Short communication

Benextramine acts as an irreversible noncompetitive antagonist of U46619-mediated contraction of the rat small mesenteric artery

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Abstract

We have studied the effects of benextramine on the U46619 (11 α,9α-epoxymethano-15S-hydroxy-prosta-5Z,13E-dienoic acid)-mediated contraction of the rat isolated small mesenteric artery. U46619 (10 nM–10 μM) produced a concentration-dependent contraction of the small mesenteric artery. The selective prostanoid TP receptor antagonist, SQ 30,741 ([1S-[1α,2α(5Z),3α,4α]]-7-[[[[(oxaheptyl)amino]acetyl]amino]-methyl]-7-oxabicyclo[2.2.1]hept-2-yl]-5-heptenoic acid; 1 μM), produced a parallel, rightward shift of the U46619 curve with an associated pA_2 value of 7.43 ± 0.09. Treatment of tissues with 100 μM benextramine depressed the maximum response to U46619 in a time-dependent manner. However, neither SQ 30,741 (10 μM) nor U46619 (10 μM) incubation significantly protected against this effect. Thus, benextramine acts as an irreversible noncompetitive antagonist of U46619. The mechanism of this action is not yet clear.

Keywords: Benextramine; Prostanoid TP receptor; (Rat); Mesenteric artery, small; SQ 30,741; U46619

1. Introduction

The tetramine disulphide, benextramine, has previously been classified and employed as an irreversible competitive antagonist of α-adrenoceptors (Melchiorre, 1981) and neuropeptide Y receptors (Tessel et al., 1993; Palea et al., 1995). In this study, we investigated the effects of benextramine on the response of the rat isolated small mesenteric artery to the 9,11-methanoepoxy analogue of prostaglandin H_2, U46619, which is believed to mediate contraction of vascular smooth muscle via a selective action at prostanoid TP receptors (Coleman et al., 1994). We show that benextramine acts as an irreversible antagonist of U46619 in the small mesenteric artery via an, as yet unknown, nonsyntopic mechanism.

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2. Materials and methods

2.1. Rat isolated small mesenteric artery preparation

Male Wistar rats (225–350 g) were killed by cervical dislocation and the mesentery was removed and placed in ice-cold modified Krebs-Henseleit solution of the following composition (mM): NaCl 119.0, $NaHCO_3$ 25.0, KCl 4.7, KH_2PO_4 1.2, $MgSO_4$ 1.2, glucose 5.5, $CaCl_2$ 2.5 and ethylenediaminetetra-acetic acid 0.026. Four arterial trees were dissected from each mesenteric vascular bed and were cleared of surrounding adipose tissue. From each arterial tree, a ~ 2 mm ring segment was mounted in a small vessel myograph (J.P. Trading, Aarhus, Denmark) with separated 6 ml organ baths (thermostatically controlled at 37 ± 0.5°C, containing modified Krebs-Henseleit solution and continuously gassed with 95% O_2 and 5% CO_2) as described by Mulvany and Halpern (1977). The endothelium was removed by gentle rubbing of the intimal surface with a scoured whisker from a cat (Wang et al., 1994). Tissue responses were measured continuously as changes in isometric tension and were displayed on potentiometric chart recorders.

2.2. Experimental protocol

Following a 30 min stabilization period, the internal diameter of each vessel was set to a tension equivalent to 0.9 times the estimated diameter at 100 mm Hg effective transmural pressure (l_{100}: 183 ± 7 μm, $n = 43$) according to the standard procedure of Mulvany and Halpern (1977). After a further 30 min stabilization period, a calibration contraction (6.6 ± 0.5 mN) was obtained to 30 μM 5-hydroxytryptamine (5-HT) in each tissue and the absence of the endothelium was then confirmed by the lack of a response to 10 μM of the muscarinic acetylcholine receptor agonist, 5-methylfurmethide.

In the first experiment, following a washout period of 15 min, the tissues were incubated for 60 min with 3 μM indomethacin to prevent synthesis of endogenous prostanoids and with 1 μM SQ 30,741 or vehicle. Single U46619 concentration-effect ($E/[A]$) curves were then obtained by cumulative additions as third-log unit concentration increments. Effect was expressed as percentage of the calibration response to 30 μM 5-HT.

In the second series of experiments, the tissues were exposed to 100 μM benextramine or vehicle for 30, 60 or 120 min and were subsequently washed for 30 min with ten solution changes. Following 60 min incubation with 3 μM indomethacin, U46619 $E/[A]$ curves were obtained as described above. It was also investigated whether SQ 30,741 or U46619 could protect against the antagonistic action of benextramine. For this purpose, the same protocol as described above was employed, but the tissues were incubated until a response plateau was achieved with 10 μM SQ 30,741 (30 min) or with 10 μM U46619, before exposing the tissue to benextramine (100 μM, 30 min) without washing SQ 30,741 or U46619. Preliminary experiments had shown that incubation with SQ 30,741 or U46619 alone (i.e. not followed by benextramine treatment) had no significant effect on the U46619 $E/[A]$ curve (data not shown).

2.3. Compounds

The following compounds, obtained from the sources mentioned, were used: [Arg[8]]vasopressin (acetate salt), benextramine tetrahydrochloride, 5-hydroxytryptamine creatine sulphate (5-HT), indomethacin and U46619 (11α,9α-epoxymethano-15S-hydroxy-prosta-5Z,13E-dienoic acid): Sigma Chemical Company, Netherlands; 5-methylfurmethide iodide: Wellcome Research Laboratories, UK; SQ 30,741 ([1S-[1α,2α(5Z),3α,4α]]-7-[[[[[(oxaheptyl)amino]acetyl]amino]-methyl]-7-oxabicyclo[2.2.1]-hept-2-yl]-5-heptenoic acid): Bristol-Myers Squibb, Princeton, NJ, USA. Indomethacin and SQ 30,741 were dissolved in absolute ethanol. U46619 was dissolved initially in 20% ethanol to give a 1 mM stock solution and subsequently diluted in distilled water. All other drugs were dissolved in distilled water.

3. Results

3.1. Interaction between U46619 and SQ 30,741

U46619 (10 nM–10 μM) produced a concentration-dependent contraction of the small mesenteric artery (Fig. 1) and the individual $E/[A]$ curve data ($n = 5$) were fitted to the Hill equation (see Jenkinson et al., 1995) to provide estimates of midpoint location ($p[A]_{50}$: 7.38 ± 0.06), midpoint slope (n_H: 1.5 ± 0.1) and upper asymptote (α: 107 ± 6%). SQ 30,741 (1 μM) produced a parallel rightward shift of the U46619 $E/[A]$ curve without significantly changing the Hill slope or maximum response ($p[A]_{50}$: 5.93 ± 0.09; n_H: 1.7 ± 0.2; α: 107 ± 7%; $n = 5$) and a pA_2 value of 7.43 ± 0.09 was estimated, indicative of an action at prostanoid TP receptors (see Schumacher et al., 1989; Ogletree and Allen, 1992).

3.2. Effect of benextramine on the U46619-induced contraction

Treatment with 100 μM benextramine for 30, 60 or 120 min, followed by washout, depressed the maximum response to U46619 in a time-dependent manner (Fig. 2). In an attempt to clarify the mechanism behind this action, we investigated whether incubation with either SQ 30,741 or U46619 could protect, without subsequent washing, against the antagonistic effect of benextramine. As shown in Fig. 2, prior incubation of the tissues with neither SQ 30,741 (10 μM, 30 min) nor U46619 (10 μM, incubated until a response plateau was achieved) produced any significant protection against the effect of subsequent exposure for 30 min to 100 μM benextramine.
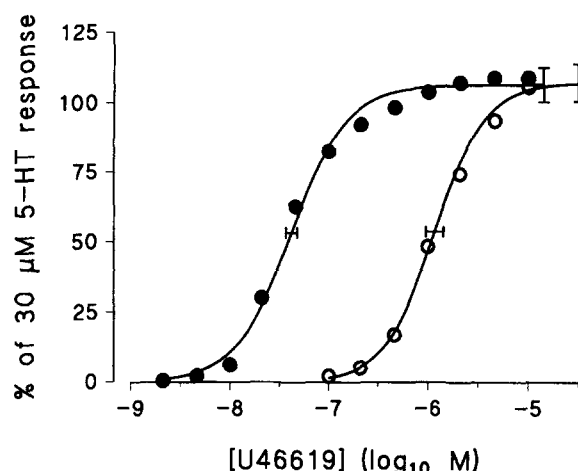
Treatment (30 min) with 100 μM benextramine had no

Fig. 1. Concentration-effect curves obtained with the rat isolated small mesenteric artery for U46619 in the absence (●) and presence (○) of 1 μM SQ 30,741. The lines shown superimposed on the mean experimental data points ($n = 5$) were simulated using the Hill equation (see text for parameter estimates). Horizontal and vertical error (S.E.M.) bars are shown on the mean midpoint and upper asymptote locations, respectively.
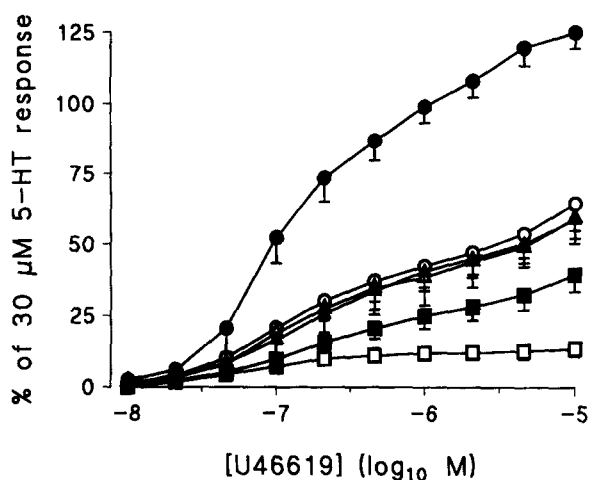
Fig. 2. Concentration-effect curves ($n = 4$–6) obtained with the rat isolated small mesenteric artery for U46619 following treatment with vehicle (●), 100 μM benextramine alone for 30 (○), 60 (■) and 120 min (□) or 100 μM benextramine for 30 min in the presence of 10 μM SQ 30,741 (▲) and 10 μM (△) U46619. Error bars indicate S.E.M.

significant effect on the Hill equation parameter estimates ($p[A]_{50}$: 8.98 ± 0.14 and 8.91 ± 0.09; n_H: 2.6 ± 0.6 and 2.1 ± 0.1; α: $129 \pm 4\%$ and $116 \pm 14\%$ for control and benextramine-treated tissues, respectively, $n = 3$) associated with the $E/[A]$ curves of another contractile agent in this assay, arginine vasopressin (0.1–100 nM). Thus, the antagonism of U46619 by benextramine appeared not to be due to a nonspecific effect.

4. Discussion

In this study, we have investigated the effects of benextramine, a ligand previously classified and employed as an irreversible competitive antagonist of α-adrenoceptors (Melchiorre, 1981) and neuropeptide Y receptors (Tessel et al., 1993; Palea et al., 1995), on the U46619-mediated contraction of the rat small mesenteric artery. Previous studies have shown that, in a variety of assays, U46619 mediates vasoconstriction via a selective action at prostanoid TP receptors (see Ogletree and Allen, 1992; Coleman et al., 1994; Baxter et al., 1995). This also appears to be the case in the rat isolated small mesenteric artery preparation, since the pA_2 estimate of 7.4 obtained for SQ 30,741 was similar to previously reported affinity values for prostanoid TP receptors in various rat smooth muscle assays (pK_B: ~ 7.8; Ogletree and Allen, 1992) and human and monkey platelets (pK_B: 7.1 and 7.2, respectively; Schumacher et al., 1989).

Treatment of small mesenteric arteries with benextramine was found to produce significant inhibition of the response to U46619. The degree of this effect was time dependent, i.e. the longer the incubation with benextramine, the greater the magnitude of the antagonism, indicative of an irreversible antagonistic mechanism

(Furchgott, 1954, 1966; Palea et al., 1995). In an attempt to elucidate the underlying mechanism of the antagonistic effect of benextramine, we performed receptor protection experiments, since it would be expected that if an irreversible antagonist inhibits the response of an agonist by reducing the number of receptors, then a reversible competitive antagonist should be able to protect receptors from such inactivation (Furchgott, 1954, 1966). However, SQ 30,741, in a concentration of 10 μM, which was ~ 250 times its apparent affinity for prostanoid TP receptors in the small mesenteric artery and thus would be expected to leave less than 0.5% of the receptors unoccupied, did not produce significant protection against the effects of benextramine treatment. Similarly, incubation with a near-maximally effective concentration (10 μM) of U46619 did not significantly inhibit the effects of benextramine. Hence, the protection experiments suggest that benextramine does not bind to the same site on the receptor as U46619 and SQ 30,741, but acts as an irreversible noncompetitive antagonist (see Jenkinson et al., 1995) of the prostanoid TP receptor-mediated contraction of the rat small mesenteric artery. Therefore, benextramine cannot be used as a tool to determine dissociation equilibrium constants for agonists at prostanoid TP receptors in a manner similar to that described previously for α_1-adrenoceptors (see, for example, Elliot and Pearce, 1994) and neuropeptide Y Y_1 receptors (Palea et al., 1995).

At present, we do not know the underlying mechanism of the antagonistic action of benextramine exposed in this study. The response to arginine vasopressin was not affected by benextramine, thus a nonspecific effect can be ruled out. Furthermore, vasopressin V_1 receptors, which are believed to mediate vasoconstriction to arginine vasopressin (Thibonnier, 1992), are coupled to the same signal transduction system as prostanoid TP receptors, namely the production of inositol-1,4,5-triphosphate (IP$_3$; Thibonnier, 1992; Coleman et al., 1994). This suggests that the antagonism was not due to a direct effect at the IP$_3$ pathway.

Previously, it has been reported that benextramine is a blocker of calcium channels in rat brain with an associated IC$_{50}$ value of ~ 10 μM (Plotek and Atlas, 1983). However, this effect was shown to be reversible (Plotek and Atlas, 1983) and thus cannot explain our data.

Overall, it remains to be clarified whether benextramine interferes with the effector pathway following prostanoid TP receptor activation by U46619 or whether it reduces agonist affinity and/or efficacy via an action at an allosteric receptor site.

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