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THE EFFECT OF NALOXONE ON SPINAL REFLEXES TO ELECTRICAL AND MECHANICAL STIMULI IN THE ANAESTHETIZED, SPINALIZED RAT

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SUMMARY

1. Previous studies of the effects of naloxone on spinal neural responses have yielded disparate results. The reasons for this remain unclear but may relate to the diversity of animal preparations used, the route of administration of naloxone, the site and modality of the stimuli and the intensity of afferent input used.

2. A model requiring little preparative surgery compared to most other electrophysiological preparations has now been used to investigate the effects of naloxone (1, 10, 20 and 50 μg kg^{-1} I.V.) on single-motor-unit flexion reflex responses to alternating mechanical and electrical stimuli in spinalized rats, anaesthetized with α-chloralose.

3. Naloxone caused a dose-dependent facilitation of reflex responses to electrical stimuli delivered at intensities sufficient to activate either A fibres alone or A and C fibre afferents together. The component of the responses presumed to be due primarily to activation of C fibres was enhanced relatively more than the A fibre component.

4. Responses evoked during high-intensity mechanical pinch stimuli were not facilitated by equivalent doses of naloxone. The post-stimulus after-discharge was, however, enhanced by a similar percentage to the response to high-intensity electrical stimuli.

5. Lowering the intensity of the mechanical stimulus led to a diminished firing rate of the units during the stimulus itself. The stimulus was, nevertheless, still noxious. Naloxone was found to have a facilitatory effect on this smaller evoked response both during the pinch stimulus and during the period of after-discharge. The apparent lack of effect of naloxone during the higher intensity mechanical stimulus may be due to neurones in the polysynaptic pathway being activated at near-maximal firing rates.

6. We conclude that the ability of naloxone to facilitate spinal reflexes is not dependent on the nature of the stimulus, at least between electrical and mechanical stimuli, but is more a function of the intensity of the applied stimulus.

INTRODUCTION

There is considerable evidence to suggest that endogenous opioid peptides play a role in modifying the transmission of nociceptive information at the level of the spinal cord. Using a variety of techniques, high concentrations of endogenous opioid peptides have been localized to the superficial layers of the spinal cord (Standaert, Watson, Houghton & Saper, 1986, and for reviews see Basbaum & Fields, 1984; Millan, Millan, Członkowski, Höllt, Pilcher, Herz & Colpaert, 1986). Administration of opioids systemically, intrathecally or by ionophoresis causes a depression of firing of spinal neurones in a naloxone-reversible manner (see Duggan & North, 1984, for a review). Evidence for a tonic inhibitory role of endogenous opioid peptides in the spinal cord comes primarily from observations of facilitatory effects on spinal reflex and neuronal responses to peripheral stimuli following the administration of opioid antagonists such as naloxone.

Different groups have, however, reported rather disparate effects of naloxone, particularly on spinal reflex and single-unit responses to 'natural' peripheral stimuli. Henry (1979) observed that the majority of cells recorded from laminae IV and V of the cat spinal cord increased their spontaneous firing rates and responded to a greater extent to uniform noxious thermal stimuli. In contrast, Duggan, Hall, Headley & Griersmith (1977) saw no clear effect of naloxone, in a similar spinal preparation, on responses either to noxious thermal or to non-noxious stimuli. No facilitatory effect of systemic naloxone was seen on the responses of motoneurones to mechanically evoked reflex responses in decerebrated, spinalized rats (Woolf & Wall, 1986) or to reflex responses in awake intact rats (Goldstein, Pryor, Otis & Larsen, 1976). Neither were there facilitatory effects, in spinally intact, awake rats, on reflex responses to noxious thermal stimuli (Woolf, 1980), vocalization threshold to mechanical stimuli (Kayser, Benoist, Neil, Gautron & Guilbaud, 1988; Kayser, Besson & Guilbaud, 1988) or thermal or mechanical stimuli in the spinalized, anaesthetized rat following naloxone (Parsons, West & Headley, 1989). However, in other studies of reflex responses, Clarke & Ford (1987), in spinal decerebrate rabbits, and Bell & Martin (1977), in spinal decerebrate cats, observed facilitatory effects of opioid antagonists on reflexes evoked by innocuous mechanical and noxious thermal stimuli respectively. Woolf (1980) saw an increase in tail-flick latency to a thermal stimulus following intrathecal administration of 25 μg naloxone but a decrease in latency following injection of 150 μg.

Results obtained from investigations utilising methods of electrical stimulation to evoke neural responses and reflexes are somewhat clearer. Naloxone has been shown to enhance electrically evoked reflexes to impulses both in large-diameter muscle afferents and in large- and small-diameter cutaneous afferents (Duggan, Morton, Johnson & Zhao, 1984; Bell, Sharpe & Pickworth, 1985; Clarke & Ford, 1987, and for a review see Duggan & North, 1984). In most studies carried out in the dorsal horn, naloxone enhanced the afferent C fibre-evoked discharge of cells in laminae IV and V (Rivot, Chaouch & Besson, 1979; Fitzgerald & Woolf, 1980; LeBars, Chitour, Kraus, Dickenson & Besson, 1981); it does not, however, always do so (Duggan *et al.* 1984). The effects of naloxone on the Aδ-evoked discharge were less clearly defined (Fitzgerald & Woolf, 1980) and no effects were observed on the Aα-evoked discharge (LeBars *et al.* 1981).

One explanation for some of the discrepancies lies in the topographical selectivity of opioid-mediated inhibition (Clarke & Ford, 1987). None the less, certain anomalies remain. First, opioid agonists have generally been shown to be relatively selective for depressing responses to noxious stimuli as opposed to non-noxious stimuli (Duggan & North, 1984). Assuming a role of endogenous opioid peptides in processing nociceptive information, why do opioid antagonists appear to be non-selective in their facilitation of responses to both low- and high-threshold electrical stimuli? Secondly, whilst the effects of opioid antagonists appear to facilitate responses to electrical stimulation, responses to naturally evoked noxious stimuli are affected less.

In this series of experiments, an electrophysiological preparation of spinalized rats requiring little surgical intervention has been used to address these points. We have investigated the possibility that the nature, intensity and synchrony of the stimuli used may affect the opioid inhibition revealed by systemic naloxone; we show that some of the anomalies between results with electrical and natural stimulation can be explained in this way. Preliminary results have been presented in abstract form (Hartell & Headley, 1991).

METHODS

Most of the methods have been described elsewhere (Hartell & Headley, 1990*a*). Briefly, male Wistar rats were anaesthetized with halothane in oxygen. Following cannulation of the trachea, carotid artery and right jugular vein, a small dorsal incision was made over the thoracic spinal column and the area around the wound surface was infiltrated with local anaesthetic (1% lignocaine with 1:200000 adrenaline). The musculature overlying thoracic 9–11 vertebrae was retracted to expose and allow subsequent removal of one or two laminae and the spinal cord was transected at the level of thoracic 10–11 vertebrae. All surgery was kept to a minimum. Following surgery, animals were given a 50 mg kg^{-1} I.V. dose of α-chloralose (dissolved in either a buffered solution of sodium tetraborate at a concentration of 30 mg ml^{-1}, or in isotonic saline at a concentration of 10 mg ml^{-1}) and the halothane discontinued. Supplementary doses of 20 mg kg^{-1} I.V. were given at intervals of approximately 1 h.

Rectal temperature was maintained at $37 \pm 0{\cdot}5$ °C. Blood pressure was monitored and experiments were terminated if the systolic pressure fell below 100 mmHg for any sustained period. Fluid (either isotonic saline or Haemaccel; Hoechst) was provided over the course of the experiment at the normal fluid intake rate in awake rats of approximately 80 ml kg^{-1} 24 h^{-1} I.V. In some cases 1–2 ml of whole blood was given (I.V.) post-operatively. The right hindlimb was immobilized in a plaster of Paris cast and a period of at least 1 h allowed before recording, so as to allow recovery from the effects both of halothane and of the spinalization.

Responses to electrical and mechanical stimuli, delivered to the right hindpaw, were recorded directly from the hindlimb flexor muscle flexor digitorum longus. Teflon-coated tungsten microwires (total diameter 75 μm; Clark Electromedical Instruments) embedded in 26 g needle electrodes were inserted through a small skin incision directly over the muscle and were positioned with a micromanipulator to obtain single-motor-unit recordings. Discriminated spikes were displayed on an oscilloscope so that spike conformation could be monitored.

The receptive fields of the units were similar in both size and location between animals. Fields generally encompassed the two most lateral toes (toes 5 and 4) and spread proximally along the lateral half of the foot up to the hock. The pinch stimuli were applied to either toe 5 or to the webbing between toes 5 and 4. Electrical stimuli were applied to the lateral edge of the foot, 4–5 mm away from the base of the 5th toe, using percutaneous needle electrodes 4 mm apart.

Two separate stimulation protocols were utilized. The first was designed to investigate the effects of naloxone on single-motor-unit responses to three different kinds of high-intensity stimuli. A 3 min cycle was established comprising (a) a noxious mechanical pinch stimulus, (b) sixteen electrical pulses, delivered at high intensity at 2 Hz and (c) at 0·3 Hz. The second protocol again used three different stimuli cycled over 3 min. A similar intensity noxious pinch stimulus to that in the first protocol was alternated with a second pinch at a lower intensity. Both stimuli were considered to be noxious since the mean force of the lower intensity stimulus was in fact twice that

required to evoke a threshold response in anaesthetized, spinalized animals and the mean forces of both stimuli were greater than the threshold required to elicit paw withdrawal using the same pincher device in conscious rats (N. A. Hartell, unpublished observations). In addition, a group of sixteen low-intensity, low-frequency (0·3 Hz) electrical stimuli was included in this cycle.

All pinch stimuli were applied for a period of 15 s using a pneumatically driven, electronically controlled pinching device (Hartell & Headley, 1990*b*) applied to a single toe or the inner-toe webbing of the ipsilateral hindpaw over a 4 mm^2 area. The onset of the stimuli was feedback controlled to follow a rapid ramp (8 N s^{-1}) and the peak force was maintained at a pre-set level (see Fig. 1 for the form of the stimulus). Chart records were made of the stimulus force, number of spikes per second and of the number of spikes evoked during three phases of the pinch stimuli. Spike counts over the first 5 s (early pinch) were separated from those over the subsequent 10 s (late pinch). It is presumed that the early phase of the pinch contains a greater component responding to rapidly adapting, low-threshold afferents. Figure 1 shows that a degree of adaptation in the rate of firing of the neurones did take place during the initial phase of the pinch for this unit, as with most of the other units recorded. A period at the end of the pinch stimulus was also counted separately to include the after-discharge which usually lasted between 5 and 15 s.

Prior to drug testing in five of the experiments, a ramped pinch was applied to the hindpaw of the rats at an onset rate of 0·15 N s^{-1}. The stimulus force was allowed to increase until the firing rates of the neurones became maximal. From this, an estimation of the mean maximum firing rates of a subset of the neurones was calculated.

Constant-voltage electrical stimuli were applied with percutaneous needle electrodes. Low-intensity electrical stimuli were delivered at 0·2 ms pulse widths at 1·5–2 times the threshold for evoking a single spike of short latency (12–16 ms). At no time were spikes evoked with latencies over 80 ms.

High-intensity stimuli were delivered at 1 ms pulse widths at 5 times the threshold (T_R) for evoking a single reflex spike of short latency (12–16 ms). It may have been more specific to have expressed stimulus strengths as multiples of the thresholds of the A and C fibre components of the afferent volley but this was not done because this would have required increased surgical trauma which has been shown to enhance opioid sensitivity in the spinal cord (see Hartell & Headley, 1990*c*; Hartell, Headley & Parsons, 1990). When the stimulus was presented at 0·3 Hz, two distinct components of the response, an early phase and a late phase, could be separated routinely since there was a clear silent period between the phases. This silent period was variable in duration (between 50 and 200 ms) but always occurred around 240 ms post-stimulus. Therefore, this cut-off latency was chosen to divide the early and late components of the response. Assuming a motoneurone conduction distance of 13 cm, a motoneurone conduction velocity of 60 m s^{-1} (Chamberlain & Lewis, 1989), a synaptic delay at the neuromuscular junction of 0·5 ms, minimum central synaptic delays of 0·5 ms, and an afferent conduction distance of 15 cm, the fastest afferent fibres capable of mediating the late part of the response had conduction velocities of around 0·63 m s^{-1}. This value falls within the range of conduction velocities for unmyelinated afferent nerve fibres found by Lynn & Carpenter (1982) in rats of similar age, although it is somewhat slower than their calculation of the fastest conducting unmyelinated fibres (0·9 m s^{-1}). Since afferent volleys were not monitored in these experiments, we cannot therefore be certain that the early and late phases of the responses relate entirely to activation of myelinated and unmyelinated afferents, although this does seem most likely. The latencies in our experiments may have been prolonged because of a lower temperature in the extremities of the limb (compared to Lynn & Carpenter, 1982), and to the fact that we did not record directly from motoneurones but from muscle fibres at an unknown distance from the neuromuscular junction.

If the frequency of electrical stimulation was increased, the number of spikes evoked with each successive stimulus increased. This phenomenon is known as wind up (Mendell, 1966). Whilst the same cut-off points were used in the analysis of the high-frequency (2 Hz) stimuli, the degree of wind up led to less clearly separated early and late responses but, none the less, it was still clear that the bulk of the presumed C fibre response was in the 241–500 ms band.

Records were kept of the number of spikes evoked and post-stimulus time histograms were collected for each group of sixteen sweeps. Separate collections were made for the earlier part of the reflex response (1–240 ms), the later part (241–500 ms) and the total responses (1–500 ms).

Microcomputers were used for the on-line calculations of drug effects (Headley, Parsons & West, 1985; Parsons & Headley, 1989); these were expressed as percentages of the mean of three stable

control responses prior to drug administration. Results were only considered to be technically acceptable if the recovery reached at least 50% of the maximum drug effect and if the time course of recovery matched that expected for the known kinetics of naloxone (Tepperman, Hirst & Smith, 1983). Naloxone was administered intravenously, at 6 min intervals, in the following cumulative increments: 1, 10, 20 and 50 μg kg^{-1}. This dose routine was chosen because it covers the range over which naloxone fully antagonizes the actions of μ-opioid agonists and produces near-complete antagonism of the action of κ-opioids on spinal motoneurones in chloralose-anaesthetized rats (Parsons *et al.* 1989). Naloxone almost always produced it maximal effect within 3 min of administration (see also Parsons *et al.* 1989) and so the first response after drug administration was usually measured.

RESULTS

Five single motor units were given a ramped stimulus prior to drug testing. The mean maximum firing rate of this subset of single motor units was $31{\cdot}3 \pm 4{\cdot}2$ spikes s^{-1} and the mean stimulus force required to elicit this maximal response was $3{\cdot}1 \pm 0{\cdot}4$ N applied over a 4 mm^2 area.

For seven of the twelve units examined in this study (one unit per rat), high-intensity pinch stimuli ($3{\cdot}0 \pm 0{\cdot}2$ N, $n = 7$) were cycled with high-intensity electrical stimuli (1 ms pulse width, $5T_R$) presented at high (2 Hz) and low (0·3 Hz) frequencies. Figure 1 shows a chart record of the effect of cumulative doses of naloxone (1, 10, 20 and 50 μg kg^{-1} I.V.) on the firing rate of a single motor unit responding alternately to such noxious mechanical and electrical stimuli. Naloxone did not affect the number of spikes evoked over the duration of the pinch stimulus although the period of firing immediately afterwards was prolonged and the number of spikes was increased. The response to electrical stimulation, at both high and low frequencies, was enhanced in a dose-dependent manner. Recovery to pre-drug control levels was followed and in this case took approximately 1 h.

Prior to drug administration, the higher rate of electrical stimulus presentation evoked a greater number of spikes, averaged over the sixteen stimuli, than did the lower frequency stimulus rate; this is a result of the wind up of the response (Mendell, 1966). The mean numbers of spikes evoked by the sixteen stimuli at each frequency, for a total of seven animals, were fifty at 0·3 Hz and eighty-two at 2 Hz. In each case, 70% of the spikes occurred in the first 240 ms post-stimulus so that wind up did not alter the proportion of shorter latency (1–240 ms) or longer latency spikes (241–500 ms). Naloxone caused a marked increase in the number of shorter and longer latency spikes at both frequencies of stimulation. The responses at presumed C fibre latencies were, however, facilitated to a greater extent than the earlier responses when calculated in terms of percentage changes from pre-drug control values (Fig. 2). Naloxone was less effective in enhancing the reflexes evoked at 2 Hz than those evoked at 0·3 Hz (measured as percentages of pre-drug control levels). In absolute terms, the numbers of extra spikes evoked by equivalent doses of naloxone in the low- and high-frequency stimulation groups were not significantly different.

In a separate set of experiments, a series of sixteen, 0·2 ms duration, low-intensity electrical stimuli was applied to the receptive field of the hindpaw at 1·5–2 times the threshold for evoking a single spike at a latency of between 12 and 16 ms. Naloxone enhanced the response to low-intensity electrical stimulation as shown in the lower panel of Fig. 2. The level of facilitation caused by naloxone was similar to that which

occurred to the first 240 ms of the response to high-intensity electrical stimulation (see Fig. 2, upper panel).

The effects of cumulative doses of naloxone were studied on a total of twelve single motor units responding to high-intensity, noxious pinch stimuli. The mean stimulus

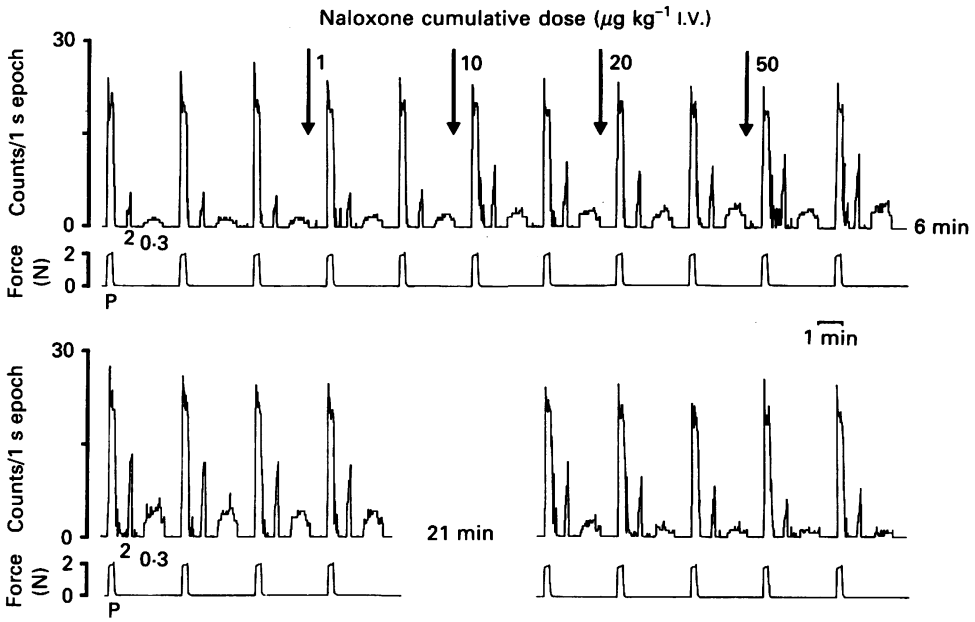

Fig. 1. Chart record displaying the effects of naloxone on the evoked firing rate of a single motor unit recorded from the flexor digitorum longus muscle in a spinalized rat anaesthetized with α-chloralose. Stable responses were obtained to constant pinch stimuli (P: 2 N over a 4 mm² area, onset rate 8 N s^{-1}) applied once every 3 min to the inter-toe webbing of the ipsilateral hindpaw. The force of the stimulus is displayed below the firing rate trace. In addition, groups of sixteen constant-voltage pulses (1 ms pulse width, 5 T_R), presented at both high (2 Hz) and low (0·3 Hz) frequencies, were applied percutaneously within the receptive field of the unit. All three stimuli were cycled every 3 min. The three sections show periods separated by the intervals indicated. Naloxone was administered intravenously at 6 min intervals in a cumulative regime from 1 to 50 μg kg^{-1}.

force applied was 2·8 ± 0·2 N. This evoked a mean firing rate during the last 10 s of the pinch (late pinch) of 26·6 ± 1·4 spikes s^{-1}. The upper panel of Figure 3 shows data for the dose-dependent effects of naloxone on the three phases of the high-intensity pinch (early, late and after-discharge) for all twelve units. No significant change with any dose of naloxone was seen over the duration of the pinch stimulus itself (either early or late phases of the response), but the after-discharge was significantly facilitated at and above doses of only 1 μg kg^{-1} I.V.

The lower panel in Fig. 3, shows a comparison of the effects of naloxone on late pinch, after-discharge and the total counts during low- and high-frequency electrical stimulation for the seven units upon which all these parameters were tested in parallel. Only the late phase of the response to pinch was unaffected by naloxone. The after-discharge to pinch stimuli and the responses to high- and low-frequency

stimulation were affected to similar degrees. Statistical analysis revealed that there was no significant difference in the effectiveness with which naloxone facilitated the responses to low-frequency electrical stimulation, high-frequency electrical stimulation and the after-discharge response to mechanical noxious pinch stimuli. There

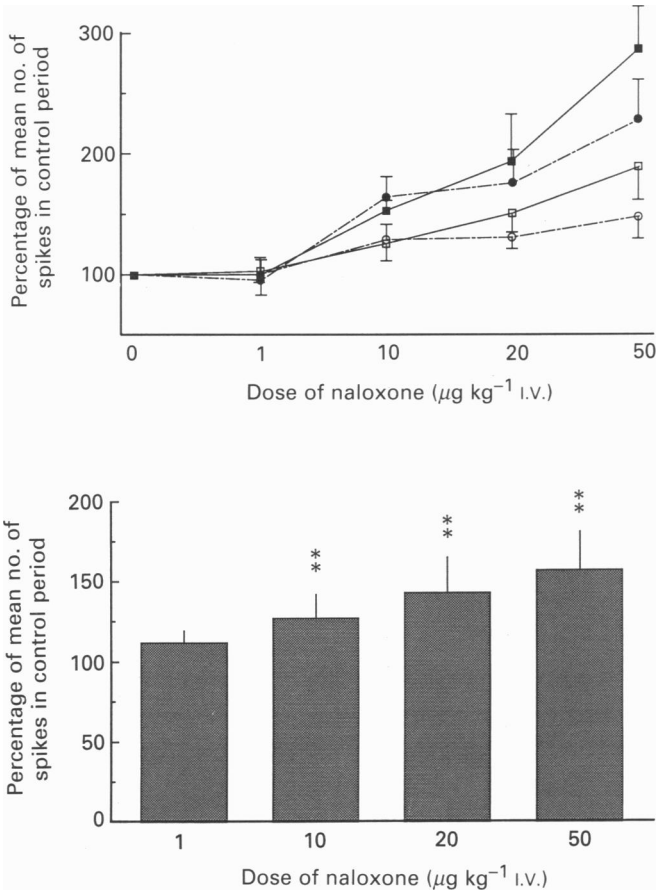

Fig. 2. The upper panel shows pooled data of the effects of I.V. naloxone on the single-motor-unit reflex responses evoked by percutaneous electrical stimulation at intensities presumed to activate afferent C fibres. These dose–response curves for naloxone display the normalized data, for seven units, expressed as percentages of the mean number of spikes evoked in the pre-drug control period. □, 1–240 ms, 0·3 Hz; ○, 1–240 ms, 2 Hz; ■, 241–500 ms, 0·3 Hz; ●, 241–500 ms, 2 Hz. The lower panel illustrates the effect of naloxone on the response of single motor units to low-intensity electrical stimulation. The bars represent pooled data of the percentage of the mean number of spikes evoked by sixteen stimuli during the control period. At all doses of naloxone, a significant difference in the number of spikes evoked compared to the control value occurred (Wilcoxon matched pairs, two tailed; $^{**}P < 0{\cdot}005$, $n = 5$).

was, however, a difference in the potency of naloxone between all three of these responses and the responses which occurred during the mechanical stimulus.

The effects of naloxone on responses to alternating higher and lower intensity noxious pinch stimuli were studied in five units. The mean high-intensity pinch force

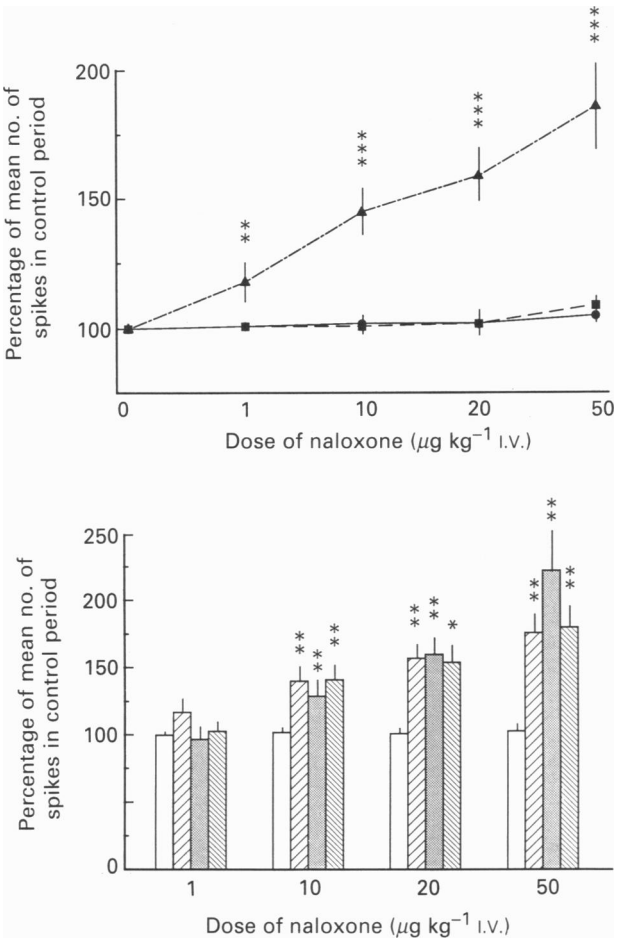

Fig. 3. In the upper panel the effects of naloxone are shown on the three phases of responses to high-intensity pinch stimuli, namely early pinch (first 5 s; ■), late pinch (subsequent 10 s; ●) and after-discharge (▲). Data from twelve units responding to high-intensity pinch stimuli have been pooled. Following naloxone administration, changes in the number of spikes evoked were expressed as percentages of pre-drug values for each phase. Means and standard errors are shown. Naloxone did not produce any significant change of the early or late phases of the response to pinch. The after-discharge was however, markedly facilitated. Statistically significant differences of the after-discharge with respect to early and late pinch were reached at all doses (Wilcoxon matched pairs test, two tailed, $^{**}P < 0{\cdot}005$ and $^{***}P < 0{\cdot}001$). In the lower panel the bar chart summarizes the effects of naloxone on the seven cells with which direct comparison was made between responses to late pinch (□), pinch after-discharge (▨), and on averaged responses to low- (▩) and high-frequency (■) electrical stimulation (up to 500 ms). Calculations were performed in the same way as for the upper panel. Statistically significant differences between late pinch and the other three measures were reached at doses of 10 μg kg^{-1} I.V. and above of naloxone (Wilcoxon matched pairs test, two tailed; $^{*}P < 0{\cdot}01$; $^{**}P < 0{\cdot}005$), but no difference was seen between any combination of after-discharge and electrical stimuli.

was $2{\cdot}5 \pm 0{\cdot}2$ N producing a late pinch mean firing rate of $28{\cdot}4 \pm 1{\cdot}9$ spikes s^{-1}. The mean low-intensity pinch force was $1{\cdot}6 \pm 0{\cdot}2$ N, producing a late pinch firing rate of $16{\cdot}3 \pm 3{\cdot}2$ spikes s^{-1}.

Figure 4 displays the effects of naloxone on the late phase of responses to low- and high-intensity pinch stimuli (upper panel) and on the after-discharge (lower panel) for the pooled data ($n = 5$). At doses of 20 and 50 μg kg^{-1} I.V., naloxone was significantly more effective in facilitating responses to the lower intensity pinch

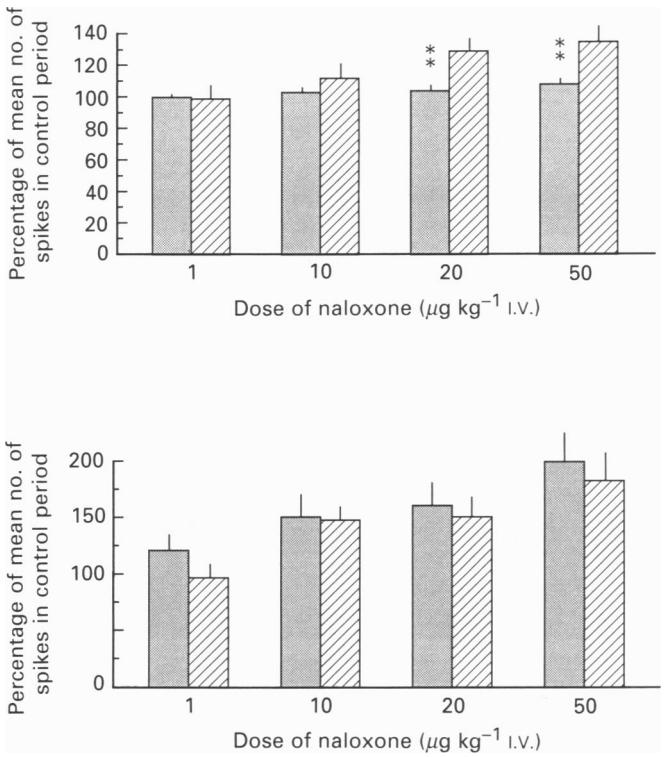

Fig. 4. Bar charts showing the effects of naloxone on responses during late pinch (upper panel) and on the after-discharge (lower panel) to lower (▨) and higher intensity (▩) pinch stimuli. Data are expressed in the same way as in Fig. 3. Asterisks mark where significant differences were reached in comparisons between responses to the low- and high-intensity pinch stimuli (Wilcoxon matched pairs; $^{**}P < 0{\cdot}005$, $n = 5$).

stimuli than those to higher intensity stimuli, when compared on the reflex during the latter part of the mechanical stimulus. Exactly the same result was obtained for the early part of the pinch stimuli. No difference was seen, however, between the effects of naloxone on after-discharges to the two types of stimuli.

DISCUSSION

This study, performed on anaesthetized, spinalized rats, using a technique that reduces the need for extensive preparative surgery, has demonstrated that naloxone, via an action at the spinal level, clearly produced a facilitatory effect upon reflexes evoked by some but not all types of noxious stimuli. This finding is in agreement with several other investigations. The most striking feature of our study was the apparent lack of facilitation produced by naloxone on responses to high-intensity, noxious

pinch stimuli compared to the clear facilitation of responses evoked by electrical stimulation at C fibre intensities.

Effects of naloxone on reflexes evoked by electrical stimulation

Much of the evidence concerning the effects of naloxone on spinal pathways has come from studies using peripheral electrical stimulation as the somatic stimulus. The advantage of this type of stimulation is that it is easily controlled and, in conjunction with nerve recordings of afferent compound action potentials, allows verification that a constant proportion of afferent nerve fibres is excited, thereby ensuring that alterations in efferent activity arise purely from central effects. In addition, graded electrical stimulation may be used so that effects on responses to large myelinated fibres may be distinguished from effects on responses to smaller myelinated and unmyelinated fibres. The obvious disadvantage of electrical stimulation is that the stimulus is artificial and there is evidence to suggest that electrical stimulation itself, can activate opioid-mediated inhibitions in the spinal cord. For instance, peripheral electrical stimulation has been shown to depress reflex responses to thermal stimuli in behavioural experiments (Woolf, Mitchell & Barrett, 1980). In electrophysiological studies (Chung, Fang, Cargill & Willis, 1983; Clarke, Ford & Taylor, 1989), high-intensity electrical stimulation produced a long-lasting inhibition of the flexion reflex via segmental mechanisms. All of these effects were naloxone reversible. The question arises, therefore, whether the clear facilitatory effect of naloxone on electrically evoked reflexes represents an action against tonic opioidergic mechanisms or simply an action against opioidergic inhibitory mechanisms activated by the electrical stimulus itself.

In our experiments, doses of naloxone as low as 1 $\mu g\ kg^{-1}$ produced a significant facilitation of the flexion reflex responses to high- and low-intensity electrical stimulation. The greatest effect of naloxone was seen on the late component of the response. Rivot *et al.* (1979) observed similar effects of naloxone on the C fibre discharge of wide dynamic range cells recorded from laminae IV and V of the dorsal horn. However, they required 20 times the dose needed in our study to produce an equivalent degree of facilitation. Fitzgerald & Woolf (1980) also saw a facilitation of C fibre evoked discharges in neurones recorded from laminae IV and V of the dorsal horn of spinalized, decerebrated rats, but again, much higher doses of naloxone were utilized.

Stimulating at a frequency of 2 Hz, at C fibre strengths, caused an increase in the number of spikes evoked with each successive stimulus (Mendell, 1966). Naloxone produced a dose-dependent facilitation of the response to both 0·3 and 2 Hz frequencies of stimulation. When measured in terms of percentage changes from their respective control value, naloxone was less effective at enhancing the response elicited at the higher frequency. In absolute terms, however, the number of extra spikes evoked as a result of successive doses of naloxone was not different, indicating that a set dose of naloxone enhanced these reflex responses by the same amount irrespective of the background level of neuronal excitability.

The effects of naloxone on the presumed A fibre component of the flexion reflex were studied in two ways: firstly, by looking at the early phase of responses to high-intensity stimulation, and secondly, by using lower intensity stimuli that did not elicit the long-latency component attributed to the activation of C fibre afferents.

Both sets of results confirmed that naloxone enhanced the low-threshold component of the reflex in a dose-dependent manner, although the extent of this enhancement was less than that for the C fibre component. This agrees in part with results obtained from ventral roots in the spinalized cat following large and small diameter afferent stimulation for both mono- and polysynaptic reflexes (Duggan, 1984; Duggan *et al.* 1984). Catley, Clarke & Pascoe (1983) also saw a facilitation of the flexion reflex to A$\alpha\beta$ strength stimulation following naloxone administration (5 μg kg^{-1}), but the effect of naloxone was found to be much greater on responses recorded from extensor muscle nerves to similar strength stimuli.

Effect of naloxone on reflexes evoked by 'natural' stimuli

Evidence concerning the effects of naloxone on naturally evoked responses of neurones in various laminae of the spinal cord is limited and often contradictory (see Introduction). In view of the facilitation of responses to electrical stimulation in the presence of naloxone, it was surprising to find that little effect of naloxone was observed on the response of single motor units to either the early or late phases of the responses to high-intensity pinch stimuli, even in the same preparation. The after-discharges to the pinch stimuli were facilitated to a similar degree to the responses to the high-intensity electrical stimulation, in a dose-dependent manner. This suggests that naloxone was in some way altering the level of tonic excitability in the spinal cord but that the effect was not seen during the duration of the mechanical stimulus itself.

Since, in this investigation, responses of cells were recorded to alternating electrical and mechanical stimulation, the differential effect of naloxone on responses to these types of stimuli must be attributed to factors related to the nature of the stimuli rather than to inter-animal or inter-cell differences.

In the comparison of higher and lower intensity pinch stimuli alternated on the same units, naloxone caused a dose-dependent facilitation on the late phase of the reflex response to pinch for the lower intensity stimulus whilst having no effect on the response to the higher intensity stimulus. One possible explanation for the facilitation of the response during only the lower intensity response during pinch stimulus might be that naloxone enhances responses to non-noxious stimuli in preference to those to noxious stimuli. This is unlikely for three reasons. First, both the lower and higher intensity stimuli were considered to be noxious (see Methods). Secondly, short-latency responses to electrical stimuli at A afferent fibre strengths (Figure 2, lower panel) were enhanced by naloxone proportionately less than were the late responses to more intense stimuli (Fig. 2, upper panel). Thirdly, if naloxone were selective, we would have expected the early phase of the pinch stimulus to be preferentially enhanced as it is presumed to have a larger low-threshold mechanoreceptive component than the late part of the pinch; this was not the case (see Fig. 4). At the same time it is clear from this study that naloxone is less effective in facilitating reflex responses to more intense noxious stimuli. This agrees with results obtained from intact mice with which it was found that the naloxone-mediated decrease in latency of onset of behavioural responses to thermal stimuli became less as the stimulus intensity was increased (Jacob, Tremblay & Colombel, 1974).

It is possible that an effect of naloxone at the higher stimulus intensity may have been masked by a near-maximal firing rate of neurones in the polysynaptic pathway.

The reflex enhancement may therefore only have been seen during the lower intensity stimulus because more, or all, of the interneurones in the reflex pathway were responding at submaximal firing rates and so had more room for increase. This idea is supported by the data concerning the maximal firing rates of the neurones. The neurones responding to the high-intensity pinch stimulus were firing with a mean rate of 28·4 spikes s^{-1} during late pinch. This rate was around 90% of the mean maximum firing rate that could be elicited by the pinching device as determined at the start of the experiments. The lower intensity pinch stimulus evoked a mean firing rate at 52% of the maximum. The maximal firing data are, however, limited to only a subset of the units recorded.

The degree of facilitation of spinal reflex responses to noxious mechanical stimuli produced by naloxone therefore appears to depend on the intensity with which the stimuli are applied. We have suggested that this effect may be related to the firing rates of neurones in the polysynaptic reflex pathway. From Fig. 1 it is clear that the mean firing rate of this typical unit, over 1 s epochs, was much lower during the periods of electrical stimulation than during the mechanical pinch stimuli. Since naloxone was very effective in facilitating the response to electrical stimuli, we might speculate that electrical stimulation does not excite the pathway maximally, so allowing naloxone to facilitate the response above control levels.

Duggan (1984), in his review, speculated that the surgery necessary to prepare animals for neurophysiological recording might be an adequate stimulus for the release of opioid peptides, and that this might be responsible for the opioidergic inhibition of spinal reflexes detected in these preparations, as revealed in the presence of naloxone. The physiological function for such a system was postulated to be immobility that might help tissue repair following injury. From these ideas, one might infer that the degree of preparative surgery would alter the degree of tonic opioidergic inhibition. Such a hypothesis might explain the lack of effect of naloxone on reflexes evoked in paraplegic patients (Willer & Bussel, 1980) compared with the often substantial effects in acute animal preparations (see Introduction).

In conclusion, naloxone, at lower doses than those used in many other studies, produced a highly significant elevation of the flexion reflex response to high- and low-intensity electrical stimulation in anaesthetized spinalized rats. Similar levels of reflex facilitation were not observed during high-intensity pinch stimuli although the post-stimulus after-discharge was enhanced to a similar degree to the response evoked by high-intensity electrical stimuli. A less intense noxious pinch stimulus evoked reflex responses at lower firing rates, and these responses were significantly facilitated by naloxone. These results provide evidence for an increased general level of neuronal excitability in the presence of naloxone which may not be seen during natural stimuli if the stimulus intensity is too high. These findings support the presence of a tonic inhibitory activity of spinal intrinsic opioid systems in the rat. However, the much smaller doses of naloxone required in this low surgery preparation to produce facilitations equivalent to those seen in several previous investigations suggest that surgical intervention itself is not the major activator of opioid-mediated inhibitions.

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REFERENCES

BASBAUM, A. I. & FIELDS, H. L. (1984). Endogenous pain control systems: brainstem spinal pathways and endorphin circuitry. *Annual Review of Neuroscience* **7**, 309–338.

BELL, J. A. & MARTIN, W. R. (1977). The effect of the narcotic antagonists naloxone, naltrexone and nalorphine on spinal cord C-fiber reflexes evoked by electrical stimulation or radiant heat. *European Journal of Pharmacology* **42**, 147–154.

BELL, J. A., SHARPE, L. G. & PICKWORTH, W. B. (1985). Electrophysiologically recorded C-fiber reflexes in intact and acute decerebrate-spinal cats: absence of naloxone facilitation in intact cats. *Neuropharmacology* **24**, 555–559.

CATLEY, D. M., CLARKE, R. W. & PASCOE, J. E. (1983). Naloxone enhancement of spinal reflexes in the rabbit. *Journal of Physiology* **339**, 61–73.

CHAMBERLAIN, S. & LEWIS, D. M. (1989). Contractile characteristics and innervation ratio of rat soleus motor units. *Journal of Physiology* **412**, 1–21.

CHUNG, J. M., FANG, Z. R., CARGILL, C. L. & WILLIS, W. D. (1983). Prolonged naloxone-reversible inhibition of the flexion reflex in the cat. *Pain* **15**, 35–53.

CLARKE, R. W. & FORD, T. W. (1987). The contributions of μ-, δ- and κ-opioid receptors to the actions of endogenous opioids on spinal reflexes in the rabbit. *British Journal of Pharmacology* **91**, 579–589.

CLARKE, R. W., FORD, T. W. & TAYLOR, J. S. (1989). Activation by high intensity peripheral nerve stimulation of adrenergic and opioidergic inhibition of a spinal reflex in the decerebrated rabbit. *Brain Research* **505**, 1–6.

DUGGAN, A. W. (1984). Inhibition in the spinal cord: its role in the response to injury. In *Advances in Pain Research and Therapy*, vol. 6, ed. KRUGER, L. & LIEBESKIND, J. C., pp. 123–134. Raven Press, New York.

DUGGAN, A. W., HALL, J. G., HEADLEY, P. M. & GRIERSMITH, B. T. (1977). The effect of naloxone on the excitation of dorsal horn neurones of the cat by noxious and non-noxious cutaneous stimuli. *Brain Research* **138**, 185–189.

DUGGAN, A. W., MORTON, C. R., JOHNSON, S. M. & ZHAO, Z. Q. (1984). Opioid antagonists and spinal reflexes in the anaesthetized cat. *Brain Research* **297**, 33–40.

DUGGAN, A. W. & NORTH, R. A. (1984). Electrophysiology of opioids. *Pharmacological Reviews* **35**, 219–281.

FITZGERALD, M. & WOOLF, C. J. (1980). The stereospecific effect of naloxone on rat dorsal horn neurones; inhibition in superficial laminae and excitation in deeper laminae. *Pain* **9**, 293–306.

GOLDSTEIN, A., PRYOR, G. T., OTIS, L. S. & LARSEN, F. (1976). On the role of endogenous opioid peptides: failure of naloxone to influence shock escape threshold in the rat. *Life Sciences* **18**, 599–604.

HARTELL, N. A. & HEADLEY, P. M. (1990*a*). Spinal effects of four injectable anaesthetics on nociceptive reflexes in rats: a comparison between electrophysiological and behavioural measurements. *British Journal of Pharmacology* **101**, 563–568.

HARTELL, N. A. & HEADLEY, P. M. (1990*b*). Electrophysiological techniques for monitoring spinal reflex responses to noxious stimuli in acutely prepared anaesthetized rats and in chronically prepared conscious rats. *Journal of Physiology* **425**, 10*P*.

HARTELL, N. A. & HEADLEY, P. M. (1990*c*). Effects of surgical trauma on the direct spinal actions of anaesthetic and analgesic agents. *Pain*, suppl. 5, S203.

HARTELL, N. A. & HEADLEY, P. M. (1991). Facilitation of spinal reflexes by naloxone. Comparison of responses to electrical and mechanical stimuli in the anaesthetized, spinalized rat. *Journal of Physiology* **435**, 71*P*.

HARTELL, N. A., HEADLEY, P. M. & PARSONS, C. G. (1990). The degree of surgical intervention influences the potency with which injectable anaesthetics depress spinal nociceptive reflexes in the adult rat. *Journal of Physiology* **420**, 28*P*.

HEADLEY, P. M., PARSONS, C. G. & WEST, D. C. (1985). A set of 'BASIC' programs for the online analysis of neuronal spike firing data. *Journal of Physiology* **364**, 7*P*.

HENRY, J. L. (1979). Naloxone excites nociceptive units in the lumbar dorsal horn of the spinal cat. *Neuroscience* **4**, 1485–1491.

JACOB, J. J., TREMBLAY, E. C. & COLOMBEL, M.-C. (1974). Facilitation de réactions nociceptives par la naloxone chez la souris et chez le rat. *Psychopharmacologia* **37**, 217–223.

KAYSER, V., BENOIST, J. M., NEIL, A., GAUTRON, M. & GUILBAUD, G. (1988*a*). Behavioural and electrophysiological studies on the paradoxical antinociceptive effects of an extremely low dose of naloxone in an animal model of acute and localized inflammation. *Experimental Brain Research* **73**, 402–410.

KAYSER, V., BESSON, J. M. & GUILBAUD, G. (1988*b*). Paradoxical effects of low doses of naloxone in experimental models of inflammatory pain. *Progress in Brain Research* **77**, 301–312.

LEBARS, D., CHITOUR, D., KRAUS, E., DICKENSON, A. H. & BESSON, J. M. (1981). Effect of naloxone upon diffuse noxious inhibitory controls (DNIC) in the rat. *Brain Research* **204**, 387–402.

LYNN, B. & CARPENTER, S. (1982). Primary afferent units from the hairy skin of the rat hind limb. *Brain Research* **238**, 29–43.

MENDELL, L. M. (1966). Physiological properties of unmyelinated fiber projection to the spinal cord. *Experimental Neurology* **16**, 316–332.

MILLAN, M. J., MILLAN, M. H., CZŁONKOWSKI, A., HÖLLT, V., PILCHER, C. W. T., HERZ, A. & COLPAERT, F. C. (1986). A model of chronic pain in the rat: response of multiple opioid systems to adjuvent-induced arthritis. *Journal of Neuroscience* **6**, 899–906.

PARSONS, C. G. & HEADLEY, P. M. (1989). Spinal antinociceptive actions of μ-and κ-opioids: the importance of stimulus intensity in determining 'selectivity' between reflexes to different modalities of noxious stimulus. *British Journal of Pharmacology* **98**, 523–532.

PARSONS, C. G., WEST, D. C. & HEADLEY, P. M. (1989). Spinal antinociceptive actions and naloxone reversibility of intravenous μ- and κ-opioids in spinalized rats: potency mismatch with values reported for spinal administration. *British Journal of Pharmacology* **98**, 533–543.

RIVOT, J. P., CHAOUCH, A. & BESSON, J. M. (1979). The influence of naloxone on the C-fiber response of dorsal horn neurons and their inhibitory control by raphe magnus stimulation. *Brain Research* **176**, 355–364.

STANDAERT, D. G., WATSON, S. J., HOUGHTON, R. A. & SAPER, C. B. (1986). Opioid peptide immunoreactivity in spinal and trigeminal dorsal horn neurones projecting to the parabrachial nucleus in the rat. *Journal of Neuroscience* **6**, 1220–1226.

TEPPERMAN, F. S., HIRST, M. & SMITH, P. (1983). Brain and serum levels of naloxone following peripheral administration. *Life Sciences* **33**, 1091–1097.

WILLER, J. C. & BUSSEL, B. (1980). Evidence for a direct spinal mechanism in morphine-induced inhibition of nociceptive reflexes in humans. *Brain Research* **187**, 212–215.

WOOLF, C. J. (1980). Analgesia and hyperalgesia produced in the rat by intrathecal naloxone. *Brain Research* **189**, 593–597.

WOOLF, C. J., MITCHELL, D. & BARRETT, G. D. (1980). Antinociceptive effect of peripheral segmental electrical stimulation in the rat. *Pain* **8**, 237–252.

WOOLF, C. J. & WALL, P. D. (1986). Morphine-sensitive and morphine-insensitive actions of C-fibre inputs on the rat spinal cord. *Neuroscience Letters* **64**, 221–225.