Journal of Physiology (1992), **449**, *pp.* 493–501
With 3 figures
Printed in Great Britain

OPIOIDERGIC INHIBITION OF FLEXOR AND EXTENSOR REFLEXES IN THE RABBIT

BY R. W. CLARKE, FIONA J. GALLOWAY, J. HARRIS, J. S. TAYLOR* AND T. W. FORD

From the Department of Physiology and Environmental Science, University of Nottingham School of Agriculture, Sutton Bonington, Loughborough, Leicestershire LE12 5RD

(*Received 6 June 1991*)

SUMMARY

1. Recordings were made from gastrocnemius medialis (GM), semitendinosus (ST) and tibialis anterior/extensor digitorum longus (TA/EDL) motor nerves during mechanical and electrical stimulation of the skin of the foot in decerebrated and spinalized rabbits.

2. GM motoneurones were excited from the heel and not from the toes, whereas TA/EDL responded to stimulation at the toes but not at the heel. ST also responded to electrical and mechanical stimulation at the toes, but there was a disparity between the effects of the two types of stimuli when they were applied at the heel: ST motoneurones fired in response to electrical stimulation of the heel but showed only an 'off' response to mechanical stimulation at this site.

3. The opioid antagonist naloxone caused a dose-dependent increase in all reflexes evoked by electrical stimulation of the skin. The heel–GM, toes–ST and toes–TA/EDL reflexes all increased to more than 3 times control levels with naloxone, which also caused significant decreases in the latencies of these reflex responses. On the other hand, the heel–ST response increased to just 1·4 times control levels and showed no decrease in latency with the opioid antagonist.

4. These data suggest that segmental withdrawal reflex pathways in the rabbit are suppressed by endogenous opioid peptides. This opioid-mediated inhibition seems to operate non-selectively on reflex pathways between cutaneous afferents and motoneurones.

INTRODUCTION

It has often been assumed that endogenous opioid peptides are concerned primarily with the control of events related to the detection of noxious stimuli. There is, however, a large body of evidence which suggests that these putative transmitters have an important role in the motor functions of the spinal cord. Opioid antagonists such as naloxone have been shown to increase spinal reflexes elicited by stimulation

* Present address: Department of Neuroscience, J.H.M. Health Center, University of Florida, Gainesville, FL 32610, USA.

MS 9460

of large and small diameter afferents from skin (McClane & Martin, 1967; Catley, Clarke & Pascoe, 1983; Duggan, Morton, Johnson & Zhao, 1984; Hartell & Headley, 1991), muscle (Goldfarb & Hu, 1975; Boureau, Willer & Dauthier, 1978; Duggan & Zhao, 1986) and viscera (Roppolo, Booth & DeGroat, 1983), thus indicating that opioid peptides exert a rather non-selective, widespread inhibition of reflex pathways to spinal motoneurones.

In the past we have suggested that there might be some topographical differences in the distribution of opioidergic inhibition of spinal reflexes in the rabbit. This idea was based largely on studies of short-latency reflexes evoked in the ankle extensor gastrocnemius medialis (GM), and the knee flexor semitendinosus (ST) by stimulation of the sural nerve. The extensor reflex was increased 6–7 times after naloxone whereas the flexor reflex increased by a factor of only 1·5–2 (Catley *et al.* 1983; Clarke & Ford, 1987). We believed that the sural–GM and sural–ST reflexes were indicators of the excitabilty of withdrawal reflex pathways from the heel. Subsequent studies have shown that selective electrical stimulation of sural nerve C fibres, or noxious mechanical stimulation within the sural receptive field (around the heel) readily activates GM motoneurones (Clarke, Ford & Taylor, 1989*b*), confirming that heel/sural–GM pathway is likely to be involved in reflex withdrawal of the heel from a noxious stimulus. Reflexes evoked in GM by stimulation of myelinated sural axons can therefore be regarded indicators of the level of excitability of one component of this pathway. Surprisingly, it is not possible to be so confident about the status of the sural input to ST motoneurones. Reflexes in this flexor muscle show only an 'off' response to pinching at the heel and are *inhibited* by stimulation of sural nerve C fibres. Thus, the short-latency sural–ST reflex does not correlate with a 'naturally' evoked reflex response. Having made this observation it became necessary to re-investigate the distribution of opioidergic inhibition in rabbit spinal cord, in particular the extent to which flexor reflexes in the rabbit are under the influence of opioid peptides. To do this it was necessary to find flexor reflex pathways which might contribute to limb withdrawal. To this end we have studied the reflex responses of GM, ST and the ankle flexor muscles to pinching at the heel and the toes, and compared these with responses elicited by electrical stimulation of the skin at the same sites. Naloxone was then tested against the electrically evoked responses so that comparisons could be made with our earlier studies. A preliminary report of these data has been published as an abstract (Clarke, Ford, Galloway & Taylor, 1989*a*).

METHODS

Experiments were performed on twenty-two New Zealand Red rabbits of either sex weighing between 1·7 and 3·1 kg, anaesthetized by intravenous injection of methohexitone sodium (Brietal, Eli Lilly, 10 mg/kg initially) and maintained, after cannulation of the trachea, on halothane (2–4 %) in $N_2O:O_2$ (70:30). Cannulae were inserted into one carotid artery and one jugular vein for recording arterial blood pressure and administration of drugs respectively. The second carotid artery was ligated. The spinal cord was sectioned between T12 and L2, and all animals were decerebrated to the pre-collicular level by suction (see Clarke, Ford & Taylor, 1988). At this point anaesthesia was discontinued and the animals paralysed with gallamine triethiodide (Flaxedil, May & Baker, 4 mg/kg initially); ventilation was maintained artificially using room air supplemented with oxygen. End-tidal CO_2 % was monitored and maintained between 3·5 and 4·5 %, and core temperature was held between 37·5 and 38·5 °C by a thermostatically controlled heating blanket. Body fluids were not systematically replaced during these experiments.

The left leg was clamped rigidly by the femur and tibia, and the popliteal fossa opened via a lateral incision. The cut edges of the posterior biceps muscle were reflected to form the walls of a pool into which warmed paraffin oil was poured to prevent desiccation of the tissues. The nerves to GM, ST and the ankle flexors tibialis anterior (TA) and extensor digitorum longus (EDL) were dissected free of connective tissue, cut, and placed over paired platinum recording electrodes between which they were crushed to give monophasic recordings. No attempt was made to separate TA from EDL, and recordings were made from these two nerves together. Reflex responses were evoked by electrical stimuli applied through paired stainless-steel electrodes inserted at the base of the toes and at the heel. Shocks of 2 ms duration and 3–12 V given at 1 Hz were employed to elicit short-latency reflex discharges in GM, ST or TA/EDL, from which the responses were averaged (eight sweeps) and integrated by computer. The stimulus intensity was set in each experiment to give reflexes of approximately equal sizes in all nerves from which recordings were made, so that the effects of naloxone could be compared directly. Electrical stimuli were used to allow comparisons with the effects of naloxone described in earlier work (e.g. Clarke & Ford, 1987), and the percutaneous method of application was chosen because there are no accessible, purely cutaneous nerves emanating from the toes of the rabbit. The patterns of reflexes evoked by firm manual pinching with serrated forceps of the skin at the heel and the toes were noted in each rabbit, to determine which electrically evoked reflexes could be associated with naturally activated withdrawal reflex pathways. Naloxone (hydrochloride, DuPont, UK) was dissolved in Ringer–Dale solution to concentrations of 0·01–2 mg/ml and administered I.V. in volumes of 0·1–1 ml. After an initial control period of 32–48 min, naloxone was administered intravenously to opiate-naive rabbits in increasing doses of 5, 10, 20, 50, 100 and 200 μg/kg to give a total cumulative dose of 385 μg/kg. Injections were separated by intervals of 24 min and dose–effect curves for naloxone were constructed as described previously (Clarke & Ford, 1987). The effects of the opioid μ-receptor agonist fentanyl (Sublimaze, Janssen, 1–100 μg/kg total dose) were investigated in four animals which had not received naloxone.

Data are presented as means ± standard errors of the mean. In previous studies it was found that the distribution of areas of reflexes recorded from a large number of animals did not differ significantly from a normal distribution (J. S. Taylor, unpublished observation), so statistical comparisons were by Student's unpaired *t* tests and a significance level of 0·05 assumed throughout. The letter '*n*' refers to the number of individual rabbits on which tests were carried out.

RESULTS

Reflex responses to stimulation at the toes and at the heel

The patterns of responses of GM, ST and ankle flexor motor nerves to noxious mechanical and low-intensity electrical stimulation at the heel and at the toes are summarized in Table 1. The toes–ST, toes–TA/EDL and heel–GM reflexes could be evoked by both electrical *and* natural stimulation, but the heel–ST reflex was activated only by electrical stimulation. The mean absolute voltage–time integrals for each electrically evoked reflex response were: heel–GM, 129 ± 18 μV ms (n = 15); heel–ST, 95 ± 19 μV ms (n = 13); toes–ST, 144 ± 21 μV ms (n = 15); and toes–TA/EDL, 132 ± 14 μV ms (n = 15). Mean latencies were 8·8 ± 0·3, 8·6 ± 0·44, 9·8 ± 0·31 and 12·1 ± 0·39 ms respectively. The heel–GM and heel–ST reflexes were not significantly different in size from responses evoked by direct electrical stimulation of all myelinated (Aβ and Aδ) sural nerve afferents (Clarke *et al.* 1988; J. S. Taylor and R. W. Clarke, unpublished observations).

The effects of naloxone

All four reflexes evoked by electrical stimulation were potentiated by naloxone (Figs 1 and 2). The increases in the heel–GM, toes–ST and toes–TA/EDL reflexes were to 390 ± 39 (n = 15), 310 ± 23 (n = 12) and 369 ± 57 % (n = 12) of pre-naloxone levels respectively, and were not statistically different from each other. The heel–ST

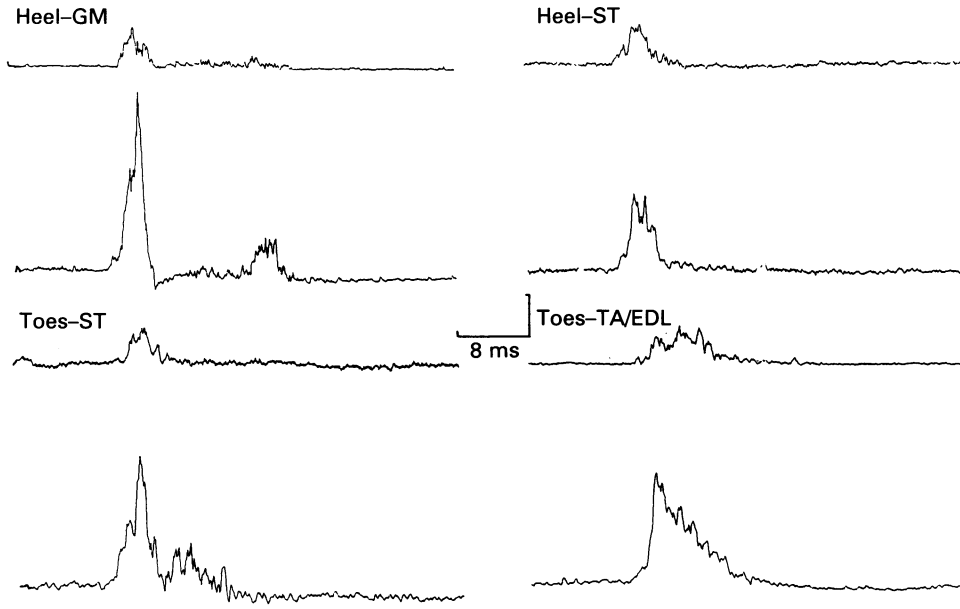

Fig. 1. Examples of heel–GM, heel–ST, toes–ST and toes–TA/EDL reflex responses in an untreated animal (upper trace of each pair), and after 385 μg/kg naloxone I.V. (cumulative dose, lower trace in each pair). Each record is the average of eight sweeps, and the stimulus was given at the beginning of each sweep. The voltage calibration is 100 μV for heel–GM, and 50 μV for all the others. All records from one animal.

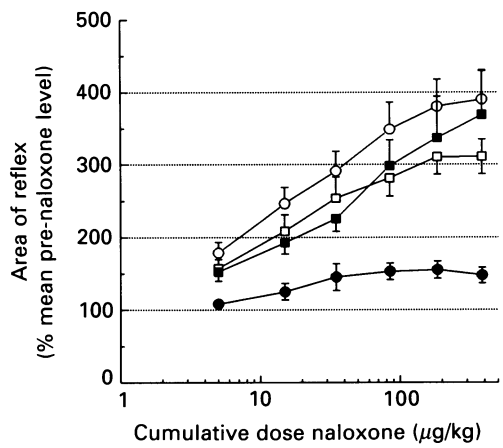

Fig. 2. Cumulative log dose–effect curves for naloxone against the heel–GM (○), heel–ST (●), toes–ST (□) and toes–TA/EDL (■) reflexes. Each point is the mean ± S.E.M.

reflex was enhanced to just 148 ± 8% (n = 10) of control levels (Fig. 2), significantly less than any of the other three reflexes studied ($P < 0{\cdot}01$ in all cases). The latencies of the heel–GM, toes–ST and toes–TA/EDL reflex responses decreased significantly with naloxone, by an average of 1·06 ± 0·20, 1·01 ± 0·15 and 1·35 ± 0·23 ms respectively. The latency of the heel–ST reflex did not change after the opioid

antagonist (Fig. 3). The opioid antagonist had no consistent effects on arterial blood pressure: prior to administration of naloxone the average mean arterial pressure was $76 \pm 3{\cdot}8$ mmHg, and after the drug it was $75 \pm 4{\cdot}2$ mmHg.

In four animals it was noticed that, after the cumulative dose of naloxone had reached 35 μg/kg, it was possible to evoke four to five rhythmic alternating bursts of firing in ankle flexor and extensor muscle nerves by pinching the heel.

TABLE 1. The patterns of responses in the GM, ST and TA/EDL motor nerves to electrical and mechanical stimulation at the heel and at the toes

	Stimulus applied to heel		Stimulus applied to toes	
Muscle nerve	Electrical (low intensity)	Mechanical (noxious)	Electrical	Mechanical
GM	Yes	Yes	No	No
ST	Yes	'Off' response	Yes	Yes
TA/EDL	No	No	Yes	Yes

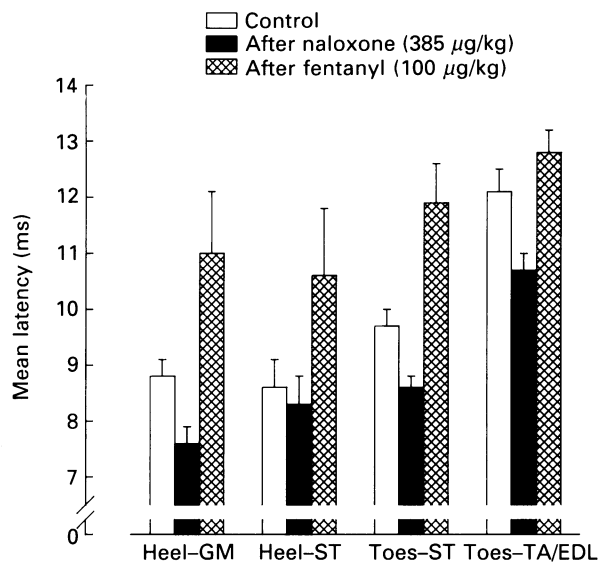

Fig. 3. The mean latencies +S.E.M. of the heel–GM, heel–ST, toes–ST and toes–TA/EDL reflexes from untreated rabbits, after naloxone, and after fentanyl.

The effects of fentanyl

Fentanyl (1–100 μg/kg) produced dose-dependent decreases in all four reflexes evoked by electrical stimulation. The drug was tested three times against each reflex. Significant decreases were seen with a cumulative dose of 10 μg/kg, and after the highest dose of fentanyl, the heel–GM, heel–ST, toes–ST and toes–TA/EDL reflexes decreased to averages of $3{\cdot}4 \pm 0{\cdot}8$, $16{\cdot}1 \pm 9{\cdot}8$, $9{\cdot}6 \pm 6{\cdot}4$ and $8{\cdot}6 \pm 1{\cdot}8$% of pre-drug controls respectively. There were concomitant increases in the latencies of all four responses. In contrast to an earlier study (Clarke & Ford, 1987), we found a

significant decrease in mean arterial blood pressure with fentanyl: from 82 ± 10 to $62 \pm 6{\cdot}3$ mmHg. All effects of fentanyl were reversed by subsequent administration of naloxone at 20 μg/kg.

DISCUSSION

Reflexes evoked by electrical stimulation of the skin

In these experiments reflexes were evoked by electrical stimulation of the skin, or by pinch. The responses to pinch showed which reflex pathways were likely to be involved in reflex withdrawal of the heel or the toes from a noxious stimulus. Where there was a coincidence between the responses to electrical and mechanical stimulation at a particular location (e.g. heel to GM), we believe that the electrically evoked reflex can be considered as a relevant indicator of the excitability of some components of the withdrawal reflex evoked from that site (see Introduction). Attempts were made to monitor in the sural nerve the afferent volley evoked by electrical stimulation at the heel. However, the waveforms so recorded were difficult to relate to the compound action potential which can be elicited by direct stimulation of the nerve, presumably because percutaneous stimulation results in excitation of afferent axons at more than one point. It is therefore not possible to describe precisely the nature of the afferents excited by our stimuli. The centripetal conduction distances were approximately 200 mm from the heel and 250 mm from the toes. Assuming a motoneurone conduction velocity of around 70–80 m/s and a central delay of 2–3 ms (see Clarke, 1982), the latencies of the reflex responses indicate that they were evoked by activity in myelinated afferents with conduction velocities between 20 and 50 m/s. It was not possible to differentiate between components due to activity in Aβ and Aδ axons. There were no long-latency discharges characteristic of reflexes evoked by non-myelinated C afferents (Clarke *et al.* 1989*b*; Hartell & Headley, 1991).

The effects of naloxone on different reflexes

Naloxone enhanced all reflexes studied in these experiments, a result which suggests that endogenous opioid peptides exert a wide-ranging suppression of withdrawal reflexes in the distal hindlimb of the rabbit. This finding is consistent with most previous studies which have indicated that opioid peptides provide, in spinalized animals, a non-selective blanket of inhibition which attenuates transmission through spinal reflex pathways to motoneurones (see Introduction). It would appear that opioid peptides are potentially very important modulators of the excitability of spinal reflexes in general. The exact mechanisms which underlie opioidergic inhibition of spinal reflexes have yet to be established, although it is almost certain that endogenous opioids do not inhibit motoneurones directly (Duggan & Zhao, 1986; Ford, Harris & Taylor, 1991). The shifts in the latencies of reflex responses which accompanied naloxone were probably due mainly to disinhibition of reflex drive from fast, low-threshold afferent fibres. This view is supported by the fact that responses of single neurones within the spinal cord show similar changes in latency with naloxone (Ford *et al.* 1991), and by the observation that the thresholds for GM reflexes elicited by sural nerve stimulation are reduced

after naloxone (Clarke & Ford, 1987). Furthermore, recent studies have shown that very weak mechanical stimuli applied to the skin at the heel do not normally generate reflex discharges in GM motoneurones, but that they will do so after blockade of opioid receptors (Harris, Ford & Clarke, 1991). Findings such as these lead us to believe that one physiological role of opioid peptides is to help to set the threshold for withdrawal reflexes so that they are not normally activated by innocuous stimuli.

The increase in the heel–GM reflex response was not as great in percentage terms as that seen for the reflex evoked by direct electrical stimulation of the sural nerve, although the absolute sizes of the reflexes recorded in the control states were similar (see Clarke *et al.* 1988). Presumably this is because the test stimuli used in the present study were delivered directly within the excitatory receptive field for GM motoneurones. Stimuli applied in this way would recruit a high proportion of reflexogenic afferents compared to stimulation of the sural nerve trunk (which includes afferents which originate outside the GM receptive field), but would not have access to as many reflexogenic fibres in total.

As ST motoneurones responded to electrical stimulation at the heel but not to noxious mechanical stimulation at the same point, the short-latency heel–ST reflex cannot, unlike the other three reflexes examined in this study, be considered as contributing to a withdrawal reflex. Perhaps it is an example of the so-called 'private line' connections of uncertain function, which exist between large diameter cutaneous fibres and motoneurones (see Lundberg, 1979). It seems likely that in the rabbit, the reflex connections between afferents from the heel and the ST motor nucleus are more limited than for the three other reflex pathways studied, so that there is less 'spare capacity' to be released by the action of naloxone (or even strychnine, see Clarke *et al.* 1989*b*). It does not therefore seem necessary to propose that there is any topographical selectivity in the tonic release of endogenous opioids in the lumbosacral spinal cord (NB the same is not true for stimulus-evoked release of opioids; see Catley, Clarke & Pascoe, 1984). The differences between reflexes cannot be due to an absence of opioid receptors in the heel–ST system as fentanyl, the opioid μ-receptor agonist, inhibited all four reflexes.

Withdrawal reflexes and opioidergic inhibition

Our earlier conclusion, that opioids are directed primarily against reflex pathways to extensor motoneurones, is no longer tenable. Our mistake was to believe that because ST motoneurones responded to electrical stimulation of the sural nerve they would also be activated by natural stimulation of the heel, as in the cat (Hagbarth, 1952) and rat (Schouenborg & Kalliomäki, 1990). It is commonly assumed that the 'flexion reflex' is a functional unity in which all flexor motoneurones are excited (and all extensor motoneurones inhibited) by noxious natural stimulation from all parts of the lower limb (Sherrington, 1910), but this is not true. Creed & Sherrington (1926) showed that different flexor muscles are recruited to different degrees according to the nerve used to evoke the reflex response. Hagbarth (1952) went one step further in demonstrating that noxious stimulation of particular areas of the skin was excitatory to some extensor muscles and inhibitory to their antagonistic flexors. Thus, the 'flexion withdrawal reflex' is in fact comprised of a family of reflex

pathways, so organized as to bring about the most appropriate limb withdrawal movements in reponse to threatening stimuli (see Creed & Sherrington, 1926; Hagbarth, 1952; Clarke *et al.* 1989*b*; Schouenborg & Kalliomäki, 1990). The heel–GM reflex pathway is one member of this family, and the present results indicate that it has more in common with other hindlimb withdrawal reflexes than we had imagined previously.

This work was supported by the AFRC: J.S.T. and J.H. were SERC scholars at the time this work was carried out. We would like to thank Gabrielle Isherwood and Caroline Northway for helping in some of these experiments. Naloxone was a generous gift of DuPont (UK) Ltd.

REFERENCES

BOUREAU, F., WILLER, J.-C. & DAUTHIER, C. (1978). Study of naloxone in normal awake man: effects on spinal reflexes. *Neuropharmacology* **17**, 565–568.

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

CATLEY, D. M., CLARKE, R. W. & PASCOE, J. E. (1984). Post-tetanic depression of spinal reflexes in the rabbit and the possible involvement of opioid peptides. *Journal of Physiology* **352**, 479–489.

CLARKE, R. W. (1982). The properties of some cutaneo-muscular reflexes in the hindlimb of the rabbit. Ph.D. Thesis, University of London.

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., GALLOWAY, F. J. & TAYLOR, J. S. (1989*a*). Naloxone enhancement of flexor and extensor reflexes in the rabbit. *Journal of Physiology* **416**, 18*P*.

CLARKE, R. W., FORD, T. W. & TAYLOR, J. S. (1988). Adrenergic and opioidergic modulation of a spinal reflex in the rabbit. *Journal of Physiology* **404**, 407–417.

CLARKE, R. W., FORD, T. W. & TAYLOR, J. S. (1989*b*). Reflex actions of selective stimulation of sural nerve C fibres in the rabbit. *Quarterly Journal of Experimental Physiology* **74**, 681–690.

CREED, R. S. & SHERRINGTON, C. S. (1926). Observations on concurrent contraction of flexor muscles in the flexor reflex. *Proceedings of the Royal Society* B **100**, 258–267.

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

DUGGAN, A. W. & ZHAO, Z. Q. (1986). Microelectrophoretic application of naloxone near motoneurones fails to reproduce the effects of systemic naloxone in anaesthetized cats. *Neuroscience Letters* **45**, 305–310.

FORD, T. W., HARRIS, J. & TAYLOR, J. S. (1991). The effects of naloxone on spinal neurones with an excitatory input from the sural nerve in the decerebrated and spinalized rabbit. *Journal of Physiology* **435**, 61*P*.

GOLDFARB, J. & HU, J. W. (1975). Enhancement of reflexes by naloxone in acute spinal cats. *Neuropharmacology* **15**, 785–792.

HAGBARTH, K.-E. (1952). Excitatory and inhibitory skin areas for flexor and extensor motoneurones. *Acta Physiologica Scandinavica* **26**, suppl. 94, 1–58.

HARRIS, J., FORD, T. W. & CLARKE, R. W. (1991) Opioidergic modulation of reflexes evoked by mechanical stimulation of the heel in the rabbit. *Society for Neuroscience Abstracts* **17**, 465.

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

LUNDBERG, A. (1979). Multisensory control of spinal reflex pathways. *Progress in Brain Research* **50**, 11–28.

MCCLANE, T. K. & MARTIN, W. R. (1967). The effects of morphine, nalorphine, naloxone and cyclazocine on the flexor reflex. *International Journal of Neuropharmacology* **6**, 89–98.

ROPPOLO, J. R., BOOTH, A. M. & DEGROAT, W. C. (1983). The effects of naloxone on the neural control of the urinary bladder of the cat. *Brain Research* **264**, 355–358.

SCHOUENBORG, J. & KALLIOMÄKI, J. (1990). Functional organization of the nociceptive withdrawal reflexes. 1. Activation of hindlimb muscles in the rat. *Experimental Brain Research* **83**, 67–78.

SHERRINGTON, C. S. (1910). Flexion-reflex of the limb, crossed-extension reflex, and reflex stepping and standing. *Journal of Physiology* **40**, 28–121.