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NALOXONE ENHANCEMENT OF SPINAL REFLEXES IN THE RABBIT

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SUMMARY

1. The effects of intravenous morphine, (−)-naloxone and (+)-naloxone have been studied on three ipsilateral cutaneo-muscular reflexes in spinal rabbits.

2. Morphine, 3 mg/kg caused a slow-onset depression of all three reflexes. This effect was naloxone reversible.

3. The ipsilateral extensor reflexes, sural to gastrocnemius medialis and saphenous to vastus lateralis were both enhanced to more than double control size following a 5 μg/kg dose of naloxone given in the absence of morphine. For the sural–gastrocnemius reflex, naloxone potentiated the reflex drive from all groups of myelinated afferent fibres.

4. The ipsilateral flexion reflex, sural to semitendinosus, was only weakly enhanced by naloxone, the 5 μg dose leading to an increase in the size of the reflex to 130% of control.

5. All observed actions of naloxone were stereospecific as the enantiomer (+)-naloxone failed to affect any of the reflexes even in a dose of 50 μg/kg.

6. We conclude from these findings that opioid peptides are tonically released in rabbit spinal cord, and that they have differential effects in control of flexion and extension reflexes. It is suggested that the ipsilateral extension reflexes are held under a more powerful opioid-mediated depression than that operating upon the flexion reflexes, and that this difference may be related to the greater inhibitory inflow to extensor motoneurones from ipsilateral skin areas.

INTRODUCTION

Naloxone is a specific opioid antagonist which has been most useful in investigations concerning endogenous opioid peptides. Actions of small doses of this drug observed in the absence of exogenous opioid administration are almost certainly the result of antagonism of endogenous opioids; particularly where the actions are specific to the (−)-enantiomer (Duggan, 1981). Preliminary reports from our laboratory have shown that in the spinally transected rabbit the short-latency reflex from sural nerve to the ipsilateral gastrocnemius medialis (g.m.) is greatly enhanced by very small doses of naloxone (Catley & Pascoe, 1978; Catley, Clarke & Pascoe, 1981). This suggests that

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the reflex, sural to g.m. is subjected to a tonic, opioid-mediated depression. It is the most potent example of reflex enhancement by an opioid antagonist yet reported (McClane & Martin, 1967; Goldfarb & Hu, 1976; Bell & Martin, 1977; Boreau, Willer & Dauthier, 1978).

This paper gives a full account of our investigations on the sural–g.m. reflex, and further studies on the role of opioids in spinal reflex control.

METHODS

Experiments were performed on New Zealand Red rabbits of both sexes weighing from 1·5 to 3·9 kg. Anaesthesia was induced by an intravenous injection of methohexitone sodium (Brietal, Eli Lilly) in a sufficient quantity, and maintained after tracheal cannulation by a mixture of halothane in oxygen delivered from a closed-circuit machine. Both carotid arteries were tied. Arterial blood pressure was monitored from a cannula in the left carotid artery and maintained between 30 and 50 mmHg during surgery by the hypotensive action of halothane. A laminectomy was made at T10 to T12 and the spinal cord transected. The rabbits were decerebrated by suction usually at the precollicular level via a mid-line craniotomy; large blood vessels were clipped. In some rabbits the whole pituitary gland was removed by suction at this stage. The preparation was then allowed to recover from the anaesthetic and the blood pressure quickly rose to between 90 and 120 mmHg. Rectal temperature was maintained between 36 and 38 °C by the heated myograph stand upon which the animals lay.

A period of at least 1 hr was allowed after cessation of anaesthesia before starting to record reflexes.

The reflexes sural to gastrocnemius and sural to semitendinosus

Reflexes were recorded as mass action potentials from the relevant muscle nerve. The preparations were paralysed with gallamine triethiodide (Flaxedil, May & Baker) in an initial dose of 4 mg/kg I.V. supplemented during the experiment as necessary, and were artificially respired with room air. Expired CO_2 was monitored continuously and used as an indicator of the rate at which to respire the rabbits. The left leg was rigidly clamped at knee and ankle, and the left popliteal fossa opened via a lateral incision in the thigh to expose the sciatic and hamstring nerves. The muscles around the fossa were retracted to form the walls of a pool into which warmed water-saturated paraffin oil was poured. The sural nerve was cut peripherally in the popliteal fossa and the de-sheathed central end placed over a pair of platinum stimulating electrodes. A single platinum electrode was placed under the nerve in continuity higher in the leg for recording afferent volleys. Reflex responses were recorded from the de-sheathed central end of the cut g.m. or semitendinosus (s.t.) nerve, which was crushed between the electrodes to give a monophasic signal. In some experiments, records were made from g.m. and s.t. nerves simultaneously. Signals were suitably amplified and reflex responses to sural stimulation were quantified from the summed responses to eight successive stimuli given at 1/sec. For this purpose a Biomac 1000 averager (Data Laboratories) or a Research Machines 380Z microcomputer were used. Data obtained from the Biomac had later to be analysed using a Linc-8 computer (Digital Equipment Corporation). The computers were programmed to calculate the voltage–time integral (area) of the reflex signal by addition of the ordinates bounded by two movable cursors and an adjustable base line. This parameter was chosen because it was found to have a lower coefficient of variation than the peak amplitude of the response. All areas were adjusted to the same gain and sampling frequency so that responses from different experiments were directly comparable.

In a few instances functionally single α and γ motor fibres were isolated for recording from the de-sheathed g.m. nerve.

The sural nerve compound action potential in the rabbit begins with a definite double peak. This we refer to as the A-$\alpha\beta$ component. In some experiments the A-δ component of the sural nerve was excited selectively using the anode-block method of Catley & Pascoe (1977).

Reflexes recorded from muscles

Some experiments on the sural–g.m. reflex and all those on the saphenous–vastus lateralis (v.l.) reflex were performed on unparalysed preparations, recording responses directly from their target muscle. The lower limb was acutely denervated in all cases leaving only the relevant muscle nerve intact. Sural or saphenous nerves were prepared for stimulation and volley recording as described above. The relevant muscle tendon was detached and connected to a strain-gauge tension transducer so that the muscle lay approximately at its resting length. Tissues were prevented from drying by covering them in warmed paraffin oil. Reflexes recorded in this way were quantified in g tension generated by the muscle response to stimulation of the cutaneous nerve at 1 Hz. Electromyographic responses were also sampled using concentric needle electrodes. These and mechanical responses were all recorded from CRO traces using Kodak RAR 35 mm film.

RESULTS

Control response

Reflex responses in the g.m. nerve were elicited by single shocks to the ipsilateral sural nerve at a strength sufficient to excite only the A-α β components of the compound action potential. This means that only fibres with a conduction velocity of above 25 m/sec were excited. The A-α component on its own did not normally set up a reflex in g.m. Higher strengths of stimulation were avoided since it has been shown previously that tetanic stimulation of the A-δ fibres produces a powerful and long-lasting depression of the reflex (Catley & Pascoe, 1978).

The reflex had a mean over-all latency of 6·7 msec ($n = 131$), and a range of 5·5–7·5 msec (Fig. 1). The peak amplitude of the reflex in g.m. was of the order of 100 μV, but showed great variability. For this reason eight reflexes were summed. At a stimulation rate of 1 Hz there was a noticeable increase in the size of the reflex to the second and sometimes the third stimuli of a series. For this reason the addition of eight reflexes was not started until the fourth stimulus in a sequence. Responses, i.e. the summed eight reflexes, were then recorded at regular intervals, usually every 2 min.

The sizes of reflexes varied greatly between preparations. We made attempts to reduce this variability but were not able to find its underlying cause. In some rabbits the left leg was extensively denervated. The rationale was that, since tetanic stimulation of A-δ and C fibres is known to inhibit the reflex (Catley & Pascoe, 1978), the denervation would remove nociceptive input from damaged tissues and so might lead to a larger, less variable reflex. The only noticeable effect of the denervation was that the reflexes were smaller. To the same end, the reduction of nociceptive inflow, the level of halothane anaesthesia was increased in some animals during the preparation and was maintained for about 30 min after all cutting had been completed. This did not noticeably affect the reflex. Finally, we considered the possibility of blood-borne endorphins from the pituitary gland (Rossier, French, River, Ling, Guillemin & Bloom, 1977) affecting the size of the reflex. Removal of the pituitary was carried out for a time but later abandoned since it appeared not to affect the size of the reflex.

In many animals there was a steady increase in the size of the responses during the control period before any drugs had been given. This was studied systematically in three rabbits in which the responses were measured every two minutes for 30, 64

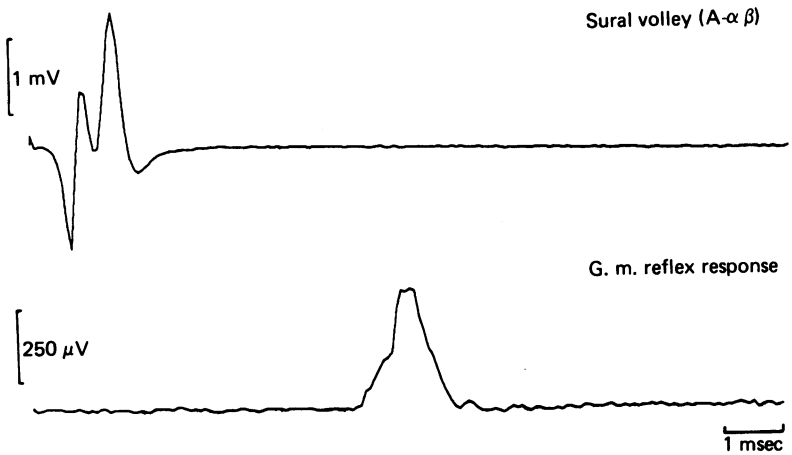

Fig. 1. Above: triphasic record of sural nerve volley used to elicit reflex showing A-α β peaks. Below: monophasic record of g.m. reflex. Both traces are averages of eight sweeps. Stimulus occurred at start of sweeps.

and 102 min respectively. In all three animals there was a steady increase in the responses. Rectilinear regression lines were fitted by the method of least squares, and from the slopes it was calculated that the increases were 41, 30 and 102%/hr respectively.

Effects of morphine and naloxone

Morphine had a depressant action upon the sural–g.m. response. In Fig. 2*A* are seen the effects of giving successively, 1 mg/kg and 2 mg/kg of morphine sulphate. The response showed a slow decline to less than 20% of the control value. This depression was more than reversed by the opiate antagonist naloxone in a dose of 0·1 mg/kg.

The effect of naloxone on its own was more dramatic. In Fig. 2*B* is seen the effect of giving 5 μg/kg of naloxone. There was a more than doubling of the response. In twenty-two experiments 5 μg/kg of naloxone resulted in a mean increase in the response to 246% (S.E. of the mean 26) of controls. The lower dose of 2·5 μg/kg gave a mean increase of 186% (S.E. of the mean 15, $n = 15$) compared to controls.

The effect of the drug developed slowly taking from 5 to 12 min to produce its maximum effect. In three experiments the times to half decay of the effect were 34, 38 and 28 min. These figures are in reasonable agreement with the values obtained in other studies (Berkowitz, Ngai, Hempstead & Spector, 1975; Markowitz, Jacobson, Bain & Kornetsky, 1976). The enhancement caused by naloxone was not noticeably affected by hypophysectomy or by acute denervation of the ipsilateral limb.

As stated in the Methods, all responses were adjusted for amplifier gain and sampling freqency so that they were directly comparable. It was interesting to see whether there was any relation between the potentiation produced by naloxone and the control response. The percentage potentiations and the absolute potentiations produced by 5 μg/kg of naloxone were plotted against the pre-drug responses in Fig. 3*A*, *B*. There was a clear trend in Fig. 3*A* such that small control responses showed

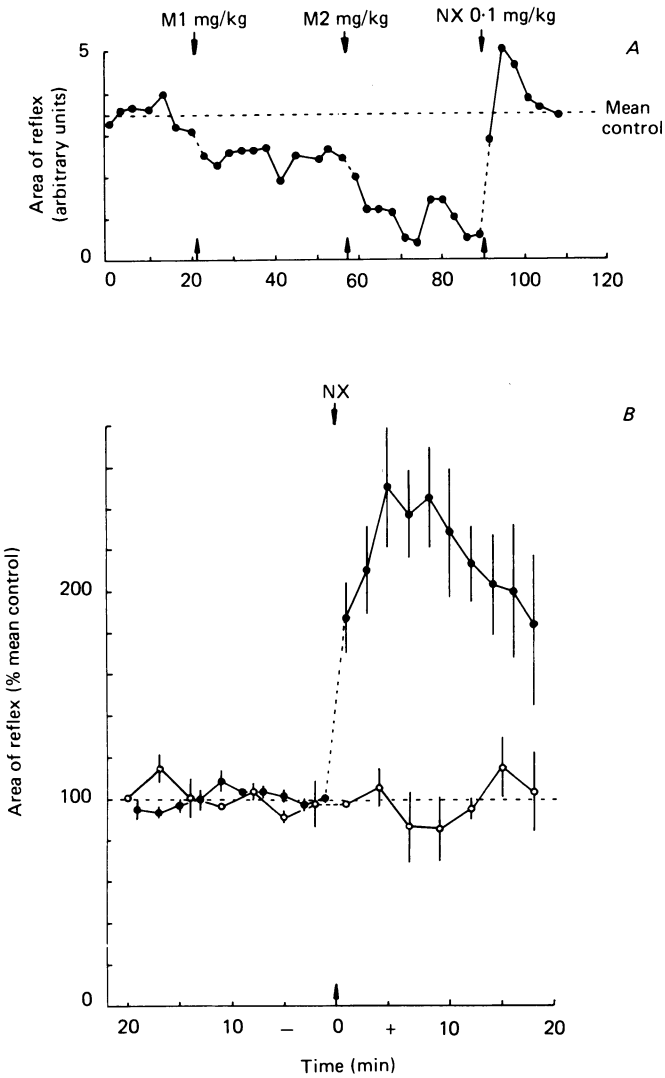

Fig. 2. *A*, effect of morphine (M) on area of sural–g.m. reflex. A total dose of 3 mg/kg I.V. gave clear depression which was reversed by naloxone (NX) 0·1 mg/kg I.V. *B*, effect of naloxone 5 μg/kg I.V. (filled circles, $n = 22$ with S.E. of the mean) and (+)-naloxone, 50 μg/kg I.V. (open circles, $n = 4$ with S.E. of the mean). Both drugs were given in the absence of any other drugs at time zero. Data normalized to assist comparison.

small potentiations. This is the reverse of what we had expected and means that small controls are not small because of a large amount of tonic opioid-mediated inhibition.

We were fortunate to be given 1 mg of (+)-naloxone which is inactive as an opioid antagonist (Iijima, Minamikawa, Jacobson, Brossi & Rice, 1978). This was without effect upon the responses even when given in a dose of 50 μg/kg (Fig. 2*B*). The low doses of naloxone required to produce the enhancement and the stereospecific nature of the effect strongly suggest that its action is due to the antagonism of opioids at stereospecific opioid receptors.

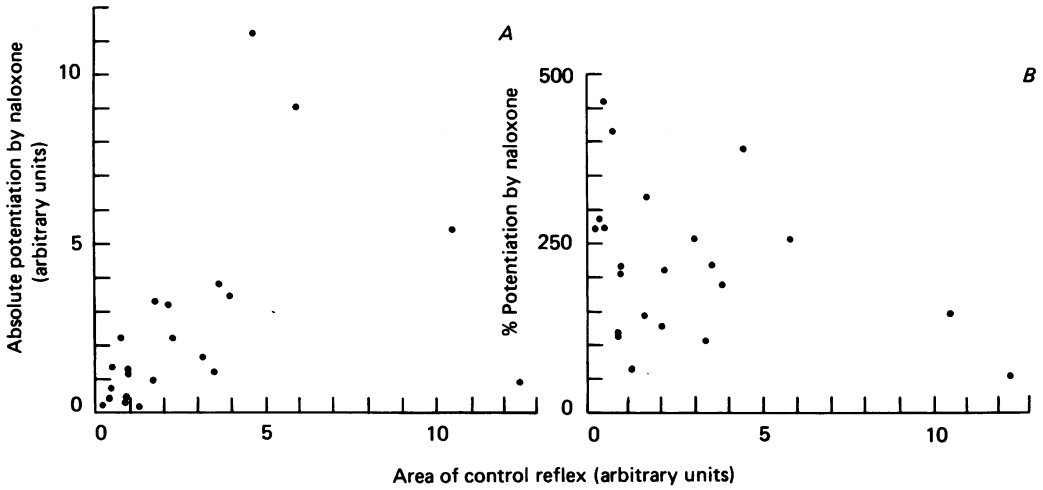

Fig. 3 Scatter plots of absolute potentiation (*A*), and percentage potentiation (*B*) of sural–g.m. reflex plotted against size of control reflex. Potentiation produced by 5 μg/kg I.V. naloxone. Note that small reflexes tend to show small absolute potentiation.

Much attention has been focused in the past upon the effect of naloxone upon nociceptive inputs. In our experiments naloxone enhanced the reflex drive from all myelinated fibres in the sural nerve on to g.m. Selective stimulation of sural afferents conducting faster than 45 m/sec (the A-α elevation of the compound action potential) did not normally cause discharge in the ipsilateral g.m. nerve, but such a stimulus became quite powerfully reflexogenic after administration of naloxone at 5 μg/kg I.V. Selective activation of sural A-δ fibres gave rise to a reflex response in g.m. with a latency of 14 to 18 msec (Fig. 4*A*) which was potentiated to 220 % of pre-drug levels after the 5 μg/kg dose of naloxone (Fig. 4*B*).

Spontaneous motor activity in g.m. nerve is usually low or undetectable in spinally transected rabbits. After naloxone 5 μg/kg, spontaneous discharges were apparent and where present before there was a clear increase in frequency. This was particularly obvious from the audio output of the amplifier when recording from the whole g.m. nerve. In addition, single fibre recordings showed that there was an increase in spontaneous firing of both α and γ motoneurones with this dose of naloxone.

In many but not all animals naloxone produced a small increase in blood pressure but this was not studied systematically.

So far we have assumed that the short latency discharge in g.m. to ipsilateral sural nerve stimulation was occurring in α motoneurones. We confirmed this in uncurarized preparations. Stimulating the sural nerve at 1 Hz at A-α β strength produced a small reflex twitch in the ipsilateral g.m. muscle. This response was, like that recorded from the muscle nerve, extremely variable in size. The latency of the electromyogram to sural stimulation was between 6·8 and 9·6 msec which is consistent with that for the reflex recorded from the nerve after allowing for the extra delay of neuromuscular transmission.

The reflex recorded from the muscle was just as potently enhanced by naloxone

as that recorded from the nerve (Fig. 5). This confirms that the small, short-latency reflex seen in g.m. after A-α β stimulation of the ipsilateral sural nerve was occurring in α motoneurones. The administration of naloxone was also followed by an increase in the resting tension of the muscle, reflecting the increase in spontaneous activity recorded from the muscle nerve.

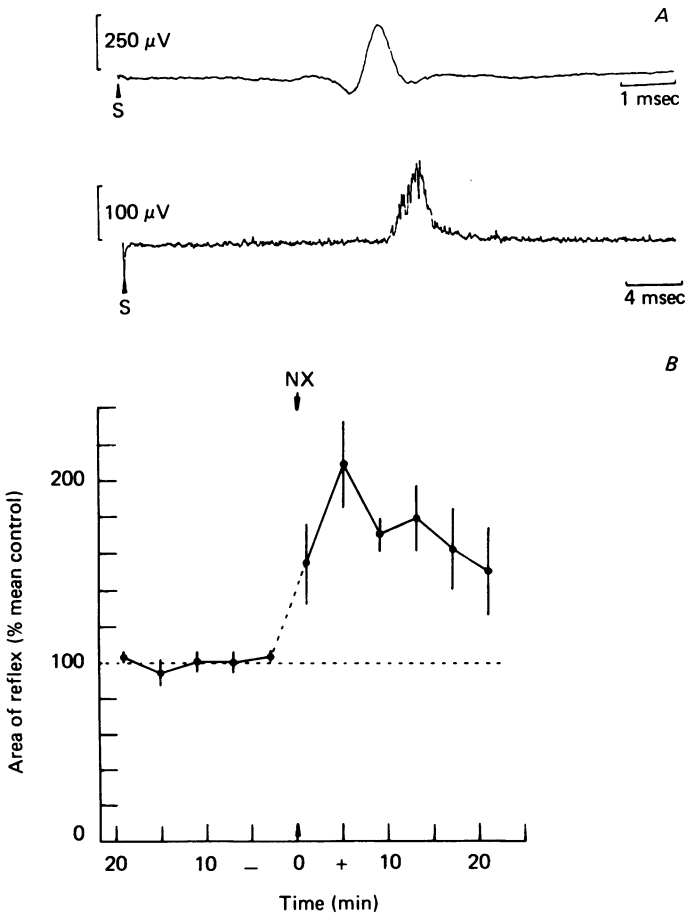

Fig. 4. *A*, above, triphasic record of selectively evoked sural A-δ volley, and below the reflex response produced in g.m. nerve. Note the different time scales. Each trace is the average of eight sweeps. *B*, effect of naloxone (NX) 5 μg/kg I.V. on the reflex, $n = 5$ with S.E. of the mean.

The reflex saphenous to v.l.

Exciting the A-α β components of the saphenous nerve resulted in a small reflex twitch in the ipsilateral v.l. muscle. The latency of the electromyographic response was from 6·1 to 9·1 msec. When morphine was given at an accumulated dose of 3 mg/kg I.V. a slow depression of the reflex was seen (Fig. 6*A*), which was rapidly reversed by naloxone. Giving the opioid antagonist alone in a dose of 5 μg/kg was followed by an increase in the reflex response to 226 % (S.E. of the mean 60, $n = 5$) of control

values, see Fig. 6*B*), The enantiomer (+)-naloxone failed to alter the reflex responses in doses of up to 50 μg/kg (Fig. 6*B*). Naloxone itself caused an increase in the resting tension of the v.l. muscle. Thus the effects of the opioid antagonist on this reflex are obviously similar to those on the sural–g.m. reflex.

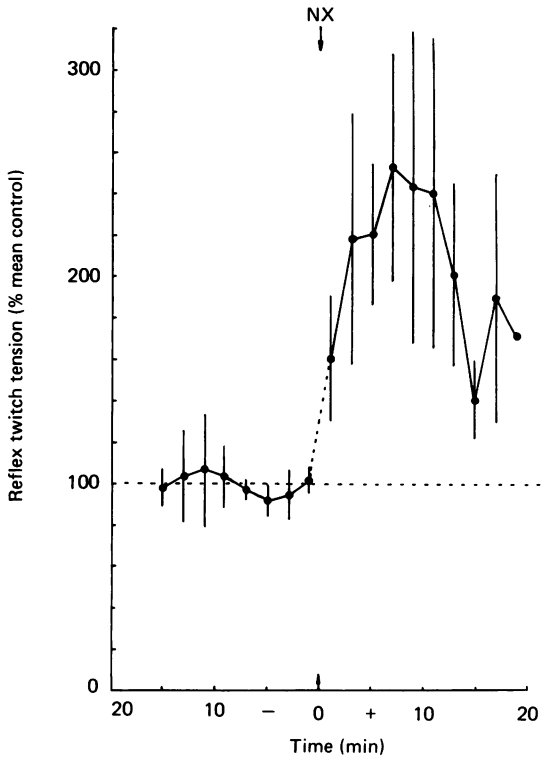

Fig. 5. Effect of naloxone (NX) 5 μg/kg, I.V. on sural–g.m. muscle-reflex tension, $n = 4$ with S.E. of the mean.

The reflex sural to s.t.

Activation of sural afferents conducting over 25 m/sec produced a reflex response of variable size in the ipsilateral s.t. nerve with a latency of 5·1 to 7·0 msec. As with the two other reflexes a cumulative dose of morphine at 3 mg/kg I.V. caused a slow onset of a naloxone-reversible depression of the s.t. response (Fig. 7*A*).

However, the effects of naloxone on its own were not as marked as with the extension reflexes. When given at 5 μg/kg I.V., naloxone resulted in a potentiation of the reflex to only 130% of controls (Fig. 7*B*). Significant effects were observed in only six of the ten preparations in which this reflex was studied, but the action was nevertheless stereospecific since (+)-naloxone had no effects at all (Fig. 7*B*). In experiments where the sural–g.m. and sural–s.t. reflexes were studied together, the effects of naloxone were always much greater on the extension than on the flexion reflex, indicating that the tonic release of endogenous opioids is greater in the sural–g.m. reflex pathway.

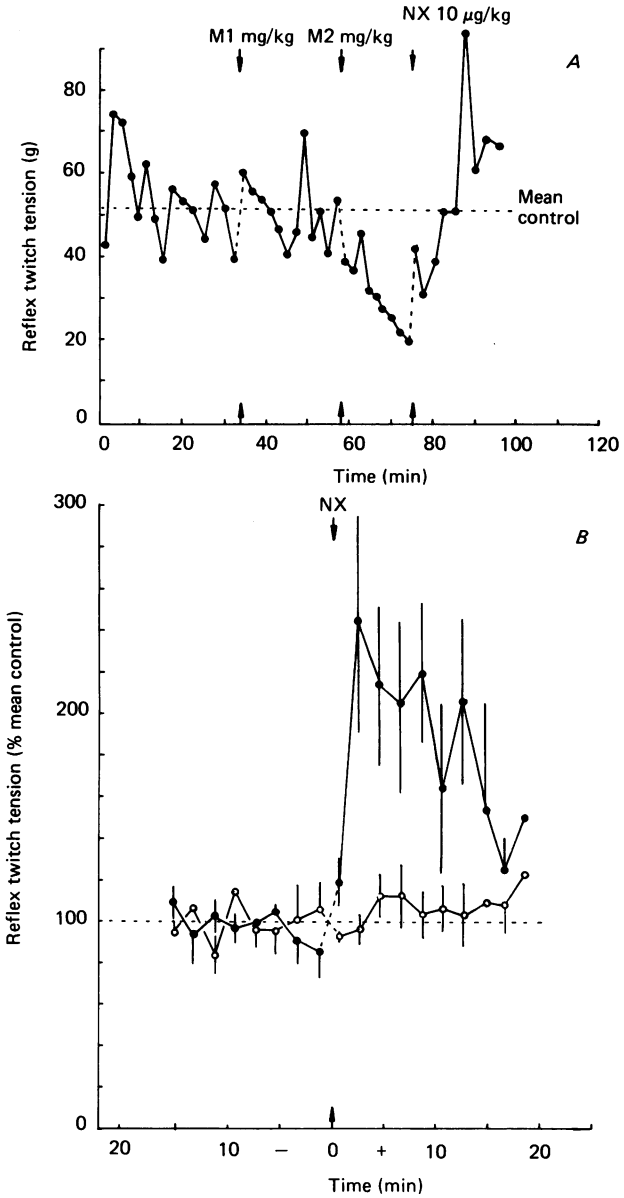

Fig. 6. *A*, effect of morphine (M) on tension of v.l. produced by stimulation of saphenous nerve. A total dose of 3 mg/kg I.V. gave clear depression which was reversed by naloxone (NX) 10 μg/kg I.V. *B*, effect of naloxone, 5 μg/kg I.V. (filled circles, $n = 5$ with S.E. of the mean) and (+)-naloxone 50 μg/kg I.V. (open circles $n = 4$ with S.E. of the mean). Both drugs given in absence of any other drugs, at time zero. Data normalized.

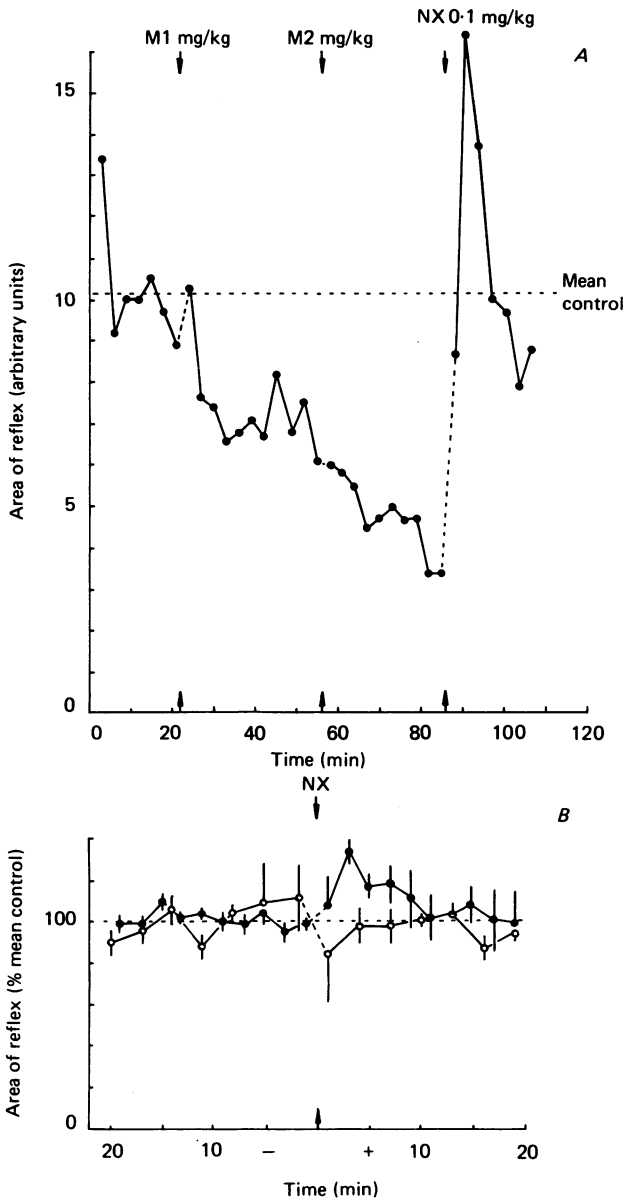

Fig. 7. *A*, effect of morphine (M) on area of sural–s.t. reflex. A total dose of 3 mg/kg I.V. gave clear depression which was reversed by naloxone (NX) 0·1 mg/kg I.V. *B*, effect of naloxone 5 μg/kg I.V. (filled circles, $n = 10$ with S.E. of the mean) and (+)-naloxone, 50 μg/kg I.V. (open circles, $n = 4$ with S.E. of the mean). Both drugs were given in the absence of any other drugs at time zero.

DISCUSSION

Naloxone, when given to morphine-naive spinally transected rabbits in a dose of 5 μg/kg, potentiated both the ipsilateral extension reflexes, g.m. and v.l., to more than double control levels. The flexion reflex, sural–s.t. was also potentiated, but to only 130% of controls. In each case the effect was stereospecific since (+)-naloxone failed to affect reflexes in doses ten times as large as those found to be effective for the (−)-enantiomer. The potency and stereospecificity shown by naloxone make it extremely unlikely that its effects were due to some non-specific action (e.g. see Dingledine, Iversen & Breuker, 1978; Fry, Zieglgänsberger & Herz, 1979; Fry, Herz & Zieglgänsberger, 1980; Duggan, 1981). We are confident that naloxone causes enhancement of these reflexes by antagonism of tonically released inhibitory opioid peptides.

Arndt & Freye (1979) showed that the hypotension and bradycardia seen during halothane administration to dogs is blocked by (−)-naloxone but not (+)-naloxone applied to the fourth ventricle. All the preparatory surgery in our experiments was done under halothane anaesthesia, but at least an hour and a half elapsed from the time that halothane administration ceased to the time of giving the first dose of naloxone. In some experiments the interval was many hours. Thus, the acute effects of halothane had passed. However, we cannot completely rule out the possibility that halothane has a long-term depressant action upon spinal reflexes and that this action is blocked by naloxone.

As naloxone enhancement of the sural–g.m. reflex was not affected by hypophysectomy, it is clear that reflex depression could not be attributed to a blood-borne opioid from the pituitary. The high potency of naloxone in enhancing the ipsilateral extensor reflexes suggests that the antagonistic effect is exerted at the μ subtype of opioid receptor, for which naloxone has a high affinity (see Kosterlitz & Paterson, 1980). Morphine also binds preferentially to the μ receptors and, when given systemically, it is approximately equipotent upon the sural–g.m. and the sural–s.t. reflexes. This suggests that the two reflex arcs have similar numbers of μ receptors. When naloxone is given by itself, however, it is much more potent upon the sural–g.m. reflex than upon the sural–s.t. reflex, yet the neuronal pathways of the two reflexes lie in the same segment of the spinal cord. Thus, it is likely that the opioid is liberated in different amounts close to its site of action.

Many previous workers claim that opioids selectively depress (and naloxone selectively enhances) the responses of spinal neurones to input from high threshold, A-δ and C fibre, cutaneous afferents (for review, see Yaksh, 1981). Thus, the present view is that opioid control of nociception is mediated by inhibition of high-threshold afferents. However, in the reflexes we have studied it is clear that naloxone in small doses enhanced reflex drive from large as well as small afferents, an observation indicating that endogenous opioids are not concerned exclusively with the suppression of nociceptive responses. It is interesting that Duggan, Hall & Headley (1977) report that iontophoretic application of enkephalins into laminae II and III of the cat dorsal horn caused a selective depression of the responses of neurones in laminae IV and V to high-threshold input, but that placing the opioids directly into the deeper laminae

reduced responses to both large and small diameter afferents. This suggests that in our experiments naloxone exerts its action in laminae IV and V.

The ipsilateral extension reflexes described here are probably manifestations of 'local sign' in withdrawal reflexes (Creed & Sherrington, 1926). Extensor muscles are usually inhibited by stimulation of ipsilateral cutaneous nerves, but individual muscles can be excited by stimulation of areas of skin (or the appropriate cutaneous nerve) which would be moved directly away from the source of the stimulus by contraction of that muscle (Hagbarth, 1952). To illustrate, contraction of g.m. in the standing animal lifts the heel (an area of skin innervated by sural) away from the ground, and might well play a role in withdrawal of the heel from a noxious stimulus applied at that point. However, the size of the extensor responses to the low intensity stimuli used in the present experiments is small and is unlikely to cause significant movement of the relevant parts of the limb; these reflexes only become significant if the stimulus to the relevant skin area or nerve excites nociceptive afferents. Extensor motor nuclei are most powerfully excited from ipsilateral skin if the stimulus applied is of a noxious nature (Hagbarth, 1952), and the sural–g.m. reflex in rabbits is massively potentiated by single conditioning shocks applied to high-threshold sural afferents (Clarke, 1982). Excitatory reflex connexions obviously exist between extensor muscles and ipsilateral skin areas, but they are normally only released upon high intensity stimulation of the skin. It is possible that these connexions are held in check by tonic opioid release.

We have studied two ipsilateral extensor reflexes and one flexor reflex. On the basis of this limited sample the differential effects of naloxone suggests that opioids are a less important factor in flexion reflex control than they are in extension reflexes. Perhaps this is a reflexion of the wider range of excitatory ipsilateral cutaneous inputs to flexor muscles. Extensors are subject to inhibitory input from most ipsilateral skin areas (Sherrington, 1910) whilst the opposite position is true for flexors. Thus the opioid-mediated depression of the extensor reflexes could arise from afferent inflow from inhibitory skin areas. The naloxone sensitivity of the sural–g.m. reflex is not affected by acute denervation of the ipsilateral limb, so that such a process would have to be independent of short-term changes in sensory inflow. It would be interesting to observe these reflexes in chronically denervated preparations to test this point.

D.M.C. and R.W.C. were M.R.C. scholars during this work, which forms part of the thesis presented by R.W.C. for the degree of Ph.D. in the University of London. The RML 380Z computer was obtained on an M.R.C. equipment grant to J. E. P. and P. H. Ellaway. We are grateful to Mrs M. Catley for expert technical assistance, and to Mr C. Makepeace (University of Bristol) and Mr J. Lockett (UCL) for the photography. The (+)-naloxone was a kind gift from Dr A. E. Jacobson, and most of the (−)-naloxone a gift fron Endo Laboratories Inc.

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