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Effect of etorphine, morphine and diprenorphine on neurones of the cerebral cortex and spinal cord of the rat

T. J. BISCOE, A. W. DUGGAN* AND D. LODGE†

Department of Physiology, University of Bristol, Bristol

Summary

1. The actions of etorphine, morphine and diprenorphine were investigated on neurones of the cerebral cortex and spinal cord of rats anaesthetized with pentobarbitone.

2. In the cerebral cortex, intravenous etorphine increased the latency of the primary evoked response to a peripheral nerve stimulus and suppressed the rhythmical after-discharge. Diprenorphine reversed this effect. These actions were demonstrated on both field potentials and unit firing.

3. Morphine had no effect on the primary response but the frequency of after-discharge bursts was reduced and there was an increase in firing between bursts.

4. In the cerebral cortex, electrophoretically applied etorphine reduced after-discharges when applied for long periods but had no effect on the depressant actions of glycine and γ-aminobutyric acid (GABA) nor on the excitant action of acetylcholine and L-glutamate. Similarly there was no alteration by etorphine of the effects of glycine, GABA and L-glutamate on spinal cord neurones.

5. It is concluded that etorphine may act pre-synaptically in the cerebral cortex.

Introduction

Etorphine is a thebaine derivative (Bentley & Hardy, 1963 ; Lister, 1964) which is about 2,000 times more potent than morphine as an analgesic in rats (Blane, Boura, Fitzgerald & Lister, 1967). However, it differs from morphine in the degree of catatonia produced by doses higher than those producing analgesia (Blane *et al.*, 1967). It is used in veterinary practice for the immobilization and transport of wild animals (Harthoorn, 1967) where its effects are antagonized by diprenorphine (Bentley, Boura, Fitzgerald, Hardy, McCoubrey, Aikman & Lister, 1965), also a thebaine derivative. This paper describes experiments on the actions of these drugs on the firing pattern of cortical cells in response to a stimulus to a peripheral nerve and on possible interactions with suspected transmitters in the central nervous system. The results indicate differences from the effects previously reported for morphine-like compounds.

Methods

Experiments were performed on male albino rats (350–500 g) anaesthetized with an initial dose of pentobarbitone sodium, 45 mg/kg intraperitoneally, anaesthesia

* C. J. Martin Travelling Fellow.
† Wellcome Trust/Animal Health Trust Research Fellow.

being maintained subsequently with regular intravenous injections. During the period of recording the animals were paralyzed with gallamine triethiodide and ventilated with humidified oxygen in order to minimize movements of the brain and spinal cord and to ensure adequate ventilation over long periods. Throughout the period of paralysis pentobarbitone was given intravenously at a frequency and in doses similar to those used prior to muscle relaxation. Blood pressure in the femoral artery was recorded in some experiments with a Consolidated Electrodynamics type 4-327-L221 strain gauge and Devices oscillographic recorder. Body temperature was maintained between 38 and 39°C.

Experiments on the cerebral cortex. The appropriate area of the right cerebral cortex was exposed by removal of a bone flap but the dura was left intact until just prior to recording. The left sural, medial popliteal and lateral popliteal nerves, or the three grouped together as the sciatic nerve, were prepared for stimulation (0·3 ms rectangular pulses) with silver wire electrodes. Silver ball electrodes were then placed on the intact dura to determine the site of maximum evoked response to stimulation of the three nerves. From its gross location and histology this area corresponded to the junction of cortical areas 2 and 3 of Krieg (1946). At that site an opening approximately 0·25–0·5 mm in diameter was made in the dura and a small area of the underlying pia removed, without damage to blood vessels. The exposed meninges were continually irrigated with Krebs solution (Krebs & Henseleit, 1932) at 38° C, equilibrated with 95% O_2 and 5% CO_2. In the early experiments, the dura was widely removed but these preparations deteriorated quickly with herniation of the exposed cerebral tissue and had respiratory movements so large as to require the use of a pressor plate to obtain stable extracellular recordings. By minimizing the area of exposed tissue, good recording conditions for 6–8 h were commonly obtained without the use of a pressor plate.

Experiments on the spinal cord. The spinal cord was exposed by removal of the laminae of the lower thoracic and upper lumbar vertebrae and was immersed in a pool of liquid paraffin maintained at 38°C throughout the experiment. The dura was opened longitudinally and a small hole was made in the pia prior to recording. The spinal cord was not cut as this had been found to result in deterioration of the preparation. The cells studied were located either by their spontaneous firing or their response to the ejection of the excitant amino-acid, DL-homocysteic acid.

Extracellular recordings were made with the 4 M NaCl-filled centre barrel of seven-barrel micropipettes. The outer barrels contained the compounds to be ejected by microelectrophoresis and these, together with concentration and pH, if adjusted were: glycine (0·5 M, pH 3), γ-aminobutyric acid (GABA, 0·5 M, pH 3), DL-homocysteate Na (0·2 M, pH 8), acetylcholine chloride (1·0 M), L-glutamate Na (1·0 M, pH 8), etorphine HCl (20 mM), diprenorphine HCl (60 mM), 2% pontamine sky blue solution in 0·5 M Na acetate, pH 7·3. A retaining voltage of 0·5 V was usually maintained across each drug-containing barrel throughout the experiment. The general principles of microelectrophoresis followed were those of Curtis (1964).

For intravenous use drugs were dissolved in 165 mM NaCl solution to give final concentrations as follows: etorphine 10 or 50 μg/ml; diprenorphine 30 or 150 μg/ml; morphine 0·5 mg/ml; pentobarbitone sodium 10 mg/ml; gallamine triethiodide 20 mg/ml.

The time constant of the recording system was varied according to whether action potentials or field potentials were being investigated. Potentials were viewed with

an oscilloscope and a pulse height selector was used to select a cell action potential for study. The output of the pulse height selector was viewed on the same oscilloscope and was also displayed as a continuous firing rate record via a rate-meter and pen recorder (Servoscribe, Goerz); alternatively, after appropriate pulse shaping, post-stimulus histograms of the selected potential were prepared with a small computer (Biomac 500, Data Laboratories). Two types of histogram were compiled; when studying the pattern of cell bursts, 500 time intervals of 1 or 2 ms were used to analyse cell firing after a stimulus but when interest was centred on the timing of the first burst the bin width was reduced to 125 or 63 μs.

The study of patterns of cortical cell firing in response to a peripheral nerve stimulus is often complicated by the superimposition of the first burst of activity upon a relatively large field potential. This is usually not the case with subsequent bursts. Accordingly, if the pulse height selector is adjusted to count the action potentials in these later bursts, the large field of the primary burst gives false additional counts. Wherever possible, these false counts were avoided by using a time constant of 2 ms, by grading the peripheral nerve stimulus and by reducing the number of nerves stimulated. Where false counts could not be avoided, the cell was either rejected or effects on after-discharges only were studied.

Results

Etorphine and diprenorphine were administered both intravenously and microelectrophoretically. According to Blane *et al.* (1967) the ED50 of the analgesic dose of etorphine given intravenously to rats is 0·23 μg/kg whereas the ED50 for catatonia is 2·2 μg/kg given subcutaneously. When used in veterinary practice for the immobilization of animals larger doses are used, e.g. 7·48 μg/kg, i.m., for the dog (Crooks, Whiteley, Jenkins & Blane, 1970). As a major aim in this study was a possible effect of etorphine on cortical synapses, the doses used in the rat were in the range of those producing catatonia (2 to 12 μg/kg, i.v.). The first series of experiments on field potentials in the cerebral cortex were performed with the higher doses but in subsequent studies on single cells a range of intravenous doses was used. As field potentials represent the summed activity of many cells and the results paralleled the findings with single cells, these experiments were not repeated with lower doses of etorphine.

Field potentials evoked in the cerebral cortex by a stimulus to a peripheral nerve

When recorded with a ball electrode, a surface positive wave could be obtained over a relatively large expanse of cortex but the area at which this was of maximum amplitude and minimum duration was limited to a patch with a diameter of 1 to 3 mm.

The primary evoked response, when recorded with a microelectrode, consisted of a small positive wave followed by a large negative wave, the latency to onset and latency to peak of the negative wave being 8–12 ms and 20–40 ms, respectively. The microelectrode was advanced until the negative wave was of maximum amplitude, which occurred at a depth of 0·5 to 0·6 mm from the cortical surface and commonly coincided with the appearance of action potentials on the negative wave.

In most preparations a repetitive response (Adrian, 1941 ; Morison & Dempsey, 1943) followed the primary evoked response, the frequency of these secondary waves being 8–10 Hz. These waves, which are pronounced in barbiturate anaesthesia, were usually absent in preparations which were considered poor by inspection of flow in the superficial cortical vessels.

Etorphine, given intravenously in a dose of 12·5 μg/kg, increased the latency of the primary evoked response. The primary negative wave became broader with little change in amplitude, the area enclosed by the wave being increased and the after-discharges reduced or more commonly abolished. These effects were seen in all eight animals studied in this way. These effects were partly reversed by diprenorphine, 37·5 μg/kg i.v., the increase in latency of the primary evoked response being fully reversed, although the after-discharges returned with a frequency of

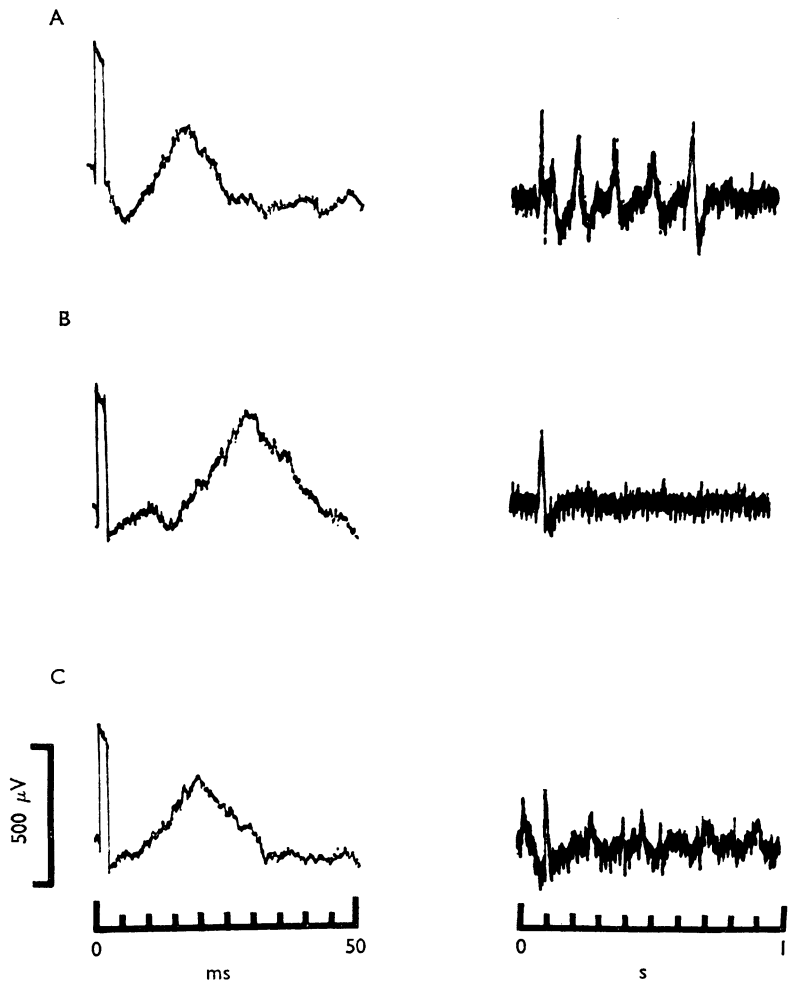

FIG. 1. The effects of etorphine and diprenorphine on field potentials recorded in the right cerebral cortex of the rat in response to stimulation (0·3 ms, 0·5 Hz) of the left sciatic nerve. The tracings on the left contain primary evoked responses alone, while those on the right show repetitive discharges. A, Control ; B, records obtained 11 min after etorphine (12·5 μg/kg, i.v.) ; C, 8 min after diprenorphine (37·5 μg/kg, i.v.) and 21·5 min after etorphine. The nerve stimulus coincides with the leading edge of the voltage calibration pulse. The artefact in the upper right hand record is due to cardiac action potentials.

waves that was usually slower than that of the control observations (Fig. 1). In this animal, the increase in the latency to onset and in the time to peak of the primary evoked response was 12 ms; shifts of this order were common with this dose of etorphine. The after-discharges were completely suppressed; they returned at a lower frequency after diprenorphine.

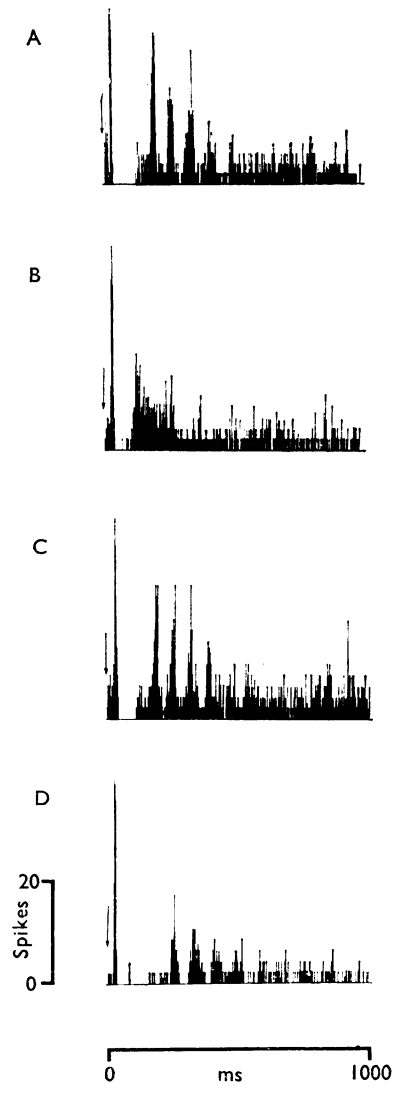

FIG. 2. The effects of etorphine, diprenorphine and pentobarbitone on the firing pattern of a cortical neurone in response to a stimulus to the left sciatic nerve. The stimulus to the nerve (0·3 ms, 0·5 Hz), marked by an arrow, was initiated 20 ms after the commencement of each sweep of the multiscaler. The firing of the cell was analysed in 500 intervals of 2 ms. Each record represents the summed responses to 45 stimuli. (A), Control; (B), computed 17 min after etorphine (2 μg/kg, i.v.); (C), computed 2 min after diprenorphine (6 μg/kg, i.v.) and 28 min after etorphine; (D), computed 2 min after pentobarbitone (5 mg/kg, i.v.) and 20 min after diprenorphine.

The responses of neurones of the deep pyramidal cell layer of the cerebral cortex to stimulation of a peripheral nerve

The cells investigated were those found in the same area of the cerebral cortex as that used for field potential studies. They were located at a depth of 0·65 to 1·17 mm from the cortical surface ; marking with pontamine sky blue (Hellon, 1971) showed that all but two cells were located in layer V. Most cells evoked in this layer exhibited an after-discharge having a similar period to that observed in the field studies (Figs. 2A, 3A, 4A and 5A) ; these alone were investigated when etorphine was administered intravenously.

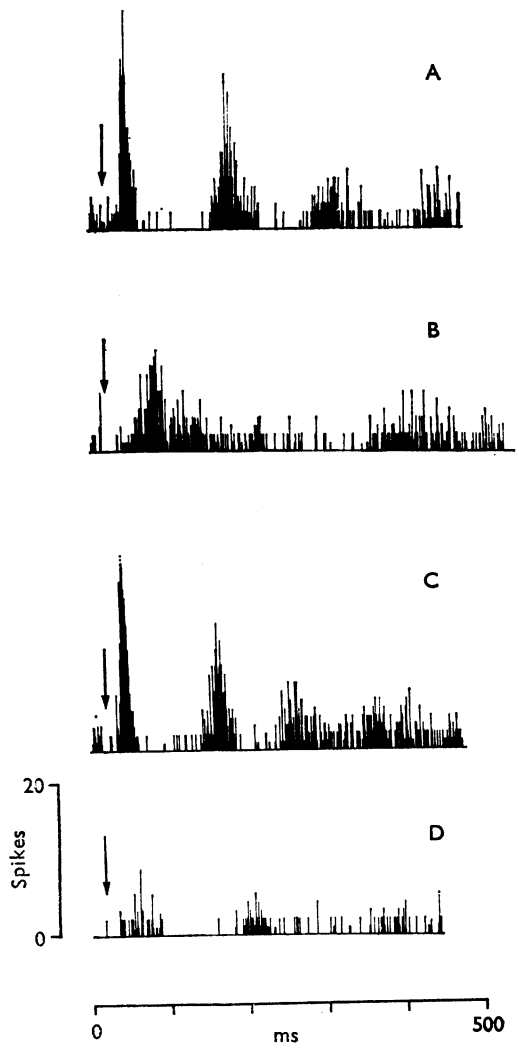

FIG. 3. The effects of etorphine, diprenorphine and pentobarbitone on the firing pattern of a cortical neurone in response to a stimulus to the left sciatic nerve (0·3 ms, 0·5 Hz). The records were obtained in a manner similar to those of Fig. 2: 32 sweeps were summed and the bin width was 1 ms. (A), Controls ; (B), 2 min after etorphine (12·5 μg/kg, i.v.) ; (C), 2 min after diprenorphine (35 μg/kg, i.v.) and 20 min after etorphine ; (D), 2 min after pentobarbitone (5 mg/kg, i.v.) and 14 min after diprenorphine.

Etorphine, morphine and diprenorphine administered intravenously. Low doses of etorphine (2 μg/kg) increased the latency of the first burst (primary evoked response), and decreased the frequency of after-discharges (Fig. 2). Higher initial doses of etorphine or additional subsequent doses up to a total dose of 12·4 μg/kg further increased the latency of the first burst and usually abolished after-discharges (Fig. 3). Both these effects could be reversed by intravenous diprenorphine in doses of 6–35 μg/kg, i.e. about three times that of the preceding dose of etorphine; a similar ratio is used in veterinary practice for the reversal of etorphine-induced immobilization (Crooks *et al.*, 1970). All nine animals from which post-stimulus histograms were prepared gave similar results.

The post-stimulus histograms compiled from one deep pyramidal cell are shown in Figure 2. This cell fired spontaneously and responded to stimulation of the sciatic nerve with an initial group of action potentials followed by a series of after-discharges (Fig. 2A). Intravenous etorphine (2 μg/kg) caused a mean drop in blood pressure of 30 mmHg. A post-stimulus histogram compiled 17 min after the administration of etorphine (Fig. 2B), at which time the blood pressure was near the pre-dose level, shows that the time at which the cell was most likely to fire in the first burst following the stimulus was slightly increased from 28·3 to 37·6 ms; synchronized after-discharges were almost completely suppressed. This increase in latency is difficult to see with a computer bin width of 2 ms, but was quite clear on records computed with a bin width of 125 μs (not illustrated). Diprenorphine (6 μg/kg) was injected intravenously 28 min after etorphine and reversed both effects of etorphine (Fig. 2C). Pentobarbitone, 5 mg/kg administered 20 min after diprenorphine, reduced the spontaneous firing of the cell and the probability of firing within each burst without abolishing the post-stimulus synchronization (Fig. 2D).

A larger dose of etorphine (12·5 μg/kg, i.v.) had a more profound effect on the time of maximum probability of firing with the first burst, increasing the latency to peak from 29 to 60 ms in the case of the cell illustrated in Figure 3. Diprenorphine (35 μg/kg, i.v.) reversed this effect and after-discharges reappeared. Additional pentobarbitone reduced the probability of firing within the first and subsequent bursts as before.

The effects of diprenorphine without previous administration of etorphine (15 and 30 μg/kg, i.v.) were studied in one animal. That this compound increased the excitability of the cell was shown by two results. First, there was an increase in the basal firing rate. Secondly, there was an increase in the probability of firing at the peaks of the synchronized bursts following a stimulus as compared with peak probabilities in the control period.

The effects of morphine on cell firing in response to a peripheral nerve stimulus were also studied. With doses of 2 to 4 mg/kg, i.v., an analgesic dose in the rat, the only constant effect was an increase in the probability of firing at each peak of the after-discharge, usually accompanied by an increase in the probability of firing between peaks. The further administration of morphine, up to a total of 12 mg/kg, i.v., had no effect on the timing or amplitude of the first burst but the frequency of the after-discharges was reduced and the peaks increased. Five of six experiments gave results of this type, the sixth animal showing no change in firing pattern. Results from one animal are shown in Figure 4. In three animals,

diprenorphine was given (45 to 90 μg/kg, i.v.), after the larger doses of morphine; there was partial reversal of the effects on after-discharges.

Etorphine and diprenorphine administered electrophoretically. Since drugs administered intravenously can affect many cells on the pathway from peripheral nerve to cerebral cortex, etorphine was also administered electrophoretically in the vicinity of cortical neurones and post-stimulus histograms prepared.

In 15 cells no consistent effect by etorphine (10–40 nA) was observed on the timing of the first burst following a stimulus. Four of these cells, together with a further 7 cells were also studied using computer bin widths of 1 or 2 ms; etorphine

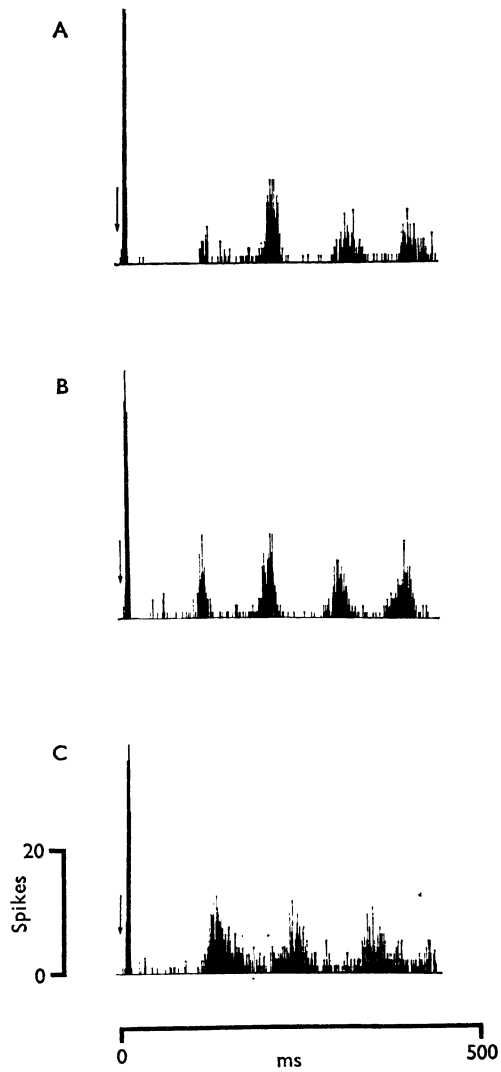

FIG. 4. The effect of morphine on the firing of a cortical neurone in response to a stimulus (0·3 ms, 0·5 Hz) to the left sciatic nerve. The method of analysis was similar to that of Fig. 2 but the bin width was 1 ms and 32 sweeps were summed. (A), Control; (B), 2 min after morphine (2 mg/kg, i.v.); (C), 2 min after a second dose of morphine (10 mg/kg) and 14 min after the first dose.

reversibly reduced after-discharges in 9 of them. When relatively large currents (30–40 nA) were passed for prolonged periods, the number of action potentials in the first burst was also reduced (Figure 5). Following the continuous ejection of etorphine, 20 nA for 4 min, after-discharges were nearly completely suppressed but complete recovery was not observed in this cell. These experiments were rendered difficult by the reduction in spike height produced by these ejecting currents, as this effect alone could cause an apparent loss of after-discharges without any effect on the primary response if the latter were superimposed on a large field potential.

Interaction of etorphine and diprenorphine with possible transmitters

Etorphine and diprenorphine were ejected electrophoretically in the vicinity of spinal cord neurones and cells of the area of cerebral cortex previously defined. Effects on the action of glycine, GABA, L-glutamate and acetylcholine were sought.

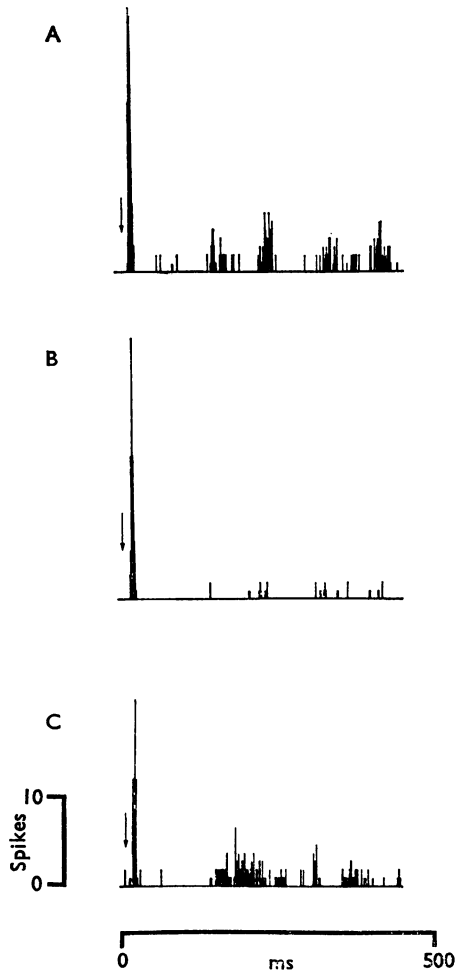

FIG. 5. The effect of electrophoretically administered etorphine on the firing pattern of a cortical cell in response to a stimulus to the left sciatic nerve. Thirty-two sweeps were summed and the bin width was 1 ms. (A), Control; (B), 4 min after the commencement of ejection of etorphine, 20 nA for 11 min; (C), 13 min after the cessation of ejection of etorphine.

On spinal neurones, ejection of etorphine (up to 80 nA) had no consistent effect on the depressant effect of glycine and GABA nor on the excitant action of glutamate. On one cell, diprenorphine blocked the depressant effect of glycine but, on other cells, it was without effect on the action of glycine, GABA and glutamate. When diprenorphine was ejected with currents greater than 50 nA, spike amplitude was usually reduced.

On 10 cortical cells the effects of acetylcholine, glutamate and GABA were not altered selectively by etorphine. High ejecting currents (30–60 nA) usually reduced spike amplitude and depressed the firing rate and then the excitant action of acetylcholine and glutamate were reduced in parallel. As in the spinal cord, the first observed effect of electrophoretically ejected diprenorphine was a reduction in spike amplitude so that the counting of cell discharge rate became impossible.

Discussion

The transmitter involved in the initial activation of cells of the cerebral cortex by a stimulus to a peripheral nerve is unknown, but there is evidence against the participation of acetylcholine (Krnjević & Phillis, 1963; Krnjević, 1964; Szerb, 1965; Spehlmann, Daniels & Smathers, 1971). Etorphine given intravenously increased the latency of this response without reducing the total number of action potentials, an effect which can be explained by a reduction in conduction velocity of the appropriate nerve fibres or a delay in the synaptic activation of cells along the pathway from periphery to cortex. Although the effect of etorphine on conduction velocity is unknown, concentrations of morphine many times those impairing transmission in hypogastric ganglia of the cat are without effect on the conduction velocity of nerve fibres (Kosterlitz & Wallis, 1964).

The failure of electrophoretically administered etorphine to affect the latency of the primary evoked response does not exclude an action by etorphine at this synapse. The complex shape and large size of these neurones renders inconclusive negative results obtained by this method. However, other substances which suppress after-discharges when given intravenously, viz. atropine and chloralose, do not affect the latency or size of the primary evoked response (Krnjević & Phillis, 1963). Further experiments to study the effects of intravenous etorphine on the activation of cells of the dorsal horn of the spinal cord, cuneate and gracile nuclei and thalamus may elucidate the site of the effect on transmission.

There is evidence that the after-discharges of cortical cells following a stimulus to a peripheral nerve involve cortical cholinergic synapses (Adrian, 1941; Chatfield & Dempsey, 1942; Krnjević & Phillis, 1963; Krnjević, 1964; Brownlee & Mitchell, 1968). After-discharges were depressed by electrophoretically administered etorphine to a greater extent than was the primary evoked response, though the excitation caused by electrophoretically administered acetylcholine was not blocked. If acetylcholine is indeed the transmitter mediating after-discharges, then this failure to block its action suggests a presynaptic action by etorphine.

In these experiments large doses of morphine reduced but did not suppress cortical after-discharges, an effect readily obtained with doses of etorphine used. Whilst this effect may be relevant to an understanding of the greater catatonia produced by etorphine, the animals were anaesthetized with barbiturate and the firing pattern in response to peripheral nerve stimulation differed from that observed in conscious animals (Baker, Fetz & Towe, 1969).

An interference with the release of acetylcholine from nerve terminals has been proposed for the action of morphine on the ileum (Paton, 1957; Schaumann, 1957), heart (Kennedy & West, 1967), sympathetic ganglia (Kosterlitz & Wallis, 1966) and spinal Renshaw cells (Duggan & Curtis, 1972). Neurochemical studies have shown an action by morphine on the levels of acetylcholine within the central nervous system (Large & Milton, 1970; Hano, Kaneto, Kakunaga & Moribayashi, 1964) and on its release (Beleslin & Polak, 1965; Sharkawi & Schulman, 1969; Jhamandas, Pinsky & Phillis, 1970; Jhamandas, Phillis & Pinsky, 1971).

Previous studies on the effect of morphine on cortical evoked responses from stimulation of the tooth pulp in the dog (2–10 mg/kg, Chin & Domino, 1961) and medial lemniscal stimulation in the cat (6 mg/kg, Fujita, Yasuhara, Yamamoto & Ogiu, 1954) showed no changes in latency or amplitude of the primary evoked response but made no reference to effects on after-discharges. The findings with etorphine, particularly on the after-discharges, suggest that this compound has an effect on cortical synapses and that this may be by reducing the amount of acetylcholine released from nerve terminals.

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REFERENCES

ADRIAN, E. D. (1941). Afferent discharges to the cerebral cortex from peripheral sense organs. *J. Physiol., Lond.*, **100**, 159–191.

BAKER, M. A., FETZ, E. E. & TOWE, A. L. (1969). Activity of single neurons in the somesthetic cortex of the awake cat. *The Physiologist*, **12**, 164.

BELESLIN, D. & POLAK, R. L. (1965). Depression by morphine and chloralose of acetylcholine release from the cat's brain. *J. Physiol., Lond.*, **177**, 411–419.

BENTLEY, K. W., BOURA, A. L. A., FITZGERALD, A. E., HARDY, D. G., MCCOUBREY, A., AIKMAN, M. L. & LISTER, R. E. (1965). Compounds possessing morphine-antagonising or powerful analgesic properties. *Nature, Lond.*, **206**, 102–103.

BENTLEY, K. W. & HARDY, D. G. (1963). New potent analgesics in the morphine series. *Proc. chem. Soc.*, p. 220.

BLANE, G. F., BOURA, A. L. A., FITZGERALD, A. E. & LISTER, R. E. (1967). Actions of etorphine hydrochloride (M99): a potent morphine-like agent. *Br. J. Pharmac. Chemother.*, **30**, 11–22.

BROWNLEE, W. G. & MITCHELL, J. F. (1968). The pharmacology of cortical repetitive after-discharges. *Br. J. Pharmac. Chemother.*, **33**, 217P.

CHATFIELD, P. O. & DEMPSEY, E. W. (1942). Some effects of prostigmine and acetylcholine on cortical potentials. *Am. J. Physiol.*, **135**, 633–640.

CHIN, J. H. & DOMINO, E. F. (1961). Effects of morphine on brain potentials evoked by stimulation of the tooth pulp of the dog. *J. Pharmac. exp. Ther.*, **132**, 74–86.

CROOKS, J. L., WHITELEY, H., JENKINS, J. T. & BLANE, G. F. (1970). The use of a new analgesic-tranquilliser mixture in dogs. *Vet. Rec.*, **87**, 498–502.

CURTIS, D. R. (1964). Microelectrophoresis. In: *Physical Techniques in Biological Research*, Vol. 5, ed. by Nastuk, W. L., pp. 144–190. New York: Academic Press.

DUGGAN, A. W. & CURTIS, D. R. (1972). Morphine and the synaptic activation of Renshaw cells. *Neuropharmacology*, **11**, 189–196.

FUJITA, S., YASUHARA, M., YAMAMOTO, S. & OGIU, K. (1954). Studies on sites of action of analgesics. 2. The effect of analgesics on afferent pathways of pain. *Jap. J. Pharmac.*, **4**, 41–51.

HANO, K., KANETO, H., KAKUNAGA, T. & MORIBAYASHI, N. (1964). Pharmacological studies of analgesics. VI. The administration of morphine and changes in acetylcholine metabolism in mouse brain. *Biochem. Pharmac.*, **13**, 441–447.

HARTHOORN, A. M. (1967). Comparative pharmacological reactions of certain wild and domestic mammals to thebaine derivatives in the M series of compounds. *Fedn Proc.*, **26**, 1251–1261.

HELLON, R. F. (1971). The marking of electrode tip positions in nervous tissue. *J. Physiol., Lond.*, **214**, 12*P*.

JHAMANDAS, K., PINSKY, C. & PHILLIS, J. W. (1970). Effects of morphine and its antagonists on release of cerebral cortical acetylcholine. *Nature, Lond.*, **228**, 176–177.

JHAMANDAS, K., PHILLIS, J. W. & PINSKY, C. (1971). Effects of narcotic analgesics and antagonists on the *in vivo* release of acetylcholine from the cerebral cortex of the cat. *Br. J. Pharmac.*, **43**, 53–66.

KENNEDY, B. L. & WEST, T. C. (1967). Effect of morphine on electrically induced release of autonomic mediators in the rabbit sinoatrial node. *J. Pharmac. exp. Ther.*, **157**, 149–158.

KOSTERLITZ, H. W. & WALLIS, D. I. (1964). The action of morphine-like drugs on impulse transmission in mammalian nerve fibres. *Br. J. Pharmac. Chemother.*, **22**, 499–510.

KOSTERLITZ, H. W. & WALLIS, D. I. (1966). The effects of hexamethonium and morphine on transmission in the superior cervical ganglion of the rabbit. *Br. J. Pharmac. Chemother.*, **26**, 334–344.

KREBS, H. A. & HENSELEIT, K. (1932). Untersuchungen über die Harnstoffbildung im Tierkörper. *Hoppe-Seyler's Z. physiol. Chem.*, **210**, 33–66.

KRIEG, W. J. S. (1946). Connections of the cerebral cortex. 1. The albino rat. A. Topography of the cortical areas. *J. comp. Neurol.*, **84**, 221–276.

KRNJEVIĆ, K. (1964). Micro-iontophoretic studies on cortical neurones. *Int. Rev. Neurobiol.*, **7**, 41–98.

KRNJEVIĆ, K. & PHILLIS, J. W. (1963). Pharmacological properties of acetylcholine-sensitive cells in the cerebral cortex. *J. Physiol., Lond.*, **166**, 328–350.

LARGE, W. A. & MILTON, A. S. (1970). The effect of acute and chronic morphine administration on brain acetylcholine levels in the rat. *Br. J. Pharmac.*, **38**, 451–452.

LISTER, R. E. (1964). Structure activity requirements in some novel thebaine-derived analgesics. *J. Pharm. Pharmac.*, **16**, 364–366.

MORISON, R. S. & DEMPSEY, E. W. (1943). Mechanism of thalamocortical augmentation and repetition. *Am. J. Physiol.*, **138**, 297–308.

PATON, W. D. M. (1957). The action of morphine and related substances on contraction and on acetylcholine output of coaxially stimulated guinea pig ileum. *Br. J. Pharmac. Chemother.*, **12**, 119–127.

SCHAUMANN, W. (1957). Inhibition by morphine of the release of acetylcholine from the intestine of the guinea pig. *Br. J. Pharmac. Chemother.*, **12**, 115–116.

SHARKAWI, M. & SCHULMAN, M. P. (1969). Inhibition by morphine of the release of ^{14}C-acetylcholine from rat brain cortex slices. *J. Pharm. Pharmac.*, **21**, 546–547.

SPEHLMANN, R., DANIELS, J. C. & SMATHERS, C. C. (1971). Acetylcholine and the synaptic transmission of specific impulses to the visual cortex. *Brain*, **94**, 125–138.

SZERB, J. C. (1965). Averaged evoked potentials and cholinergic synapses in the somatosensory cortex of the cat. *Electroenceph. clin. Neurophysiol.*, **18**, 140–146.

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