ELSEVIER

European Journal of Pharmacology 347 (1998) 201–204

ejp

Short communication

α_2-Adrenoceptor antagonists enhance responses of dorsal horn neurones to formalin induced inflammation

G. Mark Green *, Lucy Lyons, Anthony H. Dickenson

Department of Pharmacology, University College London, Gower Street, London, WC1E 6BT, UK

Received 28 January 1998; revised 9 March 1998; accepted 11 March 1998

Abstract

Intrathecally applied α_2-adrenoceptor antagonists atipamezole, idazoxan and yohimbine had no significant effect on any neuronal response in normal animals. In contrast, all three antagonists (100 μg) significantly increased the area under the curve of the total response to formalin, especially the second phase. Our results suggest the α_2-adrenoceptor-mediated noradrenergic inhibitory system in the spinal cord is dormant under normal conditions, but controls both the magnitude and duration of the neuronal responses to subcutaneous injection of formalin. © 1998 Elsevier Science B.V.

Keywords: α_2-Adrenoceptor; Inflammation; Formalin; Nociception; Spinal cord; (Rat)

1. Introduction

Descending pathways from a wide variety of midbrain and brainstem sites modulate nociceptive transmission in the dorsal horn (see Refs. in Millan, 1997). The spinal effects of descending systems are mediated by a range of transmitters including the noradrenergic system, acting spinally on inhibitory α_2-adrenoceptors. α_2-Adrenoceptors are present in high density in the superficial laminae and substantia gelatinosa of the dorsal horn (Nicholas et al., 1993). Furthermore, the predominant α_2-subtype (80–90% of total α_2-adrenoceptors) in the spinal cord is the $\alpha_{2A/D}$-subtype (Lawhead et al., 1992; Hunter et al., 1997).

The potent antinociceptive effect of spinally applied α_2-adrenoceptor agonists has been demonstrated both behaviourally and electrophysiologically (see Refs. in Millan, 1997). Several lines of evidence indicate enhanced descending inhibition following the development of peripheral inflammation (Weil-Fugazza et al., 1986; Cervero et al., 1991; Men and Matsui, 1994; Ren and Dubner, 1996; Tsuruoka and Willis, 1996).

In the present study, we have recorded the responses of nociceptive dorsal horn neurones to subcutaneous injection of formalin, an inflammatory stimulus. The involvement of α_2-adrenoceptor mediated noradrenergic inhibition in the formalin response was examined using the selective α_2-adrenoceptor antagonists, atipamezole and idazoxan, and the relatively non-selective yohimbine. The effects of the antagonists on the electrically evoked responses of dorsal horn neurones in normal animals were used as a control.

2. Materials and methods

2.1. Surgical procedure

The protocol followed is essentially that described in Dickenson and Sullivan (1987). Male Sprague–Dawley rats were anaesthetized using halothane in a 66% N_2O/33% O_2 mixture. A laminectomy was performed over the L_1–L_3 area and the dura was removed. Halothane concentration was maintained at 1.5–2% for the duration of the experiment (levels that produced complete areflexia). The UK Home Office rules and the International Association for the Study of Pain guidelines for animal care were followed.

* Corresponding author. Tel.: +44-171-491-3737; fax: +44-171-419-3742; e-mail: g.green@ucl.ac.uk

Tungsten electrodes were used to make single unit extracellular recordings of dorsal horn neurones that received afferent input from the toes of the hindpaw. Transcutaneous electrical stimulation of the receptive field at three times the C-fibre threshold was applied to produce acute neuronal responses. Post-stimulus histograms were constructed (Spike 2 software, C.E.D. 1401 interface) at 10-min intervals to a train of 16 stimuli at 0.5 Hz with a 2-ms pulse width. The resulting Aβ-, Aδ- and C-fibre-evoked responses were separated by latency (0–20 ms, 20–90 ms and 90–300 ms, respectively) and quantified. In addition, the post-discharge of the neurone between 300–800 ms was recorded and quantified.

2.2. α_2-Adrenoceptor antagonists and electrically evoked responses

We first examined the effects of the α_2-adrenoceptor antagonists atipamezole, idazoxan and yohimbine on the response to transcutaneous electrical stimulation. Doses of 1 μg, 10 μg and 100 μg were applied directly on the spinal cord in a volume of 50 μl for both idazoxan and yohimbine. Only the 10 μg and 100 μg doses were administered for experiments with atipamezole. Responses were recorded for 60 min following the administration of each dose of drug. No animal in the study received more than one of the three antagonists.

2.3. α_2-Adrenoceptor antagonists and the formalin model

A second group of animals were then used for the formalin studies where, once a C-fibre response of the neurones had been confirmed, 50 μl of 5% formalin solution was injected subcutaneously into the center of the receptive field. The formalin evoked activity was counted using a rate function. Atipamezole, idazoxan and yohimbine were administered as a pre-treatment 20 min prior to injection of formalin.

The effects of the antagonists on electrically evoked responses were expressed as a percentage of control $\pm$ the standard error of the mean (S.E.M.). The effects of drugs on the formalin response were quantified by measuring the area under the curve and also the number of action potentials in the first and second phases of the response.

2.4. Data analysis

Statistical analysis was performed using two-way analysis of variance (ANOVA). For multiple comparisons, the Fisher's PLSD (protected least squares difference) post-hoc test was used (Statview v4.0, Abacus Concepts). Significance was set at $P < 0.05$. In formalin experiments, the area under the curve was calculated using the trapezoidal rule (PCS v2.0, Springer-Verlag).

3. Results

79 neurones located deep within the dorsal horn were studied (average C-fibre threshold of 1.4 ± 0.1 mA, average depth of 758 ± 22 μm).

3.1. α_2-Adrenoceptor antagonists and electrically evoked responses

The α_2-adrenoceptor antagonists had no significant effect on the electrically evoked responses of spinal dorsal

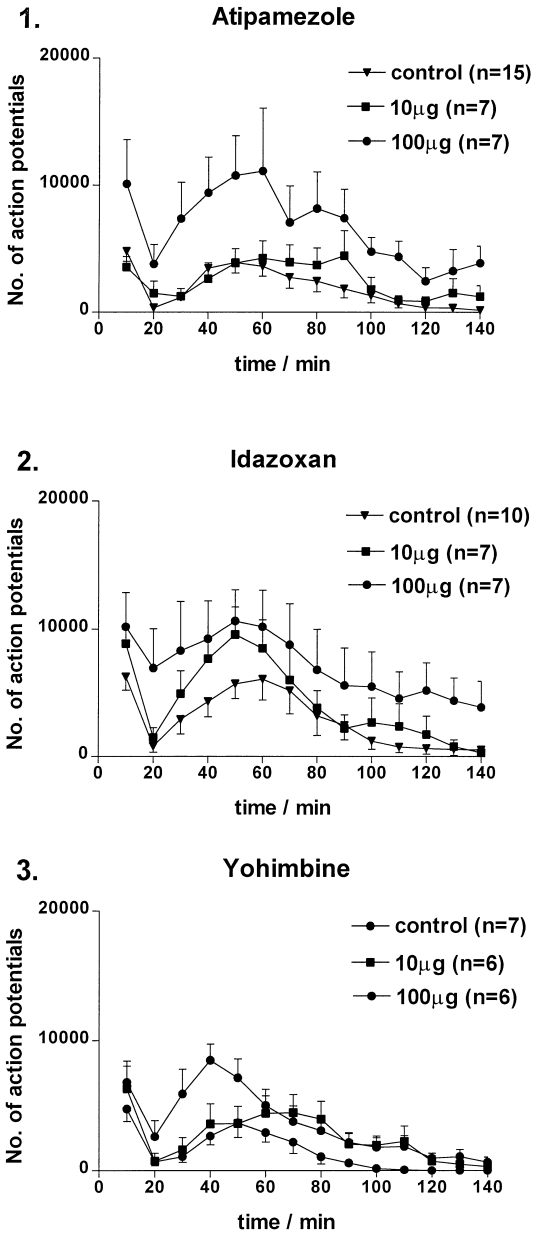

Fig. 1. The effect of (1) atipamezole, (2) idazoxan and (3) yohimbine on the formalin response. The antagonists were administered as a 20-min pre-treatment. Results presented as mean number of action potentials in 10-min time bins $\pm$ S.E.M.

horn neurones. Percent control after 100 μg dose, atipamezole = A β- 99 ± 19, A δ- 99 ± 26, C-fibre 108 ± 16, post discharge 190 ± 38, idazoxan = A β- 96 ± 15, A δ- 105 ± 15, C-fibre 130 ± 17, post discharge 198 ± 39, yohimbine = A β- 96 ± 20, A δ- 78 ± 18, C-fibre 80 ± 12, post discharge 179 ± 55.

3.2. α_2-Adrenoceptor antagonists and formalin

On injection of formalin the characteristic biphasic response was observed in all control experiments (Fig. 1). Each neurone was recorded for 2 h and 20 min. The sum of the action potentials within the first 10-min post-formalin was termed the first phase (mean control value of 5256 ± 556 spikes). Subsequent activity of the neurone (10 to 140 min) was assigned to the second phase (24,389 ± 3858 spikes).

Atipamezole, administered 20 min prior to injection of formalin, significantly increased the area under the curve of the formalin response ($F(2,26) = 10.533$, $P = 0.0004$). Post-hoc analysis revealed 10 μg atipamezole had no significant effect, whereas 100 μg atipamezole significantly increased the response ($P = 0.0001$) (Table 1 and Fig. 1). Also, activity during the silent interphase period was considerably enhanced with the 100 μg dose (mean spike count of 361 ± 77 and 3787 ± 1536 for control and 100 μg atipamezole, respectively).

Overall, idazoxan had a significant effect on the formalin response ($F(2,19) = 3.554$, $P = 0.0489$). Post-hoc analysis showed no significance after 10 μg idazoxan, but a significant increase after 100 μg ($P = 0.0163$) (Table 1 and Fig. 1). Activity during the silent interphase period was also enhanced with the 100 μg dose (mean spike count of 813 ± 486 and 6937 ± 3082 for control and 100 μg idazoxan, respectively).

Yohimbine also had a significant effect on the formalin response ($F(2,16) = 5.138$, $P = 0.0189$. Again, post-hoc analysis showed a significant increase after pretreatment with 100 μg yohimbine ($P = 0.0055$) but not 10 μg (Table 1 and Fig. 1).

A 100-μg atipamezole significantly increased the first phase ($F(2,26) = 3.485$, $P = 0.0456$) but idazoxan and yohimbine had no significant effect (Table 1). All three antagonists (100 μg) significantly increased the second phase of the response ($F(2,26) = 11.223$, $P = 0.0003$, $F(2,19) = 3.814$, $P = 0.0405$ and $F(2,16) = 4.534$, $P = 0.0275$ for atipamezole, idazoxan and yohimbine, respectively) (Table 1).

4. Discussion

Subcutaneous injection of formalin elicits a characteristic biphasic response that can be seen both neuronally and behaviourally. However, the rate of neuronal firing and nociceptive behaviour reach a peak and begin to subside within an hour (Dickenson and Sullivan, 1987; Dubuisson and Dennis, 1977) although the formalin induced inflammation and peripheral oedema continue to develop for many hours. This suggests central inhibitory mechanisms may be involved in an ongoing suppression of formalin evoked activity. Here we provide electrophysiological evidence for spinal α_2-adrenoceptor mediated noradrenergic inhibition in determining both the magnitude and the duration of the response to formalin.

In normal animals, we found no α_2-adrenoceptor mediated noradrenergic inhibitory control over dorsal horn neurones. During formalin induced inflammation however, atipamezole, idazoxan and yohimbine all significantly increased the magnitude of the second phase of the formalin response, suggesting an increase in α_2-adrenoceptor mediated noradrenergic inhibition.

The selectivity of atipamezole, idazoxan and yohimbine for the α_2-adrenoceptor is widely accepted. There are several reports from both electrophysiological and behavioural studies demonstrating atipamezole, idazoxan and yohimbine reversible, dexmedetomidine induced antinociception (Sullivan et al., 1992; Refs. in Millan, 1997). However, it is notable that the relatively less potent yohimbine is only able to partially reverse the effects of dexmedetomidine (see Refs. in Millan, 1997).

Several reports suggest an increase in descending inhibitory control following inflammation (Cervero et al., 1991; Schaible et al., 1991; Ren and Dubner, 1996). There is also evidence for increased activity of noradrenergic systems in the spinal cord in response to inflammatory pain (Weil-Fugazza et al., 1986; Brandt and Livingstone,

Table 1
The effect of α_2-adrenoceptor antagonists on the area under the curve of the formalin response (results presented as mean area under the curve ± S.E.M. as % of control) and the number of action potentials in the 1st phase and 2nd phase of the response (results presented as mean number of action potentials ± S.E.M. as % of control)

	Area under curve (as % control)	1st phase (as % control)	2nd phase (as % control)
Atipamezole 10 μg	135 ± 46	74 ± 17	142 ± 51
Atipamezole 100 μg	357 ± 73[a]	210 ± 72[a]	379 ± 73[a]
Idazoxan 10 μg	137 ± 32	141 ± 24	152 ± 36
Idazoxan 100 μg	256 ± 72[a]	162 ± 43	295 ± 86[a]
Yohimbine 10 μg	172 ± 35	132 ± 45	181 ± 33
Yohimbine 100 μg	267 ± 44[a]	143 ± 26	288 ± 51[a]

[a] Denotes $P < 0.05$ compared with control.

1990; Men and Matsui, 1994; Tsuruoka and Willis, 1996). Behavioural studies have shown atipamezole and idazoxan have no effect on nociceptive thresholds in normal animals suggesting there is little or no tonic α_2-adrenoceptor mediated inhibitory control, in agreement with our electrophysiological study (Hylden et al., 1991; Nagasaka and Yaksh, 1990). In addition, other studies have demonstrated the antinociceptive potency of clonidine is increased during the earlier stages of inflammation, when administered either systemically (Kayser et al., 1992) or spinally (Hylden et al., 1991). Furthermore, after carrageenan induced inflammation idazoxan, but not atipamezole, significantly increased electrically evoked C-fibre responses (Stanfa and Dickenson, 1994). The lack of effect of atipamezole after carrageenan induced inflammation may be a result of a minor activation of descending systems by carrageenan compared to the barrage of neuronal activity produced by formalin.

After 100 μg of idazoxan and yohimbine, a similar increase in the total response to formalin and the second phase was seen, although both failed to have a significant effect on the first phase. A 100-μg atipamezole, however, produced a greater increase in the total formalin response compared to idazoxan and yohimbine, and caused a significant increase in both the first and second phases. This is most likely due to the greater selectivity of atipamezole for the $\alpha_{2A/D}$-subtype, which predominates in the spinal cord, relative to idazoxan and yohimbine. In addition, yohimbine probably had a lesser effect than atipamezole because of its lower potency. However, idazoxan, closer in potency to atipamezole than yohimbine, caused a very similar increase to yohimbine in the formalin response.

In addition to their relative selectivity for the $\alpha_{2A/D}$-subtype, the relative selectivity of the antagonists for the α_2-adrenoceptor over the α_1-adrenoceptor is likely to influence the results of our study. Excitatory α_1-activity would oppose inhibitory α_2-activity, decreasing the potentiation of the formalin response evoked by antagonising spinal α_2-adrenoceptors. Thus, the far lower α_2/α_1 selectivity of idazoxan and yohimbine relative to atipamezole (Virtanen et al., 1989) may well be a significant influence on our results.

In summary, we have provided electrophysiological evidence for α_2-adrenoceptor mediated noradrenergic inhibitory control of formalin induced noxious transmission in the spinal cord, which is dormant under normal conditions. However, during formalin induced inflammation, this system is activated, controlling both the magnitude and duration of the neuronal responses to the peripheral inflammatory stimulus.

Acknowledgements

G.M. Green is supported by a BBSRC Studentship and this work is supported by the Wellcome Trust and Biomed II.

References

Brandt, S.A., Livingstone, A., 1990. Receptor changes in the spinal cord of sheep associated with exposure to chronic pain. Pain 42, 323–329.

Cervero, F., Schiable, H.G., Schmidt, R.F., 1991. Tonic descending inhibition of spinal cord neurones driven by joint afferents in normal cats and in cats with an inflamed knee joint. Exp. Brain Res. 83, 675–678.

Dickenson, A.H., Sullivan, A.F., 1987. Subcutaneous formalin-induced activity of dorsal horn neurones in the rat: differential response to an intrathecal opiate administered pre and post formalin. Pain 30, 349–360.

Dubuisson, D., Dennis, S.G., 1977. The formalin test: a quantitative study of the analgesic effects of morphine, meperidine, and brain stem stimulation in rats and cats. Pain 4, 161–174.

Hunter, J.C., Fontana, D.J., Hedley, L.R., Jasper, J.R., Lewis, R., Link, R.E., Secchi, R., Sutton, J., Eglen, R.M., 1997. Assessment of the role of α_2-adrenoceptor subtypes in the antinociceptive, sedative and hypothermic action of dexmedetomidine in transgenic mice. Br. J. Pharmacol. 122, 1339–1344.

Hylden, J.L.K., Thomas, D.A., Iadarola, M.J., Nahin, R.L., Dubner, R., 1991. Spinal opioid analgesic effects are enhanced in a model of unilateral inflammation/hyperalgesia: possible involvement of noradrenergic mechanisms. Eur. J. Pharmacol. 194, 135–143.

Kayser, V., Guilbaud, G., Besson, J.M., 1992. Potent antinociceptive effects of clonidine systemically administered in an experimental model of clinical pain, the arthritic rat. Brain Res. 593, 7–13.

Lawhead, R.G., Blaxall, H.S., Bylund, D.B., 1992. α_{2A} is the predominant α_2 adrenergic receptor subtype in human spinal cord. Anesthesiology 82, 741–748.

Men, D., Matsui, Y., 1994. Activation of descending noradrenergic system by peripheral nerve stimulation. Brain Res. Bull. 34, 177–182.

Millan, M.J., 1997. The role of descending noradrenergic and serotonergic pathways in the modulation of nociception: focus on receptor multiplicity. In: Dickenson, A.H., Besson, J.M. (Eds.), Handbook of Experimental Pharmacology, Vol. 130, The Pharmacology of Pain. Springer-Verlag, Berlin, pp. 385–446.

Nagasaka, H., Yaksh, T.L., 1990. Pharmacology of intrathecal adrenergic agonists: cardiovascular and nociceptive reflexes in halothane-anaesthetized rats. Anesthesiology 73, 1198–1207.

Nicholas, A.P., Pieribone, V.A., Hokfelt, T., 1993. Distributions for mRNA's for alpha-2 adrenergic receptor subtypes in rat brain: an in situ hybridization study. J. Comp. Neurol. 328, 575–594.

Ren, K., Dubner, R., 1996. Enhanced descending modulation of nociception in rats with persistent hindpaw inflammation. J. Neurosci. 76, 3025–3037.

Schaible, H.-G., Neugebauer, V., Cervero, F., Schmidt, R.F., 1991. Changes in tonic descending inhibition of spinal neurons with articular input during the development of acute arthritis in the cat. J. Neurophysiol. 66, 1021–1032.

Stanfa, L.C., Dickenson, A.H., 1994. Enhanced α_2-adrenergic controls and spinal morphine potency in inflammation. Neuroreport 5, 469–472.

Sullivan, A.F., Kalso, E.A., McQuay, H.J., Dickenson, A.H., 1992. The antinociceptive actions of dexmedetomidine on dorsal horn neuronal responses in the anaesthetized rat. Eur. J. Pharmacol. 215, 127–133.

Tsuruoka, M., Willis, W.D., 1996. Descending modulation from the region of the locus coeruleus on nociceptive sensitivity in a rat model of inflammatory hyperalgesia. Brain Res. 743, 86–92.

Virtanen, R., Savola, J.M., Saano, V., 1989. Highly selective and specific antagonism of central and peripheral alpha 2-adrenoceptors by atipamezole. Arch. Int. Pharmacodyn. Ther. 297, 190–204.

Weil-Fugazza, J., Godefroy, F., Manceau, V., Besson, J.M., 1986. Increased norepinephrine and uric acid levels in the spinal cord of arthritic rats. Brain Res. 374, 190–194.