ELSEVIER

European Journal of Pharmacology 333 (1997) 9–15

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BU-224 produces spinal antinociception as an agonist at imidazoline I_2 receptors

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Received 18 February 1997; revised 5 June 1997; accepted 27 June 1997

Abstract

In this electrophysiological study, the effect of BU-224 (2-(4,5-dihydroimidazol-2yl)-quinoline hydrochloride)), a novel high affinity imidazoline I_2 receptor ligand, was tested on the responses of nociceptive neurones in the spinal dorsal horn. When applied spinally, akin to an intrathecal application (i.t.), BU-224 (5-250 μg) reduced the nociceptive responses of dorsal horn neurones, producing a dose-dependent inhibition of C-fibre evoked responses, postdischarge and wind-up of the cells. A complete block of the antinociceptive effects was produced when idazoxan (100 μg), with both α_2-adrenoceptor and imidazoline I_2 receptor antagonist actions, was administered i.t. 10 min prior to the maximal dose of BU-224 tested. The nonselective α_2-adrenoceptor antagonist, yohimbine (150 μg) only partially attenuated the inhibitory effects of BU-224 when administered i.t. 10 min prior. The highly selective α_2-adrenoceptor antagonist, atipamezole (100 μg) produced no greater reversal than yohimbine under the same conditions. Although BU-224 has been reported to possess high affinity for imidazoline I_2 receptors, a minor action at spinal α_2-adrenoceptor receptors cannot be discounted. These results demonstrate that BU-224 is an agonist and that imidazoline I_2 receptors, present in the dorsal horn, might play a role in spinal nociception, although further studies are needed to fully elucidate their functional roles. © 1997 Elsevier Science B.V.

Keywords: Imidazoline receptor; BU-224 (2-(4,5-dihydroimidazol-2yl)-quinoline hydrochloride)); Nociception; Spinal cord; (Rat)

1. Introduction

It is now well established from radioligand studies that imidazoline receptors and α_2-adrenoceptors are discrete entities (Parini et al., 1989; Michel et al., 1990; Regunathan and Reis, 1996). Two different subtypes of imidazoline sites have already been described based on ligand selectivity and regional distributions (Michel and Insel, 1989; Michel and Ernsberger, 1992). Sites which show high affinity for [^{3}H]clonidine have been classed as imidazoline I_1 receptors, while those showing high affinity for [^{3}H]idazoxan have been termed imidazoline I_2 receptors (Michel and Ernsberger, 1992). Imidazoline I_2 receptors have been further subclassified into I_{2A}- and I_{2B}-subtypes with high and low affinity for the guanidine amiloride, respectively (Diamant et al., 1992; Miralles et al., 1993). Recently, a putative endogenous ligand for imidazoline receptors was isolated from mammalian brain tissues (family (Li et al., 1994; Raasch et al., 1995) and named agmatine; agmatine may act as a neurotransmitter or neuromodulator at some members of this receptor family (Li et al., 1994: Piletz et al., 1995). Although agmatine also recognizes a α_2-adrenoceptor binding sites, it appears to be devoid of pharmacological activity at either peripheral or central α_2-adrenoceptors (Pinthong et al., 1995).

Imidazoline receptors are widely distributed both centrally and peripherally, and are reportedly involved in mediation of a number of responses either in peripheral tissues or in the central nervous system (Regunathan and Reis, 1996). From radioligand studies, it is known that imidazoline I_1 receptors have a limited distribution in brain, being found mainly in the brainstem (Ernsberger et al., 1987). This localization can explain some of the actions of clonidine and related imidazoline drugs, mediated by these imidazoline I_1 receptors (Regunathan and Reis, 1996). In contrast, imidazoline I_2 receptors are widely distributed in the central nervous system, but their functional role is still not fully established. A recent finding concerning the functional role of imidazoline sites relates to an association with monoamine oxidase. It has been suggested that some forms of monoamine oxidase may

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have binding sites for imidazoline I_2 receptor drugs (Carpene et al., 1995). Some of the physiological effects of idazoxan and other imidazoline receptor ligands, such as actions on food and water intake as well as urine output, may also be mediated by imidazoline I_2 receptors (Jackson et al., 1991; Brown et al., 1995; Regunathan and Reis, 1996).

However, until now the role of imidazoline receptors in antinociception has not been fully determined, and few studies have addressed this question, in part due to a lack of available selective ligands. Monroe et al. (1995) have recently demonstrated, using radioligand studies, the presence of imidazoline receptors in the rat spinal cord. They found that the antinociceptive effect of intrathecal clonidine was only due to its action on spinal α_2-adrenoceptors since both idazoxan (an α_2-adrenoceptor/imidazoline I_2 receptor antagonist) and yohimbine (an α_2-adrenoceptor antagonist) completely blocked clonidine-induced antinociception in a similar fashion, excluding the involvement of imidazoline receptors and in particular imidazoline I_1 receptors. Some studies have investigated the effects of the putative endogenous ligand, agmatine in nociception. Agmatine has been shown to produce inhibition of the reflex responses to noxious stimuli in spinal rats via non- α_2-adrenoceptor actions (Bradley and Headley, 1996). Moreover, Kolesnikov et al. (1996) have also demonstrated that imidazoline receptors are responsible for the potentiation of intrathecal opioid analgesia. Recently, new imidazoline receptor ligands such as BU-224 and RS45041-190 have become available, and in binding studies they have been reported to posses high affinity and selectivity for imidazoline I_2 receptors (Hudson et al., 1996; MacKinnon et al., 1995). RS-45014.190 has been used to assess the role of imidazoline I_2 receptors in some physiological functions (Brown et al., 1995) but until now no in vivo studies have been carried out with the high affinity imidazoline I_2 ligand BU-224 and its receptor actions are not known.

Therefore, in this study, we have evaluated the effects of BU-224 on the responses of dorsal horn neurones following transcutaneous electrical stimulation to determine the possible functional role of spinal imidazoline receptors on nociceptive transmission.

2. Materials and methods

2.1. Animals and surgical procedure

Experiments were performed on a total of 23 Sprague–Dawley rats (200–250 g) following methods described previously (Dickenson and Sullivan, 1986). They were initially anaesthetized with 3.5–2.5% halothane in a gaseous mix (66% N_2O/33% O_2) and then maintained on 2% halothane for the cannulation of the trachea and subsequent surgery. The rat was held in a stereotaxic frame and a laminectomy was performed removing L1–L3 vertebrae to expose the spinal cord. Temperature was maintained at 36 ± 0.5°C and details of other physiological parameters have been previously described (Dickenson and Le Bars, 1987).

2.2. Electrophysiological study

A parglene-coated tungsten electrode was lowered into the dorsal horn and extracellular recordings were made from convergent neurones within the dorsal horn which responded to both noxious (pinch, prod and heat) and innocuous (touch) stimuli applied on the ipsilateral hind paw. During recording, the animals were spontaneously breathing and the level of halothane was reduced to a 1.5–2% to maintain complete areflexia. Recording commenced about one hour after surgery. The responses of the neurones were studied by applying acute nondamaging stimuli (electrical pulses 2 ms wide at 0.5 Hz) via a pair of needles inserted transcutaneously. After determining the latency and threshold for the respective C-fibre, A δ- and A β-evoked responses, 16 electrical stimuli were delivered at $3\times$ the threshold to activate C-fibres for each particular neurone. Poststimulus histograms (number of action potentials against latency of evoked responses after the trial stimulation) were constructed and quantified the neuronal responses by means of a 1401 interface (CED, UK) and MRATE software. All the cells studied showed a clear short latency A β- (0–20 ms after stimulus), A δ-responses (20–90 ms after stimulus) and long latency C-fibre responses (90–300 ms after stimulus) followed by a postdischarge period (quantified from 300 to 800 ms). The degree of 'wind-up' of the cells (the enhanced response to repeated constant stimuli) was also quantified, being calculated as the total number of spikes to the 16 stimuli minus the initial response of the cell to the first stimulus $\times 16$. The depth of the cells (μm) was established using a SCAT microdriver (Digitimer, UK).

2.3. Pharmacological treatments

At least three stable control responses to electrical stimulation were established before the application of each drug. Cumulative dosing was used so that dose-response relations could be determined for each individual neurone. Four different doses of BU-224 were administered: 5, 25, 125 and 250 μg. In separate studies, yohimbine, a non selective α_2-adrenoceptor antagonist (150 μg), atipamezole a selective α_2-adrenoceptor antagonist or idazoxan, an α_2-adrenoceptor/imidazoline I_2 receptor antagonist (100 μg), were administered 10 min prior to the highest dose of BU-224. All the drugs were dissolved in saline and applied to the surface of the spinal cord (akin to intrathecal injection) in a 50 μl volume. The response of the neurones after the administration of each dose was followed for 40 min, tests being performed every 10 min. Spike height was monitored throughout the experiments and no changes in

amplitude of action potentials were observed. The numbers of neurones in each experimental group are given in the results section ($n = 6–9$); on occasions more than one neurone per rat was studied but intervals of up to 5 h were used between studies.

2.4. Drugs

BU-224 (2-(4,5-dihydroimidazol-2yl)-quinoline hydrochloride)) was purchased from Tocris Cookson (UK). Idazoxan ((±)-2-(1,4-benzodioxan-2-yl)-2-imidazoline hydrochloride)) and yohimbine (17-hydroyyohimban-16-carboxylic acid methyl ester hydrochloride) were obtained from Research Biochemicals International (UK). Atipamezole (4-(2-ethylindan-2-yl)imidazole) was from FARMOS (Finland).

2.5. Data analysis

Results were calculated as percentages of the control values for each neurone and the overall results expressed as mean ± standard error of the mean (S.E.M.). The effect of cumulative doses of BU-224 on the neuronal responses were analysed statistically with one-way ANOVA (repeated measures) and the Newman–Keuls post hoc test.. The correlation coefficient of the dose response relationship of BU-224 was determined with linear regression analysis, and thus possible dose-dependent effects were evaluated. Statistical analysis of the antagonist studies was performed with the Mann–Whitney *U*-test. A P value less than 0.05 was considered statistically significant.

3. Results

A total number of 30 neurones were studied. The results were obtained from neurones which received both Aβ- and C-fibre afferent inputs and responded to both innocuous (touch and prod) and noxious (heat and pinch) peripheral stimuli. All the neurones were located deep within the dorsal horn (mean depth 718 ± 24 μm).

3.1. Effect of BU-224 on the electrically-evoked neuronal responses

Four different doses of BU-224 (5, 25, 125 and 250 μg) were tested upon the electrically evoked responses of dorsal horn neurones ($n = 9$). The intrathecal administration of BU-224 produced a significant dose-dependent inhibition of the C-fibre evoked responses ($y = (-0.99)x + 347.05$, $r^2 = 0.69$, $P < 0.0001$). Whereas 5 μg and 25 μg had no effect on C-fibre responses, the two highest doses, 125 and 250 μg, significantly reduced the total number of C-fibre evoked action potentials when compared to control responses, producing inhibitions of 32.7 ± 11% ($P < 0.01$) and 70.3 ± 10% ($P < 0.001$), respectively. As shown in Fig. 1, this inhibitory action was selective for noxious-related responses since no significant changes were observed for innocuous Aβ-evoked responses. The postdischarge following the main C-fibre responses as well as the wind-up of the cells were also affected by BU-224, being inhibited in parallel with C-fibre evoked responses, again only after 125 and 250 μg doses. The postdischarge was inhibited by 51.0 ± 17% and 77.1 ± 12% for the population of cells with 125 and 250 μg, respectively ($P < 0.05$ for 125 μg and $P < 0.001$ for 250 μg) (Fig. 1). Interestingly, the initial response of the cells (input), the response to the first stimulus prior to the occurrence of wind-up, was also reduced (Fig. 2) to 29.4 ± 10.1% of control values by the highest dose ($P < 0.01$).

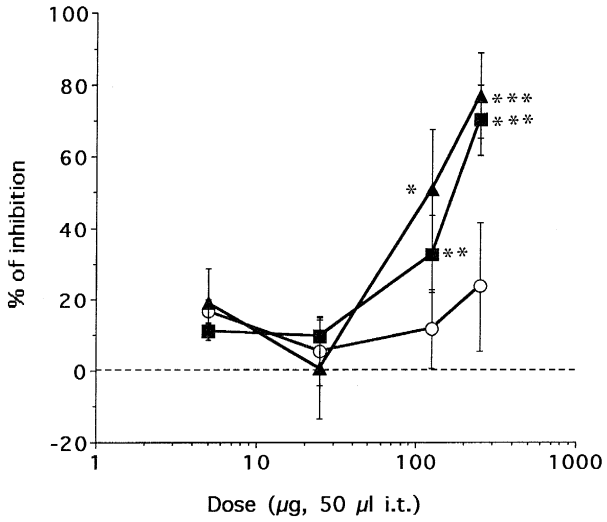

Fig. 1. The dose-response curves for inhibition of (■) C- and (O) Aβ-fibre-evoked responses and (▲) postdischarge by cumulative doses of i.t. BU-224. Using ANOVA and Newman–Keuls post hoc test, * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$.

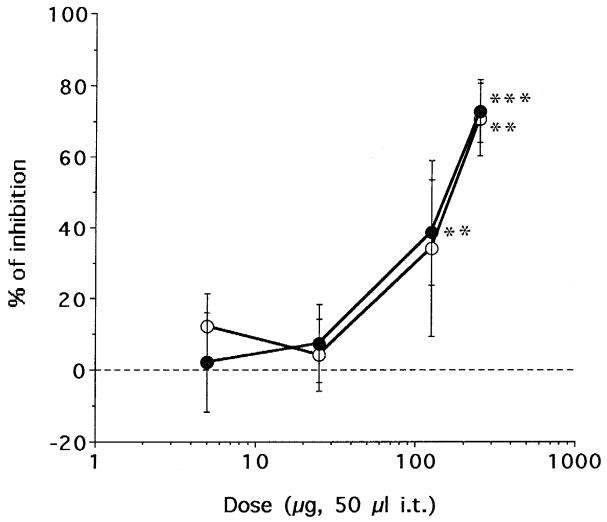

Fig. 2. The dose-response curves for inhibition of the (○) initial input (number of action potentials evoked by the first electrical stimulus) and (●) wind-up of the cells by cumulative doses of i.t. BU-224. Using ANOVA and Newman–Keuls post hoc test, ** $P < 0.01$ and *** $P < 0.001$.

As shown also in Fig. 2, wind-up, the increase in the number of C-fibre evoked response per stimulus during a train of stimuli, was also affected by the drug, reaching $38.7 \pm 15\%$ and $72.9 \pm 9\%$ inhibitions after 125 and 250 μg doses, respectively ($P < 0.01$ for 125 μg and $P < 0.001$ for 250 μg). In Fig. 3, an example of the wind-up of the C-fibre evoked response in a single neurone is shown, before and after four cumulative doses of BU-224. The Aδ-responses of the neurones were also inhibited in a similar manner to the other noxious-related measures, with the 125 μg and 250 μg doses reducing the responses to 49.8 ± 8 and $26.0 \pm 12\%$ of control values ($P < 0.01$ and $P < 0.001$, respectively). The mean peak effect of BU-224 on all the neuronal responses was 24 ± 2 min justifying the cumulative doses being given at 40 min intervals.

3.2. Effect of the α_2-adrenoceptor-antagonists, yohimbine and atipamezole on BU-224 inhibitions

A high dose of the nonselective α_2-adrenoceptor antagonist yohimbine (150 μg) was applied intrathecally 10 min prior to the spinal administration of the maximal dose of BU-224 tested (250 μg) ($n = 9$). Atipamezole, the highly selective α_2-adrenoceptor antagonist, (100 μg) was tested in the same way ($n = 6$). Yohimbine and atipamezole both only partially attenuated the inhibitory effects of BU-224 on both C-fibre responses or postdischarge (Fig. 4). The C-fibre response was reversed from 29.7 ± 10 to $58.0 \pm 10\%$ of control values and the postdischarge from 22.9 ± 12 to $54.9 \pm 17\%$ of control after yohimbine pretreatment and to $67.5 \pm 18\%$ and $46.0 \pm 14\%$ respectively, by atipamezole. None of these effects reached statistical significance. The degree of inhibition of wind-up of the cells following 250 μg BU-224 alone or after yohimbine or atipamezole was also unchanged. These results might suggest that a minor component of the action of the high dose of BU-224 could be via α_2-adrenoceptors.

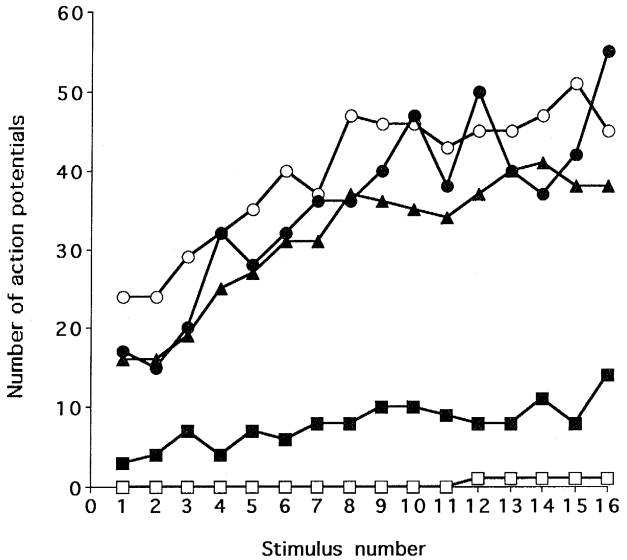

Fig. 3. Example of the wind-up of the C-fibre-evoked response in a single deep dorsal neurone before (control) and after the i.t. administration of different doses of BU-224: (○) control, (●) 5, (▲) 25, (■) 125 and (□) 250 μg. Note the reduction in the response to the first few stimuli in the presence of 5, 25 and 125 μg BU-224 but the subsequent wind-up occurs; only after the 250 μg dose is wind-up completely abolished.

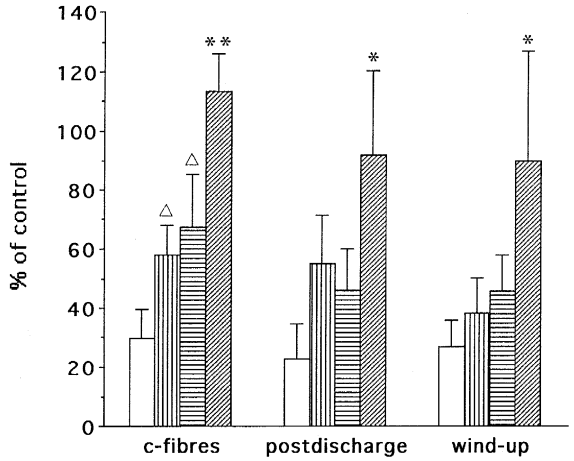

Fig. 4. Partial reversal of the inhibitory effects of 250 μg BU-224 (blank bars) on C-fibre-evoked responses, postdischarge and wind-up of the cells by 150 μg of the nonselective α_2-adrenoceptor antagonist, yohimbine (vertical bars) ($n = 9$) or 100 μg of the selective α_2-adrenoceptor antagonist, atipamezole (horizontal bars) ($n = 6$). By contrast, 100 μg of the α_2-adrenoceptor/imidazoline I_2 receptor antagonist idazoxan (hatched bars) ($n = 6$) completely reversed the inhibitions. Using Mann–Whitney U-test, * $P < 0.05$ vs. BU-224, ** $P < 0.01$ vs. BU-224 and $^{\triangle}$ $P < 0.05$ vs. Idazoxan + BU-224 group.

3.3. Effect of the α_2-adrenoceptor / imidazoline I_2 receptor antagonist, idazoxan on BU-224 inhibitions

The effect of the highest dose of BU-224 alone and then following preadministration of α_2-adrenoceptor/imidazoline I_2 receptor antagonist idazoxan, was studied on the C-fibre evoked response, postdischarge and wind-up of the neurones ($n = 6$). Pretreatment with 100 μg idazoxan 10 min prior to 250 μg BU-224 completely reversed the inhibition of all the neuronal responses produced by BU-224 (Fig. 4). The C-fibre evoked responses returned to control values, $113.4 \pm 13\%$ ($P < 0.01$ vs. 250 μg BU-224 alone). The postdischarge and wind-up of the cells also reversed to values similar to control responses, $92.0 \pm 28\%$ and $89.8 \pm 37\%$ of control values, respectively ($P < 0.05$ for both). The reversal of the effects of BU-224, analysed for the C-fibre responses, by idazoxan was significantly different from that produced by atipamezole and yohimbine ($P < 0.05$ for both measures). These results indicate that the major component of the inhibitory effects of BU-224 at the level of the spinal cord is mediated by imidazoline I_2 receptors.

4. Discussion

These results demonstrate that the spinal administration of the imidazoline I_2 receptor ligand, BU-224, inhibits the response of dorsal horn neurones in a dose-dependent manner. This antinociceptive action is selective for C-fibre and other nociceptive responses since no significant changes were seen in the A β-fibre evoked responses. BU-224 reduced the C-fibre responses, A δ-fibre evoked responses, postdischarge and wind-up of the neurones to a comparable degree. Moreover, BU-224-induced antinociception was completely reversed by the α_2-adrenoceptor/imidazoline I_2 receptor antagonist, idazoxan. The inhibitions were only partly reversed by both the nonselective α_2-adrenoceptor-antagonist yohimbine and the highly selective α_2-adrenoceptor antagonist, atipamezole.

The static nature of the preparation of the anesthetized animal with the dura overlying the spinal cord removed, precludes diffusion to supraspinal sites. The direct spinal administration of the drugs and the rapid onset of drug action suggests that the actions observed are mediated by receptors at the spinal cord level. The presence of imidazoline receptors in the rat spinal cord (Monroe et al., 1995) supports this proposition.

In our study, it seems that the reduction in the total number of action potentials evoked by C-fibre activation is mainly a consequence of a reduction in the nociceptive input into the dorsal horn rather than alterations in the spinal processing of nociceptive messages. Nitric oxide has been implicated in the wind-up of spinal nociceptive neurones and the subsequent central hyperexcitability, possibly acting as a retrograde neurotransmitter which leads to further activation of NMDA receptors (Haley et al., 1990; Sorkin, 1993). It has been found that agmatine, the putative endogenous imidazoline receptor ligand, can inhibit nitric oxide formation in supernatants of brain or kidney and therefore could influence the nitric oxide synthase pathway (Morrisey et al., 1995). In our study, BU-224 inhibited both the postdischarge and the wind-up of the cells. Nevertheless this effect seems to be more a consequence of the overall general reduction in the evoked nociceptive responses in the dorsal horn rather than a selective effect on those mechanisms (NMDA-receptor-dependent) underlying wind-up.

There is recent evidence for the presence of nonadrenergic imidazoline receptors within the spinal cord, but whether they are presynaptic- or postsynaptically localized is still unknown (Monroe et al., 1995). According to the above findings, it might be suggested that some of the effects of BU-224 could be due to its action on receptors localised presynaptically which are probably controlling/modulating the release of neurotransmitters from the primary afferents. Additional postsynaptic actions of the drug cannot be excluded since the wind-up of the cells was completely abolished by the high dose of BU-224. It could also be speculated that spinal imidazoline receptors could contribute to the inhibitory descending pathways in a similar way to α_2-adrenoceptors. The profile of BU-224 against C-fibre responses and wind-up of the cells resembles, albeit with much lower potency, that seen after intrathecal administration of the potent α_2-adrenoceptor agonist, dexmedetomidine in the same electrophysiological model (Sullivan et al., 1992). Interestingly, there is recent biochemical evidence indicating that [^{3}H]dexmedetomidine binds to a nonadrenergic site in the adult rat spinal cord, a binding site that has been proposed to be a novel type of imidazoline receptor for which cimetidine has relatively high affinity (Savola and Savola, 1996).

In our study, the inhibitory actions of BU-224 were completely reversed by idazoxan, but not yohimbine or atipamezole. Yohimbine has no effect on the neuronal responses evoked by transcutaneous stimulation (M. Green, personal communication). Also, we have already reported the lack of significant effects of idazoxan and atipamezole alone on C-fibre evoked responses in normal animals, although following carrageenan inflammation idazoxan produced significant facilitations (Stanfa and Dickenson, 1994). Thus, the reversal of BU-224 effects by idazoxan must reflect a competitive antagonism at the same receptor rather than being simply due to enhanced neuronal activity. In radioligand binding studies, BU-224 has high affinity for brain nonadrenoceptor idazoxan binding sites (imidazoline I_2 receptors) with considerable selectivity with respect to α_2-adrenoceptors (K_i α_2-adrenoceptor/I_2 receptor = 4172) (Hudson et al., 1996). However, in the antagonism experiments, the partial reversal by yohimbine and atipamezole leaves open the possibility of some action of the highest doses of BU-224 on α_2-adrenoceptors. This is not surprising considering that the two receptor classes (α_2-adrenoceptor and imidazoline receptors) have several common structural features, and furthermore both receptors can mediate identical physiological responses (Hieble and Ruffolo, 1995). The distinction between idazoxan and yohimbine/atipamezole suggests that the actions of BU-224 are mostly mediated through spinal imidazoline receptors. It has been reported that imidazoline I_1 receptors are not involved in nociception since the antinociceptive effect of intrathecal clonidine is mediated exclusively by spinal α_2-adrenoceptors, and a lack of imidazoline I_1 binding sites in the spinal cord has been reported (Monroe et al., 1995). These facts, together with the high affinity of BU-224, suggest its actions are mediated through the I_2-subtype of imidazoline receptors, and due to the complete reversal by idazoxan it is not unlikely that BU-224 is acting as an agonist at these receptors. This is therefore the first demonstration that BU-224 acts as an agonist at the imidazoline I_2 binding site in vivo.

There is also recent evidence in the literature supporting the possible role of spinal imidazoline receptors in controlling/modulating spinal nociception. Agmatine, the putative endogenous imidazoline receptor ligand, (Li et al.,

1994) has been found to recognize not only imidazoline but also α_2-adrenoceptor binding sites, although it completely failed to activate either peripheral or central α_2-adrenoceptors (Pinthong et al., 1995). Interestingly, agmatine dose-dependently potentiated the analgesic effect of intrathecal morphine, and idazoxan, but not yohimbine, reversed this enhanced analgesia, suggesting that this action of agmatine may be mediated through imidazoline receptors (Kolesnikov et al., 1996). Agmatine has been also shown to produce, at high i.v. doses, an inhibition of the reflex responses to noxious stimuli (pinch and electrical stimulation) in spinally intact rats. Although the receptor mechanism of these actions remained unclear, α_2-adrenoceptors were not involved since atipamezole was unable to reverse inhibitions of the reflex responses to noxious stimuli (Bradley and Headley, 1996). Recently, RS-45041-190 with selectivity and high affinity for imidazoline I_2 receptor (MacKinnon et al., 1995), has been shown to potentiate hyperalgesia in a model of acute arthritis when injected intrathecally, consequently suggesting a role of spinal imidazoline I_2 receptors in controlling hyperexcitability in inflammation (Houghton and Westlund, 1996).

These results demonstrate that imidazoline I_2 receptors are present in the dorsal horn and might play a role in spinal nociception. Further studies using selective ligands might allow a better understanding of the functional roles of spinal imidazoline receptors in nociceptive pathways. Moreover, there is still a great need for functional studies to clarify or define whether imidazoline drugs that bind to imidazoline receptors are full or partial agonists or antagonists. It is also important to assess the effectiveness of selective imidazoline ligands as potential pharmacological agents in the control of nociception under certain pathophysiological conditions, such as inflammation/hyperalgesia.

Acknowledgements

This work was supported by The Wellcome Trust, UK and EEC Biomed II.

References

Bradley, K.J., Headley, P.M., 1996. Effects of agmatine on spinal nociceptive responses in anaesthetized rats. J. Physiol. 495, 46P.

Brown, C.M., MacKinnon, A.C., Redfern, W.S., Williams, A., Linton, C., Stewart, M., Clague, R.U., Clark, R., Spedding, M., 1995. RS-45041.190: A selective, high affinity ligand for I_2 imidazoline receptors. Br. J. Pharmacol. 116, 1737–1744.

Carpene, C., Collon, P., Remaury, A., Cordi, A., Hudson, A., Nutt, D., Lafontan, M., 1995. Inhibition of amine oxidase activity by derivatives that recognize imidazoline I_2 sites. J. Pharmacol. Exp. Ther. 272, 681–688.

Diamant, S., Eldar-Geva, T., Atlas, D., 1992. Imidazoline binding sites in human placenta: evidence for heterogeneity and a search for physiological function. Br. J. Pharmacol. 106, 101–108.

Dickenson, A.H., Le Bars, D., 1987. Supraspinal morphine and descending inhibitions acting on the dorsal horn of the rat. J. Physiol. 384, 81–107.

Dickenson, A.H., Sullivan, A.F., 1986. Electrophysiological studies on the effects of intrathecal morphine on nociceptive neurones in the rat dorsal horn. Pain 24, 211–222.

Ernsberger, P., Meeley, M.P., Mann, J.J., Reis, D.J., 1987. Clonidine binds to imidazoline binding sites as well as alpha$_2$-adrenoceptors in the ventrolateral medulla. Eur. J. Pharmacol. 134, 1–13.

Haley, J.E., Sullivan, A.F., Dickenson, A.H., 1990. Evidence for spinal *N*-methyl D-aspartate receptor involvement in prolonged chemical nociception in the rat. Brain Res. 518, 218–226.

Hieble, J.P., Ruffolo, R.R. Jr., 1995. Possible structural and functional relationships between imidazoline receptors and alpha$_2$-adrenoceptors. Ann. N.Y. Acad. Sci. 763, 8–21.

Houghton, A.K., Westlund, K.N., 1996. An I_2 imidazoline ligand, RS 45041, potentiates hyperalgesia in acute arthritis. Neuroreport 7, 1497–1501.

Hudson, A.L., Husbands, S., Lewis, J.W., Nutt, D.J., 1996. Affinity and selectivity of BU-224 and BU-239 for rat brain nonadrenoceptor idazoxan binding sites (I_2-sites). Br. J. Pharmacol. 112, 320P.

Jackson, H.C., Griffin, I.J., Nutt, D.J., 1991. The effects of idazoxan and other alpha$_2$-antagonists on food and water intake in the rat. Br. J. Pharmacol. 104, 258–262.

Kolesnikov, Y., Jain, S., Pasternak, G.W., 1996. Modulation of opioid analgesia by agmatine. Eur. J. Pharmacol. 296, 17–22.

Li, G., Regunathan, S., Barrow, C.J., Eshraghi, J., Cooper, R., Reis, D.J., 1994. Agmatine: An endogenous clonidine-displacing substance in the rat brain. Science 263, 966–969.

MacKinnon, A.C., Redfern, W.S., Brown, C.M., 1995. [^{3}H]-RS-45041-190, a selective high-affinity radioligand for I_2 imidazole receptors. Br. J. Pharmacol. 116, 1729–1736.

Michel, M.C., Ernsberger, P., 1992. Keeping an eye on the I-site: Imidazoline-preferring receptors. Trends Pharmacol. Sci. 13, 369–370.

Michel, M.C., Insel, P.A., 1989. How many imidazoline-preferring binding sites are there?. Trends Pharmacol. Sci. 10, 342–344.

Michel, M.C., Regan, J.W., Gerhardt, M.A., Neubig, R.R., Insel, P.A., Motulsky, H.J., 1990. Nonadrenergic [^{3}H]idazoxan binding sites are physically distinct from alpha$_2$-adrenergic receptors. Mol. Pharmacol. 37, 65–68.

Miralles, A., Olmos, G., Sastre, M., Barturen, F., Martin, I., Garcia-Sevilla, J.A., 1993. Discrimination and pharmacological characterization of I_2-imidazoline sites with [^{3}H]idazoxan and alpha$_2$-adrenoceptors with [^{3}H]RX821002 (2-methoxy idazoxan) in the human and rat brains. J. Pharmacol. Exp. Ther. 264, 1187–1197.

Monroe, P.J., Smith, D.L., Kirk, H.R., Smith, D.J., 1995. Spinal nonadrenergic imidazoline receptors do not mediate the antinociceptive action of intrathecal clonidine in the rat. J. Pharmacol. Exp. Ther. 273, 1057–1062.

Morrisey, J., McCracken, R., Ishidoya, S., Klahr, S., 1995. Partial cloning and characterization of an arginine decarboxylase in the kidney. Kidney Int. 47, 1458–1461.

Parini, A., Coupry, I., Grahams, R.M., Uzielli, I., Atlas, D., Lanier, S.M., 1989. Characterization of an Imidazoline/guanidinium receptive site distinct from the alpha$_2$-adrenergic receptor. J. Biol. Chem. 264, 11874–11878.

Piletz, J.E., Chikkala, D.N., Ernsberger, P., 1995. Comparison of the properties of agmatine and endogenous clonidine-displacing substance at imidazoline and alpha$_2$-adrenergic receptors. J. Pharmacol. Exp. Ther. 272, 581–587.

Pinthong, D., Wright, I.K., Hanmer, C., Millns, P., Mason, R., Kendall, D.A., Wilson, V.G., 1995. Agmatine recognizes alpha$_2$-adrenoceptor binding sites but neither activate nor inhibits alpha$_2$-adrenoceptors. Naunyn-Schmiedebergs Arch. Pharmacol 35, 10–16.

Raasch, W., Regunathan, S., Li, G., Reis, D.J., 1995. Agmatine, the bacterial; amine, is widely distributed in mammalian tissues. Life Sci. 56, 2319–2330.

Regunathan, S., Reis, D.J., 1996. Imidazoline receptors and their endogenous ligands. Annu. Rev. Pharmacol. Toxicol. 36, 511–544.

Stanfa, L.C., Dickenson, A.H., 1994. Enhanced alpha$_2$-adrenergic controls and spinal morphine potency in inflammation. Neuroreport 5, 469–472.

Savola, M.K.T., Savola, J.M., 1996. [^{3}H]Dexmedetomidine, an α_2-adrenoceptor agonist, detects a novel imidazoline binding site in adult rat spinal cord. Eur. J. Pharmacol. 306, 315–323.

Sorkin, L.S., 1993. NMDA evokes an L-NAME sensitive spinal release of glutamate and citrulline. Neuroreport 4, 479–482.

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.