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α_2-adrenoceptor antagonist properties of OPC-28326, a novel selective peripheral vasodilator

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1 Antagonistic properties of OPC-28326 ([4-(*N*-methyl-2-phenylethylamino)-1-(3,5-dimethyl-4-propionyl-aminobenzoyl)] piperidine hydrochloride monohydrate), a selective peripheral vasodilator, were investigated by analysing the data from functional studies in various tissues from the rat and binding studies of the drug to α_2-adrenoceptor subtypes.

2 Using a human recombinant receptor and rat kidney cortex, we found that OPC-28326 displays affinities to α_{2A}-, α_{2B}- and α_{2C}-adrenoceptors with K_i values of 2040, 285, and 55 nM, respectively. The K_i values of yohimbine for α_{2A}-, α_{2B}-, and α_{2C}-adrenoceptors were 3.0, 2.0 and 11.0 nM, respectively.

3 B-HT 920, an α_2-adrenoceptor agonist, produced a pressor response *via* peripheral postsynaptic α_2-adrenoceptor stimulation (thought to be an α_{2B}-subtype) in a reserpine-pretreated pithed rat preparation. OPC-28326 (3–30 mg kg^{-1}, i.v.) and yohimbine (0.3–3 mg kg^{-1}, i.v.) caused dose-dependent rightward shift in the pressor dose-response curve induced by B-HT 920. The apparent pA_2 values were 1.55 (0.87–2.75, 95% confidence interval) and 0.11 (0.06–0.21) mg kg^{-1}, respectively. The potency of OPC-28326 was about 14 times less than that of yohimbine.

4 Clonidine inhibited the tension developed by electrical stimulation, of the rat vas deferens, by its peripheral presynaptic $\alpha_{2A/D}$-adrenoceptor action. OPC-28326 (1–100 μM) and yohimbine (10–1000 nM) caused a rightward shift in the concentration-response curve of clonidine. The pA_2 values were 5.73 (5.54–5.91) and 7.92 (7.84–8.01), respectively, providing evidence for a potency of OPC-28326 of about 155 times less than that of yohimbine.

5 Mydriasis was induced by brimonidine *via* stimulation of central $\alpha_{2A/D}$-adrenoceptors in anaesthetized rats. Intravenous OPC-28326 had no effect on this action, even at a very high dose of 10 mg kg^{-1} i.v., while yohimbine (0.1–0.3 mg kg^{-1} i.v.) inhibited mydriasis in a dose-dependent manner, indicating that OPC-28326 was at least 100 times less potent than yohimbine in regard to the anti-mydriatic effect.

6 These data suggest that OPC-28326 preferentially exerts peripheral and postsynaptic antagonistic actions on the α_{2B}- and α_{2C}-adrenoceptor subtypes.
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Keywords: OPC-28326; peripheral vasodilator; α_2-adrenoceptor; antagonist; postsynaptic; yohimbine; vas deferens; pithed rat; blood pressure; mydriasis

Abbreviations: CI, confidence interval; CNS, central nervous system; CR, concentration ratio; dBP, diastolic blood pressure; DR, dose ratio

Introduction

OPC-28326 (4-(*N*-methyl-2-phenylethylamino)-1-(3,5-dimethyl-4-propionyl-aminobenzoyl) piperidine hydrochloride monohydrate, Figure 1) is a newly developed selective vasodilator of hindlimb blood vessels; the drug increases femoral artery blood flow, but exerts little effect on other cardiovascular parameters (Orito *et al*., 1999). OPC-28326 inhibited blood flow reduction induced by brimonidine, a selective α_2-adrenoceptor agonist (Guimaraes & Nunes, 1990; Thomas *et al*., 1994), in the rat hindlimb preparations (Orito *et al*., 1999). Thus, one of the vasodilator mechanisms of this compound probably involves an antagonistic action on α_2-adrenoceptors (Orito *et al*., 1999).

The α_2-adrenoceptors have been subdivided into three subtypes, α_{2A}-, α_{2B}-, and α_{2C}-, based on ligand binding and molecular cloning studies (Harrison *et al*., 1991; MacDonald *et al*., 1997). In mice lacking α_{2B}-adrenoceptors, the pressor response induced by an α_2-adrenoceptor agonist was absent (Link *et al*., 1996). The central hypotensive action of the α_2-adrenoceptor agonist was absent in mice lacking the α_{2A}-adrenoceptors (MacMillan *et al*., 1996). It has also been reported that presynaptic α_2-adrenoceptors are similar to α_{2A}-adrenoceptors (Smith & Docherty, 1992; Daniel *et al*., 1995). It has been suggested that each subtype of α_2-adrenoceptors has a distinctive role in haemodynamics. Thus, information on the exact subtype of the α_2-adrenoceptor that OPC-28326 may preferably binds is very important and bears on the

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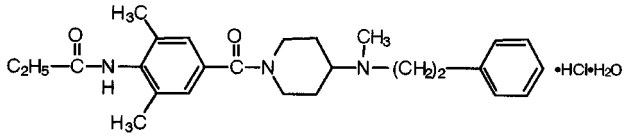

Figure 1 Chemical structure of OPC-28326.

drug's mechanism of action as a selective peripheral vasodilator.

The α_2-adrenoceptors are anatomically located in both pre- and post-synaptic areas and their effects on haemodynamics are different from each other (Docherty, 1998). The central α_2-adrenoceptors have a distinctively different function from the peripheral one (Docherty, 1998). Thus, it is important to elucidate the mechanisms by which OPC-28326 acts as an α_2-adrenoceptor antagonist at these sites.

In the present study, the α_2-adrenoceptor blocking property of OPC-28326 is demonstrated in terms of its pre- and postsynaptic and central antagonistic actions, and compared with those of yohimbine. The relationship of the receptor subtype selectivity with α_2-adrenoceptors and the antagonistic action of OPC-28326 are discussed.

Methods

Receptor binding assay

The α_{2A}- and α_{2C}-adrenoceptor binding assays were performed by a modified method previously reported (Uhlén *et al*., 1994). Membranes expressing the human α_{2A}- and α_{2C}-adrenoceptor, respectively were prepared in 75 mM Tris-HCl buffer containing 12.5 mM $MgCl_2$ and 2 mM EDTA, pH 7.4. A 6 μg aliquot of membranes was incubated with 1 nM [^{3}H]-MK-912 for 60 min at 25°C. Non-specific binding was estimated in the presence of 10 μM WB 4101. Membranes were filtered and washed four times and the filters were counted to determine the amount of [^{3}H]-MK-912 specifically bound. The α_{2B}-adrenoceptor binding assay was performed by a modified method as described previously (Connaughton & Docherty, 1990). Kidney cortical membranes of male Wistar rats weighing 175 $\pm$ 25 g were prepared in 50 mM Tris-HCl buffer containing 5 mM EDTA, pH 7.4. A 7.5 mg aliquot of membranes was incubated with 2 nM [^{3}H]-yohimbine for 30 min at 25°C. Nonspecific binding was estimated in the presence of 10 μM phentolamine. The membranes were filtered and washed three times and the filters were counted and the effect of OPC-28326 on [^{3}H]-yohimbine specific binding was quantitated.

'Specific binding' was defined as the difference between total and nonspecific binding. Under above incubation conditions, specific binding was 90–95% of total binding in each assay. Concentration-response curves for the inhibition of radioligand binding were constructed from the data derived from experiments in which duplicate samples were incubated in the presence of 10 nM – 100 μM of OPC-28326. Three separate experiments were performed for each ligand. K_i values were obtained from the IC_{50} using the equation

$$K_i = IC_{50}/(1 + [L]/K_D), \quad (1)$$

where [L] = concentration of radioligand and K_D = affinity constant of radioligand (Cheng & Prusoff, 1973). The binding of OPC-28326 to the α_2-adrenoceptor subtypes were carried out at Panlabs Taiwan, Ltd. (Taipei, Taiwan, Republic of China).

Functional properties of OPC-28326 as an α_2-adrenoceptor antagonist

In the following experiments, Male Sprague-Dawley rats (SLC, Shizuoka, Japan), weighing 215–373 g, were used. Three to five rats each were housed in individual cages under the following conditions: 23 $\pm$ 2°C, 60 $\pm$ 10% humidity, and lit daily from 07:00 to 19:00 h in a controlled room. The rats received laboratory chow and water *ad libitum*. The care and handling of the animals were in accordance with 'The Guidelines for Animal Experimentation in Otsuka Pharmaceutical Co., Ltd.; October 01, 1994'.

Peripheral postsynaptic α_2-adrenoceptor blocking action – pithed rats

We examined the effect of OPC-28326 on pressor response induced by B-HT 920, an α_2-adrenoceptor agonist, and compared with that of yohimbine in reserpine-pretreated pithed rats. The B-HT 920-induced pressor response was produced *via* an action on peripheral postsynaptic α_2-adrenoceptors (thought to be the α_{2B}-subtype) stimulation. Rats were treated with reserpine (5 mg kg^{-1}, i.p.) and anaesthetized with diethyl ether 16–24 h later. After cannulating the trachea, animals were pithed by inserting a steel rod into the spinal canal *via* the orbit. Artificial ventilation was started with a tidal volume of 10 ml kg^{-1} at a rate of 60 min^{-1}. A catheter was placed in the carotid artery and connected to an amplifier (2238, NEC Medical Systems, Tokyo, Japan) *via* a pressure transducer (SPB-105, NEC Medical Systems) to measure blood pressure. Another catheter was positioned in the jugular vein for intravenous administration of drugs. Atropine (1 mg kg^{-1}) and propranolol (1 mg kg^{-1}) were administered intravenously. After the animal had stabilized, OPC-28326, yohimbine, or its vehicle (distilled water) was administered intravenously. Diastolic blood pressure (dBP) was used for evaluating pressor responses. Two minutes after the administration of the drugs or its vehicle, a dose-response curve for B-HT 920, a selective agonist for the α_2-adrenoceptor (van Meel *et al*., 1981; Kobinger & Pichler, 1981), was generated. Because the pressor response of B-HT 920 was long lasting, the drug was administered cumulatively. Blood pressure was recorded on a thermal pen recorder (Recti-Horiz-8K, NEC Medical Systems).

Peripheral presynaptic α_2-adrenoceptor blocking action – isolated rat vas deferens

In the rat vas deferens, clonidine inhibited the tension developed by electrical stimulation, by an action on peripheral presynaptic $\alpha_{2A/D}$-adrenoceptors. We examined the effect of OPC-28326 on concentration-response curves for clonidine and compared the data with that of yohimbine. Rats were anaesthetized with sodium pentobarbital (50 mg kg^{-1}, i.p.) and exsanguinated. Both vasa deferentia

were excised and bisected. The prostatic portion of the vasa was suspended in a water-jacketed organ bath filled with modified Krebs-Henseleit solution (mM): NaCl 119, KCl 4.7, $MgSO_4$ 1.0, KH_2PO_4 1.2, $NaHCO_3$ 25, $CaCl_2$ 2.5, and glucose 11. Propranolol was routinely added to the solution at a final concentration of 1 μM and the solution was gassed with 95% O_2+5% CO_2 at 37°C. The preparation was stimulated *via* platinum electrodes with square wave pulses of 10 V and 1 ms duration at a frequency of 0.1 Hz. The resting tension was set at 1.0 g. Developed tension was recorded by a thermal pen recorder (Recti-Horiz-8K, NEC Medical Systems) by means of strain-gauge transducers (UL-20GR, Shinko, Tokyo, Japan). The inhibitory response to clonidine, a preferential α_2-adrenoceptor agonist, was expressed as a percentage of initial contractile force induced by electrical stimulation.

When the force of contraction had stabilized, a concentration-response curve was first obtained for the inhibition of contraction produced by clonidine. The drug was removed by three washouts at intervals of >20 min. After reequlibration, OPC-28326 (1–100 μM), yohimbine (10–1000 nM), or vehicle (distilled water) was added to the bath. Twenty minutes after application, a concentration-response curve was again obtained for the inhibition of the contraction produced by clonidine. At least two concentration-response curves for the inhibition of clonidine-induced contraction were obtained for each tissue; time control studies demonstrated that agonist sensitivity remained constant (data not shown).

Clonidine, when added at concentrations above 10 nM, may adhere to the glass and/or epoxy resin material of the bath and to the electrode assembly, resulting in contamination of the Krebs solution in subsequent experiments (MacDonald & McGrath, 1980). Thus, particular attention was paid to decontamination by washing the bath and electrode assembly thoroughly with hydrochloric acid and detergent after every experiment.

Central α_2-adrenoceptor blocking action–mydriasis in rats

Mydriasis was induced by brimonidine, an α_2-adrenoceptor agonist, *via* stimulation of central $\alpha_{2A/D}$-adrenoceptors in anaesthetized rats. We examined the effect of OPC-28326 on concentration-response curve for brimonidine and compared the data with that of yohimbine. Rats were anaesthetized with sodium pentobarbital (50 mg kg^{-1}, i.p.) and a polyethylene catheter was inserted into the femoral vein for drug administration. After induction of mydriasis by intravenous administration of brimonidine (100 μg kg^{-1}), OPC-28326 (0.3–10 mg kg^{-1}), yohimbine (0.1–3 mg kg^{-1}), or vehicle (distilled water) was administered *via* the catheter, and the change in pupil diameter was measured. Alterations in pupil diameter are expressed as a percentage of the increase in the diameter induced by brimonidine. The mydriasis elicited by brimonidine (100 μg kg^{-1}) was stable from 3 to 20 min after administration (data not shown), hence the dose-response to test compounds was also obtained during same time period after administration.

Pupil diameter was measured by means of a magnifier lens (PEAK, Japan), with a 10×magnification. The lens was held close to, but not touching the corneal surface. Changes in pupil diameter induced by drugs were measured using a graticule in the magnifiers lens (0.1 mm increments). The lighting was kept at steady intensity throughout the experiments.

Drugs

OPC-28326 (synthesized at Otsuka Pharmaceutical Co., Ltd., Tokyo, Japan), yohimbine (Sigma Co., MO, U.S.A.), B-HT 920 (Sigma), clonidine (Sigma) and atropine (Sigma) were dissolved respectively, and diluted in distilled water to obtain the desired concentrations. With regard to the binding assay, OPC-28326 was dissolved in 0.5% dimethylsulfoxide (Wako Pure Chemicals, Osaka, Japan). Propranolol was dissolved in distilled water, or modified Krebs-Henseleit solution. Brimonidine (Sigma) was dissolved in dimethylformamide (Wako Pure Chemicals). Reserpine (Sigma) was dissolved in citric acid solution (4%, w w^{-1}).

Statistical analysis

Data are expressed as mean±s.e.mean. Slope of the Schild plot, pA_2, apparent pA_2, and IC_{50}, and their 95% confidence intervals (95% CI) were estimated. Differences were considered statistically significant at $P<0.05$. The differences between basal values of all groups were analysed by one-way ANOVA. In experiments using pithed rats, the apparent pA_2 values for OPC-28326 and yohimbine were estimated by plotting log (dose ratio (DR) −1) against the negative log of the antagonist dose (Arunlakshana & Schild, 1959). The DR was obtained using the equation

$$DR = ([ED_{50\%}] \text{ after drug administration})/([ED_{50\%}] \text{ after vehicle administration}), \quad (2)$$

where $ED_{50\%}$ is the dose at which produced a 50% of maximum pressor response induced by B-HT 920 in control group. In the experiments with rat vasa deferentia, the pA_2 values for OPC-28326 and yohimbine were estimated by plotting log (concentration ratio (CR) −1) against the negative log of the molar concentration of antagonist. CR was obtained using the equation

$$CR = ([IC_{50}] \text{ after drug application})/([IC_{50}] \text{ before drug application}), \quad (3)$$

where IC_{50} is the dose of clonidine which induced a half maximal inhibition of the tension developed by electrical stimulation. For the mydriasis experiments, the IC_{50} and its 95% CI were determined by log-logit regression analysis.

Results

Receptor binding assay

The binding affinities of OPC-28326 for the α_2-adrenoceptor subtypes were determined by radioligand displacement assays. The K_i values and slope factors (in parenthesis) of OPC-28326 for α_{2A}-, α_{2B}-, and α_{2C}-adrenoceptors were 2040±40 nM (0.8±0.1), 285±43 nM (0.9±0.06), and 55±8 nM (1±0.2), respectively. The K_i values of yohimbine for α_{2A}-, α_{2B}-, and α_{2C}-adrenoceptors were 3.0, 2.0 and 11.0 nM, respectively.

Peripheral postsynaptic α_2-adrenoceptor blocking action–pithed rats

The basal dBP in all groups examined are shown in Table 1. There were no statistical differences among the basal values of OPC-28326 (3, 10, and 30 mg kg^{-1}) and its vehicle groups, or among those of yohimbine (0.3, 1, and 3 mg kg^{-1}) and its vehicle groups. As shown in Figure 2, B-HT 920 dose-dependently increased dBP and almost maximally by approximately 80 mmHg at the highest dose of 1000 μg kg^{-1}. The $ED_{50\%}$ for B-HT 920 in the control group was 8.1$\pm$1.7 μg kg^{-1} (n=5). The dose-response curve for B-HT 920 was shifted rightward in a dose-dependent manner by pretreatment with OPC-28326 (3, 10, and 30 mg kg^{-1}). Yohimbine also shifted the dose-response curve of B-HT 920 dose-dependently (Figure 2). Dose-response curves for OPC-28326 (3, 10, and 30 mg kg^{-1}) and its vehicle and yohimbine (0.3, 1, and 3 mg kg^{-1}) and its vehicle were parallel. Schild plot analysis showed that the slopes (95% CI) of OPC-28326 and yohimbine were 1.12 (0.81–1.42) (n=5) and 1.10 (0.81–1.39) (n=5), respectively. These values were not statistically different from unity (Figure 3), suggesting that antagonism by these compounds is of a competitive nature despite the fact that maximum responses were not reached with each concentration of antagonist. The apparent pA_2 (95% CI) of OPC-28326 and yohimbine are 1.55 (0.87–2.75) and 0.11 (0.06–0.21) mg kg^{-1}, respectively.

Table 1 Basal diastolic blood pressure (dBP) in pithed rats (mean$\pm$s.e.mean, n=5)

Group	*dBP* (mmHg)	*One-way ANOVA* P *value*
Vehicle (distilled water)	57.2$\pm$3.5	
OPC-28326 (3 mg kg^{-1})	62.0$\pm$5.4	
OPC-28326 (10 mg kg^{-1})	58.6$\pm$2.8	
OPC-28326 (30 mg kg^{-1})	60.1$\pm$3.1	0.8362
Yohimbine (0.3 mg kg^{-1})	59.2$\pm$3.5	
Yohimbine (1 mg kg^{-1})	62.0$\pm$5.8	
Yohimbine (3 mg kg^{-1})	60.8$\pm$3.8	0.8674

Basal values were not statistically significant among OPC-28326 (3, 10 and 30 mg kg^{-1}) and its vehicle and among yohimbine (0.3, 1 and 3 mg kg^{-1}) and its vehicle (one-way ANOVA).

Peripheral presynaptic α_2-adrenoceptor blocking action–rat isolated vas deferens

Table 2 shows the basal contractile force of rat vas deferens induced by field stimulation of control and each dose in the OPC-28326 and yohimbine groups. There were no statistical differences among the basal contractile force in any group (Table 2). The contractile force was not different among any groups, even after application of distilled water (control) or at all doses of OPC-28326 and yohimbine (Table 2). As shown in Figure 4, clonidine inhibited the contraction induced by electrical stimulation in a concentration-dependent manner and reached almost complete inhibition at 30 nM. The concentration-response curve for clonidine was shifted right-

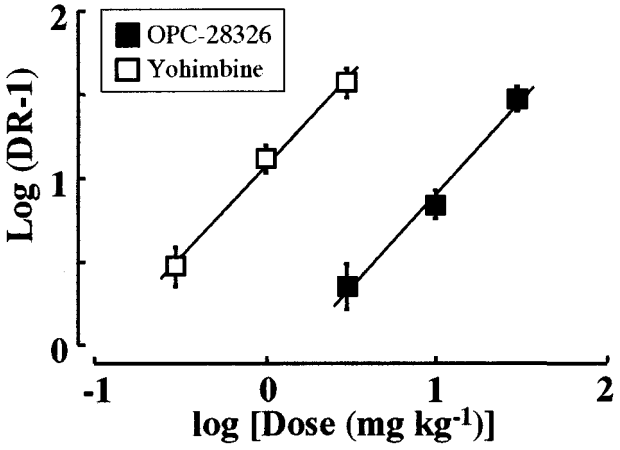

Figure 3 Schild plot illustrating the antagonism by OPC-28326 and yohimbine of the pressor response induced by B-HT 920 in pithed rats. Each point is mean$\pm$s.e.mean of five animals. DR: dose ratio. The slopes of regression lines of both compounds are not statistically different from unity. The calculated apparent pA_2 (95% CI) of OPC-28326 and yohimbine are 1.55 (0.87–2.75) mg kg^{-1} and 0.11 (0.06–0.21) mg kg^{-1}, respectively.

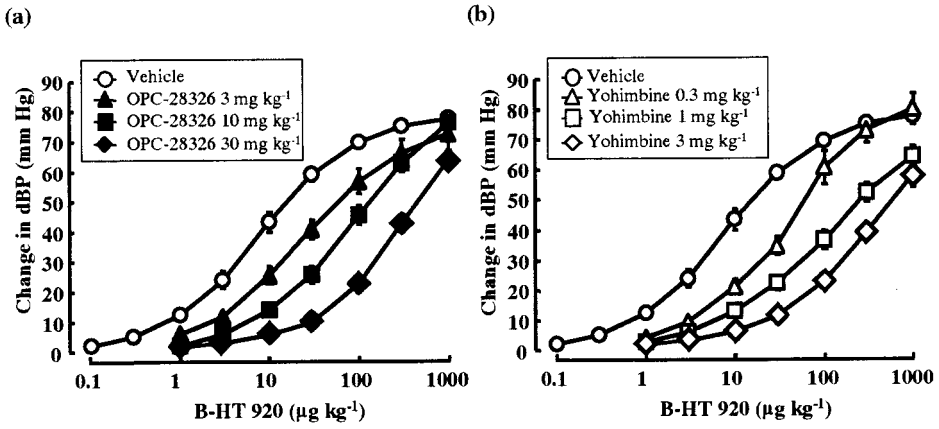

Figure 2 (a) Effect of intravenous OPC-28326 (3, 10, and 30 mg kg^{-1}) and its vehicle (distilled water) on the B-HT 920-induced pressor response in pithed rats. (b) Effect of intravenous yohimbine (0.3, 1, and 3 mg kg^{-1}) and its vehicle on the B-HT 920-induced pressor response in pithed rats. Values are expressed as mean$\pm$s.e.mean of five animals.

ward by pretreatment with OPC-28326 (1, 10, and 100 μM), or yohimbine (10, 100, and 1000 nM) in a concentration-dependent manner (Figure 4). OPC-28326 and yohimbine data exhibited linear Schild plots with a slope (95% CI) of 0.95 (0.80 – 1.10) ($n=6$), and of 1.03 (0.95 – 1.10) ($n=6$), respectively (Figure 5). These values are close to the theoretical value of unity, suggesting that these compounds antagonize presynaptic α_2-adrenoceptor action in a competitive manner. The pA_2 values (95% CI) for OPC-28326 and yohimbine were 5.73 (5.54 – 5.91) and 7.92 (7.84 – 8.01), respectively.

In a preliminary study, the inhibitory action of clonidine against the tension developed by electrical stimulation was not affected by 10 nM prazosin, an α_1-adrenoceptor antagonist, or by 100 nM desipramine, a noradrenaline uptake inhibitor (data not shown).

Central α_2-adrenoceptor blocking action – mydriasis in rats

Pupil diameter was 0.2 ± 0.0 mm ($n=6$) under stable light conditions in our laboratory. Stable mydriasis was obtained following intravenous administration of brimonidine (100 μg kg^{-1}), and was maintained for 20 min; the pupil diameter increased to 3.9 ± 0.0 mm ($n=6$), 3.9 ± 0.1 ($n=6$), and 3.9 ± 0.1 mm ($n=6$) in the vehicle, OPC-28326, and yohimbine groups, respectively. When OPC-28326 was administered intravenously to rats, the mydriatic response induced by brimonidine was unaffected, even at the highest dose of 10 mg kg^{-1} (Figure 6). Yohimbine (0.3, 1, and 3 mg kg^{-1}, i.v.), on the other hand, reversed the mydriatic response to brimonidine dose-dependently (Figure 6); pupil diameter decreased to 0.5 ± 0.0 mm at 3 mg kg^{-1}. The IC_{50} (95% CI) was 0.21 (0.13 – 0.33) mg kg^{-1}. The vehicles for OPC-28326 and yohimbine did not affect the brimonidine-elicited mydriasis (data not shown).

Table 2 Basal and drug-treated contraction of electrically stimulated (10 V, 1 ms, 0.1 Hz) and prostatic rat vas deferens (mean $\pm$ s.e.mean, $n=6$)

Group	*Basal value* (mg)	*After drug application* (mg)
Vehicle (distilled water)	800 ± 125	811 ± 131
OPC-28326 (1 μM)	873 ± 25	877 ± 25
OPC-28326 (10 μM)	753 ± 158	712 ± 139
OPC-28326 (100 μM)	759 ± 88	778 ± 91
Yohimbine (10 nM)	831 ± 162	865 ± 172
Yohimbine (100 nM)	738 ± 76	763 ± 92
Yohimbine (1000 nM)	893 ± 118	901 ± 121
P value	0.9449	0.9117

Basal values were not statistically significant among all groups (one way ANOVA). Values after respective drug application were not statistically significant among all groups (one way ANOVA).

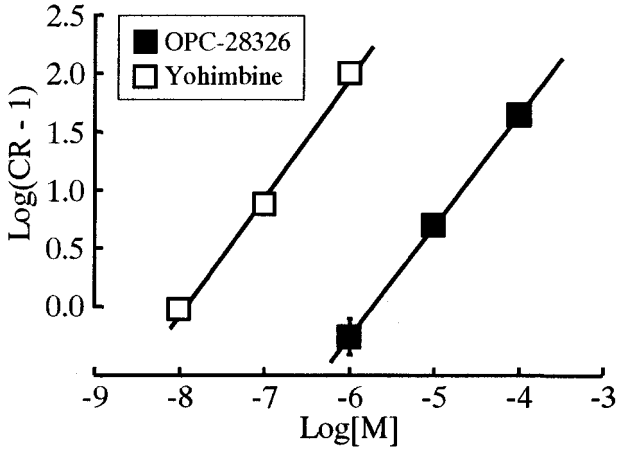

Figure 5 Schild plot illustrating the antagonism by OPC-28326 and yohimbine of the inhibitory effect of clonidine on the electrically-stimulated prostatic portion of isolated rat vas deferens. Each point is mean $\pm$ s.e.mean of six preparations. CR: concentration ratio. The slopes of regression lines of both compounds are not statistically different from unity. The pA_2 (95% CI) of OPC-28326 and yohimbine were ascertained to be 5.73 (5.54 – 5.91) and 7.92 (7.84 – 8.01), respectively.

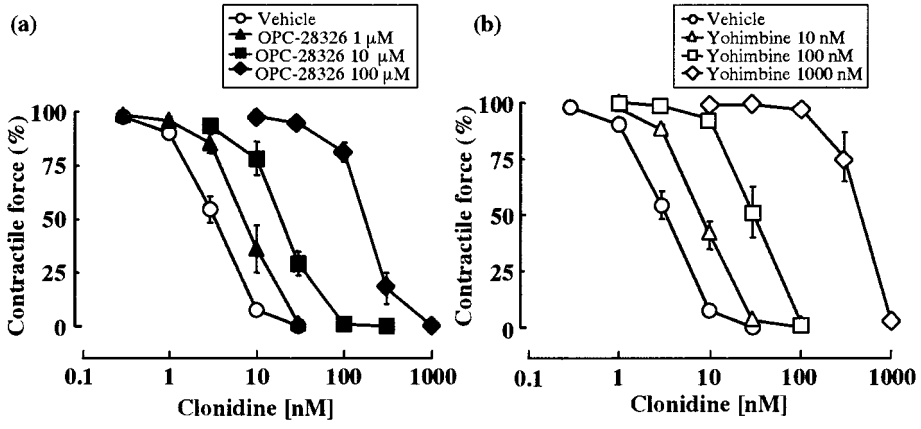

Figure 4 (a) Effect of OPC-28326 (1, 10, and 100 μM) and its vehicle (distilled water) on clonidine's inhibitory action against contraction induced by electrical stimulation (10 V, 1 ms, 0.1 Hz) in the prostatic portion of isolated rat vas deferens. (b) Effect of yohimbine (10, 100, and 1000 nM) and its vehicle on clonidine's inhibitory action against contraction induced by electrical stimulation (10 V, 1 ms, 0.1 Hz) in the prostatic portion of isolated rat vas deferens. Values are expressed as mean $\pm$ s.e.mean of five preparations.

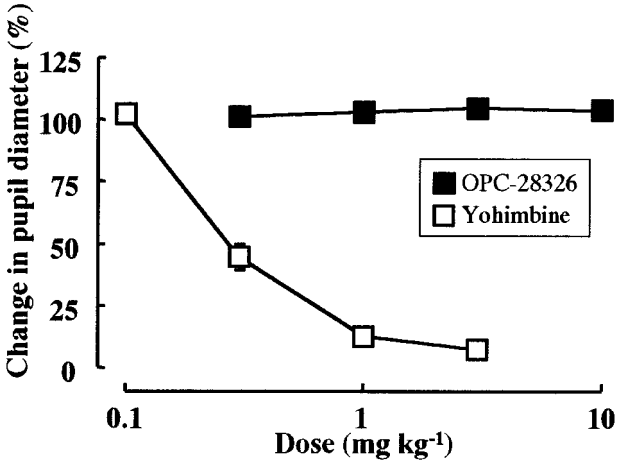

Figure 6 Effects of OPC-28326 and yohimbine on brimonidine-elicited mydriasis. OPC-28326 did not affect on the mydriasis. On the other hand, yohimbine inhibited the mydriatic action of brimonidine, dose-dependently. Values are expressed as mean±s.e.mean of six animals.

Discussion

In the present study, the α_2-adrenoceptor subtype binding property of OPC-28326 was revealed with K_i values of OPC-28326 for the α_{2A}-, α_{2B}-, and α_{2C}-adrenoceptors of 2040±40, 285±43 and 55±8 nM, respectively. The rank order of the potency of affinity was α_{2C}- > α_{2B}- > α_{2A}-adrenoceptors. The K_i values of yohimbine for α_{2A}-, α_{2B}-, and α_{2C}-adrenoceptors were 3.0, 2.0 and 11.0 nM, respectively (data of Panlabs) The K_i values of yohimbine were consistent with previously reported values cited in The IUPHAR Compendium of Receptor Characterization and Classification (1998).

In reserpine-pretreated pithed rats, yohimbine, at a dose of 1 mg kg^{-1}, shifted the dose-response curve for the B-HT 920-induced pressor response to the right approximately 15 fold; van Meel *et al*. (1981) in their investigation of the peripheral α_2-adrenoceptor action of some compounds using pithed rats found that yohimbine, at a dose of 1 mg kg^{-1}, shifted the dose-response curve of B-HT 920 to the right by 20 fold. The latter result is consistent with that found in the present study. Thus, the postsynaptic α_2-adrenoceptor antagonistic action of OPC-28326 and yohimbine could be quantitatively evaluated in the present study. In α_{2B}-adrenoceptors knockout mice, the pressor response induced by α_2-adrenoceptors agonist was absent (Link *et al*., 1996), suggesting that postsynaptic α_{2B}-adrenoceptors are responsible for the vascular contraction induced by α_2-adrenoceptors agonists. In this sense, it was suggested that the pressor response of B-HT 920 may be induced *via* an α_{2B}-adrenoceptor action in the pithed rat. Because both OPC-28326 and yohimbine inhibited the B-HT 920-induced pressor response in a dose-dependent manner with slopes of the Schild plots not different from unity, we conclude that these compounds antagonize postsynaptic α_{2B}-adrenoceptor activity in a competitive manner. Judging from the apparent pA_2 values, the antagonistic activity of OPC-28326 was about 14 times less potent than that of yohimbine. This potency ratio is consistent with previously obtained results showing that the inhibitory action of OPC-28326 (1 μM) against brimonidine-induced flow reduction was almost identical to that of yohimbine (100 nM) in rat perfused hind limb preparations (Orito *et al*., 1999). From the K_i values for α_{2B}-adrenoceptor binding, we conclude that OPC-28326 is about 140 times less potent than yohimbine.

In isolated vas deferens, contraction may be elicited by field stimulation that causes a release of neurotransmitters such as ATP and/or noradrenaline (Major *et al*., 1989). If noradrenaline were released from nerve terminals, it would act on presynaptic autoreceptors and inhibit further noradrenaline release. The released noradrenaline would also act on postsynaptic α_1-adrenoceptors and facilitate contractile action. Thus, 'pure' presynaptic α_2-adrenoceptor antagonistic actions of test compounds cannot be determined in this type of experimental condition. Brown *et al*. (1983) have reported that the non-adrenergic component is dominant in the contraction induced by electrical stimuli in the prostatic portion of the vas deferens when stimulation was carried out at a lower frequency, viz., 0.1 Hz. Thus, the same stimulation frequency was used in the present study. Although an α_1-adrenoceptor antagonist and uptake inhibitor would affect electrically induced contraction if noradrenaline acted as a transmitter, we ascertained that prazosin and desipramine did not affect contraction. These results then suggest that noradrenaline may not contribute to the contraction elicited by electrical field stimulation under our experimental conditions. Thus, the potency of action on the presynaptic α_2-adrenoceptor was evaluated based on the effect against the inhibitory action of the field stimulation-induced contraction of the α_2-adrenoceptor antagonist. The presynaptic α_2-adrenoceptors of the rat vas deferens are reported to be of the α_{2A}-subtype (Smith & Docherty, 1992; Molderings & Göthert, 1995). In the present study, both OPC-28326 and yohimbine shifted the concentration-response curve of clonidine rightward and the slopes of the Schild plots were not different from unity. These results suggest that OPC-28326 and yohimbine act on the α_{2A}-adrenoceptor in a competitive manner. However the pA_2 values of OPC-28326 was about 155 times less potent than that of yohimbine and the K_i value of OPC-28326 for binding to the α_{2A}-adrenoceptor indicates that OPC-28326 is about 680 times less potent than that of yohimbine.

Although the ratio of the K_i values and the ratio of functional antagonistic actions in the *in vivo* or *in vitro* experiments do not exactly correspond with each other, as described above, the selectivity of OPC-28326 to postsynaptic α_2-adrenoceptors may be delineated by the selectivity to the α_{2B}-adrenoceptor. We do not have an explanation at this time for this difference between the potency ratios in binding and the functional studies but it is possible that as with other drugs, protein binding and/or distribution of compounds may play a role.

The preferential antagonistic action of OPC-28326 to the α_{2B}-adrenoceptors compared to the α_{2A}-adrenoceptors provides evidence that this drug may be beneficial as a peripheral vasodilator. Hentrich *et al*. (1986) reported that electrically evoked overflow of transmitter was inhibited by an α_2-adrenoceptor agonist and was facilitated by antagonists in human pulmonary arteries. Thus, the vasodilatory action *via* the α_{2B}-adrenoceptor is limited by vasoconstriction due to facilitation of transmitter release *via* presynaptic antagonistic action. However, OPC-28326 may exert only a minor effect

due to its weak antagonist potency at the presynaptic α_{2A}-adrenoceptor.

It is well known that mydriasis may be induced by α_2-adrenoceptor agonists *via* stimulation of central nervous system (CNS) α_{2D}-adrenoceptors (Heal *et al*., 1995). The latter are a species orthologue of the human α_{2A}-adrenoceptor (Lanier *et al*., 1991) in the rat. Yohimbine dose-dependently inhibited the brimonidine-induced mydriatic response, which is probably due to an α_{2A}- or α_{2D}-adrenoceptor antagonistic action of this drug. OPC-28326, on the other hand, did not affect the mydriasis induced by the α_2-adrenoceptor agonist even when used at doses as high as 10 mg kg^{-1}. This might be explained by the selectivity to the adrenoceptor subtypes and/or the low level of distribution in the CNS.

Stimulation of central postsynaptic α_2-adrenoceptors induces hypotension *via* a reduction of sympathetic outflow and an augmentation of parasympathetic outflow (Ruffolo *et al*., 1993). Conversely, a vasopressor response may be elicited by the central action of an α_2-adrenoceptor antagonist (Goldberg *et al*., 1983). In mice with a point mutation of α_{2A}-adrenoceptors, the sustained decrease in blood pressure was absent (MacMillan *et al*., 1996), suggesting that centrally-induced hypotension is induced *via* stimulation of α_{2A}-adrenoceptors. Because of the very low affinity of OPC-28326 for α_{2A}-adrenoceptors, the central antagonistic action-induced pressor response may be weak. It is apparent that OPC-28326 exerts a preferentially vasodilatory action *via* an α_{2B}-adrenoceptor antagonistic action and has only a weak vasocontractile effect induced by presynaptic and central α_{2A}-adrenoceptor antagonism.

In the present study, OPC-28326 showed the highest affinity to the α_{2C}-adrenoceptor subtype, among all three subtypes. Although the role of the α_{2C}-adrenoceptor in haemodynamics is still not fully understood, α_2-adrenoceptor-induced vasoconstriction is augmented by cooling in the peripheral arteries (Flavahan *et al*., 1985). Recently, Chotani *et al*. (2000) reported that α_{2C}-adrenoceptors are silent at 37°C, but contribute to the cold-induced enhancement of α_2-adrenoceptor-induced vasoconstriction. The α_2-adrenoceptors in the arterial smooth muscle of the limbs are more prominent in the distal arteries than the proximal (Flavahan *et al*., 1987). This evidence suggests that vasoconstriction may be induced by α_{2C}-adrenoceptor action in the limb extremities, especially in a cold environment (Chotani *et al*., 2000). In addition to the α_{2B}-adrenoceptor antagonistic action, OPC-28326 may dilate peripheral vascular beds through an antagonistic action on α_{2C}-adrenoceptors that are located in the postsynaptic area.

The preferential antagonistic action of OPC-28326 on α_{2B}- or α_{2C}-adrenoceptors compared to the α_{2A}-adrenoceptor lends encouragement that this drug may be beneficial as a peripheral vasodilator. The overall profile of OPC-28326 provides evidence that this drug should be a useful therapeutic agent in the treatment of peripheral vasospasm, such as Raynaud's phenomena and may also be useful in the treatment of peripheral occlusive disease, such as intermittent claudication.

In conclusion, the data in the present study indicates that OPC-28326 has a preferred antagonist action on the postsynaptic α_2-adrenoceptors (both α_{2B}- and α_{2C}-adrenoceptor). These properties are important and show that the drug may be useful clinically as a peripheral vasodilator because it may not induce any increase in catecholamine release from nerve endings in blood vessels and may not facilitate noradrenaline release in the CNS.

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