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α_1-Adrenoceptor subtypes and effect of α_{1A}-adrenoceptor agonist NS-49 on guinea pig nasal mucosa vasculature

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Abstract

It is now clear that α_1-adrenoceptors comprise a heterogeneous family. In the present study, we characterized the α_1-adrenoceptor subtype in the nasal mucosa vasculature of guinea pigs. A rectangular strip of guinea pig nasal mucosa was suspended in an organ bath containing Krebs' bicarbonate solution. Changes in tension were recorded isometrically. Concentration–response curves for agonists were obtained in a cumulative manner. Noradrenaline produced the greatest contraction of the nasal mucosa vasculature. NS-49 ((*R*)-(−)-3′-(2-amino-1-hydroxyethyl)-4′-fluoromethane sulfonanilide hydrochloride) and oxymetazoline worked as partial agonists. The intrinsic activities of NS-49 and oxymetazoline were 0.50 ± 0.22 and 0.29 ± 0.17, respectively, compared with noradrenaline (= 1.00). Prazosin and the putative α_{1A}-adrenoceptor antagonists WB-4101 (2-(2,6-dimethoxyphenoxyethyl)aminomethyl-1,4-benzodioxane) and 5-methylurapidil antagonized the response to noradrenaline competitively (pA_2 for prazosin < 9.0). Conversely, putative α_{1B} and α_{1D}-adrenoceptor antagonists (spiperone and BMY7378 (8-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-8-azaspiro[4,5]decane-7,9-dione), respectively) did not antagonize competitively. These results suggest that the α_{1A}-subtype is predominant and that the α_{1L} (or α_{1N}) subtype may also be present in the guinea pig nasal mucosa vasculature. Furthermore, NS-49 might prove to be a nasal mucosa vasoconstrictor, which will improve nasal obstruction.

Keywords: Nasal mucosa vasculature; Noradrenaline; NS-49; α_1-Adrenoceptor subtype; Vasoconstrictor response

1. Introduction

α_1-Adrenoceptors play a number of important roles in many physiological processes, and agonists and antagonists interacting with α_1-adrenoceptors have proved useful in the treatment of a variety of diseases. Recently, researchers have shown that α_1-adrenoceptors comprise a heterogeneous family: α_{1A}, α_{1B}, and α_{1D}, and their cDNA have been identified (Ford et al., 1994; Hieble et al., 1995; Bylund et al., 1998). The existence of α_{1L} and α_{1N}, which have low affinities for prazosin ($pA_2 < 9.0$), is also hypothesized (Muramatsu et al., 1990a).

Differences in the distribution of α_1-adrenoceptor subtypes depend on tissues and species and it is an interesting task to describe their distribution and physiological function. There are clinical implications, especially in the field of urology, where the α_{1A}-adrenoceptor-selective antagonist tamsulosin is prescribed as a new drug for benign prostatic hypertrophy, reportedly with less hypotension than the usual drugs with a similar affinity for all α_1-adrenoceptor subtypes (Chapple, 1996).

Researchers in the field of otorhinolaryngopharyngology have found that the vasoconstricting effect of adrenoceptor agonists on the nasal vasculature is mainly mediated by α_1-adrenoceptors (Jackson, 1979). However, there is little information regarding subtypes of α_1-adrenoceptors in the nasal mucosa vasculature. Therefore, in the present study, we attempted to characterize the α_1-adrenoceptors, and to examine the effects of oxymetazoline (an imidazoline class α_{1A}-adrenoceptor-selective agonist) and NS-49 (a newly synthesized phenethylamine class α_{1A}-adrenoceptor-selective agonist (Obika et al., 1995)) on the nasal mucosa vasculature of guinea pigs by using an in vitro bioassay method.

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2. Materials and methods

2.1. Materials

Sources of drugs were as follows: (−)-noradrenaline bitartrate, desmethylimipramine hydrochloride, yohimbine hydrochloride, oxymetazoline hydrochloride (Sigma, St. Louis, USA); (±)-propranolol hydrochloride, WB4101 (2-(2,6-dimethoxyphenoxyethyl)aminomethyl-1,4-benzodioxane) hydrochloride, 5-methylurapidil, chloroethylclonidine dihydrochloride, spiperone hydrochloride, BMY7378 (8-[2-[4-methoxyphenyl)-1-piperazinyl]ethyl]-8-azaspiro-[4,5]decane-7,9-dione) dihydrochloride (Research Biochemicals, Natic, USA); hydrocortisone (Wako Pure Chemical Industries, Osaka, Japan) and NS-49, (*R*)-(−)-3′-(2-amino-1-hydroxyethyl)-4′-fluoromethane sulfonanilide hydrochloride (Nippon Shinyaku, Kyoto, Japan).

2.2. Animal and tissue preparation

This study was approved by the Animal Welfare Committee of Hiroshima University School of Medicine. Male and female Hartley guinea pigs, weighing 300–500 g, were anesthetized with pentobarbital sodium (35 mg/kg, i.p.) and then killed by exsanguination. The nasal mucosa was carefully dissected from the nasal septum with a sharp blade (Watanabe et al., 1998). Tissue strips of approximately 15 mm × 5 mm were vertically fixed with hooks in an organ bath containing Krebs' bicarbonate solution, which was aerated with a mixture of 95% O_2 + 5% CO_2 and maintained at 37°C. Constituents of the solution were as follows (mM): NaCl 119, KCI 4.7, $CaCl_2$ 2.5, KH_2PO_4 1.2, $MgSO_4$ 1.2, $NaHCO_3$ 25.0, and glucose 11.1.

2.3. Recording of the responses

We used an in vitro method for detecting changes in the muscle tension of the nasal mucosa vasculature (Jackson, 1979). The isolated canine nasal mucosa strip prepared as described below contracts to α-adrenoceptor agonists and sympathetic nerve stimulation (Ichimura and Jackson, 1984) and relaxes well to nitroxidergic nerve stimulation although endothelium-dependent relaxation to exogenous acetylcholine is obscure (Watanabe et al., 1998). Thus, it is conceivable that contractile and relaxant responses of the preparation reflect functional changes of vascular smooth muscles contained in the nasal mucosa strip.

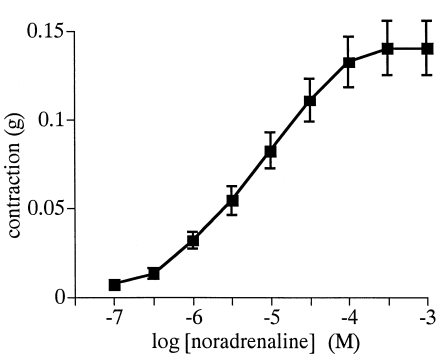

Fig. 1. Mean concentration–response curve for noradrenaline in guinea pig nasal septum mucosa. Each symbol and bar indicates the mean ± S.E.M. of six experiments.

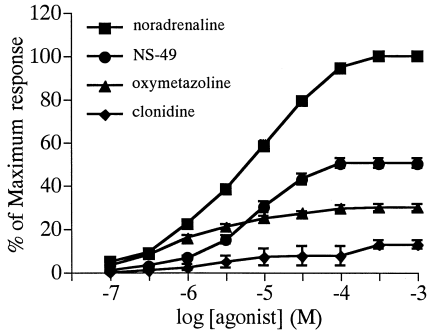

Fig. 2. Concentration–response curves of agonists for contraction in guinea pig nasal septum mucosa compared with that of noradrenaline. Maximum contraction induced by noradrenaline was taken as 100%. Each symbol and bar indicates the mean ± S.E.M. of six experiments for noradrenaline, five for NS-49 and oxymetazoline, and four for clonidine. The order of maximum contractions produced is: noradrenaline > NS-49 > oxymetazoline > clonidine.

The hook anchoring the upper end of the strip was connected to the lever of a force displacement transducer (model TB612T; Nihon-Koden Kogyo, Tokyo). Resting tension was adjusted at an optimal tension of 0.5 g. Isometric contractions and relaxations were displayed on a pen-writing oscillograph (model SR 6211; Graphtech, Tokyo). Before the start of experiment, the strips were left to equilibrate for 60 min. During this period, the bathing medium was replaced about every 15 min.

2.4. Experiments with agonists

Noradrenaline was cumulatively (0.1 μM–1 mM) added to the organ bath in the presence of neuronal and extraneuronal uptake inhibitors and a β-adrenoceptor antagonist — desmethylimipramine (0.3 μM), hydrocortisone (8.7 μM) and propranolol (0.3 μM), respectively (Bevan and Tsuru, 1981). The concentration–response curves were obtained five times from the same strip at about 60-min intervals. During those intervals, preparations were washed with fresh Krebs' bicarbonate solution five times at 5-min intervals and equilibrated for 30 min. By this method, it was confirmed that concentration–response curves for noradrenaline were reproducible at least five times. The second concentration–response curve for noradrenaline was used as the standard and control in the following experiments. In the experiment with the agonists oxymetazoline and NS-49, after recording the second concentration–response curve for noradrenaline as the control, washing the preparations five times, and equilibrating for 30 min, the

Table 1
pD_2 values and the intrinsic activity (noradrenaline = 1.00) of agonists in guinea pig nasal septum mucosa
Values are mean ± S.E.M.

Agonist	*n*	pD_2	Intrinsic activity
noradrenaline	6	5.17 ± 0.11	1.00
oxymetazoline	5	6.05 ± 0.14	0.291 ± 0.017
NS-49	5	5.14 ± 0.06	0.501 ± 0.022

agonist was cumulatively (0.1 μM–1 mM) added in a manner similar to that with noradrenaline. Because the responses to oxymetazoline and NS-49 were not reproducible with the same preparation, we used noradrenaline as the agonist in the drug antagonism experiment.

2.5. Drug antagonism experiments

After recording the control response to noradrenaline, preparations were washed and equilibrated as above and treated with each antagonist for 30 min. Then, a concentration–response curve for noradrenaline in the presence of an antagonist was obtained. This process was repeated at different antagonist concentrations. The pA_2 values for the antagonists were estimated according to the Schild method (Arunlakshana and Schild, 1959). Briefly, the concentration of noradrenaline necessary to give a half-maximal contraction in the presence of the antagonist was divided by the concentration generating a half-maximal response in the control to determine the agonist concentration-ratio (CR). After data were plotted as the log antagonist concentration (M) vs. the log (CR-1), pA_2 values and the slopes of the regression lines were obtained based on Schild plots.

2.6. Effect of chloroethylclonidine treatment

In the experiment to determine chloroethylclonidine antagonism, preparations were incubated with 100 μM chloroethylclonidine for 30 min (Han et al., 1987), after obtaining the control concentration–response curve for noradrenaline. Then, noradrenaline was added as described above after washing chloroethylclonidine out three times with a chloroethylclonidine-free solution and subsequently equilibrating for 30 min.

2.7. Statistical analysis

Experimental data are expressed as means ± S.E.M., with the exception of the slopes. Slopes are presented as mean values with 95% confidence limits. Results were analyzed using Student's *t*-test (unpaired or paired comparison), and a probability of less than 0.05 was considered significant.

3. Results

3.1. Effects of α_1-adrenoceptor agonists on resting tone

The guinea pig nasal mucosa vasculature showed a contractile response to noradrenaline, depending on concentration (Fig. 1). The maximum contraction induced by noradrenaline was 0.14 ± 0.02 g and the pD_2 value was 5.17 ± 0.11 (n = 6). It was confirmed that the response to noradrenaline was reproducible at least five times in each preparation.

Oxymetazoline and NS-49 also induced contractile responses in guinea pig nasal septum mucosa depending on concentration (Fig. 2). Intrinsic activities and pD_2 values are shown in Table 1. The maximum contractions in second concentration–response curves for oxymetazoline and NS-49, in contrast to noradrenaline, were remarkably less than those of the first curves and the pD_2 values were not stable. Thus, the contractile responses induced by these drugs were not reproducible.

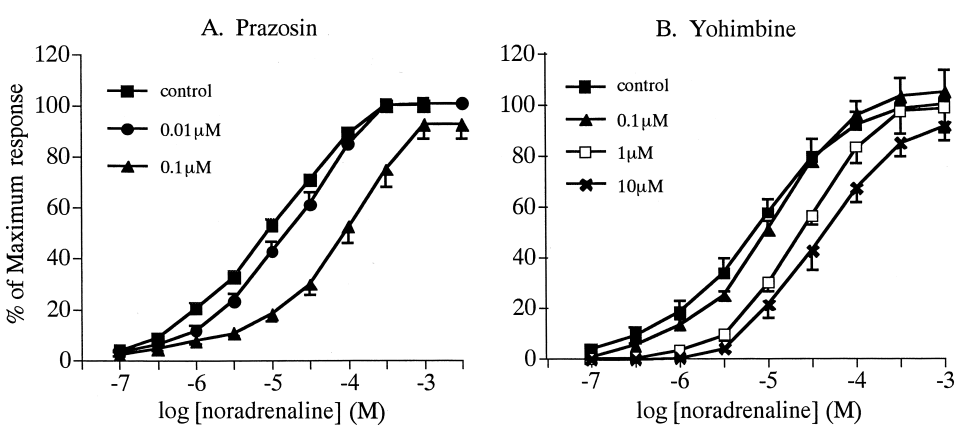

Fig. 3. Effects of prazosin (A) and yohimbine (B) on the concentration–response curve for noradrenaline in guinea pig nasal septum mucosa. Experiments were carried out in the absence and presence of the indicated concentrations of prazosin or yohimbine as described in Materials and methods. Maximum contraction in the controls was taken as 100%. Each symbol and bar indicates the mean ± S.E.M. of six and four experiments for prazosin and yohimbine, respectively.

Table 2
Antagonism of noradrenaline-induced contractions by α_1-adrenoceptor antagonists in guinea pig nasal septum mucosa
pA_2 values are mean ± S.E.M. Slopes are given with mean values and 95% confidence limit (95% CL, in parentheses).

Antagonist	*n*	pA_2	slope (95% CL)
prazosin	6	8.04 ± 0.10	0.92 (0.79–1.06)
yohimbine	5	6.69 ± 0.27	0.39[a] (0.20–0.58)
WB4101	5	7.84 ± 0.07	0.95 (0.85–1.06)
5-methylurapidil	5	7.86 ± 0.08	0.94 (0.82–1.06)
spiperone	6	6.58 ± 0.09	0.77[a] (0.69–0.85)
BMY7378	6	6.10 ± 0.13	0.82[a] (0.74–0.89)

[a] Significantly differs from unity.

In the experiment with clonidine, the guinea pig nasal mucosa showed a contractile response too small to measure. Therefore, the pD_2 value of clonidine could not be calculated (Fig. 2).

3.2. Effects of antagonists on noradrenaline-induced contraction

3.2.1. Prazosin and yohimbine

The contractile response of guinea pig nasal septum mucosa to noradrenaline was antagonized by prazosin and yohimbine, resulting in a parallel shift to the right of the control concentration–response curve (Fig. 3). The slope of the Schild plot for yohimbine was significantly less than unity, but that for prazosin was close to unity (Table 2). In other words, prazosin competitively antagonized the noradrenaline contractile response, but yohimbine did so uncompetitively.

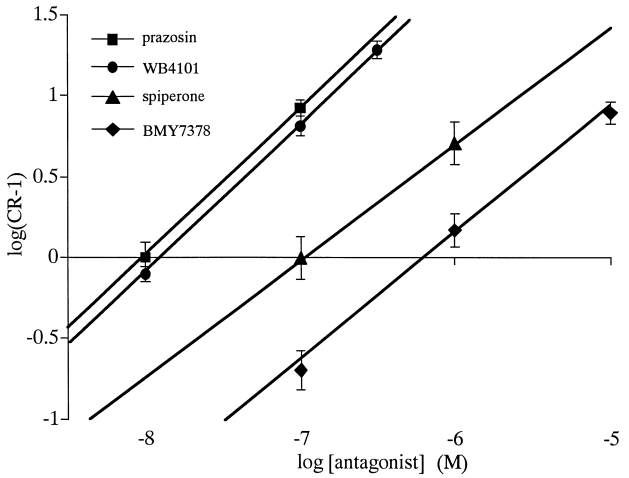

Fig. 5. Schild plots for inhibition of noradrenaline-induced contraction by antagonists in guinea pig nasal septum mucosa. Yohimbine and 5-methylurapidil are not shown. Each point and bar indicates the mean ± S.E.M. of six experiments. For pA_2 values and slopes, see Table 2.

3.2.2. WB4101, 5-methylurapidil, spiperone and BMY7378

The concentration–response curve for noradrenaline was shifted in parallel to the right by the antagonists WB4101, 5-methylurapidil, spiperone, and BMY7378 (Fig. 4). In the Schild plots (Fig. 5), however, the slopes of spiperone and BMY7378 were significantly less than unity (Table 2).

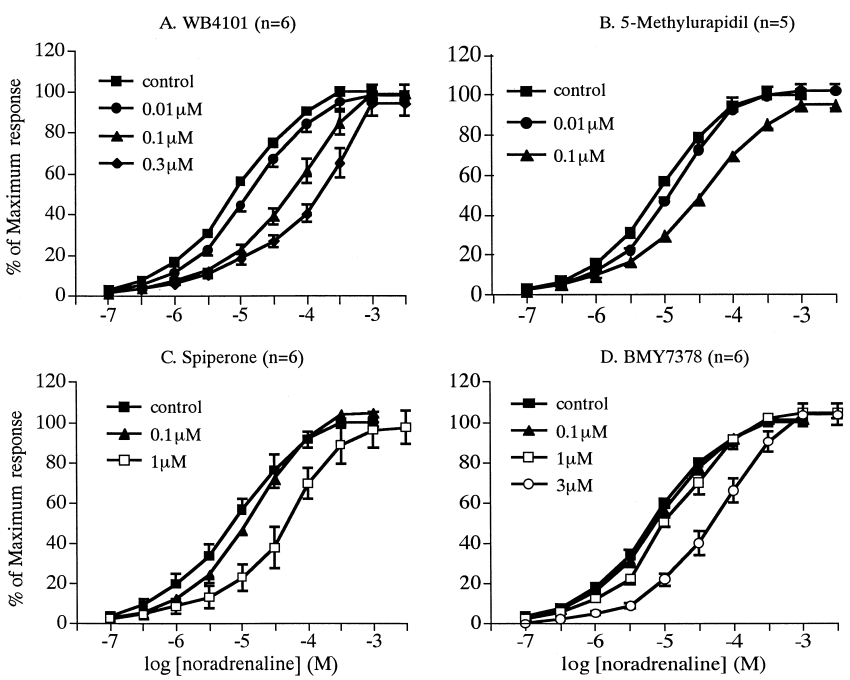

Fig. 4. Effects of WB4101, 5-methylurapidil, spiperone, and BMY7378 on the concentration–response curves for noradrenaline in guinea pig nasal septum mucosa. Experiments were carried out in the absence and presence of the indicated concentration of each antagonist as described in Materials and methods. Each symbol and bar indicates mean ± S.E.M. of six experiments for WB4101, spiperone, and BMY7378 and five experiments for 5-methylurapidil.

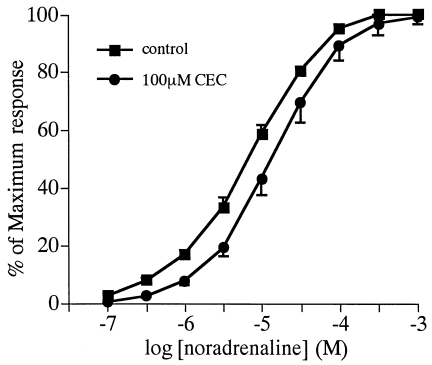

Fig. 6. Effect of chloroethylclonidine on the concentration–response curve for noradrenaline in guinea pig nasal septum mucosa. After recording the control noradrenaline response the preparation was treated with 100 μM chloroethylclonidine (CEC) for 30 min and then repeatedly washed. Maximum concentration in the controls was taken as 100%. Each point and bar indicates the mean ± S.E.M. of four experiments.

Therefore, the latter two antagonists appear to inhibit noradrenaline-induced contraction uncompetitively. The pA_2 values of both spiperone and BMY7378 were significantly less than those of prazosin, WB4101, and 5-methylurapidil.

3.2.3. Chloroethylclonidine

Pretreatment with 100 μM chloroethylclonidine for 30 min had no effect on the maximal contractile response but induced only a slight parallel shift to the right of the concentration–response curve for noradrenaline (Fig. 6). Apparent pK_B value, which was obtained after washing chloroethylclonidine out as described in Materials and methods and calculated as the $-\log$ of the dissociation constant (Kenakin, 1987), was 3.78 ± 0.40. In other words, the noradrenaline-induced contractile response of the guinea pig nasal septum mucosa resisted inactivation by chloroethylclonidine.

4. Discussion

The α_2-adrenoceptor-selective agonist clonidine produced only a limited contraction in guinea pig nasal septum mucosa (Fig. 2). While α_2-adrenoceptors exist in the nasal mucosa of dogs, their role is limited. The contractile response to clonidine is principally mediated by α_1-adrenoceptors and the role of α_2-adrenoceptors is limited (Ichimura and Jackson, 1984). This also seems to be the case in guinea pig nasal mucosa. In the experiment with the α_2-adrenoceptor-selective antagonist yohimbine, the noradrenaline-induced contractile response was antagonized only with a concentration greater than 10^{-6} M of yohimbine ($pA_2 = 6.69 \pm 0.27$), with a slope remarkably less than unity (Table 2). Yohimbine antagonizes not only the α_2-adrenoceptor but also the α_1-adrenoceptor at high concentrations above 1 μM (Tsuru et al., 1983). So this inhibition by yohimbine was a noncompetitive antagonism and may be effective against not only α_2-adrenoceptors but also α_1-adrenoceptors. In contrast, prazosin competitively antagonized noradrenaline-induced contraction. These results suggest that the contractile response of the guinea pig nasal septum mucosa to noradrenaline is mainly mediated through α_1-adrenoceptors and that α_2-adrenoceptors do not play a significant role. Because vascular smooth muscle is the only tissue that possess contractility in the nasal mucosa layer (Ichimura and Jackson, 1984), it is reasonable to assume that the contractile response is mediated through α_1-adrenoceptors in vascular smooth muscle.

In the experiment with α_1-adrenoceptor agonists, the newly synthesized phenethylamine class α_{1A}-adrenoceptor-selective agonist NS-49 and the imidazoline class α_{1A}-adrenoceptor-selective agonist oxymetazoline both induced contractile responses, depending on their concentrations. The pD_2 value of NS-49 was almost identical to that of noradrenaline. Oxymetazoline was more potent than noradrenaline and NS-49 (Table 1). The intrinsic activity was approximately 0.5 for NS-49 and less than 0.3 for oxymetazoline, indicating that these were partial agonists. In Chinese hamster ovary cells expressing cloned human α_1-adrenoceptor subtypes, both oxymetazoline and NS-49 worked as a selective and partial agonist at α_{1A}-subtype; however, NS-49 was more efficacious than oxymetazoline (Obika et al., 1995).

Muramatsu et al. (1990a) have subclassified α_1-adrenoceptors of blood vessels into three subtypes (α_{1H}, α_{1L}, and α_{1N}) according to different affinities for prazosin, yohimbine, WB4101, and HV723. The α_{1H} subtype has high affinity for prazosin ($pA_2 > 9.5$), and yohimbine (> 6.5), while the α_{1L} subtype has lower affinities for both antagonists, as well as for WB4101 and HV723 (8.0–9.0). The α_{1N} subtype is highly selective for HV723 (> 9.0), WB4101 (> 8.4), and yohimbine (> 6.5) but shows relatively low affinity for prazosin (< 9.0).

In the present study, the pA_2 value for prazosin in the guinea pig nasal mucosa vasculature was significantly less than 9 (Table 2), indicating that the subtype of this tissue was not α_{1H}, but α_{1L} or α_{1N}. We did not examine the effect of α_{1N}-adrenoceptor-selective antagonist HV723. In addition, the antagonism of noradrenaline-induced contraction by yohimbine was not competitive and the pA_2 value for WB4101 was less than 8.0. Therefore, we could not distinguish these two (α_{1L} and α_{1N}) subtypes in guinea pig nasal mucosa. Evidence for the existence of α_{1L} and α_{1N} subtypes is based only on pharmacological data; the cDNA for these subtypes has not been identified. It has been reported that these subtypes may represent a particular, energetically favorable conformational state of the α_{1A} subtype (Ford et al., 1997).

WB4101 and 5-methylurapidil antagonized noradrenaline-induced contractile responses and had Schild plot slopes close to unity. However, in the experiments using spiperone and BMY7378, the slopes were significantly less

than unity. The potencies of the former two drugs were about 10 times higher than those of the latter two drugs. By contrast, noradrenaline-induced contractile response was not influenced by chloroethylclonidine. These results suggest that α_{1A} subtypes play the most important role (using the α_{1A}, α_{1B}, and α_{1D} subclassification) in noradrenaline-induced contractile responses in guinea pig nasal mucosa. It may be that more than one subtype of the α_1-adrenoceptor exists in the same tissue. Correlations of subtype and physiological function depend on the species and organ. For example, in both the rat and rabbit thoracic aorta, the α_{1A}, α_{1B}, and α_{1D} subtypes are all present at the mRNA level (Piascik et al., 1995). The α_{1B} and α_{1D} subtypes mediate the contractile response in the rat thoracic aorta (Muramatsu et al., 1991; Buckner et al., 1996), while the α_{1B} and α_{1L} subtypes do so in the rabbit (Muramatsu et al., 1990b; Oshita et al., 1993). It appears that noradrenaline-induced contractile response is mediated through α_{1A} subtypes, though other subtypes may exist in the vascular smooth muscle of guinea pig nasal mucosa.

In conclusion, the present results suggest that noradrenaline-induced contraction of guinea pig nasal mucosa is mainly mediated through the α_1-adrenoceptor. The predominant α_1-adrenoceptor subtype responsible for this physiological function is the α_{1A} subtype, though the α_{1L} (or α_{1N}) subtype may also be present. Performing this kind of experiment using human nasal mucosa will clarify which subtype mediates vascular smooth muscle contraction in this tissue. A nasal mucosa vasoconstrictor selective for the adrenoceptor subtype, which mediates the contractile response of human nasal mucosa vasculature would ameliorate nasal obstruction with minimal adverse effects on systemic circulation, effects which can include hypertension and increased heart rate. NS-49 is a candidate worthy of further study.

Acknowledgements

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