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BLOCKADE OF STRIATAL NEURONE RESPONSES TO MORPHINE BY AMINOPHYLLINE: EVIDENCE FOR ADENOSINE MEDIATION OF OPIATE ACTION

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1 The responses of cortical and striatal neurones to morphine and adenosine applied iontophoretically have been studied in the male rat.

2 The majority of cells (57%) within the corpus striatum were profoundly inhibited, and a smaller proportion (18%) excited by morphine. Adenosine depressed the firing rate of 30/44 cells in the striatum, excitation never being observed. In contrast, the responses of cortical cells to morphine were typically weak and required longer ejection pulses to effect comparable changes in firing rate.

3 Aminophylline applied iontophoretically, as an anion, proved able to antagonize reversibly both morphine and adenosine in parallel.

4 On a number of cells, γ-aminobutyric acid (GABA) was used as a control depressant but aminophylline did not appear to reduce these responses.

5 The responses to morphine (both inhibitory and excitatory) were not easily antagonized by naloxone. Typically, excitatory responses were easier to antagonize than the inhibitory ones.

6 It is concluded that a consequence of the interaction of morphine with its receptors may be the release of adenosine which subsequently produces the inhibition observed with morphine.

Introduction

The discovery of enkephalins and endorphins within the central nervous system and their relevance to analgesia (Buscher, Hill, Romer, Cardinaux, Closse, Hauser & Pless, 1976) has stimulated interest in the physiology and pharmacology of both opiates and these endogenous opioid peptides. However, the precise mechanisms involved in the interaction of opiates with their receptors remains unclear. There is evidence that adenosine may be involved in the responses to morphine. Both morphine and adenosine inhibit transmitter release (Ginsborg & Hirst, 1972; Hedqvist & Fredholm, 1976; Hayashi, Kunitoko, Mori, Shinozuka & Yamada, 1978) and both drug actions can be blocked by theophylline (Sawynok & Jhamandas, 1976). Conversely, dipyridamole, an inhibitor of the adenosine uptake (Huang & Daly, 1974) potentiates the inhibition of transmitter release by both adenosine and morphine (Gintzler & Musacchio 1975; Hayashi *et al.*, 1978). In addition, methylxanthine derivatives, thought to act as phosphodiesterase inhibitors, can both potentiate morphine withdrawal symptoms (Collier & Francis, 1975) and induce a quasi-morphine withdrawal syndrome in naive or morphine-dependent rats (Francis, Roy & Collier, 1975; Collier & Francis, 1975; Butt, Collier, Cuthbert, Francis & Saeed, 1979).

At the cellular level, the responses of single neurones to morphine and enkephalins applied iontophoretically have been investigated extensively (Gent & Wolstencroft, 1976a, b; Zieglgänsberger & Fry, 1976; Zieglgänsberger & Tulloch, 1979; Dafny, Brown, Burks & Rigor, 1979). Of particular interest to us is the depression of neuronal firing by morphine and endogenous opioid peptides, an action shown to be stereospecific (Gayton & Bradley, 1976), and blocked by naloxone (Bradley, Bramwell & Dray, 1976; Bradley, Briggs, Gayton & Lambert, 1976; Dafny *et al.*, 1979) and reduced or abolished in morphine-tolerant rats (Dafny *et al.*, 1979). This inhibitory action is shared by adenosine and other purines (Phillis, Kostopoulos & Limacher, 1974; Taylor & Stone, 1978; Scholfield, 1978; Stone & Taylor, 1979). The present experiments were therefore undertaken to determine whether the inhibitory actions of morphine following combination with the opiate receptor, are due to a release of adenosine. The areas chosen for investiga-

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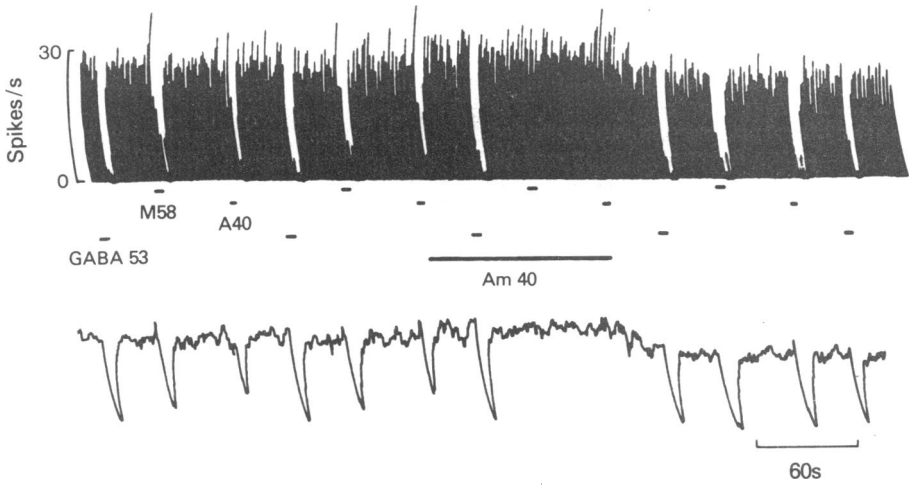

Figure 1 Ratemeter records of a striatal neurone illustrating depression of firing rate by morphine (M), γ-aminobutyric acid (GABA) and adenosine (A) and the blockade of the morphine and adenosine by aminophylline (Am). The bars indicate time and duration of drug application and the numbers the ejection current in nA. In this case the aminophylline was subjected to a 'resting' negative current of 12 nA (see text).

tion were the corpus striatum, an area rich in opiate receptors (Pert, Snyder & Kuhar, 1976) and for comparison, the cerebral cortex which has few such receptors.

Methods

Male Porton-Wistar rats weighing between 200 and 350 g were anaesthetized with urethane (1.3 g/kg, i.p.) and placed in a stereotaxic frame. Body temperature was maintained automatically at 37°C. The skull was exposed and a burr hole drilled to allow electrode penetration into the somatosensory cortex or the corpus striatum (DeGroot's (1959) co-ordinates: AP 6.6–7.4, L 2.5, H 5.0). Drugs were applied by microiontophoresis from 6 or 7 barrelled micropipettes with tip diameters 4 to 12 μm. The drugs used were: morphine sulphate 50 or 100 mM pH 5.5; adenosine hemisulphate 100 mM pH 4.5; adenosine 5'-monophosphate sodium salt (AMP) 200 mM pH 4.0; γ-aminobutyric acid (GABA) 100 mM pH 4.0; naloxone HCl 50 mM, pH 4.0, aminophylline (theophylline ethylenediamine) 50 or 100 mM, pH 9.

The drugs were all ejected as cations except aminophylline which was expelled with a negative (inward) current. The latter drug usually dissolved in distilled water to give a stable solution of pH 9 but on occasions when the pH of the distilled water was 5.5 or below, adjustment of the pH was made with 0.5 M NaOH. One barrel of the micropipette always contained 165 mM NaCl and this was used for current balancing in order to ensure zero net current flow at the electrode tip. This therefore minimized the possibility of current effects during drug ejection. Drug ejection was performed in a constant time cycle controlled by a Neurophore unit which also automatically regulated the current balancing.

Unit activity was recorded through either one barrel of the microelectrode containing 3 M NaCl or through a single microelectrode containing 1 M potassium acetate and glued alongside the multibarrelled pipette. Spikes were amplified, displayed on an oscilloscope and gated with a window discriminator. The output pulses of the latter were integrated over 1 s and plotted out on a polygraph. The discriminator pulses were also used to generate an instantaneous rate meter record which was sometimes easier to interpret. When deemed desirable, action potentials were also recorded on magnetic tape.

Results

Morphine

In the corpus striatum 127 cells were tested for their response to morphine applied iontophoretically with currents ranging from 50 to 170 nA (mean ± s.e. 109 ± 50 nA). Of these, 72 (57%) were inhibited by morphine, 23 (18%) displayed an increase in firing rate and the remaining 32 (25%) were unresponsive with ejection currents up to 170 nA. The inhibition was usually rapid in onset, profound in nature and with quick recovery within 5 to 8 s (see Figure 1).

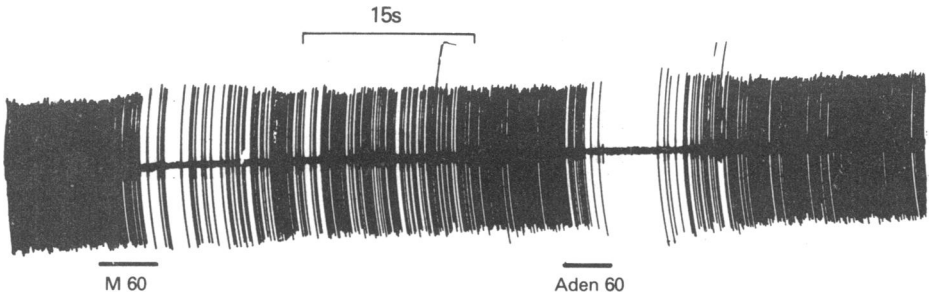

Figure 2 Spike records of a striatal neurone replayed from magnetic tape showing rapid inhibitory responses to morphine (M) and adenosine (Aden), together with increase in spike height suggestive of hyperpolarization. The numbers indicate the current in nA.

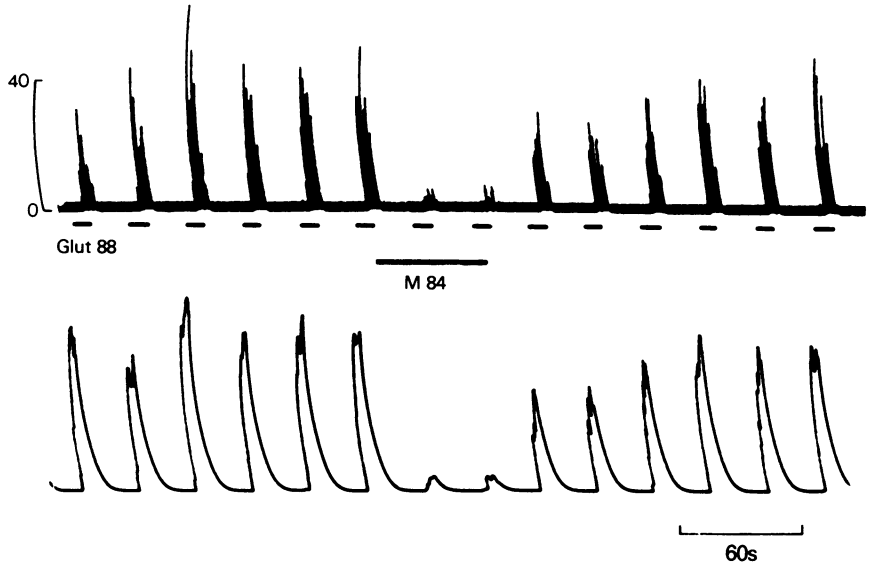

Figure 3 Ratemeter records of a striatal neurone showing antagonism of glutamate (Glut)-evoked activity by application of morphine (M). The bars indicate time and duration of drug application and the numbers the current in nA. Ordinate scale: spikes/s.

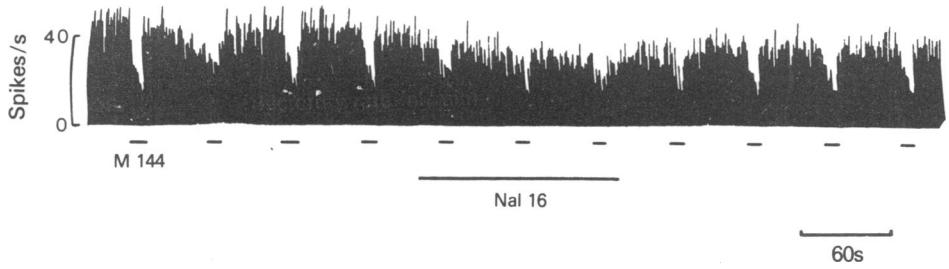

Figure 4 Ratemeter record illustrating partial antagonism of morphine (M)-evoked inhibition of a striatal cell during concurrent application of naloxone (Nal). The bars indicate time and duration of ejection of drug and the numbers the current in nA.

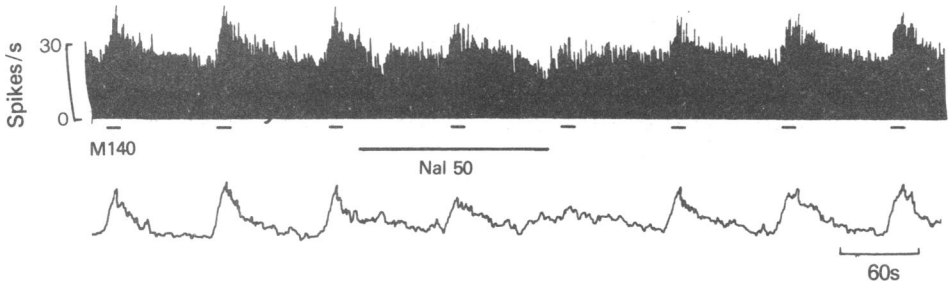

Figure 5 Ratemeter records of a striatal cell illustrating antagonism of morphine (M) excitation with naloxone (Nal). The bars indicate time and duration of ejection and the numbers the current in nA.

Spike records (Figure 2) showed an increase in spike height following morphine application suggestive of hyperpolarization of the cell. However, a few cells (8) showed a much slower time course of response, the inhibition taking up to 10 s to reach a maximum and recovery lasting as long as 30 s.

Excitation was also observed, though less frequently and this was usually of a short latency although return of the firing rate to baseline levels was typically slower than with the inhibitory responses (Figure 5). Both inhibition and excitation were observed with the same electrode.

In the cortex the responses to morphine were markedly different from those obtained in striatum: 13 out of 29 cells were inhibited, 5 were excited and 11 unresponsive. However, the inhibitions observed were typically weak, of longer latencies (up to 10 s) and required higher ejection currents for longer periods of time (up to 150 nA for 30 s). Similarly the excitations observed were slight and slow in onset, requiring maintained application for up to 15 s.

This variation in the character of the response between cortex and striatum was not merely a result of different electrode properties in different experiments, as both the weak cortical responses and the much clearer striatal responses were observed in a single electrode penetration. Most of the cells discussed above were spontaneously active, but in a few experiments glutamate was included in the multibarrel in order to study morphine responses on quiescent cells. On 17 cells studied in this way, morphine reduced glutamate-evoked activity. The depressions were rapid in onset and recovery, as with spontaneously active cells (Figure 3).

Naloxone

Neither the inhibitory nor the excitatory responses were consistently or readily antagonized by naloxone when applied either iontophoretically, intraperitoneally or intravenously. The inhibitory responses were typically very resistant to naloxone blockade. Figure 4 shows some weak antagonism of morphine inhibitory responses following naloxone applied iontophoretically. This record also illustrates the fact that naloxone produced a direct depression of cell firing, which complicated interpretation of the results. The use of high ejecting currents for shorter times was also tried but proved equally unsatisfactory.

Figure 5 illustrates the much clearer antagonism of excitatory responses due to morphine, by naloxone applied iontophoretically. Interestingly, in 5 cases naloxone reversibly antagonized the inhibition due to γ-aminobutyric acid (GABA).

Adenosine and aminophylline

The iontophoresis of adenosine with currents of 40 to 160 nA depressed the firing rate of 30 out of 44 cells tested. The responses were usually rapid in onset (within 5 s) and outlasted the ejection pulse by no more than 10 s (Figures 1 and 2). Aminophylline reduced these depressions when applied with inward (negative) currents of 20 to 80 nA (Figure 1). As with morphine, the spike records may be indicative of hyperpolarization of the cell following drug application (Figure 2).

Inhibitory responses to morphine were also observed on 22 units depressed by adenosine as described above. The iontophoresis of aminophylline abolished the adenosine and morphine responses in parallel, whilst having no apparent effect on control depressions produced by GABA (Figure 1). This selective blockade was seen in all 22 cells studied.

Discussion

The responses of neurones in the brain to morphine applied iontophoretically have been studied extensively, although the published accounts sometimes differ as to the type of response observed. In the brain

stem both predominantly excitatory (Bradley & Dray, 1974; Bradley *et al.*, 1976b; Davies & Dray, 1978) and predominantly inhibitory (Gent & Wolstencroft, 1976a, b; Gent, Smyth, Snell & Wolstencroft, 1977) effects have been observed as well as some, rare, biphasic responses (Gent & Wolstencroft, 1976b; Bradley *et al.*, 1976a, b). More anteriorly, in the striatum, inhibition of neuronal firing appears to be the predominant morphine response (Zieglgänsberger & Fry, 1976; Dafny *et al.*, 1979) and the results presented here agree with those observations.

The poor responses obtained in the cortex are consistent with the low density of opiate receptors found in this area (Pert, Kuhar & Snyder, 1975; Pert *et al.*, 1976) although again there is no agreement as to whether inhibition (Satoh, Zieglgänsberger & Herz, 1975; Zieglgänsberger & Fry, 1976) or excitation (Davies & Dray, 1978) predominates; our results favour the former workers.

The difficulty encountered with naloxone antagonism of the morphine responses was at first surprising in view of the literature describing a clear reversal of some morphine responses on single cells (Bradley *et al.*, 1976a, b; Zeiglgänsberger & Fry, 1976; Dafny *et al.*, 1979). However, a close examination of the literature reveals a wealth of inconsistencies. In the brain stem it appears to be the depression of neuronal firing by morphine that is naloxone-sensitive (Bradley *et al.*, 1976a, b) whereas other workers found that it is the excitation that can be reversed by naloxone (Davies & Dray, 1978). Some workers have found that the actions of morphine and/or endogenous opioids, the enkephalins, when applied iontophoretically can be easily reversed by naloxone (Bradley *et al.*, 1976a, b) whilst others have only achieved a partial antagonism of the enkephalins (Hill, Pepper & Mitchell, 1976; Zieglgänsberger & Tulloch, 1979). There are also reports of failures to reverse the responses to enkephalins and morphine (Gent & Wolstencroft 1976b; Gent *et al.*, 1977; Dingledine, Iversen & Breuker, 1978). In addition, Buscher *et al.* (1976) had to use very high doses of naloxone to block the analgesic activity of enkephalins.

The interaction of naloxone with opiate and opioid compounds is therefore much more complex than it is often considered to be. In particular it should not be considered that the failure of naloxone to reverse a neuronal response to morphine necessarily indicates that the opiate response is 'non-specific'. The role of opiate receptors and endogenous enkephalins in many parts of the brain, including the striatum, is still unclear, and the only real conclusion to be drawn from naloxone insensitivity is that the neuronal response under consideration probably does not contribute to those behavioural effects of morphine which are naloxone-sensitive.

Biochemical studies have, in fact, demonstrated several opiate/opioid receptors in brain, at least one of which is not readily blocked by naloxone (the δ-enkephalin receptor) (Lord, Waterfield, Hughes & Kosterlitz, 1977). As the corpus striatum is an area rich in enkephalins, and able to synthesize them (Hughes, Kosterlitz & McKnight, 1978) it may be that they have a physiological role in this region and that most of the local receptors are therefore of this δ, naloxone-resistant, species.

The antagonism of GABA by naloxone which was occasionally seen is interesting in the light of recent work showing not only a similar antagonism by microiontophoresis, but also an ability of naloxone to potentiate bicuculline-induced convulsions, the latter being prevented by diazepam (Dinglédine *et al.*, 1978). This raises doubts as to the value of naloxone as a specific opiate antagonist in single cell studies.

The main finding of the present study was the rapid and reversible blockade of the inhibitory responses to morphine by aminophylline. Methylxanthines such as aminophylline are now widely recognised as potent and relatively specific antagonists of the effects of adenosine on extracellularly directed receptors (Blume *et al.*, 1973; Gintzler & Musacchio, 1975; Sawynok & Jhamandas, 1976; Green & Stanberry, 1977; Okwuasaba, Hamilton & Cook, 1977; Stone & Taylor, 1977) and our results are therefore consistent with the hypothesis that a consequence of morphine's interacting with its receptor is a release of adenosine, which then combines with its receptor to produce the well-documented depression of firing observed here (Phillis *et al.*, 1974; Kostopoulos & Phillis, 1977; Stone & Taylor, 1977; Taylor & Stone, 1978).

Although methylxanthines do have the property of inhibiting phosphodiesterase this is a weak action (Sawynok & Jhamandas, 1976; Okwuasaba *et al.*, 1977) compared to the specific blockade of adenosine at its receptor (Blume, Dalton & Sheppard, 1973; Green & Stanberry, 1977). It is therefore highly probable that the effects seen here are due to antagonism of the neuronal effects of adenosine, especially in view of the low doses of aminophylline required, rather than phosphodiesterase inhibition.

It might also be argued that, since adenosine and morphine are potent inhibitors of calcium-dependent neurotransmitter release (Ginsborg & Hirst, 1972; Gintzler & Musacchio, 1975; Hedqvist & Fredholm, 1976; Sawynok & Jhamandas, 1976; Okwuasaba *et al.*, 1977) that such an action could contribute to their depressant effects on neuronal firing. Aminophylline might then reduce that action by increasing calcium availability in the presynaptic terminals. However, the only relevant work seems to be that of Edstrom & Phillis (1976) who reported that adenosine hyperpolarized cortical neurones with no change of membrane conductance. While vaguely suggestive, this finding certainly does not prove a presynaptic site of

action of adenosine. On the contrary, we have demonstrated that the depressant effects of adenosine and morphine are exhibited against excitatory responses to glutamate (Figure 3) a compound known to act postsynaptically by increasing sodium conductance (Zieglgänsberger & Puil, 1973).

The action of morphine suggested here is consistent with other evidence implicating adenosine in the mechanisms of the effects of opiates and opioids. The reduction of morphine analgesia by methylxanthines (Ho *et al.*, 1973) and their ability to induce a quasi-morphine withdrawal syndrome (Francis *et al.*, 1975; Collier & Francis, 1975; Butt *et al.*, 1979) can be explained in terms of a blockade of adenosine released by morphine. In addition, with respect to the inhibition of transmitter release by adenosine and morphine (Ginsborg & Hirst, 1972; Hedqvist & Fredholm, 1976) the degree of inhibition produced by both these compounds is inversely related to calcium concentration and stimulation frequency implying a similar mode of action (Gintzler & Musacchio, 1975).

In conclusion, the work presented here provides evidence for the mediation by adenosine of the inhibitory effects of morphine upon neuronal firing and suggests that this may be an alternative explanation to that of phosphodiesterase inhibition for some of the reported actions of methylxanthines.

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