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Short communication

SDZ NVI 085, an α_{1A}-adrenoceptor agonist with 5-HT_{2A} receptor antagonist properties

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Abstract

(±)-SDZ NVI 085 (3,4,4*a*5,10,10*a*-hexahydro-6-methoxy-4-methyl-9-methylthio-2*H*-naphth [2,3-*b*]-1,4-oxazine hydrochloride), an α_1-adrenoceptor agonist, produced a concentration-dependent relaxation (pIC_{50} of 7.2 ± 0.1) in the isolated caudal artery of rat precontracted with serotonin (5-hydroxytryptamine, 5-HT, 1 μM). (±)-SDZ NVI 085 had no effect upon caudal arteries precontracted with vasopressin or U46619 (9,11-dideoxy-11α,9α-epoxymethano-prostaglandin $F_{2\alpha}$). In other studies, (±)-SDZ NVI 085 shifted 5-HT concentration-effect curves to the right, in a concentration-dependent manner, and Schild regression gave a pA_2 estimate of 8.0 (slope of 1.0). Experiments using pharmacological resultant analysis indicated a syntopic interaction of (±)-SDZ NVI 085 with ketanserin (a 5-HT_2 receptor antagonist) toward 5-HT-induced contractions. It is concluded that (±)-SDZ NVI 085 behaves as a reversible competitive 5-HT_{2A} receptor antagonist, a property which may be of importance regarding its pharmacological effects in vivo.

Keywords: Blood vessel; (±)-SDZ NVI 085; 5-HT_{2A} receptor; α_1-Adrenoceptor

1. Introduction

The α-adrenoceptor agonist, (±)-SDZ NVI 085 (3,4,4*a*5,10,10*a*-hexahydro-6-methoxy-4-methyl-9-methylthio-2*H*-naphth [2,3-*b*]-1,4-oxazine hydrochloride), is a rigid structural analog of methoxamine with selectivity for the α_{1A}-adrenoceptor subtype (Eltze and Boer, 1992; Buscher et al., 1994). (±)-SDZ NVI 085 is being assessed currently as a potential treatment for central nervous system (CNS) indications characterized by symptoms of noradrenergic insufficiency, such as senile dementia and severe depression (Dravid et al., 1991). Ligands at α-adrenoceptors often interact with 5-HT_2 receptors and vice-versa (see Leysen, 1985). Therefore, the aim of the present study was to determine the potential effect of (±)-SDZ NVI 085 at 5-HT_{2A} receptors, in vitro, using the caudal artery of rat. The results demonstrate that (±)-SDZ NVI 085 behaves as a reversible competitive 5-HT_{2A} receptor antagonist.

2. Materials and methods

2.1. Tissue preparation

Male Sprague Dawley rats (200–350 g) were killed by asphyxiation with CO_2. The caudal artery was removed carefully and cleaned of adhering tissue. The endothelium was removed and the artery cut into helical strips (5–10 mm long, 1 mm wide). Strips were suspended in water-jacketed tissue baths (37°C) for measurement of isometric tension in oxygenated (95% O_2, 5% CO_2) Krebs' solution (containing (mM): Na^+ (143.5), K^+ (6.0), Ca^{2+} (2.5), Mg^{2+} (1.2), Cl^- (125.8), HCO_3^- (25), $H_2PO_4^-$ (1.2), SO_4^{2-} (1.2)) supplemented with the following: 100 μM ascorbate (to minimize oxidation of noradrenaline), 30 μM cocaine, 30 μM corticosterone (to block neuronal and extraneuronal uptake of noradrenaline respectively), 10 μM indomethacin (to inhibit prostanoid production), 1 μM propranolol (to block β-adrenoceptors), 300 nM idazoxan (to block α_2-adrenoceptors) and 1 μM prazosin (to block α_1-adrenoceptors). The concentration of prazosin selected is at least 1000-fold above its equilibrium

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dissociation constant for α_1-adrenoceptors. Resting tension was set to approximately 4 mN at the beginning of the experiment and tissues were equilibrated for 30 min.

2.2. Inhibition curves

Strips of caudal artery were pre-contracted with either 1 μM 5-HT, U46619 (9,11-dideoxy-11α,9α-epoxymethano-prostaglandin $F_{2\alpha}$) (1 μM; a stable thromboxane analog), or vasopressin (10 nM), and cumulative (±)-SDZ NVI 085 concentration-inhibition curves were obtained at 0.5 log M increments. Concentrations of (±)-SDZ NVI 085 producing half-maximal inhibition (IC_{50}) were interpolated from iterative non-linear fitting of inhibition curves and estimates of pK_B were calculated according to Cheng and Prusoff (1973): $K_B = [IC_{50}]/(1 + ([A]/[A_{50}])$ (Craig, 1993), where A is the 5-HT concentration and A_{50} is the 5-HT concentration producing half-maximal effect. In additional experiments, noradrenaline, phenylephrine, and methoxamine were added in 0.5 log M increments to strips of caudal artery that were pre-contracted with 1 μM 5-HT. Control experiments were performed in the same tissues consecutively with cumulative additions of vehicle to estimate any time-dependent relaxation following precontraction with 1 μM 5-HT.

2.3. Schild analyses

Two concentration-effect curves (E/[A]) to 5-HT were constructed in each tissue, the first serving as the control and the second, test curve, constructed 60 min following incubation with ketanserin (a reference 5-HT_2 receptor antagonist) or (±)-SDZ NVI 085. Concentration ratios for the two curves were determined at concentrations of 5-HT producing half-maximal response (EC_{50}) and were used for construction of Schild regressions for estimation of pA_2 values. Control experiments were performed to estimate time-dependent changes in agonist sensitivity.

2.4. Pharmacological resultant analysis

Two E/[A] curves to 5-HT were constructed. (±)-SDZ NVI 085 (100 or 300 nM) was present during the construction of the first E/[A] curve, whereas the second curve was constructed in the presence of ketanserin (10–300 nM) plus (±)-SDZ NVI 085 (100 or 300 nM). Concentration ratios for the two curves were determined for concentrations of 5-HT producing half-maximal response (EC_{50}), and were used for construction of Schild regressions for ketanserin. Estimates of pK_B for (±)-SDZ NVI 085 were calculated according to the equation: $K_B = [(\pm)\text{-SDZ NVI 085}]/(CR\text{-}1 - [\text{ketanserin}]/K_{B\ \text{ketanserin}})$ (Black et al., 1986), where CR is the ratio of equiactive concentrations of ketanserin in the presence and absence of (±)-SDZ NVI 085. Control experiments were performed to estimate time-dependent changes in agonist sensitivity.

All values presented are expressed as mean ± S.E.M.

2.5. Chemicals

5-HT hydrochloride, methoxamine hydrochloride, cocaine hydrochloride, idazoxan hydrochloride, and prazosin hydrochloride were obtained from Research Biochemicals (Natick, MA, USA). (±)-Noradrenaline, phenylephrine, corticosterone, vasopressin, and U46619 were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Ketanserin, cyanopindolol and (±)-SDZ NVI 085 were synthesized in the Chemistry Department at Roche Bioscience (Palo Alto, CA, USA). Solutions were prepared in deionised water or dimethyl sulphoxide (corticosterone, prazosin, ketanserin, and (±)-SDZ NVI 085). The final concentration of dimethyl sulphoxide in the bathing solution did not exceed 0.1% and had no effect on muscle contraction.

3. Results

(±)-SDZ NVI 085, in the presence of prazosin (1 μM) and idazoxan (0.3 μM), produced a concentration-dependent relaxation (pIC_{50} of 7.2 ± 0.1) in caudal artery strips precontracted with 1 μM 5-HT (Fig. 1). Using the Cheng and Prusoff (1973) correction, a pK_B value of 7.6 ± 0.1 was calculated. Relaxation by (±)-SDZ NVI 085 was not mediated by activation of

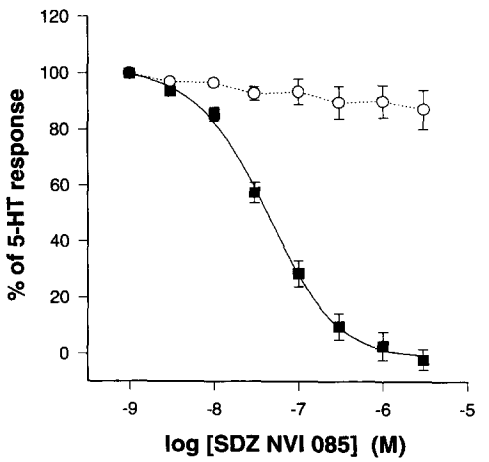

Fig. 1. Concentration-inhibition curve for (±)-SDZ NVI 085 in the rat caudal artery after precontraction with 1 μM 5-HT (■). The dashed line shows the response to vehicle (○). Curves were obtained in the presence of 1 μM idazoxan and 1 μM prazosin. Plot displays data from a representative experiment performed in quadruplicate, and is expressed as percentage of the response to 5-HT (mean ± S.E.M.; $n = 4$).

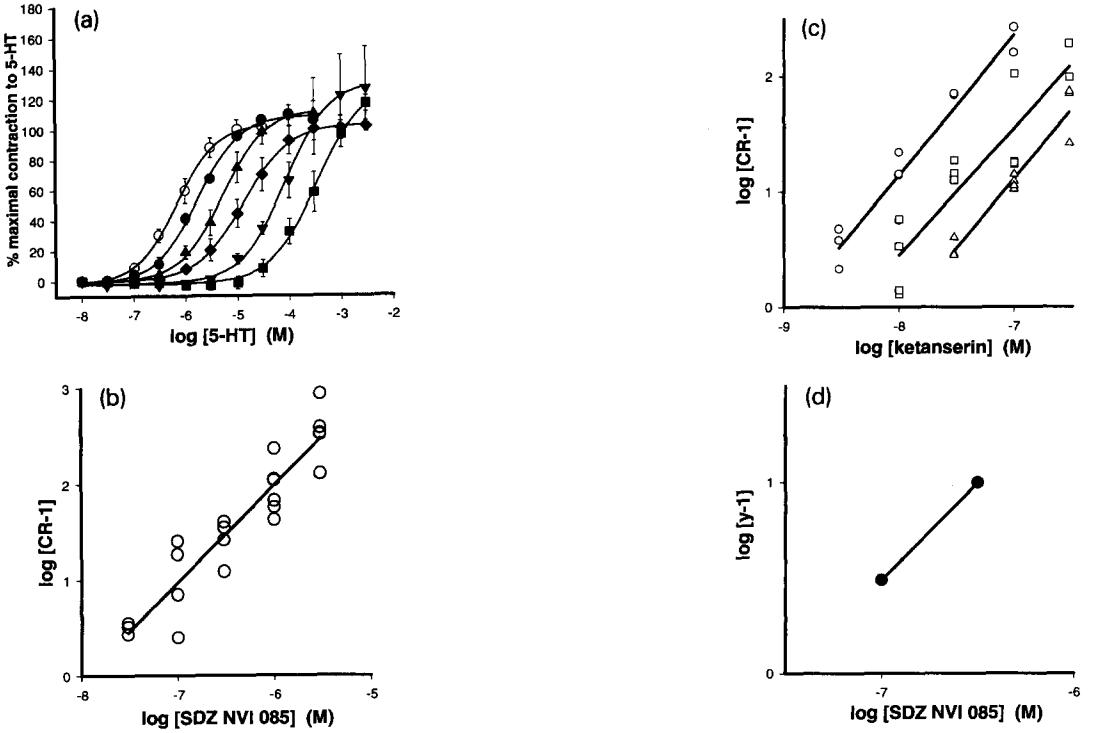

Fig. 2. (A) The effect of the α_1-adrenoceptor agonist, (±)-SDZ NVI 085, on contractile responses to 5-HT in rat caudal artery. Concentration-response curves are shown for 5-HT in the absence of (±)-SDZ NVI 085 (○, $n = 10$) and in the presence of 30 nM (●, $n = 4$), 100 nM (▲, $n = 4$), 300 nM (◆, $n = 4$), 1.0 μM (▼, $n = 6$), and 3.0 μM (■, $n = 5$) of (±)-SDZ NVI 085. Values presented are mean ± S.E.M., expressed as a percentage of the maximal contraction of the first curve. (B) Schild regression for antagonism of 5-HT-induced contraction of rat caudal artery by the (±)-SDZ NVI 085. $pA_2 = 8.0$ (slope = 1.0; 95% confidence limits = 0.83–1.20). (C) Resultant analysis of (±)-SDZ NVI 085 and ketanserin versus antagonism of 5-HT-induced contraction of rat caudal artery. Schild regressions for the reference antagonist, ketanserin, in the absence (○; $pA_2 = 9.0$, slope = 1.2) and presence of (±)-SDZ NVI 085 (100 (□; $pA_2 = 8.4$, slope = 1.1) and 300 nM (△; $pA_2 = 7.9$, slope = 1.2)). (D) Resultant plot of the regressions in (C) for (±)-SDZ NVI 085 ($pA_2 = 7.5$, slope = 1.2) where y is the ratio of equiactive concentrations of ketanserin in the presence and absence of (±)-SDZ NVI 085.

β-adrenoceptors, because 1 μM cyanopindolol failed to inhibit (±)-SDZ NVI 085-induced relaxation (data not shown). The inhibitory effect of (±)-SDZ NVI 085 appeared specific, as phenylephrine and noradrenaline did not inhibit the contractile response to 5-HT. Methoxamine (an α_1-adrenoceptor agonist related structurally to (±)-SDZ NVI 085) elicited a concentration-dependent relaxation at high concentrations only ($IC_{50} > 300$ μM). In other studies, (±)-SDZ NVI 085-induced relaxation was shown to be specific for 5-HT, as it failed to cause a significant relaxation in strips precontracted with U46619 or vasopressin (data not shown).

In order to study the nature of the inhibitory effect of (±)-SDZ NVI 085 toward 5-HT, monophasic $E/[A]$ curves to 5-HT were produced by cumulative additions of the amine. (±)-SDZ NVI 085 (30 to 3000 nM) caused surmountable, parallel, rightward, concentration-dependent displacements of $E/[A]$ curves to 5-HT (Fig. 2A). The corresponding Schild regression yielded a slope not significantly different from 1 (Fig. 2B). Assuming competitive antagonism, the estimated pA_2 value for (±)-SDZ NVI 085 is 8.0 (slope = 1.0; 95% confidence limits = 0.84–1.16).

Ketanserin (a reference 5-HT_2 receptor antagonist) also elicited parallel, rightward shifts of $E/[A]$ curves to 5-HT without a decrease in maximal response. Schild analysis yielded a pA_2 estimate of 9.0 and a slope that is not significantly different from 1 (slope = 1.2; 95% confidence limits = 0.97–1.45; Fig. 2C). Fig. 2C also shows that (±)-SDZ NVI 085 (100 and 300 nM) caused concentration-dependent, rightward shifts in the Schild regression to ketanserin. The displacement of the Schild regressions, as a function of the (±)-SDZ NVI 085 concentrations, yielded a resultant regression with a pK_B of 7.5 (Fig. 2D).

4. Discussion

(±)-SDZ NVI 085 (a structural analog of the commonly known α_1-adrenoceptor agonist, methoxamine) exerts selective agonistic activity for the α_{1A}-adrenoceptor subtype (Renaud et al., 1991; Eltze and Boer, 1992; Buscher et al., 1994). (±)-SDZ NVI 085 has been reported to produce significant alerting effects in animals via stimulation of central α_1-adrenoceptors without imparting notable blood pressure effects

(Nozulak et al., 1992), and is currently being assessed as a potential treatment for CNS indications characterized by symptoms of noradrenergic insufficiency, such as senile dementia and severe depression (Dravid et al., 1991). The aim of the current study was to investigate the specificity of this agonist by studying its interaction with 5-HT_2 receptors using an in vitro preparation of rat caudal artery. In this preparation, 5-HT-induced contraction is mediated by 5-HT_{2A} receptors (Feniuk and Humphrey, 1989).

Results (obtained in the presence of adrenoceptor blockade) show that (±)-SDZ NVI 085 produced a specific concentration-dependent relaxation of caudal artery strips precontracted with 5-HT. Furthermore, (±)-SDZ NVI 085 caused parallel, concentration-dependent, rightward shifts of concentration-effect curves to 5-HT without a decrease in maximal response. Schild regression analysis supports an interaction consistent with simple competitive antagonism for (±)-SDZ NVI 085 versus 5-HT, yielding a pK_B estimate of 8.0.

Ketanserin (a reference 5-HT_2 receptor antagonist) caused surmountable antagonism of the contractile effect of 5-HT and elicited parallel, rightward shifts of the concentration-effect curves to 5-HT without a decrease in the maximal response. Schild regression analysis supports an interaction with a single population of 5-HT receptors yielding a pK_B of 9.0 and a slope that is not significantly different from 1. This result supports a 5-HT_{2A} receptor designation (Feniuk and Humphrey, 1989; Bonhaus et al., 1995). (±)-SDZ NVI 085 (100 and 300 nM) also caused concentration-dependent, rightward shifts in the Schild regressions to ketanserin. This pharmacological resultant analysis (a technique which allows the measurement of the affinity of an antagonist for a receptor when that antagonist may have other confounding actions, Black et al., 1986) yielded a pK_B estimate of 7.5 for (±)-SDZ NVI 085.

The estimates of affinity for (±)-SDZ NVI 085 from Schild regression and resultant analyses (8.0 and 7.5, respectively) agree with the affinity (pK_B) estimated from inhibition curves versus precontraction of caudal arteries with 1 μM 5-HT ($pK_B = 7.6$). Thus, three distinct lines of assessment provide strong support for an antagonistic interaction of (±)-SDZ NVI 085 with the 5-HT_{2A} receptor.

It is concluded that (±)-SDZ NVI 085, in addition to its α_{1A}-adrenoceptor agonist properties, behaves as a potent reversible competitive 5-HT_{2A} receptor antagonist. Therefore, the pharmacological effects of (±)-SDZ NVI 085 may reflect a resultant interaction of α_{1A}-adrenoceptor agonism and 5-HT_{2A} receptor antagonism. Whether this combination represents an advance in the treatment of certain CNS disorders remains for clinical evaluation.

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