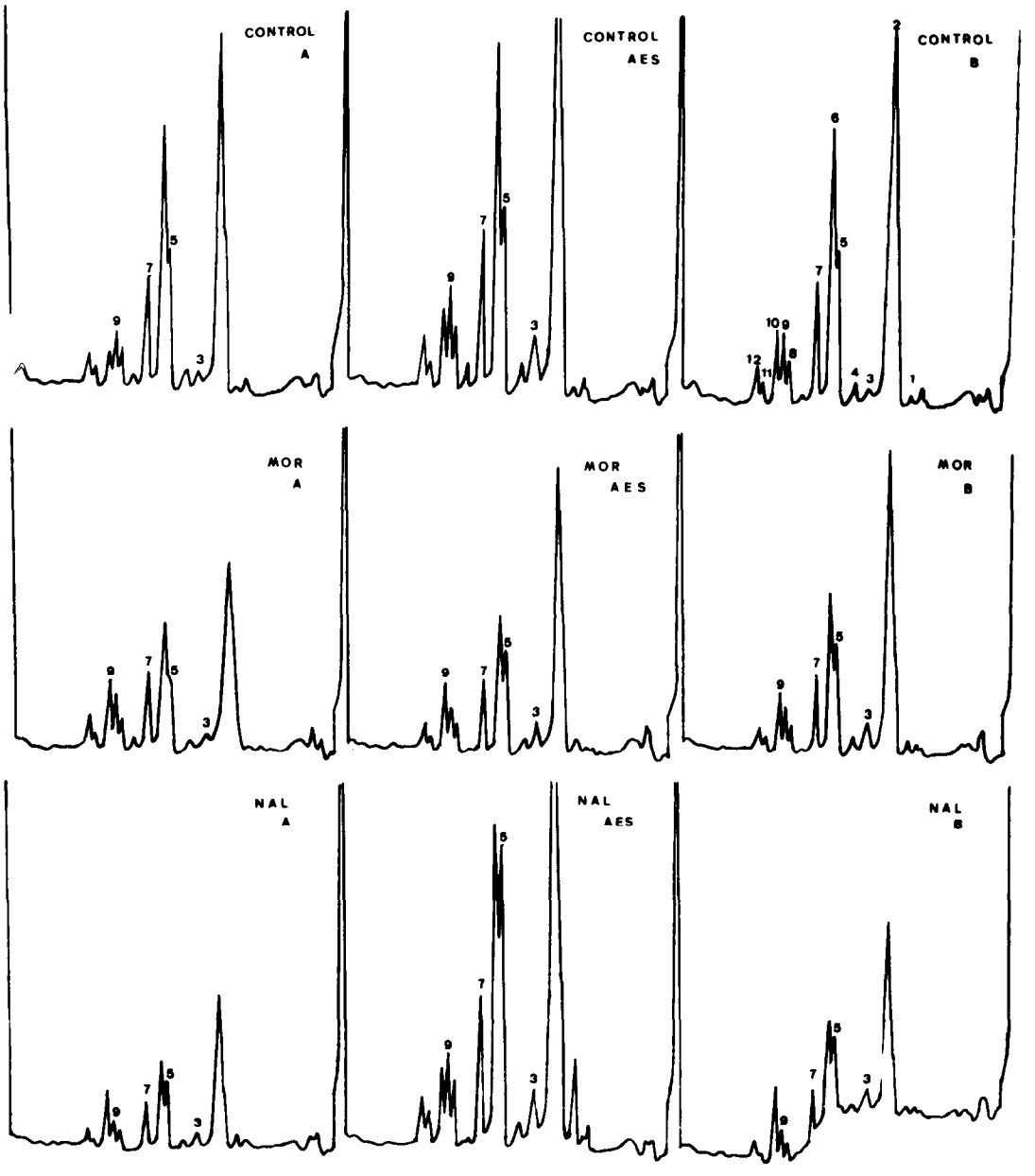

Fig. 2. Patterns of amino acid release from superfused sensorimotor cortex at rest with or without drug treatments and submitted to periods of sensory stimulation via the brachial plexus (see Materials and Methods). Representative chromatograms of controls, morphine (MOR) and naloxone (NAL) treated animals are shown. Amino acids: 1, aspartate; 2, threonine, serine and glutamine; 3, glutamate; 4, citrulline; 5, glycine; 6, alanine; 7, valine; 8, isoleucine; 9, leucine; 10, norleucine (50 pmoles/100 μl of sample); 11, tyrosine; and 12, phenylalanine. Code is as follows: B, before stimulation; AES, during afferent electrical stimulation; A, after stimulation.

othane gas mixtures) the observed responses to stimulation were greatly depressed and not easily detected.

DISCUSSION

Morphine and other opiates have been shown to depress the spontaneous release of acetylcholine *in vitro* [1–5] and *in vivo* [6–8], and to depress the release of dopamine [9], and turnover of biogenic amines [10]. Also GABA release from amacrine cells of the goldfish retina *in vitro* is prevented by morphine [17].

We have previously reported [12] that morphine given systemically completely suppressed the large degree of release of glutamate and acetylcholine evoked by direct application of a depolarizing scorpion venom toxin (Tityustoxin) to the sensorimotor cortex. This effect was entirely prevented by naloxone. We now report that morphine has a virtually

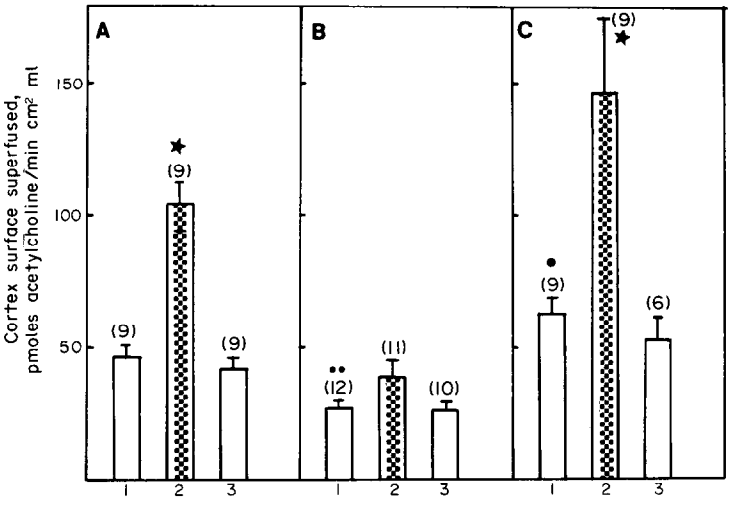

Fig. 3. *In vivo* release of acetylcholine from superfused sensorimotor cortex in the drug-free condition and after administration of morphine and naloxone; the details are exactly as described in the legend to Fig. 1. Histobars represent the mean ± S.E.M. for the number of observations indicated above them; these were obtained from 4 animals. The star indicates ACh release in A and C are significantly different from ACh release in B with $P > 0.001$. The black spot in C indicates naloxone effect in releasing ACh in C is significantly different from control (A) with $P > 0.01$. The two black spots in B indicate that spontaneous release is reduced due to morphine with $P > 0.001$.

identical action on neurotransmitter release evoked by stimulation through physiological pathways. Once again naloxone prevents this inhibitory action of morphine.

Thus, this inhibitory action of morphine extends from suppression of the massive degree of neurotransmitter release evoked by chemical depolarizing agents, to the relatively low levels evoked by stimulation of normal neural inputs to the cerebral cortex. The latter, in particular, shows that morphine at these doses (10–20 mg/kg) is greatly dampening down sensory inputs and other neural activity of the cortex, and this is directly reflected in the reduction in the levels of spontaneous release of ACh. We believe that spontaneous levels of glutamate release are not effected because a large part of the background release of this amino acid which were detected was from non-synaptic compartments [12].

The mechanism by which morphine produces these potent inhibitory effects is not clear. It seems unlikely that it could prevent the large depolarizing action of Tityustoxin or spike invasion of nerve-terminals, but narcotic analgesics including met- and leu-enkephalin hyperpolarise a proportion of neurones in both guinea-pig myenteric plexus [18] and frog sympathetic ganglion [19]. This could be the basis for its inhibitory action on the firing of neurones induced by a wide range of stimuli [20–24].

Instead, morphine could block the influx of calcium. Such an action of morphine has been demonstrated on the calcium current associated with action potentials [25], though such an effect in our experiments would have to be mediated by opiate receptor interaction since the total prevention of the morphine effect by naloxone emphasises the primary involvement of such receptors.

Alternatively, the morphine could be operating inhibitory presynaptic opiate receptors which control neurotransmitter release. Evidence for such receptors in spinal cord and pituitary gland now exists [26, 27].

Since naloxone itself shows some propensity to enhance ACh release (Fig. 3C, [11]) it seems likely that endogenous opioid peptides are normally exerting an inhibitory action on transmitter release. Such a concept is complicated by the finding that two classes of receptor may exist in the CNS, namely μ-receptors which interact with equal affinity for morphine and enkephalins, and γ-receptors which interact preferentially with enkephalin [28–32]. Whilst the functional significance of these two sub-populations is not yet clear, nonetheless the morphine type (μ) receptors abound in cortex, though they are not most enriched in this region [33]. It is via these morphine-type receptors presumably positioned at pre-synaptic sites that morphine and its endogenous counterpart exert their profound influence on neurotransmitter release.

In other CNS regions such as spinal cord morphine has been shown to exert a direct naloxone-sensitive action in both mimicking and reinforcing synaptic depression mediated by enkephalins [34].

Acknowledgements—This work was supported by an MRC Programme Grant. J. Coutinho-Netto was supported by an FAPESP Fellowship of the State of São Paulo, Brazil.

REFERENCES

1. H. Kaneto, in *Narcotic Drugs: Biochemical Pharmacology* (Ed. D. H. Clonet), p. 300. Plenum Press, New York (1971).
2. M. Weinstock, in *Narcotic Drugs: Biochemical Pharmacology* (Ed. D. H. Clonet), p. 254. Plenum Press, New York (1971).
3. W. Schaumann, *Nature, Lond.* **178**, 1121 (1956).

4. W. Schaumann, *Br. J. Pharmac. Chemother.* **12**, 115 (1957).
5. W. D. M. Paton, *Br. J. Pharmac. Chemother.* **12**, 119 (1957).
6. K. Jhamandas, C. Pinsky and P. W. Phillis, *Nature, Lond.* **228**, 176 (1970).
7. J. D. Matthews, G. Labrecque and E. F. Domino, *Psychopharmacologia* **29**, 113 (1973).
8. K. Jhamandas and M. Sutak, *Br. J. Pharmac.* **50**, 57 (1974).
9. G. A. Gudelsky and J. C. Porter, *Life Sci.* **25**, 1697 (1979).
10. A. E. Takemori, *Biochem. Pharmac.* **24**, 2121 (1975).
11. A.-S. Abdul-Ghani, J. Coutinho-Netto and H. F. Bradford, *Biochem. Pharmac.* **29**, 2197 (1980).
12. J. Coutinho-Netto, A.-S. Abdul-Ghani and H. F. Bradford, *Biochem. Pharmac.* **29**, 2777 (1980).
13. P. J. Dodd and H. F. Bradford, *Biochem. Soc. Trans.* **4**, 13 (1976).
14. J. Dobkin, *J. Neurochem.* **17**, 237 (1970).
15. W. D. M. Paton and M. Aboo Zar, *J. Physiol., Lond.* **194**, 13 (1968).
16. H. F. Bradford and A. J. Thomas, *J. Neurochem.* **16**, 1459 (1969).
17. M. B. A. Djamjoz, Chen-An Chin, D. M. K. Lam and W. K. Stell, *Nature, Lond.* **292**, 620 (1981).
18. R. A. North and M. Tonini, *Br. J. Pharmac.* **61**, 541 (1977).
19. W. Wourters and J. Van den Bercken *Nature, Lond.* **277**, 53 (1979).
20. O. Calvillo, J. L. Henry and R. S. Neuman, *Can. J. Physiol. Pharmac.* **52**, 1207 (1974).
21. R. Dingledine and A. Goldstein, *Life Sci.* **17**, 57 (1975).
22. J. Dostrovsky and B. Pomeranz, *Nature New Biol.* **246**, 222 (1973).
23. A. W. Duggan, J. G. Hall and P. M. Headly, *Br. J. Pharmac.* **61**, 65 (1977).
24. W. Zieglgänsberger and H. Bayerl, *Brain Res.* **115**, 111 (1976).
25. A. W. Mudge, S. E. Leeman and G. D. Fishbach, *Proc. natn. Acad. Sci. U.S.A.* **76**, 526 (1979).
26. L. L. Iversen, S. D. Iversen and F. E. Bloom, *Nature, Lond.* **284**, 350 (1980).
27. C. Lamotte, C. B. Pert and S. S. Snyder, *Brain Res.* **112**, 407 (1976).
28. J. A. H. Lord, A. A. Waterfield, J. Hughes, H. W. Kosterlitz and G. Henderson, *Nature, Lond.* **267**, 495 (1977).
29. H. L. Fields, P. C. Emson, B. K. Leigh, R. F. T. Gilbert and L. L. Iversen, *Nature, Lond.* **284**, 351 (1980).
30. S. R. Childers, in *Neurotransmitter Receptors* (Eds. S. J. Enna and H. I. Yamamura), p. 105. Chapman & Hall, London (1980).
31. K. J. Chang and P. Cuatrecasas, *J. biol. chem.* **254**, 2610 (1979).
32. S. R. Childers, F. Creese, A. M. Snowman and S. H. Snyder, *Eur. J. Pharmac.* **55**, 11 (1979).
33. K. J. Chang, B. R. Cooper, E. Hazum and P. Cuatrecasas, *Molec. Pharmac.* **16**, 91 (1979).
34. D. Le bars, G. Guilbaud, D. Chitour and J. M. Besson, *Brain Res.* **202**, 223 (1981).