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Selective antinociceptive effects of tizanidine (DS 103–282), a centrally acting muscle relaxant, on dorsal horn neurones in the feline spinal cord

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1 The effects of the centrally acting muscle relaxant tizanidine (DS 103–282) have been examined on the responses of laminae IV and V dorsal horn neurones to peripheral noxious and non-noxious stimuli in cats spinalized at L1.

2 Iontophoretic ejection of tizanidine near the cell bodies of the recorded neurones or more dorsally into laminae II–III resulted in a marked and prolonged depression of excitation of laminae IV and V neurones evoked by noxious stimuli. Spontaneous firing was also depressed in many neurones but responses to innocuous stimuli were unaffected.

3 Intravenous administration of tizanidine also produced a long lasting and selective reduction in responses of laminae IV and V neurones to noxious stimuli and depressed the long latency excitation of these neurones evoked by electrical stimulation of small diameter unmyelinated primary afferents.

4 In contrast to the selective antinociceptive effect of tizanidine, ejection of γ-aminobutyric acid (GABA) near laminae IV and V neurones or isoguvacine into laminae II–III produced parallel reductions in responses to noxious and non-noxious stimuli. Furthermore, ejections of the excitant amino acid kainate into laminae II–III produced parallel enhancement of responses induced by both types of stimuli.

5 The site and mechanism of the antinociceptive action of tizanidine is not known but does not appear to involve an interaction with opiate receptors as it was not antagonized by naloxone. The possibility is discussed that tizanidine acts at synapses formed between excitatory interneurones in lamina II or III and laminae IV and V neurones, either interfering with transmitter release or its postsynaptic action.

6 The effects of iontophoretically administered tizanidine are quite distinct from those of baclofen, which produced non-selective depression of responses to both noxious and innocuous stimuli, but were similar to those of noradrenaline. This raises the possibility that noradrenaline and tizanidine may act at a common site in the spinal cord.

Introduction

Tizanidine (5-chloro-4-(2-imidazolin-2yl-amino) - 2,1,3-benzothiodazole (DS 103–282) is a new centrally acting muscle relaxant (Sayers *et al.*, 1980). This agent preferentially reduces polysynaptic rather than monosynaptic reflexes and polysynaptic compared with monsynaptic excitation of spinal neurones evoked from low threshold primary afferents, effects which may account for its myotonolytic activity (Davies, 1982). In addition to its muscle relaxant action, tizanidine may have analgesic activity as it increases response latencies in the tail flick and hot plate tests on mice (Sayers *et al.*, 1980). These behavioural analgesic tests involve spinal reflexes evoked from high threshold afferent fibres. It therefore seemed pertinent to examine the effects of tizanidine on the excitatory responses of spinal neurones evoked by activation of high threshold primary afferents. To this end the effects of iontophoretically and systemically administered tizanidine have been determined in the cat spinal cord on dorsal horn neurones which were excited by noxious and non-noxious peripheral stimuli.

In the iontophoretic studies, drugs were administered from conventional multibarrelled micropipettes either positioned in the vicinity of the cell bodies of the recorded neurones in laminae IV and V or dorsal to these in the regions of laminae II–III. The reason for the latter more dorsal ejections was that small diameter primary afferents conveying nociceptive information terminate in the superficial dorsal laminae (Brown, 1982) and certain substances exert selective antinociceptive effects on spinal neurones only when ejected at these sites (Duggan *et al.*, 1977a; Davies & Dray, 1978; Headley *et al.*, 1978). Since the absence of effects of iontophoretically applied drugs can be misleading particularly when ejections are made at some distance from the recorded neurone, due possibly to inappropriate placement of the drug delivery pipette, it was considered advisable to compare the effects of tizanidine with a compound known to exert antinociceptive actions when ejected into laminae II–III. Noradrenaline was chosen as the reference compound as it has antinociceptive effects which are relatively rapidly reversible when ejected near cell bodies of dorsal horn neurones or more dorsally into laminae II–III (Headley *et al.*, 1978). The effects of tizanidine were also compared on many neurones with those of another muscle relaxant, baclofen, as the latter agent has been demonstrated to exert spinal antinociceptive effects (Wilson & Yaksh, 1978; Henry, 1982).

A preliminary account of some of the present data has already been published (Davies & Johnston, 1983).

Methods

Experiments were performed on 16 cats of either sex anaesthetized with α-chloralose (50 mg kg^{-1} i.v.) following induction with halothane and supplemented when necessary with α-chloralose 10 mg kg^{-1} i.v. Three additional cats were anaesthetized with halothane throughout the experiment. A cannula was inserted into the brachial vein for the intravenous administration of drugs. Blood pressure was continually monitored on a chart recorder via an indwelling carotid cannula and body temperature was maintained between 37–38°C by means of a thermostatically controlled heating blanket. Experiments were terminated if systolic pressure fell below 95 mmHg.

The spinal cord was exposed by lumbar laminectomy and following section of the cord at L1, the L7 and S1 ventral roots were cut ipsilaterally. Recordings were obtained from neurones located in laminae IV and V of the dorsal horn via either the central barrel (4 M NaCl) of a seven barrelled microelectrode or a single glass microelectrode (4 M NaCl or pontamine sky blue in 2% sodium acetate). Neurones were identified as being in laminae IV or V by noting their depth from the surface of the cord and determining the position of the tip of the recording electrode in histological sections subsequent to the deposition of dye at the termination of the experiment. The majority of neurones studied responded to noxious and innocuous stimuli and hence fall into the category of class 2 cells described by Iggo (1974). None of the neurones encountered responded only to noxious stimuli, although many cells were found that did not respond to noxious stimuli but were excited by low threshold mechanical stimuli. These latter non-nociceptive cells were probably similar to the class 1 neurones described by Iggo (1974).

Noxious stimuli consisted of the application of radiant heat to a small blackened area of the ipsilateral foot (usually a toe pad) via a projector lamp with feedback control from a thermocouple placed on the foot in the centre of the light beam. Cells were classed as nociceptive cells if their excitation threshold exceeded 43°C. Innocuous stimuli consisted of displacing interdigital hairs on the foot by means of an electronically controlled air jet. Responses to both types of stimuli were usually induced alternately by an automatic timing device employing a cycle length of 1.7 min or more.

In a few experiments neurones were excited by electrical stimulation of the central ends of the cut sural or tibial nerve in the ipsilateral leg via bipolar stimulating electrodes. Stimulus intensities sufficient to excite unmyelinated fibres, many of which convey nociceptive information to the spinal cord (Bessou & Perl, 1969; Beck *et al.*, 1974), were employed (1 ms pulses, 0.5 Hz, 30–100 times the threshold intensity required to excite the neurone) which evoked groups of action potentials at a latency consistent with activation of unmyelinated primary afferents (conduction velocity of 0.2–1.0 ms^{-1}). The remaining leg nerves were sectioned to prevent reflex movements.

In all instances neuronal responses were displayed on an oscilloscope, electronically counted and either recorded on chart paper or fed through a small computer for the compilation of peristimulus time histograms (PSTH). In addition, the total number of action potentials (spikes) evoked by each cutaneous stimulus was counted by a gated electronic counter. These counts were corrected for background firing rate and used to determine the maximum change in an evoked response following drug administration by expressing the count as a percentage of the mean count from at least three control responses preceding the drug. Changes of 15% or more in the evoked response were regarded as significant.

The peripheral barrels of the multibarrelled microelectrodes were filled with various combinations of the following drugs which were ejected using standard iontophoretic techniques: Na L-glutamate

(0.2 M, pH 7.2), Na DL-homocysteate (DLH) (0.2 M, pH 7.2), Na quisqualate (0.02 M in 0.165 M NaCl, pH 7.0), Na N-methyl-D-aspartate (NMDA) (0.05 M in 0.165 M aCl, pH 7.0), Na kainate (0.02 M in 0.165 M NaCl, pH 7.0), L-noradrenaline bitartrate (0.2 M, pH 4.0), tizanidine (0.1 M, pH 5.5), (−)-baclofen (0.05 M in 0.165 M NaCl, pH 3.5), γ-aminobutyric acid (GABA) (0.5 M, pH 3.2), isoguvacine (0.2 M, pH 3.0) and naloxone HCl (0.1 M, pH 5.0). One barrel of each electrode contained pontamine sky blue in Na acetate which was used for current balancing and for marking the position of the microelectrode tip in the spinal cord. Currents of 10–15 nA of appropriate polarity were used to retain drugs between ejections.

Drugs were either iontophoresed into the vicinity of the cells from which recordings were made or, where a single electrode was used for recording, the multibarrelled electrode was positioned in laminae II–III using a second micromanipulator. The vertical displacement of the two electrodes was of the order of 300–850 μm. This technique has been described in detail by Duggan *et al.* (1977a). Tizanidine and naloxone were also administered intravenously in some experiments, the doses being expressed in terms of the salt concentration.

Results

The effects of drugs were only determined on neurones that responded consistently and reproducibly to peripheral stimuli. For this reason drug effects were not examined on responses of all nociceptive cells to non-noxious stimuli as such responses were sometimes variable. The effects of drugs on lamina IV neurones were similar to those on lamina V neurones; thus the results from neurones in these laminae have been combined. In the three cats anaesthetized with halothane the effects of the drugs tested were qualitatively similar to those observed in cats anaesthetized with α-chloralose and therefore are not presented separately.

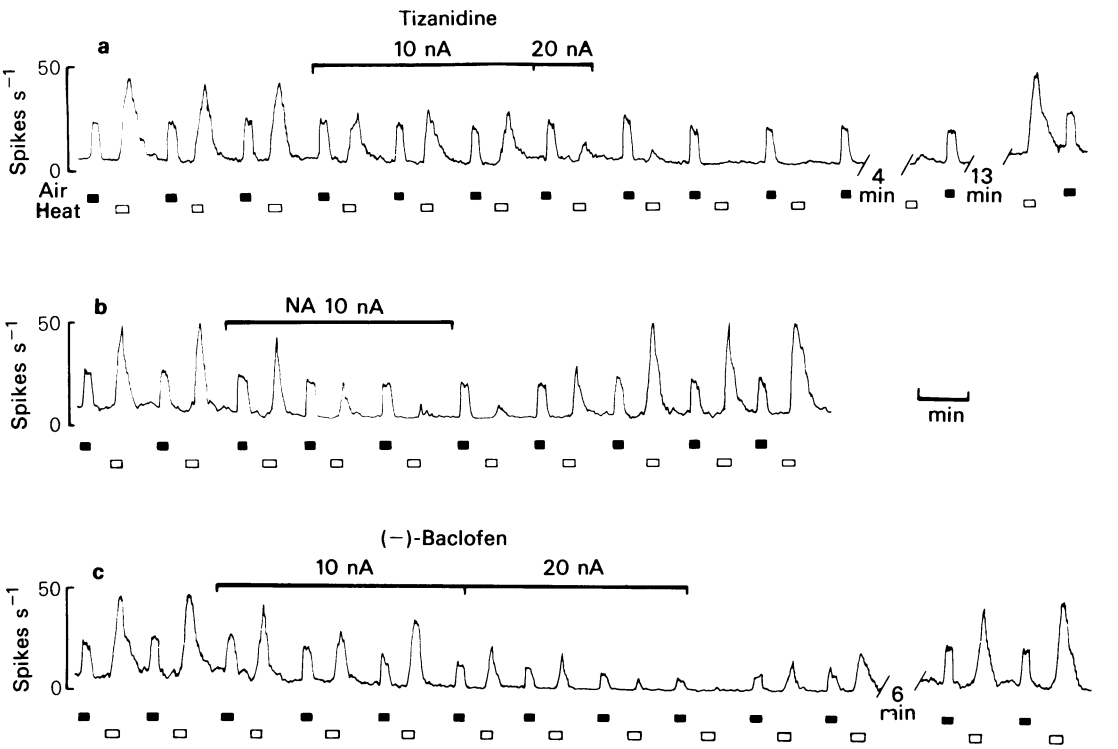

Figure 1 Effects of (a) tizanidine (10–20 nA), (b) noradrenaline (NA, 10 nA) and (c) (−)-baclofen (10–20 nA) on the responses of the same lamina V neurone induced by noxious (heat 47°C) and innocuous (air jet) stimuli. All drugs were ejected in the vicinity of the cell body of the neurone. Tizanidine and noradrenaline reduced nociceptive responses without affecting non-nociceptive responses. In contrast, (−)-baclofen reduced both types of responses concurrently. All three agents reduced spontaneous firing.

Table 1 Comparisons between the depressant effects of tizanidine and those of noradrenaline or (−)-baclofen on responses of the same dorsal horn neurones to noxious and innocuous stimuli and spontaneous firing rate when administered iontophoretically into laminae IV–V of the lumbar spinal cord

	Depressant effect on: *Response to noxious stimuli (heat)*[b]			*Response to innocuous stimuli (air-jet)*[b]			*Spontaneous firing rate*[b]		
Drug[a]	*No. dep. No. tested*	*% dep.*	*Recovery time* (min)	*No. dep. No tested*	*% dep.*	*Recovery time* (min)	*No. dep. No. tested*	*% dep.*	*Recovery time* (min)
Tizanidine (25 ± 3.5 nA for 3.4 ± 0.4 min)	16/16	69 ± 6%	19 ± 6.0	0/6			8/12	100%	17 ± 4.0
Noradrenaline (17.3 ± 1.7 nA for 3.9 ± 0.6 min)	16/16	78 ± 6%^{NS}	5 ± 0.6***	1/6	25%		9/12	88 ± 9%^{NS}	6 ± 1.0***
Tizanidine (21.1 ± 3.0 nA for 4.1 ± 0.6 min)	16/18	77 ± 5%	20 ± 2.3	1/7	60%		11/14	85 ± 6%	17 ± 2.6
(−)-Baclofen (29.6 ± 4.5 nA for 3.5 ± 0.3 min)	16/18	65 ± 8%^{NS}	9 ± 1.6**	7/7	70 ± 5%***	11 ± 2.2	10/14	86 ± 6%^{NS}	10 ± 1.9*

N.B. In many cases tizanidine, noradrenaline and (−)-baclofen were tested on the same neurones.

[a]The numbers in parentheses shows for each drug the mean ± s.e.mean ejecting current (nA) and duration (min) of ejection.

[b]The numbers in each column refer to the number of neurones on which responses were depressed (dep.) expressed as a ratio of the number of neurones on which direct comparisons were made, the mean percentage depression of the firing rate ± s.e.mean for the cells affected and the mean ± s.e.mean time (min) for recovery of the response.

**** indicate that the value shown is significantly different from the corresponding value obtained during the ejection of tizanidine on the same neurones: $^{*}P<0.05$; $^{**}P<0.01$; $^{***}P<0.001$ (Student's t test); NS, not significant.

Iontophoretic ejection near cell bodies

Tizanidine ejected with currents of 5–50 nA (21.3 ± 1.9 nA, mean ± s.e.mean) for 1–11 min (4 ± 0.29 min) consistently and reproducibly reduced responses to noxious heat by 15–100% (66 ± 4%) on 23 of 25 laminae IV and V neurones. The characteristics of this depressant action of tizanidine are illustrated for one lamina V neurone in Figure 1. Here, 10–20 nA tizanidine markedly depressed nociceptive responses and spontaneous firing. The progressive decline in responses to noxious stimuli for a few min after terminating the tizanidine ejection illustrated in this figure was a consistent feature of this drug's action. The depressant effect of tizanidine was prolonged (Figure 1) and, generally, recovery occurred 11 to sometimes more than 25 min (20 ± 4 min) after the maximum effect had been achieved. Spontaneous firing was unaffected on 8 nociceptive neurones but was reduced on 13 by 40–100% (76 ± 8%), the time-course of this effect being similar to that on responses to noxious stimuli. By contrast, responses evoked by non-noxious stimuli (air jets) were unaffected on 7 neurones in which responses to noxious stimuli were reduced by 80 ± 5.3% (e.g. Figure 1).

Comparisons between tizanidine, noradrenaline and (−)-baclofen Comparisons between the effects of tizanidine with those of (−)-baclofen or noradrenaline on the same nociceptive neurones are summarized in Table 1. Nordadrenaline had essentially similar effects to those of tizanidine, responses to noxious stimuli (and spontaneous firing) being preferentially and reversibly reduced with respect to responses to innocuous stimuli. However, the duration of action of noradrenaline was considerably shorter than that of tizanidine. (−)-Baclofen also depressed responses to noxious stimuli and spontaneous firing, but in contrast to the effects of the other two substances it also reduced responses to non-noxious stimuli in parallel with the nociceptive responses (Table 1). The actions of all three agents on the same neurone are shown in Figure 1. It can be seen that the duration of action of (−)-baclofen was significantly shorter than that of tizanidine although both drugs produced a similar depression of responses to noxious stimuli (see also Table 1) and responses did not progressively decline after terminating the (−)-baclofen ejection. The effects of GABA were examined on 4 nociceptive neurones. This agent had a non-selective action identical to that of baclofen except that the effects of GABA were fully reversible within 2 min of terminating the ejection.

Effects on responses to excitant amino acids In an attempt to determine whether the antinociceptive action of tizanidine was mediated pre- or postsynaptically the effects of this agent were examined on responses induced by excitatory amino acids on nociceptive neurones. The effects of noradrenaline and (−)-baclofen were also examined on some of these neurones. The results are summarized in Table 2.

Table 2 Depressant effect of tizanidine on responses of laminae IV–V neurones to noxious stimuli and excitatory amino acids when administered iontophoretically into laminae IV–V of the lumbar spinal cord

Tizandine	*Response induced by:*	*No. Depressed / No. neurones tested*	*% depression*[a]	*Recovery time* (min)
19.8 ± 4.0 nA for 4.2 ± 0.5 min	Noxious heat	7/7	85 ± 5%	19 ± 2.4
	L-Glutamate	6/7	74 ± 7%^{NS}	12 ± 2.2*
24.5 ± 4.4 nA for 4 ± 0.3 min	Noxious heat	9/9	52 ± 9%	20 ± 2.6
	DL-Homocysteate	7/9	44 ± 6%^{NS}	11 ± 2.8*
23 ± 5.0 nA for 3.5 ± 0.4 min	Noxious heat	10/10	64 ± 10%	22 ± 2.4
	N-methyl-D-aspartate	7/10	52 ± 6%^{NS}	8 ± 1.4**
21.4 ± 7.0 nA for 4.2 ± 1.0 min	Noxious heat	5/5	72 ± 5%	21 ± 3.6
	Quisqualate	4/5	60 ± 6%^{NS}	12 ± 2.8^{NS}
22.7 ± 7.6 nA for 3.9 ± 1.5 min	Noxious heat	4/4	68 ± 11%	23 ± 4.1
	Kainate	2/4	70%	11

All values are expressed as mean ± s.e.mean

Asterisks indicate that the value shown is significantly different from the corresponding value obtained from responses evoked by noxious stimuli ($^{*}P < 0.05$; $^{**}P < 0.01$, Student's t test); NS not significant

[a]Cells where no depression was observed were not included in the calculation of percentage depression.

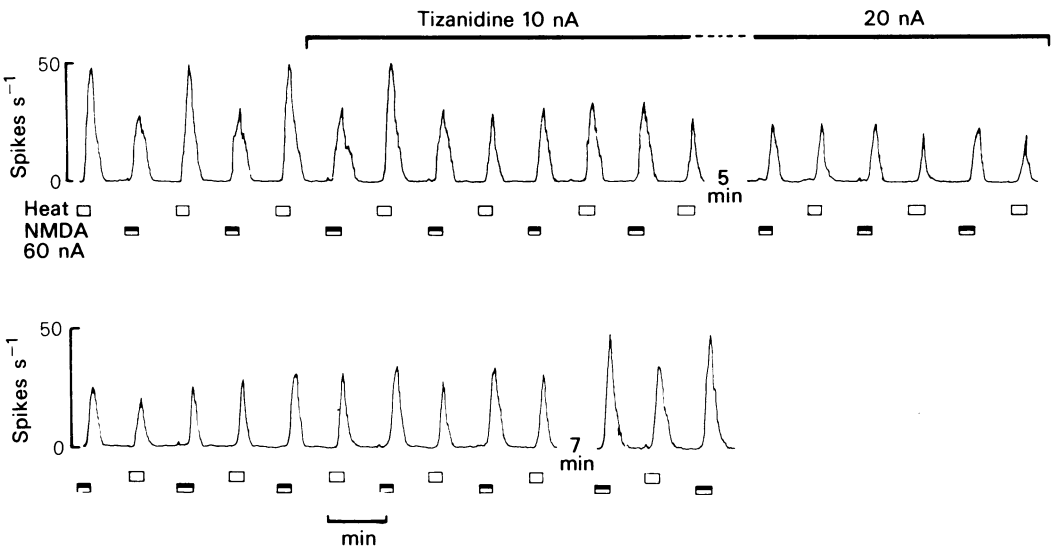

Figure 2 Effects of tizanidine ejected first at 10 nA then at 20 nA near the cell body of a lamina V neurone which was excited by noxious heat stimuli and iontophoretic N-methyl-D-aspartate (NMDA). The smaller current of tizanidine reduced responses to noxious stimuli without affecting those to NMDA. The larger current also depressed NMDA-induced firing. Note reversal of the depression of NMDA-induced responses was more rapid than reversal of responses to noxious heat. The upper and lower records in this figure are continuous.

Currents of tizanidine sufficient to reduce responses of 18 laminae IV and V neurones to noxious stimuli by 30–100% (75±6%) also reduced responses to a range of excitatory amino acids on many neurones (Table 2). On cells where the effects of tizanidine were determined on responses to more than one amino acid no differential depression of any particular amino-acid-induced response was observed. However, responses to excitatory amino acids appeared to be less sensitive to tizanidine than responses to noxious stimuli in that firstly, depression of amino acid-induced responses was more rapidly reversible (Table 2), and secondly, in tests on 8 neurones included in Table 2 responses to noxious stimuli were depressed by 30–100% (69±11%) in the absence of any significant effect on responses to DLH (5 cells), NMDA (4 cells), L-glutamate (3 cells) or quisqualate (2 cells). However, responses to these amino acids were depressed in most instances when the ejecting current of tizanidine was increased (e.g. Figure 2). The effects of tizanidine on spontaneous activity and excitation induced by amino acids seemed to be correlated, responses to excitatory amino acids being unaffected on 2 neurones on which spontaneous activity was unaffected and depressed on 11 neurones where the spontaneous firing rate was also depressed. Amino acid-induced responses were never reduced in the absence of depression of spontaneous activity, although spontaneous firing rate was reduced on 3 neurones in the absence of effects on responses to excitatory amino acids. Noradrenaline reduced responses to DLH, NMDA and L-glutamate on 7 neurones by 43±15% on which spontaneous firing was markedly depressed but had no effect on similar responses on 5 other neurones, 3 of which were not spontaneously active and 2 on which spontaneous firing was depressed. However, responses to noxious stimuli were reduced by 73±9% on all 12 neurones. (−)-Baclofen reduced the responses to these excitant amino acids by 25–100% (80±9%) on 8/10 neurones whereas responses to noxious heat were reduced on 9 of the cells by 15–95% (57±8%). Thus the responses to excitant amino acids seemed to be more sensitive to the depressant effects of baclofen than the responses to noxious heat, in contrast to the effects of tizanidine and noradrenaline. However, responses to amino acids recovered more rapidly from the effects of (−)-baclofen than those to noxious stimuli (5.2±2.1 min compared with 8.8±2.3 min, respectively), although this difference was not significant ($P > 0.05$ paired Student's t test).

Effects of tizanidine on neurones not responding to noxious heat stimuli While searching the dorsal horn for neurones responding to noxious heat many cells were encountered (mainly in laminae IV) that responded to hair movement but not to radiant heat (up to 50°C), strong pinch of the foot or joint-movement. The majority of these class 1 neurones (see Methods)

Table 3 Comparison between the depressant effects of tizanidine and those of noradrenaline or (−)-baclofen on responses of the same dorsal horn neurones to noxious and innocuous stimuli and spontaneous firing rate when administered iontophoretically into laminae II–III of the lumbar spinal cord

	Depressant effect on: *Response to noxious stimuli (heat)*[b]			*Response to innocuous stimuli (air-jet)*[b]			*Spontaneous firing rate*[b]		
Drug[a]	*No. dep. / No. tested*	*% dep.*	*Recovery time* (min)	*No. dep. / No. tested*	*% dep.*	*Recovery time* (min)	*No. dep. / No. tested*	*% dep.*	*Recovery time* (min)
Tizanidine (28.8 ± 3.5 nA for 5.5 ± 0.9 min)	14/15	76 ± 7%	36 ± 7.7	0/15	–		10/14	63 ± 11%	36 ± 9.0
Noradrenaline (31.0 ± 6.0 nA for 5.2 ± 0.5 min)	15/15	60 ± 6%^{NS}	13 ± 2.9*	1/15	25%	2	12/14	59 ± 11%^{NS}	11 ± 2.7**
Tizanidine (30.0 ± 5.7 nA for 6.7 ± 1.6 min)	6/6	74 ± 6%	38 ± 9.0	0/6			4/6	54 ± 10%	37 ± 6.0
(−)-Baclofen (57.5 ± 7.5 nA for 5.7 ± 1.1 min)	6/6	67 ± 9%^{NS}	26 ± 3.2^{NS}	6/6	55 ± 8%	29 ± 4	6/6	60 ± 13%^{NS}	26 ± 3.2^{NS}

[a] The numbers in parentheses show for each drug the mean ± s.e.mean ejecting current and duration of ejection.
[b] As for Table 1.
Asterisks indicate that the value shown is significantly different from the corresponding value obtained during the ejection of tizanidine on the same neurones: $^{*}P < 0.05$; $^{**}P < 0.01$ (Student's t test); NS, not significant.

were ignored but the effects of tizanidine, and sometimes noradrenaline and (−)-baclofen, were determined on some of them. Tizanidine up to 80 nA (62 ± 6.7 nA for 4.6 ± 0.56 min) had no effect on responses to air jets on 6 or 7 of these neurones. These responses were weakly reduced (by 16%) on 1 neurone, this depression being reversible within 2 min of terminating the tizanidine ejection. Spontaneous firing evident in 2 of these neurones was not depressed and responses to NMDA were only reduced in 1 out of 4 neurones when 80 nA tizanidine was ejected and this effect was fully reversible 1 min after completing the ejection. Noradrenaline (40–80 nA) also had no effect on responses to air jets on 4 of these neurones, or on spontaneous firing evident in 1 of them. Responses to NMDA were reduced in 1 or 2 cells by noradrenaline but as with tizanidine recovery was complete 1 min after terminating the ejection. The effects of (−)-baclofen were examined on 5 of the neurones previously tested with tizanidine. In contrast to the effects of the latter, (−)-baclofen 10–50 nA (26 ± 6.7 nA) depressed responses to hair movements by 25–90% (50 ± 8%) on all 5 neurones, reduced spontaneous firing on 1 of 2 of them and NMDA-induced excitation on 2 of 3 cells by 50 and 75%. These effects of (−)-baclofen were fully reversible 5–15 min after terminating the ejecting current.

Effects of naloxone Naloxone ejected with currents of up 100 nA before, during and after the ejection of tizanidine, noradrenaline or (−)-baclofen failed to modify the effects of these drugs on responses of 5 laminae IV and V neurones to noxious stimuli.

Iontophoretic ejection into laminae II–III

The effects of ejection of noradrenaline into laminae II–III were first determined on responses of laminae IV and V neurones to noxious stimuli, positive effects with this agent being taken as an indication that the multibarrelled electrode was positioned in the vicinity of laminae II–III (Headley *et al.*, 1978). Subsequently, the effects of tizanidine and (−)- baclofen were determined. Table 3 summarizes the results obtained in these experiments. The effects of

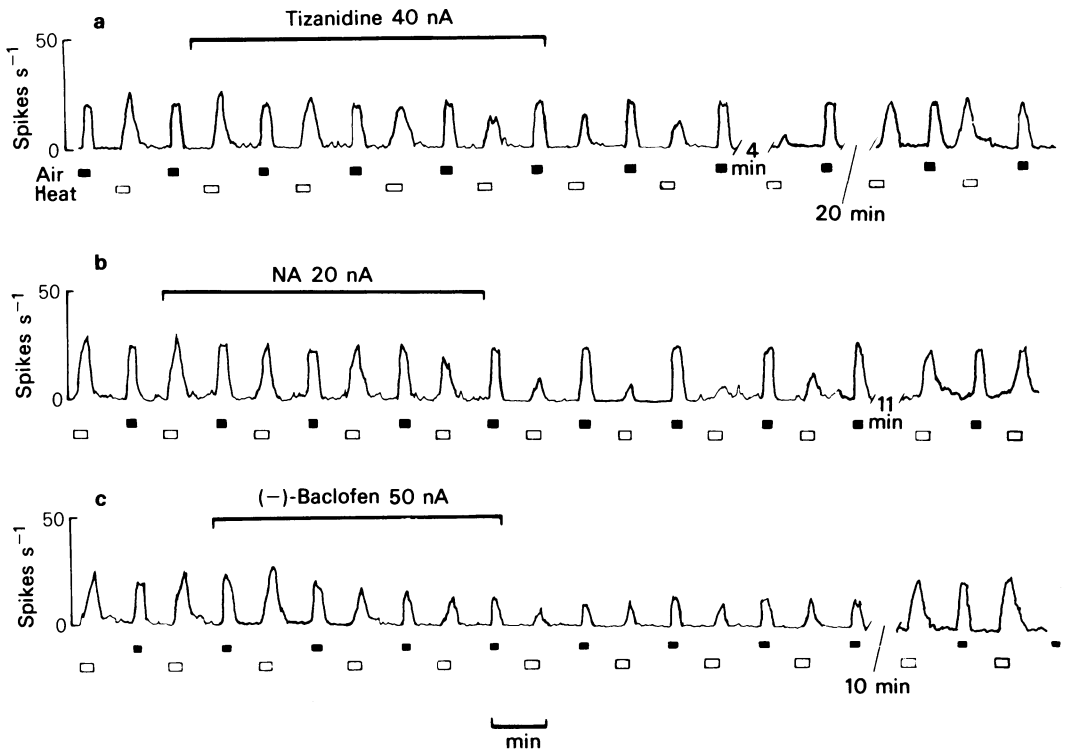

Figure 3 Effects of ejections into laminae II–III of (a) tizanidine (40 nA), (b) noradrenaline (NA 20 nA) and (c) (−)-baclofen (50 nA) on the responses of the same lamina IV neurone induced by noxious heat (46.5°C) and non-noxious stimuli. Tizanidine and noradrenaline produced a marked depression of the nociceptive response but did not affect the non-nociceptive response. (Note the long duration of action of tizanidine). (−)-Baclofen produced a parallel reduction in the responses to both types of stimuli.

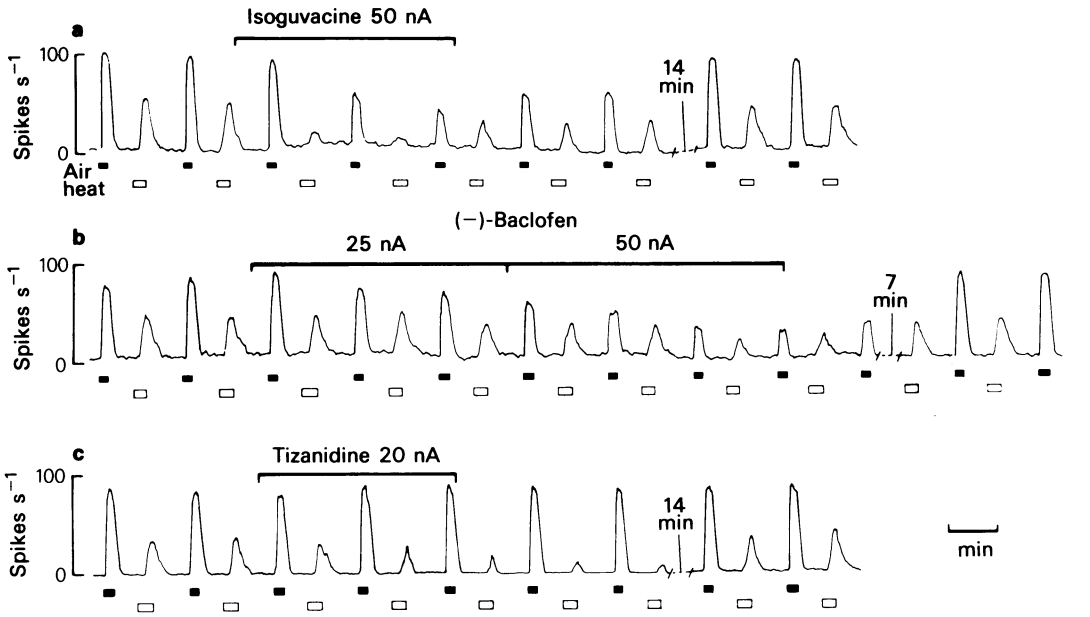

Figure 4 Effects of (a) isoguvacine (50 nA), (b) (−)-baclofen (25–50 nA) and (c) tizanidine (20 nA) on responses of a lamina V neurone induced by noxious (radiant heat 48°C) and non-noxious (air-jet) stimuli. All three drugs were ejected into laminae II–III. Isoguvacine reduced both types of responses as did baclofen. Tizanidine selectively reduced responses to noxious stimuli on the same cell.

tizanidine administered 300–850 μm above the recording site were essentially similar to those observed when it was ejected near the cell bodies of laminae IV and V neurones (cf. Table 1 and 3). Thus, tizanidine depressed responses to noxious heat and spontaneous firing by 20–100% whereas responses to non-noxious stimuli were unaffected (Table 3). Typically, maximum depression of responses to noxious stimuli was apparent 4–9 min after terminating the tizanidine ejection. Selective depression of nociceptive activation by tizanidine ejected into laminae II–III is shown in Figure 3. During the ejection of 40 nA tizanidine there was approximately a 40% reduction in firing to noxious heat but no change in the response to hair deflection. Subsequent to the termination of the ejection, responses to noxious heat declined to about 10% of the control response over a period of 7–8 min (Figure 3). The main difference between the effects of tizanidine ejected into laminae II–III and ejected near cell bodies of the recorded neurones was that the effects of the former were considerably longer lasting, recovery sometimes not occurring for more than an hour after maximal effects had been attained (compare Table 1 and 3), although this was not the case in the example shown in Figure 3. The effects of noradrenaline and (−)-baclofen were similar to those observed following ejection near cell bodies (e.g. Figure 3) except higher ejecting currents were required to produce approximately comparable reductions in firing to noxious heat (and hair deflection in the case of baclofen) and the duration of these effects was more prolonged (see Table 3).

Ejection of GABA or glutamate (up to 200 nA of each) into laminae II–III had no significant effect on responses of 5 laminae IV and V neurones to noxious or non-noxious stimuli. However, 30–50 nA of the more potent GABA agonist isoguvacine (Krogsgaard-Larsen *et al.*, 1977) ejected into laminae II–III depressed spontaneous firing and that induced by radiant heat and hair deflection in parallel, recovery occurring 15–25 min after terminating the ejecting current (Figure 4). The potent excitatory amino acid kainate (15–40 nA) also had non-selective effects when ejected into laminae II–III, enhancing responses to both types of sensory stimuli and background firing in tests on 4 cells.

Iontophoretic ejection of the opiate antagonist naloxone (up to 100 nA for 10–25 min) into laminae II–III failed to influence the selective depressant effect of tizanidine ejected into laminae II–III on responses of 5 laminae IV and V neurones to noxious stimuli. Intravenous injection of naloxone 0.5 mg kg^{-1} also failed to influence the selective antinociceptive action of tizanidine ejected into laminae II–III (3 cells).

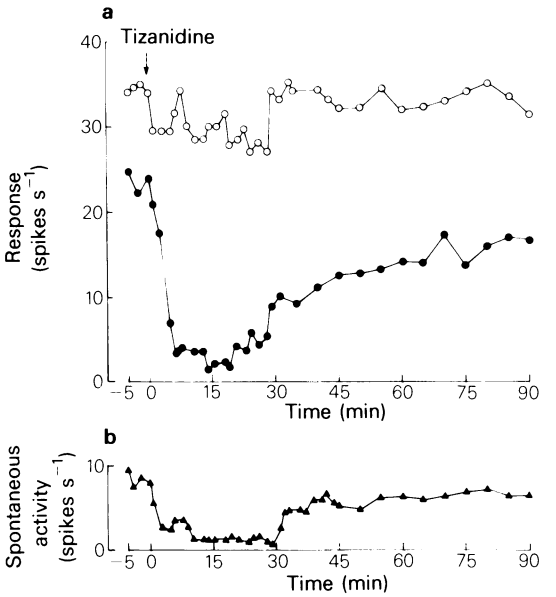

Figure 5 Effects of intravenous tizanidine (0.1 mg kg^{-1}) on the responses of a lamina V neurone to noxious heat (48°C) (○) and innocuous skin stimuli (●). (a) Shows the firing rate (spikes s^{-1}), corrected for spontaneous firing, evoked by each skin stimulus; (○) hair deflection; (●) noxious heat. (b) Spontaneous firing rate in spikes s^{-1} is also plotted. Nociceptive responses were almost abolished following the injection of tizanidine and spontaneous firing was similarly reduced. Responses to non-noxious stimuli were minimally affected.

Effects of intravenously administered tizanidine

The effects of intravenously administered tizanidine were examined on the responses of 12 laminae IV and V neurones in 11 cats to noxious and non-noxious peripheral stimuli. Immediately following the injection of tizanidine there was an initial 5–40% increase in mean arterial blood pressure with all doses used. This lasted 0.5–2 min being followed by a progressive 5–10% fall in pressure over the next few min with subsequent recovery to preinjection pressure 4–15 min after completing the injection. No attempt was made to assess the effects of tizanidine until arterial blood pressure had stabilized. Doses of 0.025 mg kg^{-1} enhanced spontaneous firing rate and that induced by noxious heat (by 100%) on 1 neurone but had no effect on nociceptive or non-nociceptive responses or spontaneous activity on two other neurones. With doses of 0.05 mg kg^{-1} responses to noxious heat were depressed by 59% on 2 cells, enhanced on 2 cells by 75% and unaffected on 2 cells. Responses induced by air jets were unaffected on all 6 of these neurones and spontaneous firing was reduced on 1 and unaffected on 4 neurones. Doses of 0.1 mg kg^{-1} tizanidine depressed responses to noxious heat on 7 out of 10 neurones tested by 60–100% (86 ± 9%), these responses being enhanced on 1 neurone and unaffected on 2. Responses to air jets were only slightly depressed (15%), with this dose of tizanidine, on 2 cells whereas spontaneous firing was reduced by 40–100% (77 ± 14%) on 4 cells and was

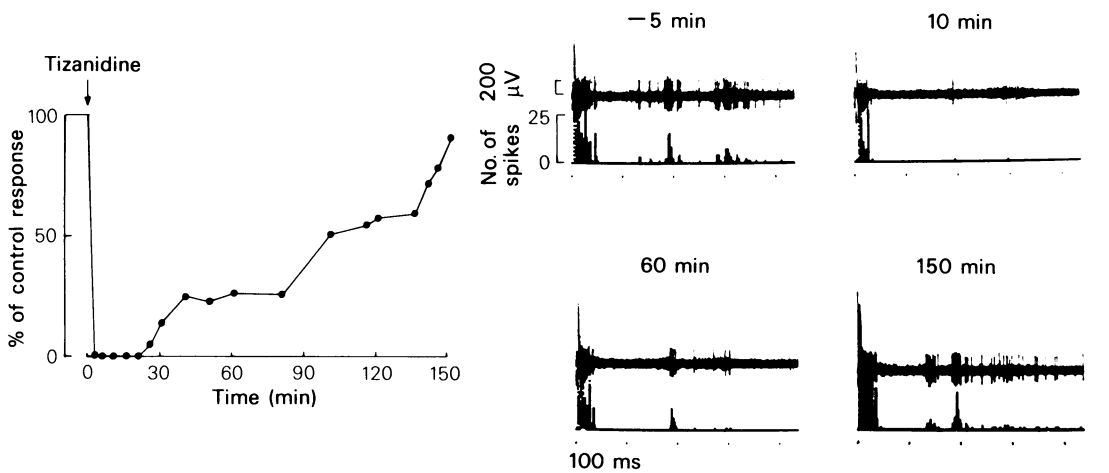

Figure 6 Effects of intravenous tizanidine (0.1 mg kg^{-1}) on the excitation of a lamina V neurone evoked by electrical stimulation of the sural nerve adequate to excite unmyelinated fibres. Responses to thirty-two stimuli were used to compile the peristimulus time histogram (PSTH) (0.4 Hz, 1 ms pulses; responses analysed in 5 ms intervals). The records on the right of this figure illustrate examples of superimposed oscilloscope sweeps of the evoked response (top) and the PSTHs (bottom) computed 5 min before, then 10, 60 and 150 min after the administration of tizanidine. The number of action potentials (spikes) occurring between 170–400 ms after the stimulus (evoked by C fibre primary afferents) were counted by a gated electronic counter. From these counts the C fibre response is plotted on the left of the figure as a percentage of the control response (i.e. before the tizanidine injection) against time.

unaffected on 2 cells. The depressant effect of intravenous tizanidine on nociceptive responses and spontaneous firing rate was long lasting, recovery never occurring in less than 60 min of completing the injection (e.g. Figure 5). However, on cells where responses to noxious stimuli were enhanced recovery occurred within 10–30 min of the injection.

Responses to the excitatory amino acids DLH and NMDA were monitored on 5 neurones where responses to noxious stimuli were reduced by tizanidine. The excitant responses to these amino acids were unaffected on 4 neurones and slightly enhanced on 1 neurone. None of these neurones was spontaneously active.

Peripheral circulatory changes may produce large variations in the firing of peripheral nociceptors (Duggan *et al.*, 1978; Davies & Dray, 1980), although such changes can be minimized by accurately controlling the surface skin temperature. Thus it is possible that some of the effects described above could be secondary to changes in peripheral blood flow. In an attempt to overcome this possibility the effects of intravenously administered tizanidine were examined on excitatory responses of 5 laminae IV and V neurones evoked by electrical stimulation of the ipsilateral sural or tibial nerve at sufficient intensity to activate small diameter unmyelinated afferents (C fibres, see Methods). In each of these 5 neurones tizanidine 0.05–0.1 mg kg^{-1} markedly reduced (70–100%) the long latency excitation evoked by such stimuli. This effect was prolonged, the duration of action of tizanidine being similar to that seen on responses to noxious stimuli. This action of tizanidine on 1 neurone is illustrated in Figure 6. The threshold intensity necessary to evoke the C fibre response in this neurone was 20 V (1 ms pulse width). Ten min after the injection of tizanidine when the response was abolished, increasing the stimulus intensity to 100 V did not result in the reappearance of the synaptic response.

Discussion

The present results demonstrate that tizanidine produces a marked and prolonged selective depression of excitatory responses, induced in laminae IV and V neurones by noxious peripheral stimuli, when it is administered iontophoretically either in the vicinity of the laminae IV and V cell bodies or more superficially into laminae II–III or intravenously. The occasional enhancement of responses to noxious stimuli following intravenous injection of tizanidine is not necessarily at variance with an antinociceptive action of this drug, as this effect was probably secondary to peripheral circulatory changes which have previously been shown to alter the firing of nocireceptors (Duggan *et al.*, 1978; Davies & Dray, 1980). The finding that excitatory responses of laminae IV and V neurones evoked by electrical stimulation of small diameter primary afferents of the type that convey nociceptive information to the spinal cord, were consistently depressed by systemic tizanidine agrees with this suggestion. Noradrenaline also produced selective antinociceptive effects when administered at the same sites as tizanidine, whereas iontophoretic ejection of (−)-baclofen at either site depressed responses to both noxious and innocuous stimuli.

As the effects of iontophoretically administered tizanidine at two different sites were similar to those seen when this agent was given systemically it could be argued that the site(s) and mechanism(s) of action of tizanidine may be identical in each case. It is possible that substances ejected into laminae II–III may diffuse vertically and act near the laminae IV and V cell bodies. However, this seems unlikely in view of the 300–850 μm distance between ejecting and recording sites and because the duration of action of all three drugs used was at least twice as long when ejected into laminae II–III compared with ejections near laminae IV and V cell bodies (Tables 1 and 3). However, slightly higher ejecting currents of tizanidine (and noradrenaline and (−)-baclofen) may have been required to depress responses to noxious stimuli on administration into laminae II–III compared with that into laminae IV and V, simply because it is necessary to affect structures distributed through a greater volume of tissue surrounding the microelectrode tip at the former site (Duggan *et al.*, 1977b). It is unlikely that the selective effects of tizanidine observed on ejection into laminae II–III resulted from depression of excitatory interneurones or excitation of inhibitory interneurones located in this region since the potent depressant isoguvacine, a GABA agonist (Krogsgaard-Larsen *et al.*, 1977), and the excitant kainate ejected into laminae II–III both produced non-selective effects on responses of laminae IV and V neurones to cutaneous stimuli. The inactivity of GABA and glutamate ejected into laminae II–III may be due to the fact that the actions of these amino acids are limited by neuronal uptake which is less effective at removing isoguvacine and kainate (Krogsgaard-Larsen *et al.*, 1977; Lodge *et al.*, 1978; Stallcup *et al.*, 1979). The most plausible explanation for the selective effects of tizanidine is that it acts at some excitatory synapse or receptor linking nociceptive afferents with laminae IV and V neurones.

Many Aδ and C nociceptor primary afferents terminate in laminae II (Light & Perl, 1979a,b; Ralston & Ralston, 1979) and may synapse here with the dendrites of laminae IV and V neurones either directly or indirectly via laminae II or III interneurones (Scheibel & Scheibel, 1968; Rethelyi & Szen-

togathai, 1973; Suguira, 1975; Mannen & Suguira, 1976; Willis & Coggleshall, 1978). Any of these possible mono- or polysynaptic connections could represent the site of action of tizanidine ejected in the vicinity of laminae II but not when ejected into laminae IV and V. Anatomically, laminae III neurones appear to be ideally situated to have synaptic contacts with the dendrites of laminae IV and V neurones in the region of their cell bodies and in laminae II–III (Willis & Coggleshall, 1978; Brown, 1982). Thus, an action of tizanidine at these synapses could account for its effects when ejected at either site and when administered systemically. While the present results do not distinguish between any of these possibilities, an effect of tizanidine on excitatory synapses between laminae II–III neurones and laminae IV and V neurones would be consistent with previous findings that polysynaptic excitation of spinal interneurones is more susceptible to the depressant effects of tizanidine than monosynaptic excitation (Davies, 1982). On this basis the absence of effects of tizanidine on responses induced by innocuous stimuli could be explained if large diameter primary afferents from low threshold mechanoreceptors synapse monosynaptically with laminae IV and V neurones. Indeed, large diameter afferents innervating hair follicles terminate in laminae III–VI (Brown, 1977; Brown *et al.*, 1977). Hence, anatomically such monosynaptic connections are possible. Alternatively, synapses on laminae IV and V neurones mediating responses to hair movement may be beyond the sphere of action of iontophoretic tizanidine, although such synapses would be expected to be accessible to systemically administered drug.

The antinociceptive action of tizanidine may be due to selective interference with excitatory transmitter release as suggested previously (Davies, 1982; Davies *et al.*, 1983). On the other hand, a postsynaptic mechanism of action may be inferred from the present findings that tizanidine depressed responses to a range of excitatory amino acids. However, these depressions were less marked and more rapidly reversible than depression of responses to noxious stimuli on the same cells and were correlated with, and therefore possibly secondary to, a reduction in background activity. The source of spontaneous activity is not known but it may be related to the nociceptive input as it was only apparent on many neurones after the initiation of noxious stimuli. Thus, depression of responses to amino acids and spontaneous activity is not necessarily indicative of a postsynaptic action for tizanidine. While we would not deny the possibility that tizanidine may act postsynaptically it seems unlikely that it acts at N-methyl-D-aspartate (NMDA) receptors as suggested by Curtis *et al.* (1983) because (a) tizanidine does not selectively depress responses to NMDA relative to other excitant amino acids, and (b) responses of spinal neurones to noxious heat are insensitive to selective NMDA antagonists, (Davies & Dray, 1979; Anis *et al.*, 1982; Davies & Johnston, unpublished observations). With respect to the effects of tizanidine on excitatory amino acids, it is interesting to note that neither tizanidine nor noradrenaline had any effect on responses to these (including NMDA) or on spontaneous activity of neurones insensitive to noxious heat (class I cells). Responses of these neurones to air jets were also insensitive to these drugs suggesting that they lack synapses or receptors that are sensitive to tizanidine and noradrenaline.

The action of tizanidine is quite different from that of the muscle relaxant baclofen which did not differentiate between responses to noxious and non-noxious stimuli. Indeed, the effects of (−)-baclofen were similar to those of GABA ejected near cell bodies or isoguvacine ejected into laminae II–III and are consistent with a general postsynaptic depressant action on central neurones. Thus, although (−)-baclofen has been found to exert spinal antinociceptive effects at doses below those necessary to produce muscle relaxation (Wilson & Yaksh, 1978) the present data offer no explanation for such a selective action. An interaction of tizanidine either directly or indirectly with opiate receptors is unlikely in view of the failure of the opiate antagonist naloxone to influence its selective depressant action. However, tizanidine and noradrenaline may act at similar sites since noradrenaline also produced selective antinociceptive effects on laminae IV and V neurones when ejected into laminae II–III or near cell bodies in the present experiments and in those described by Belcher *et al.* (1978) and Headley *et al.* (1978).

Finally the importance of the present experiments lies in the demonstration that ejecting currents and systemic doses of tizanidine producing selective antinociceptive effects on laminae IV and V neurones in the present study are similar to those previously found to depress preferentially polysynaptic excitation of spinal interneurones and polysynaptic reflexes evoked by stimulation of leg nerves (Davies, 1982). As the latter effects are thought to be responsible for the muscle relaxant action of tizanidine, the present data suggest that this drug may produce significant analgesia at therapeutic dose levels.

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