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Effect of BU98008, an imidazoline$_1$-binding site ligand, on body temperature in mice

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Abstract

Previous studies using the novel imidazoline$_1$-binding site ligand 1-(4,5-dihydro-1H-imidazol-2-yl)isoquinoline hydrochloride, BU98008, have shown it induces a hypothermic response in rodents following intraperitoneal administration. Radioligand binding data has shown that BU98008 is a highly selective imidazoline$_1$-binding site ligand with 300 fold selectively for the imidazoline$_1$-binding site relative to α_2-adrenoceptors. However, α_2-adrenoceptor agonists are known to induce hypothermia, therefore, the present study has investigated the ability of the selective α_2-adrenoceptor antagonist, RX811059 (2-ethoxy idazoxan) and the mixed imidazoline$_1$-binding site/α_2-adrenoceptor antagonist, efaroxan, to attenuate the BU98008-induced hypothermia. Preliminary experiments confirmed that BU98008 induced a dose-dependent decrease in body temperature in mice at 10 and 20 mg/kg. The response was not affected by pre-treatment with RX811059 but was significantly attenuated following pre-treatment with efaroxan. These data suggest that BU98008-induced hypothermia is mediated by activation of imidazoline$_1$-binding site. Body temperature may therefore provide a novel assay for investigating agonist and antagonist action at the imidazoline$_1$-binding site.

Keywords: Imidazoline$_1$-binding site; α_2-Adrenoceptor; Body temperature

1. Introduction

The imidazoline binding sites were first characterised some 20 years ago following the observation that certain α_2-adrenoceptor ligands bound to a catecholamine insensitive binding site (for review see Eglen et al., 1998). Imidazoline-binding sites are now known to exist as three distinct subtypes based on their pharmacology and functional effects. The imidazoline$_1$-binding site displays high affinity for the α_2-adrenoceptor agonist clonidine and is believed to mediate the hypotensive effects of this ligand as well as the more selective compounds, rilmenidine and moxonidine (Bousquet et al., 1984; Ernsberger et al., 1987; Eglen et al., 1998). The imidazoline$_2$-binding site is recognised by the α_2-adrenoceptor antagonist, idazoxan and is associated with the regulation of monoamine turnover and control of food intake (Hudson et al., 1999). The imidazoline$_2$-binding site has been shown to be associated with monoamine oxidase (MAO) although data suggests that a proportion of these sites are a non-MAO population (Eglen et al., 1998). The final subtype is the putative imidazoline$_3$-binding site, which displays a distinct pharmacology and is associated with the secretion of insulin. It has been proposed that the imidazoline$_3$-binding site may be linked to the K_{ATP} channel (Chan et al., 1991).

The imidazoline$_1$-binding site has been a particular area of interest because of the potential for ligands acting through this mechanism to reduce blood pressure without the sedative side effects observed with α_2-adrenoceptor agonists such as clonidine (for review see Bousquet, 2001). However, these ligands still display moderate affinity for α_2-adrenoceptors, activation of which may contribute to their hypotensive effects. Evidence from studies in $\alpha_{2A/D}$-adrenoceptor mutant mice has been controversial with

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central and peripheral administration of moxonidine yielding different results (Zhu et al., 1999; Tolentino-Silva et al., 2000). The conclusion drawn from these studies was that peripheral administration of moxonidine requires the presence of a functional α_2-adrenoceptor whilst the imidazoline$_1$-binding site-specific effect is only observed following central administration. Whatever the relative effects associated with the α_2-adrenoceptor and imidazoline$_1$-binding site activation in relation to hypotension, moxonidine has been shown to induce a robust hypotensive effect in patients with a reduced side-effect profile (for review see Szabo, 2002).

BU98008, 1-(4,5-dihydro-1H-imidazol-2-yl)isoquinoline hydrochloride, was developed in our laboratory and radioligand binding studies revealed a high affinity and selectivity for imidazoline$_1$-binding sites relative to both imidazoline$_2$-binding sites and α_2-adrenoceptors (Robinson et al., 2003a). During preliminary investigations into the potential application of this ligand in the treatment of hypotension, a hypothermic response at 10 mg/kg was observed. Although the dose used was unlikely to affect α_2-adrenoceptors, the hypothermic effect suggested activation of α_2-adrenoceptors. In order to further investigate the mechanisms underlying this response, the present investigation has determined whether the α_2-adrenoceptor antagonist, RX811059 (Robinson et al., 2004), blocks the hypothermia induced by BU98008. In addition, the mixed imidazoline$_{1/3}$-binding site/α_2-adrenoceptor antagonist, efaroxan, (De Vos et al., 1991; Eglen et al., 1998) has also been evaluated. The results presented in this paper suggest that imidazoline$_1$-binding sites may mediate a hypothermic response and, as such, this may represent a novel assay for the in vivo characterisation of imidazoline$_1$-binding site activation.

2. Methods

2.1. Acute effects of imidazoline ligands on body temperature in mice

Male CD1 mice (Harlan, UK, 24–34 g) were group housed in standard laboratory plastic cages with metal grid lids at 22 °C±2 °C on a 12 h light dark cycle (lights on at 06:00). Animals were provided with free access to standard rat and mouse diet (Bantin and Kingman, UK) and water.

On the day of the experiment, groups of 4–6 mice were separated and transferred to a separate cage in the testing room. Animals were allowed to acclimatise to the room for at least 1 h prior to the start of the experiment. Rectal temperature readings were made using a modified food temperature probe (Hanna Instruments, UK) inserted to 1.5 cm. The animals were lightly restrained during temperature readings and for intraperitoneal drug administration. Drugs were dissolved in saline (0.9%) and administered intraperitoneal at a dose volume of 10 ml/kg. BU98008 was administered at 10 and 20 mg/kg based on the results from previous experiments (Robinson et al., 2003a). For the pre-treatment experiments, RX811059 (3 mg/kg) and efaroxan (5 mg/kg) were administered 10 min prior to BU98008. These doses were based on the relative affinities of each drug at the α_2-adrenoceptor, ~1 nM for RX811059 (Robinson et al., 2004) and ~4 nM for ±efaroxan (Tyacke et al., 1997). Previous studies using in vivo microdialysis have also shown that these doses are equipotent in vivo, evoking an equivalent maximal increase in noradrenaline release in the frontal cortex (Data not shown). At the end of the experiment all animals were killed by stunning and cervical dislocation. All experiments were carried out in accordance with the University's policy on animal research and Home Office legislation.

2.2. Drugs and chemicals

BU98008, 1-(4,5-dihydro-1H-imidazol-2-yl)isoquinoline hydrochloride, was kindly synthesised by Dr. Stephen Husbands, University of Bath. RX811059 and efaroxan were gifts from Reckitt and Coleman.

2.3. Statistical analysis

Results for body temperature are presented as the mean±S.E.M. for a minimum of six animals per treatment group. Statistical comparisons were made using a one-way analysis of variance. Where a significant effect of treatment was observed, post hoc analysis using Dunnett's test for comparison to control group or Tukey's test for comparison between all groups was used. A significant effect was reported where $P<0.05$.

3. Results

3.1. Acute effects of BU98008 on body temperature

BU98008 induced a significant reduction in rectal temperature in mice at a dose of 10 and 20 mg/kg when compared to the saline control treated animals (Fig. 1). The effects of BU98008 at 10 mg/kg, although significant, were small and short lasting. Lower doses of BU98008, 1 and 3 mg/kg had no effect on body temperature (data not shown). Based on the results obtained a dose of 20 mg/kg, inducing a 2.0 °C reduction in body temperature, was selected for the antagonist studies.

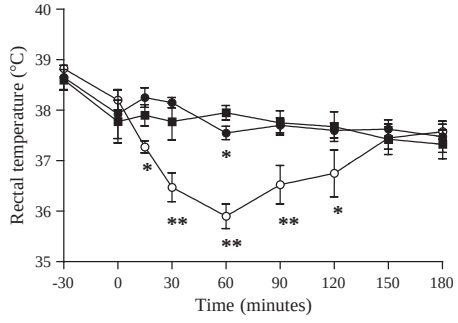

Fig. 1. Effects of BU98008 (administered at time zero) on rectal temperature in male CD1 mice. Data presented as mean±S.E.M. for vehicle (■ 0.9% saline), 10 mg/kg (●) and 20 mg/kg (○) BU98008, $n=6$ animals per group. Results were analysed using a one-way ANOVA with Dunnett's post hoc test, *$P<0.05$, **$P<0.01$.

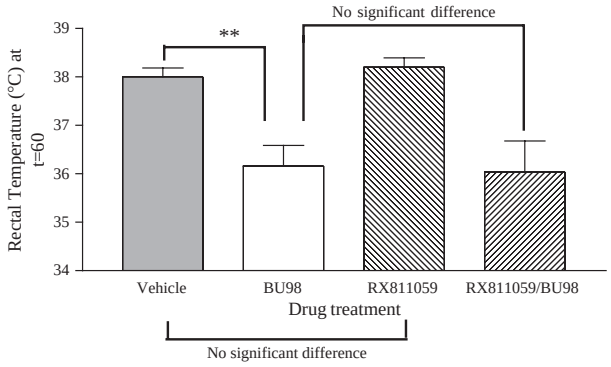

Fig. 2. Effect of pre-treatment of RX811059 ($t=-10$ min, 3 mg/kg) on BU98008 ($t=0$ min, 20 mg/kg)-induced hypothermia 60 min after drug administration in male CD1 mice. Data presented as mean±S.E.M. for 6 animals per group. Results were analysed using a one-way ANOVA with Tukey's post hoc test, $^{**}P<0.01$.

3.2. Effects of RX811059 and efaroxan on BU98008-induced hypothermia

Acute administration of BU98008 (20 mg/kg) following vehicle pre-treatment induced a robust hypothermic response with a mean reduction in body temperature of 1.8 °C at $t=60$ min following i.p. administration. Pre-treatment with RX811059 (3 mg/kg) had no significant affect on rectal temperature with the values obtained similar to those for the saline controls. Pre-treatment with RX811059 did not significantly attenuate the hypothermic response to BU98008 with both groups showing an equivalent reduction in rectal temperature (Fig. 2). In contrast, efaroxan pre-treatment significantly attenuated the hypothermic response to BU98008 (20 mg/kg) by 1.1 °C at $t=60$ min (Fig. 3). There was no significant difference between the efaroxan pre-treated group receiving saline or the saline control group suggesting these effects were not the result of physiological antagonism.

4. Discussion

Previous studies have established that BU98008 is a highly selective imidazoline$_1$-binding site ligand. Radioligand binding studies have shown that BU98008 binds with nanomolar affinity at the rat kidney imidazoline$_1$-binding sites but has micromolar affinity for α_2-adrenoceptors and imidazoline$_2$-binding sites (Robinson et al., 2003a). Following the observation that BU98008 induced a hypothermic response after intraperitoneal administration, the present study aimed to confirm this finding and establish whether the response involved the activation of α_2-adrenoceptors. This study has demonstrated that BU98008 induces hypothermia in mice and the effect is significantly attenuated by pre-treatment with efaroxan. However, the lack of effect of the selective α_2-adrenoceptor antagonist, RX811059, suggests it is the affinity of efaroxan for imidazoline$_1$-binding sites rather than α_2-adrenoceptors that resulted in the attenuation of the response.

Hypothermia is a complex physiological response, which can be modulated by central and peripheral mechanisms including the activation of specific neurotransmitter receptors. For example, agonists at α_2-adrenoceptors and 5-hydroxtryptamine$_{1A}$ receptors have both been shown to specifically affect temperature regulation (Bill et al., 1989a,b; Millan et al., 1993). Therefore, compounds that interact with any of the receptors involved in temperature regulation, or modulate levels of their endogenous ligands, can induce hypothermia. In these preliminary studies, the mechanisms underlying the effects seen with BU98008 have not been fully elucidated but the following discussion aims to provide an explanation for the results observed in the context of the known pharmacology for BU98008, efaroxan and RX811059.

In vivo effects of imidazoline$_1$-binding sites have primary focused on the regulation of blood pressure using agonists such as moxinidine and rilmendine and antagonists such as efaroxan (Bousquet, 2001). The quantification of imidazoline$_1$-binding site-mediated effects on blood pressure is relatively complex requiring central administration of the ligand into anaesthetised animals and using a pharmacological approach to demonstrate a specific site of action. However, blood pressure does not provide an assay suitable for evaluating a large number of different ligands. Based on the results from the present study, rectal body temperature may provide a valid and appropriate alternative. These data have shown that hypothermia occurs following administration of a highly selective imidazoline$_1$-binding site ligand and unlike the hypothermia induced by drugs such as moxonidine or rilmenidine, this effect is unlikely to be due to α_2-adrenoceptor activation.

The lack of involvement of α_2-adrenoceptors in this response was confirmed by the fact that the highly selective α_2-adrenoceptor antagonist, RX811059, failed to attenuate the response. In contrast, efaroxan, which displays ~10 fold selectivity for α_2-adrenoceptors relative to imidazoline$_1$-binding sites (Tyacke et al., 1997; Eglen et al., 1998)

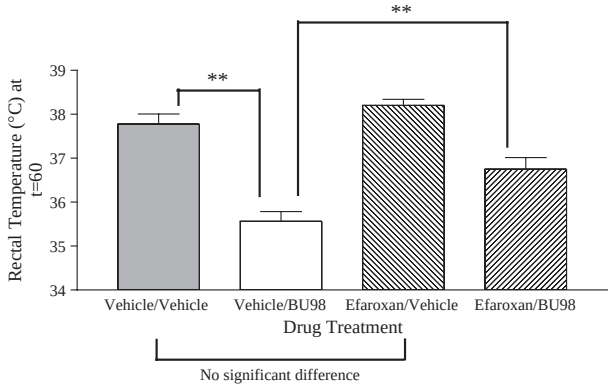

Fig. 3. Effect of pre-treatment with efaroxan ($t=-10$ min, 5 mg/kg) on BU98008 ($t=0$ min, 20 mg/kg)-induced hypothermia 60 min after drug administration in male CD1 mice. Data presented as mean±S.E.M. for 6 animals per group. Results were analysed using a one-way ANOVA with Tukey's post hoc test, $^{**}P<0.01$.

significantly attenuated the response at an equivalent α_2-adrenoceptor blocking dose. As such, the interaction of BU98008 with α_2-adrenoceptors to induce hypothermia has been excluded. The dose of efaroxan used in the present study was based on α_2-adrenoceptor affinity rather than imidazoline$_1$-binding site occupancy and this may account for the fact that only a partial reversal of BU98008-induced hypothermia was observed. A full screen of BU98008 has not been performed therefore, the involvement of a non-imidazoline$_1$-binding sites cannot be precluded. The results obtained for the two antagonists, RX811059 and efaroxan, however limit the likelihood that BU98008 is acting at a non-imidazoline binding site. RX811059 and efaroxan are structurally related compounds that differ in their ability to interact with the imidazoline$_1$-binding site. RX811059 is a highly selective α_2-adrenoceptor antagonist whilst efaroxan interacts with α_2-adrenoceptors and imidazoline$_1$/imidazoline$_3$-binding sites (Eglen et al., 1998). Efaroxan is widely used as an antagonist in imidazoline$_1$-binding site studies to separate responses mediated through α_2-adrenoceptors and imidazoline$_1$-binding sites (for example see Ernsberger, 2003, Musgrave and Badoer, 2000). Thus, efaroxan's ability to attenuate the response observed in the present study indicates the imidazoline$_1$-binding site as the common site of action. These data do not, however, preclude the possibility that activation of the imidazoline$_1$-binding site leads to the release of endogenous mediators that can activate receptors to induce a hypothermic response. Further studies are required before the mechanisms involved in this response can be fully elucidated.

Hypothermia following administration of ligands with affinity for imidazoline-binding sites has previously been reported from our group in relation to the endogenous ligand, harmane and other β-carbolines that display affinity at imidazoline-binding sites (Robinson et al., 2002, 2003b). Harmane was first characterised in relation to its ability to inhibit MAO-A, however, results obtained by our group have shown that harmane and the related β-carboline, harmalan are the active constituents of the extract clonidine displacing substance (Atlas and Burnstein, 1984). A previous paper by Adell et al. (1996) showed that harmane could induce a hypothermic response in rats *via* a mechanism other than MAO-A inhibition. Studies in our group showed that the hypothermic response to harmane was attenuated by pre-treatment with the imidazoline$_{1/2}$-binding site ligand, 2BFI (Robinson et al., 2003b).

In light of the findings presented in this paper and the high affinity of harmane for imidazoline$_1$-binding sites (Hudson et al., 1999; Husbands et al., 2001), these data further support the imidazoline$_1$-binding site as the common site of action. Therefore, we propose that body temperature may provide a novel assay for characterising in vivo activation of imidazoline$_1$-binding sites and as such will be a useful tool for identification of novel imidazoline$_1$-binding site ligands. Further studies are required to elucidate whether the hypothermia is mediated through activation of a specific, as yet uncharacterised protein, for example imidazoline receptor antisera selected (Piletz et al., 2000) or as a consequence of modulation of some other endogenous substance, which is involved in the regulation of temperature homeostasis. Several possible candidates exist including the opioid system and monoamine neurotransmitters. A more extensive investigation using specific antagonists at these receptors would address these questions.

In summary, these data confirm that the hypothermia induced by BU98008 does not occur through activation of α_2-adrenoceptors. The lack of effect at the α_2-adrenoceptor may also account for the failure of BU98008 to elicit a fall in blood pressure following intraperitoneal administration. Previous studies in α_{2A}-adrenoceptor mutant mice have shown that α_2-adrenoceptor agonism is required for the hypotensive effects of drugs like moxonidine following peripheral but not central administration (Zhu et al., 1999; Tolentino-Silva et al., 2000). The specific site of action of the ligands in relation to temperature regulation has not been elucidated but may involve interaction with an endogenous system such as the opioids or monoamine transmitters.

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