Br. J. Pharmac. (1980), **69**, 461–466

MORPHINE AND SUPRASPINAL INHIBITION OF SPINAL NEURONES: EVIDENCE THAT MORPHINE DECREASES TONIC DESCENDING INHIBITION IN THE ANAESTHETIZED CAT

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1 A study was made in cats anaesthetized with barbiturate or α-chloralose, of the excitation of dorsal horn neurones by impulses in unmyelinated (C) primary afferent fibres of the tibial nerve.

2 Block of conduction in the first lumbar segment by cooling produced large increases in the number of action potentials evoked by C fibre afferents in neurones of more caudal segments.

3 Morphine (0.3 to 1.0 mg/kg) reduced the excitation of neurones by C fibre afferents and also reduced the increase produced by blocking conduction in the spinal cord. Naloxone (0.1 to 0.3 mg/kg) reversed these effects of morphine.

4 This decrease in descending inhibition supports findings in the decerebrate cat but gives no support to the hypothesis that an important component of morphine analgesia is an activation of descending inhibitory pathways.

Introduction

It has been proposed that an important component of morphine analgesia is the activation of brain stem neurones which project to the spinal cord and ultimately inhibit the spinal transmission of nociceptive information (see reviews by Besson, Le Bars & Oliveras, 1978; Fields & Basbaum, 1978; Yaksh & Rudy, 1978). A number of indirect methods have been used to investigate this hypothesis. They include (a) the effects of supraspinal and limited spinal lesions on morphine analgesia, (b) comparisons of the effect of morphine on the activity of dorsal horn neurones in spinal animals and those with intact central nervous systems and (c) the effects of intravenous morphine and naloxone on the firing of neurones in supraspinal areas believed to produce inhibition of dorsal horn neurones. The implications of some of these methods for the results of the present experiments will be discussed subsequently.

The most direct method of investigating this problem is to measure descending inhibition of spinal neurones activated by peripheral noxious stimuli before and after the administration of morphine and naloxone. There is only one report of such measurements, that of Jurna & Grossman (1976) who reversibly blocked conduction in the spinal cord by cooling. In decerebrate cats, morphine decreased tonic descending inhibition of dorsal horn neurones which had axons ascending in the antero-lateral spinal fasciculus. The decerebrate preparation is characterized by powerful tonically active inhibition of the spinal transmission of impulses in flexor reflex afferents (Lundberg, 1964), an effect which may occlude any possible added inhibition by morphine. Thus, although unanaesthetized, there are difficulties in relating the effects of morphine on descending inhibition in such preparations to those in the normal animal. The present experiments have examined the effects of doses of morphine known to be analgesic in normal cats (McKenzie & Beechey, 1962) on descending inhibition of spinal neurones excited by impulses in C fibre primary afferents in cats lightly anaesthetized with pentobarbitone or α-chloralose. The results support those obtained in decerebrate cats; morphine decreased supraspinal inhibition of the cells studied.

Methods

Experiments were performed on 11 cats. Six were anaesthetized with pentobarbitone sodium (35 mg/kg i.p. initially), 5 with α-chloralose (50 to 60 mg/kg i.p.). All animals were artificially ventilated with air following paralysis with gallamine triethiodide; end tidal CO_2 levels were kept at approximately 4%. A cannula in a carotid artery was used to measure blood pressure and experiments were terminated if systolic pressure, in the absence of any temporary procedure such as spinal cold block, fell below 100 mmHg. The spinal cord was exposed by lumbar laminectomy and a small metal chamber, with a concave lower surface,

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was placed on the dorsal surface of the first lumbar segment. An ethylene glycol/water mixture (−2°C) was circulated through the chamber to block conduction in the spinal cord. The cooling chamber was surrounded with silicone grease and, when care was taken to prevent contact with the warm liquid paraffin bathing the rest of the spinal cord, evidence of blockade of conduction was obtained within 2 min. A thermistor incorporated in the cooling chamber, and in contact with the dorsal surface of the spinal cord usually indicated a stable temperature of 3 to 5°C within 5 min. Conduction in the spinal cord was restored by circulating water at 37°C through the metal chamber. Descending inhibition of spinal neurones always returned to near control levels within 2 min of warming. The duration of cold block was 7 to 10 min and was usually repeated twice before morphine was administered, 4 or 5 times after morphine and twice after naloxone. If return of descending inhibition is a satisfactory measure (see Figure 2), this repetition of cold block did not damage the spinal cord.

Dorsal horn neurones were excited by electrical stimulation of the ipsilateral common tibial nerve. In preliminary experiments natural stimulation of the skin with radiant heat was used as the noxious stimulus. However, this was abandoned when it was clear that the circulatory disturbances of spinal cold block (Duggan, Griersmith, Headley & Maher, 1978) when added to those of morphine (Schmidt & Livingston, 1933) changed the rate of rise of skin temperature during each period of heating. Previous experiments (Duggan & Griersmith, 1979) have shown that such changes produce large variations in the firing of cutaneous nociceptors. Since many unmyelinated primary afferent fibres convey nociceptive information to the spinal cord (Bessou & Perl, 1969; Beck, Handwerker & Zimmermann, 1974) the tibial nerve was stimulated with a strength at least 5 times threshold for C fibre activation (stimulus frequency, 0.2 Hz, pulse width 0.5 ms).

Extracellular recordings were obtained from dorsal horn neurones of the lower lumbar segments using glass microelectrodes filled with 2% pontamine sky blue in 2.0 M sodium acetate. All cell positions were confirmed by histological location of deposits of pontamine sky blue. Cell firing was counted with a window discriminator. The number of action potentials evoked by impulses in C fibre afferents following each electrical stimulus to the tibial nerve was determined by gating an electronic counter. The gate was timed to include only those action potentials which, by their latency and stimulus threshold, were produced by C fibre afferent impulses (see Figure 1).

At intervals of 4 min, 16 responses were used to compile a peristimulus histogram. The means and s.e. means of the number of C fibre-evoked action potentials following each stimulus were subsequently determined for the 16 responses contained within each histogram.

The counting of action potentials evoked by impulses in faster conducting fibres was often not accurate because of superimposition on field potentials even though the counting amplifier had time constants of 1 or 2 ms. The position of these action potentials has been retained on the histograms of Figure 1, but the column has been truncated because of the inaccuracy in counting.

Results

In each experiment, the neurone studied was excited by impulses in both myelinated and unmyelinated primary afferent fibres. Three were in lamina IV, 6 in lamina V, 1 in lamina VI and 1 in lamina VII.

The firing pattern of a lamina V neurone is shown in the peristimulus histograms of Figure 1. The later group of action potentials only appeared with a stimulus strength of 80 times threshold for the fastest conducting fibres in the tibial nerve and were maximal with a stimulus strength of approximately 200 times threshold. The latency to the peak of the later spikes in the peristimulus histogram indicates a conduction velocity of approximately 1.5 m per s assuming no interposed synapses. Thus these later spikes were produced by impulses in C fibre primary afferents. With some neurones, low stimulus strengths elicited action potentials with latencies similar to those produced by impulses in C fibre primary afferents but these were not included in this study since these later spikes probably represent firing by long polysynaptic pathways activated by low threshold afferents.

In cats anaesthetized with α-chloralose the number of action potentials evoked by impulses in C fibres was relatively small. Indeed it was difficult to locate any neurone excited by C fibres for 6 to 8 h after the anaesthetic was administered. In animals anaesthetized with barbiturate, firing by C fibre afferents was more readily observed both in terms of time after induction of anaesthesia and the number of spikes elicited by each stimulus (see for example Figure 1). The large variation between cells in the number of evoked spikes precluded pooling of the data which have been analysed as separate experiments.

Tonic descending inhibition

With 10 of the 11 neurones studied, firing evoked by impulses in C fibre primary afferents increased significantly when conduction in the spinal cord was blocked by cooling (see Figures 1 and 2). However, with both α-chloralose and pentobarbitone anaesthetized preparations there was considerable variation in the amount of descending inhibition revealed in this

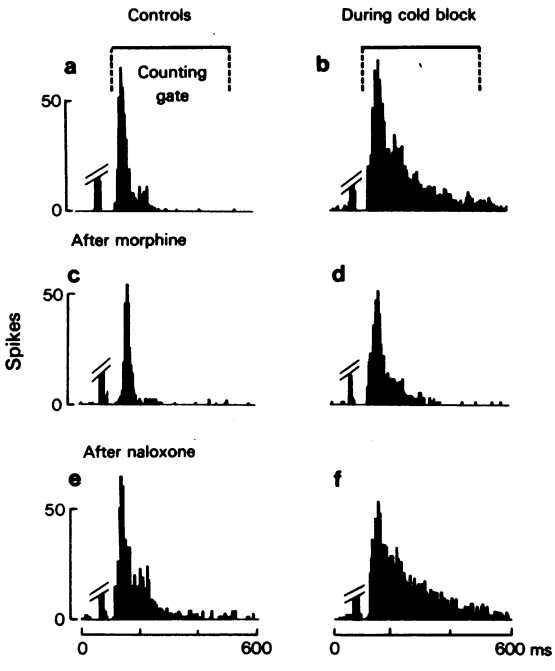

Figure 1 Peristimulus histograms of the excitation of a lamina V neurone by tibial nerve stimulation and the changes produced by cold block of the spinal cord before and after the administration of morphine and naloxone. Each histogram (address dwell time 5 ms) represents the sum of 16 responses to tibial nerve stimulation, with a stimulus strength 200 times threshold for the fastest conducting fibres. The early evoked action potentials from impulses in myelinated fibres were inaccurately counted because of a superimposed field potential and hence these columns have been truncated. Because of the slow time base, the stimulus coincides with the first bin of the truncated column.

The histograms (b), (d) and (f) were obtained after spinal conduction was blocked by cooling: (a and b) control observations; (c and d) records obtained 12 and 24 min after a total of 1 mg/kg (i.v.) of morphine had been administered; (e and f) records obtained 4 and 16 min after naloxone 0.3 mg/kg (i.v.) had been administered 60 min after the last dose of morphine.

way. With the neurone, results from which are illustrated in Figures 1 and 2 (anaesthetized with barbiturate), the counts within the period of the counting gate increased from 13.50 ± 0.61 (s.e. mean) (the 'normal' response) to 42.44 ± 0.54 following cold block of the cord. In determining the amount of inhibition on each neurone the increase in the total number of action potentials per stimulus was considered the important measure. Percentage changes can be misleading since they are markedly affected by the level of the normal response. For this reason also, each experiment has been analysed separately.

In the experiment illustrated, observations of C fibre-evoked activity were made 2, 6 and 10 min after the onset of cooling and there was no significant difference between the second and third sets of data. Thus with this neurone it was probable that descending inhibition was fully blocked after approximately 5 min of cooling. At this time it was also usual that both the hypotension and the surface temperature of the dorsal surface of the cooled segment of the spinal cord had reached plateau levels. Because of this uncertainty over the rate of onset of block of descending inhibition, in all experiments both the duration of cold block and the timing of observations were kept constant before and after the administration of morphine and naloxone. Hypotension was not the cause of decreased descending inhibition following cold block since sectioning the contralateral dorsolateral segment of the spinal cord immediately produced increases in firing similar to cold block and before any changes in blood pressure were detected.

Effects of intravenous morphine and naloxone

Morphine was administered intravenously using different dose schedules. With 6 cats, an initial dose of 0.3 to 0.5 mg/kg was followed 20 to 40 min later by a second dose of 0.5 to 1.0 mg/kg. With the remaining 5, two smaller doses were given initially (0.1 to 0.25 mg/kg) at intervals of 10 to 30 min followed by a third dose of 0.5 to 0.7 mg/kg.

The different dose schedules were used since it has been reported that relatively low doses of morphine activate descending inhibition of spinal neurones in the rabbit while higher doses are required to have direct spinal effects (Takagi, Satoh, Doi, Kawasaki & Akaike, 1976).

In no experiment was any evidence obtained that morphine increased tonic descending inhibition of the neurones studied. In 9 of 11 experiments the doses of morphine used reduced the excitation of neurones by impulses in C fibre afferents. A reduction was observed after the first dose in all 6 experiments in which this relatively high dose (0.3 to 0.5 mg/kg) was used; lower initial doses were without effect in 3 of 5 animals. After these initial doses of morphine, measurements of the change in firing produced by cold block showed a reduction in descending inhibition in all 8 animals in which the normal C fibre responses had been reduced and in one in which the initial dose of morphine was without effect. Following subsequent doses of morphine, tonic descending inhibition was reduced in 10 of the 11 experiments.

Results from one experiment are illustrated in Figures 1 and 2. With this neurone, cold block before the administration of morphine increased the mean C fibre response by 28.94 ± 1.00 spikes. Following morphine, 0.3 mg/kg, the normal response decreased from

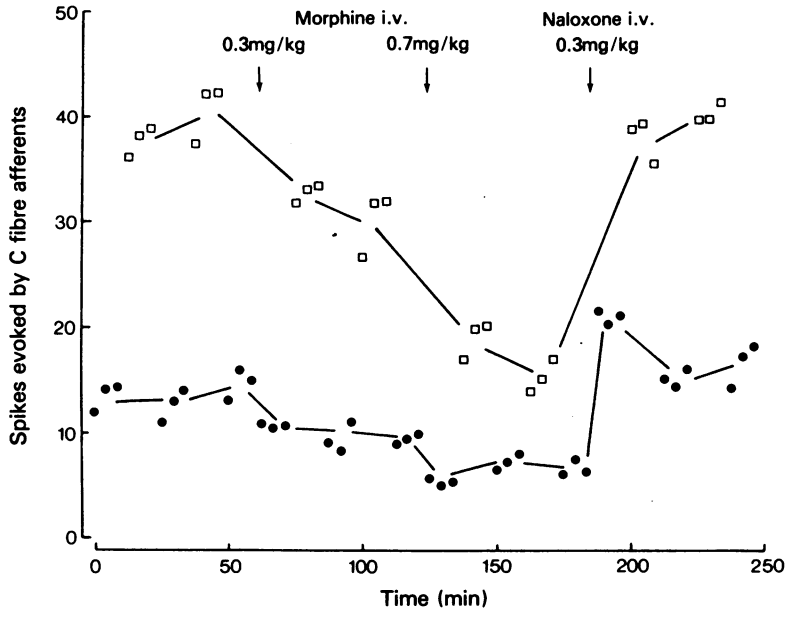

Figure 2 Reduction by morphine of descending inhibition of excitation of a dorsal horn neurone by unmyelinated primary afferents. Each point is the mean (n = 16) of the number of action potentials evoked by C fibre primary afferents following electrical stimulation of the tibial nerve. The period of counting to obtain these points is the counting gate illustrated in Figure 1; s.e. mean are not plotted since in no case were they greater than 2 spikes; (●) observations with normal conduction in the spinal cord; (□) observations with spinal conduction blocked by cooling the first lumbar segment. The neurone was located in lamina V of the seventh lumbar segment. The histograms of cell firing are illustrated in Figure 1.

13.50 ± 0.61 to 9.31 ± 0.42 and following a dose of 0.7 mg/kg to 6.50 ± 0.47. The increases produced by cold block after these two doses of morphine fell from 22.94 ± 0.52 to 10.75 ± 0.84 respectively. These changes in the effectiveness of cold block are readily seen in the graph of Figure 2. Even when these changes produced by cold block are calculated as a percentage of normal responses, it is clear that morphine decreased supraspinal inhibition of this neurone.

The observations on the neurone illustrated required 4 h and it was not possible to maintain satisfactory recording conditions for this period with all neurones, particularly when hypotension and transient hypertension were produced by cooling and warming the spinal cord. Naloxone (0.1 to 0.3 mg/kg) was therefore administered in only 7 of 11 experiments. With 5, complete reversal of both normal and cold blocked C fibre responses was observed; with 2 the depression of normal responses was reversed by naloxone but not the decreased effectiveness of cold block.

Discussion

The present experiments have shown that, in anaesthetized cats, analgesic doses of morphine decrease descending inhibition of spinal transmission of impulses in unmyelinated primary afferents. Since this result is opposed to the hypothesis that morphine analgesia, in part, results from an increase of such inhibition, it is necessary to discuss briefly the possible reasons for this difference.

Firstly, it should be emphasized that descending inhibition is tonically present in anaesthetized cats. Moreover, the hyperalgesia produced by medullary lesions of the rat (Proudfit & Anderson, 1975) and spinal lesions of the cat (Kennard, 1950) and monkey (Vierck, Hamilton & Thornby, 1971) suggest that descending inhibition is present in conscious animals. Failure to recognize the presence of tonic inhibition can result in a false interpretation of the effects of spinal and supraspinal lesions. Thus, Satoh & Takagi (1971) showed that intravenous morphine reduced the amplitude of a short latency potential recorded in the

ventrolateral funiculus of the spinal cord following electrical stimulation of a splanchnic nerve and that spinal transection abolished this action. The effect of spinal transection alone was not examined and hence it is not possible to conclude that morphine increased descending inhibition.

An interference with tonic descending inhibition can complicate the interpretation of experiments in which lesions of the medulla and spinal funiculi have reduced the apparent potency of morphine in reducing behavioural reflexes believed to be conducted only through the spinal cord. Thus, if animals are made hyperalgesic by the particular lesion, then this alone may explain the apparent decrease in potency of morphine following the lesion. Such hyperalgesia was produced by ablation of the nucleus raphé magnus of the rat in the experiments of Proudfit & Anderson (1975) but not in those of Yaksh, Plant & Rudy (1977) and Chance, Krynock & Rosecrans (1978). It is difficult to interpret the experiments of Basbaum, Marley, O'Keefe & Clanton (1977) in which lesions of the dorsolateral spinal funiculus of the rat reduced the ability of morphine to suppress withdrawal of a limb following application of an alligator clip since data on the effects of the lesion alone were not supplied.

If morphine increases descending inhibition of dorsal horn neurones then this drug should have a greater effect on the responses of these cells when the cord is intact than in spinal preparations. The present experiments produced the opposite result, large decreases in responses were caused by morphine when conduction in the spinal cord was blocked when compared with effects produced in the normal animal. This difference is attributable to the decrease in descending inhibition produced by morphine. In support of this finding it has been found that, in decerebrate cats, morphine readily depressed the responses of dorsal horn neurones to noxious skin stimuli (Le Bars, Menétrey, Conseiller & Besson, 1975) and electrical stimulation of peripheral nerve (Le Bars, Guilbaud, Jurna & Besson, 1976a) when the spinal cord was divided but had little effect in preparations with the spinal cord intact (Le Bars, Menétrey & Besson, 1976). However, it should be pointed out that Hanaoka, Ohtani, Toyooka, Dohi, Ghazisaidi, Taub & Kitahata (1979) did not confirm these findings, morphine being a more effective depressant of the nociceptive responses of dorsal horn neurones in decerebrate cats with the spinal cord intact. No explanation can be offered for this difference.

A decreased effectiveness of morphine in spinal rabbits was reported by Satoh, Nakamura & Takagi (1971) and Takagi, Satoh, Doi, Kawasaki & Akaike (1976). In these experiments the effectiveness of morphine in reducing excitation of dorsal horn neurones by intra-arterial bradykinin was compared before and after spinal transection. The distribution of intra-arterial bradykinin to receptors will be affected by changes in local circulation. For example, changes in the patency of arteriovenous anastomoses, known to be in part under spinal control (Hales, Fawcett & Bennett, 1975) will affect the population of receptors reached. Morphine and particularly spinal transection (Duggan *et al.*, 1978) affect the peripheral circulation and hence the firing of nociceptors by bradykinin will almost certainly change with these procedures. It is therefore not possible to attribute a changed effectiveness of morphine solely to actions on the central nervous system when using intra-arterial bradykinin as a noxious stimulus. It should be pointed out that noxious heat was abandoned in the present experiments since it was not a quantitatively accurate stimulus with the use of reversible spinal block.

Thus, experiments which purport to support an increase in descending inhibition following analgesic doses of morphine are largely based on indirect methods. The two series of experiments in which this inhibition has been measured, those of Jurna & Grossman (1976) and the present experiments, have shown that both in the decerebrate and anaesthetized cat this inhibition is decreased by morphine. Results of such experiments cannot indicate the relative importance of the spinal and supraspinal actions of morphine in producing analgesia, but they do suggest that it is improbable that activation of descending inhibition is responsible for the spinal actions of morphine.

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(Received June 15, 1979.
Revised September 26, 1979.)