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Short communication

The α_1-adrenoceptor agonist, SDZ NVI-085, behaves as a potent, competitive antagonist of 5-hydroxytryptamine-induced contraction of rat aorta

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Abstract

In this study we investigated the actions of SDZ NVI-085 ((−)-(4*aR*,10*aR*)-3,4,4*a*,5,10,10*a*-hexahydro-6-methoxy-4-methyl-9-(methylthio)-2*H*-naphth[2,3-*b*)-1,4-oxazine hydrogen malonate), previously classified and employed as a selective α_1-adrenoceptor agonist, in the rat isolated aorta assay. It was shown that, in addition to its α_1-adrenoceptor agonistic action, SDZ NVI-085 behaves as a competitive antagonist of 5-hydroxytryptamine (5-HT)-induced contraction of rat aorta ($pK_B = 8.13 \pm 0.08$). The structurally related α_1-adrenoceptor agonist, SK&F 89748-A (*l*-1,2,3,4-tetrahydro-8-methoxy-5-(methylthio)-2-naphthalenamine hydrochloride), produced inhibition of the 5-HT response only at the highest concentration tested (1 μM) with an associated pA_2 value of 6.0 ± 0.1. These findings suggest that the affinity of SDZ NVI-085 for 5-HT_2 receptors is considerably higher than for α_1-adrenoceptors, which may have implications for its use as a pharmacological tool.

Keywords: α_1-Adrenoceptor; 5-HT (5-hydroxytryptamine, serotonin); 5-HT_2 receptor; Aorta, rat; SDZ NVI-085; SK&F 89748-A

1. Introduction

The naphthoxazine, SDZ NVI-085, has been classified as a selective α_1-adrenoceptor agonist in the rabbit isolated ear artery preparation and the pithed rat (Nozulak et al., 1992), rat vas deferens and perfused kidney (Eltze and Boer, 1992) and rat renal cortex slices (Büscher et al., 1994). It has been suggested that lipophilic, centrally acting α_1-adrenoceptor agonists, like SDZ NVI-085, may be of therapeutic value in some disorders of the central nervous system characterised by symptoms of noradrenergic insufficiency, notably dementia, depression and narcolepsy (Dravid et al., 1991; Renaud et al., 1991; Nozulak et al., 1992; Nishino et al., 1993). Furthermore, various groups have suggested that SDZ NVI-085 may be a useful pharmacological tool for studying α_1-adrenoceptors because of its ability to discriminate between α_1-adrenoceptor subtypes (Renaud et al., 1991; Eltze and Boer, 1992; Nishino et al., 1993; Büscher and Michel, 1994; Michel and Insel, 1994; Büscher et al., 1994).

We now report that SDZ NVI-085, in addition to its α_1-adrenoceptor agonistic action, behaves as a competitive antagonist of 5-hydroxytryptamine (5-HT)-induced contraction of rat aorta. Our findings suggest that the affinity of SDZ NVI-085 for 5-HT_2 receptors is considerably higher than that for α_1-adrenoceptors, which may have implications for the use of this ligand as a pharmacological and therapeutic tool.

2. Materials and methods

2.1. Isolated rat aorta ring preparation

Male Wistar rats (200–350 g) were killed by cervical dislocation and the thoracic aorta was dissected. The aorta was mounted on a length of scoured polythene tubing and placed in a Petri dish containing modified Krebs-Henseleit solution (KHS) of the following (mM) composition: NaCl 119.0, $NaHCO_3$ 25.0, KCl 4.7, KH_2PO_4 1.2, $MgSO_4$ 1.2, glucose 11.0, $CaCl_2$ 0.25 and

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ascorbic acid 0.1 to prevent oxidation of ligands added to the solution. The Ca^{2+} concentration for this assay was thus one-tenth of that of standard KHS, in order to eliminate the spontaneous phasic contractions of the aorta seen in standard KHS (Chemtob et al., 1992; Van der Graaf et al., 1993). The aorta was cleared of surrounding adipose tissue and the endothelium was removed by gentle rubbing of the intimal surface with the polythene tube. The effectiveness of this procedure was confirmed after completion of each concentration-effect curve by the lack of relaxant response to 10 μM of the muscarinic acetylcholine receptor agonist, 5-methylfurmethide. Six to nine ring segments ($\sim$ 4 mm length) were prepared from each aorta and were mounted between two stainless-steel wires in 15 ml organ baths, thermostatically controlled at 37 $\pm$ 0.5°C, containing modified KHS and continuously gassed with 95% O_2 and 5% CO_2. Tissue responses were measured as changes in isometric tension (g) and displayed on potentiometric chart recorders.

2.2. Experimental protocol

Following application of 2 g resting tension, the tissues were allowed to stabilise for 60 min during which time the organ bath fluid was replaced 4 times with pre-warmed KHS at regular intervals. The resting tension was re-established after each replacement of KHS. Single agonist concentration-effect (E/[A]) curves were obtained by cumulative additions as half-log unit concentration increments. Cocaine (30 μM) was present in all experiments to block neuronal uptake.

In the experiments in which the contractile effects of noradrenaline and SDZ NVI-085 were investigated, the tissues were incubated for 90 min before the start of the E/[A] curves with timolol (6 μM) to block β_1/β_2-adrenoceptors and with prazosin (3 nM) or vehicle. In the experiments in which the antagonistic effect of SDZ NVI-085 on the contractile response to 5-HT was investigated, the tissues were incubated for 90 min with pargyline (10 μM) to inhibit monoamine oxidases, prazosin (0.1 μM) and with SDZ NVI-085 or vehicle. Preliminary experiments had shown that the presence of 0.1 μM prazosin had no effect on the 5-HT E/[A] curve (data not shown) and prevented the tissues from contracting in response to SDZ NVI-085, even at the highest concentration (1 μM) tested.

2.3. Analysis

Individual agonist curve data were fitted to the Hill equation using an iterative, least-squares method to provide estimates of midpoint slope (n_H), midpoint location (EC_{50}, estimated as a logarithm) and upper asymptote (α). The effect of drug treatment on these parameters was assessed by one-way analysis of variance (ANOVA) or Student's t-test, as appropriate. Values of $P < 0.05$ were considered to be significant.

When the minimum criteria for competitive antagonism were satisfied, that is when the antagonist produced a parallel rightward shift of the agonist E/[A] curves with no change in upper asymptote, pK_B values were obtained by fitting the individual midpoint location values obtained in the absence and presence of antagonist to the derivation of the Schild equation described by Black et al. (1985). When the Schild plot slope parameter (b) was not significantly different from unity, the data were re-fitted with b constrained to unity so that the antagonist dissociation equilibrium constant (K_B) could be estimated as log $K_B \pm$ S.E. For purposes of display, a Schild plot was then made with slope of unity and intersection of the abscissa scale at the log K_B calculated by the method above.

2.4. Compounds

Compounds were as follows and were obtained from the following sources: cocaine hydrochloride, 5-hydroxytryptamine creatinine sulphate (5-HT), *l*-noradrenaline hydrochloride, pargyline hydrochloride, prazosin hydrochloride and timolol maleate: Sigma Chemical Company, Netherlands; 5-methylfurmethide iodide: Wellcome Research Laboratories, UK; SDZ NVI-085 ((−)-(4*aR*,10*aR*)-3,4,4*a*,5,10,10*a*-hexahydro-6-methoxy-4-methyl-9-(methylthio)-2*H*-naphth[2,3-*b*)-1,4-oxazine hydrogen malonate): generous gift from Sandoz, Switzerland; SK&F 89748-A (*l*-1,2,3,4-tetrahydro-8-methoxy-5-(methylthio)-2-naphthalenamine hydrochloride): generous gift from SmithKline Beecham Pharmaceuticals, USA. Noradrenaline was dissolved and diluted in stoichiometric, aqueous ascorbic acid solution. Prazosin and SK&F 89748-A were dissolved initially in 50% ethanol to give 0.1 mM stock solutions and subsequently diluted in distilled water. All other drugs were dissolved in distilled water. Noradrenaline and 5-HT solutions were made up each day. All other drug stock solutions were stored below −20°C and diluted on the day of the experiment. The maximum volume of drug solution added to the 15 ml organ baths did not exceed 750 μl, corresponding to 5% of the bath volume.

3. Results

3.1. Noradrenaline and SDZ NVI-085 concentration-effect relations

Noradrenaline (1 nM–10 μM) produced a concentration-dependent contraction of the aorta (Fig. 1) and the individual agonist E/[A] curves ($n = 5$) were fitted

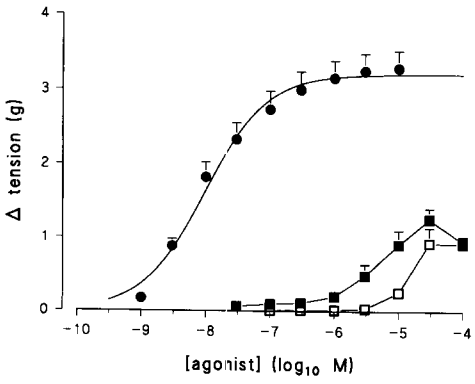

Fig. 1. Concentration-effect curves ($n = 5$) obtained with rat aorta for noradrenaline (●) and SDZ NVI-085 in the absence (■) and presence (□) of 3 nM prazosin. The line shown superimposed on the mean experimental data point for noradrenaline was simulated using the Hill equation (see text for parameter estimates). Error bars indicate S.E.M.

to the Hill equation to obtain estimates of the midpoint location ($pEC_{50} = 8.02 \pm 0.05$), midpoint slope ($n_H = 0.90 \pm 0.06$) and upper asymptote ($\alpha = 3.2 \pm 0.2$ g). SDZ NVI-085 (0.1–30 μM) also produced concentration-dependent contraction (Fig. 1). However, because SDZ NVI-085 produced significant relaxation at higher concentrations, it was not possible to fit the $E/[A]$ data to the Hill equation (Fig. 1). Prazosin (3 nM) produced a significant rightward shift of the contraction phase of the SDZ NVI-085 $E/[A]$ curves and although, due to the limited experimental window, it was not possible to test whether prazosin was behaving as a competitive antagonist, the shift was consistent with a pA_2 value of ~9.1.

3.2. Effect of SDZ NVI-085 on the response to 5-HT

In the presence of 0.1 μM prazosin, 5-HT (0.01–30 μM) produced a concentration-dependent contraction ($pEC_{50} = 7.08 \pm 0.07$), $n_H = 1.37 \pm 0.16$, $\alpha = 1.4 \pm 0.2$ g, $n = 6$, Fig. 2A). SDZ NVI-085 (0.01–1 μM), which had no effect on the basal tone in the presence of 0.1 μM prazosin, produced a concentration-dependent, parallel rightward shift of the 5-HT $E/[A]$ curve without changing the upper asymptote (Fig. 2A). Individual log EC_{50} values were fitted to the competitive model and the Schild slope parameter ($b = 0.97 \pm 0.06$, d.f. = 35) was found to be not significantly different from unity. Because the behaviour of SDZ NVI-085 was consistent with a simple competitive antagonism of 5-HT, a pK_B value (8.13 ± 0.08, d.f. = 36) was estimated with b constrained to unity (Fig. 2B).

The effect on the response to 5-HT of another ligand previously classified as a selective α_1-adrenoceptor agonist, SK&F 89748-A (DeMarinis and Hieble, 1983), was also investigated. This tetralin formed the starting point for the development of SDZ NVI-085 and is closely related to SDZ NVI-085 in terms of chemical structure (Nozulak et al., 1992). In contrast to SDZ NVI-085, SK&F 89748-A only produced a small, but significant, rightward shift of the 5-HT $E/[A]$ curve at the highest concentration tested (1 μM) and a pA_2 value of 6.0 ± 0.1 (d.f. = 14) was estimated. It should be noted that in order to abolish the contractile effect of SK&F 89748-A it was necessary to increase the concentration of prazosin 10-fold (1 μM) for this experiment. However, this had no significant effect on the control 5-HT curve ($pEC_{50} = 6.91 \pm 0.06$, $n_H = 1.88 \pm 0.18$, $\alpha = 1.8 \pm 0.1$ g, $n = 8$).

4. Discussion

In this study, we investigated the actions of SDZ NVI-085, previously classified as a selective α_1-adrenoceptor agonist (see Introduction), in the rat isolated aorta assay. In contrast to Eltze and Boer (1992), we found that 0.1–30 μM SDZ NVI-085 caused concentration-dependent contraction of the aorta. This contraction appeared to be mediated by α_1-adrenoceptors

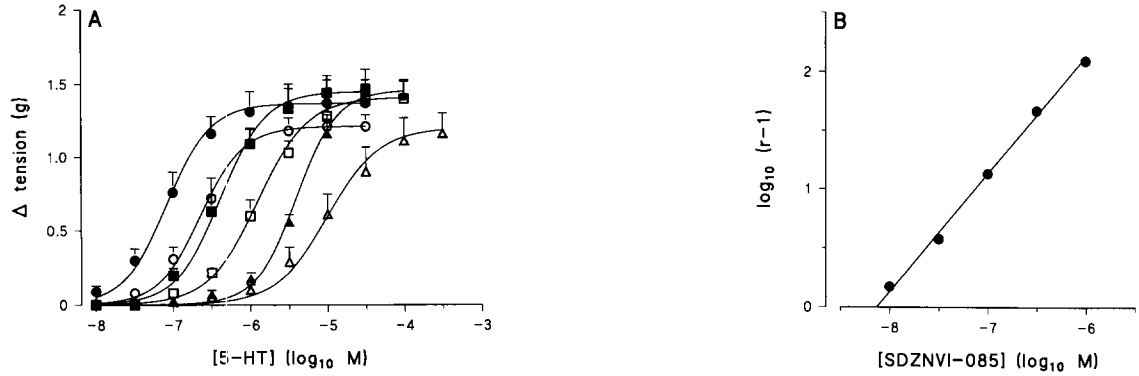

Fig. 2. (A) Concentration-effect curves ($n = 6$–7) obtained with rat aorta for 5-HT in the absence (●) and presence of 0.01 (○), 0.03 (■), 0.1 (□), 0.3 (▲) and 1 μM (△) SDZ NVI-085. The lines shown on the mean (±S.E.M.) experimental data points were simulated using the Hill equation. (B) Associated Schild plot for the interaction between 5-HT and SDZ NVI-085.

because 3 nM prazosin inhibited the SDZ NVI-085 $E/[A]$ curve with an associated pA_2 value of ~ 9.1, consistent with pK_B values for prazosin measured against other α_1-adrenoceptor agonists in this tissue (see, for example, Digges and Summers, 1983; Van der Graaf et al., 1993). An explanation for the difference between Eltze and Boer's (1992) and our results may be the fact that noradrenaline was $\sim$ 7-fold less potent in their assay ($pEC_{50} = 7.20$) than in our assay ($pEC_{50} = 8.02$). This reduced agonist potency could suggest a reduced receptor density or less efficient coupling of occupied receptors in their assay, which could be mirrored by the complete loss of expression of efficacy by SDZ NVI-085.

We showed that SDZ NVI-085, in addition to its action at α_1-adrenoceptors, antagonises the response of rat aorta to 5-HT in a simple, competitive manner. Since the 5-HT-induced contraction of the rat aorta is believed to be mediated by a homogeneous population of 5-HT_2 receptors (Cohen et al., 1981; Killam et al., 1990), this result indicates that SDZ NVI-085 is a potent 5-HT_2 receptor antagonist. In fact, comparison of the pK_B estimate of 8.1 obtained in this study with reported pK_I values at cloned α_1-adrenoceptor subtypes expressed in COS cells (α_{1a}: 6.46; α_{1b}: 4.91; α_{1d}: 5.71; Büscher et al., 1994) suggests that the affinity of SDZ NVI-085 for 5-HT_2 receptors is considerably higher than that for α_1-adrenoceptors. Hence, the classification 'selective α_1-adrenoceptor agonist' appears to be inappropriate in the case of SDZ NVI-085 and the relatively high affinity for 5-HT_2 receptors may have implications for its use as a pharmacological and therapeutic tool. We believe that the possibility of an interaction with 5-HT receptors should be kept in mind when SDZ NVI-085 is employed as a tool to study α_1-adrenoceptors.

To our knowledge, SDZ NVI-085 is the first example of a potent 5-HT_2 receptor antagonist from the class of the hexahydronaphthoxazines. Interestingly, the structurally related α_1-adrenoceptor agonist, the 2-aminotetralin, SK&F 89748-A, displayed an approximately 100-fold lower affinity for the 5-HT_2 receptors in the aorta. Thus, methylation and incorporation of the primary amine of this tetralin into an oxazine ring appear to have enhanced the affinity for 5-HT_2 receptors. It may therefore be suggested that rigidified 2-aminotetralin systems could provide interesting starting points for the development of new 5-HT_2 receptor antagonists.

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