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# Effect of JTH-601, a putative $\alpha_{1L}$-adrenoceptor antagonist, on guinea pig nasal mucosa vasculature

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**Abstract**

The existence of $\alpha_1$-adrenoceptors with low affinity for prazosin, an $\alpha_{1L}$ subtype, has been proposed in addition to $\alpha_1$-adrenoceptor subtypes with high affinity for prazosin, i.e. the $\alpha_{1H}$ group: $\alpha_{1A}$, $\alpha_{1B}$ and $\alpha_{1D}$ subtypes. In the present study, we investigated the effect of JTH-601 (3-(*N*-[2-(4-hydroxy-2-isopropyl-5-methylphenoxy)ethyl]-*N*-methylaminomethyl)-4-methoxy-2,5,6-trimethylphenol hemifumarate), a putative $\alpha_{1L}$-adrenoceptor antagonist, on the isolated guinea pig nasal mucosa vasculature. JTH-601 (0.01–0.03 μM) competitively antagonized the noradrenaline-induced contraction of the tissue in a concentration-dependent manner. The $pA_2$ value for JTH-601 was $8.14 \pm 0.04$ (means ± SEM, $n = 6$). The data suggests that the $\alpha_{1L}$-subtype is involved in the noradrenaline-induced contraction of the guinea pig nasal mucosa vasculature. 

*Keywords:* Nasal mucosa vasculature; $\alpha_{1L}$-Adrenoceptor subtype; JTH-601; Drug antagonism

## 1. Introduction

Adrenoceptors are found in nearly all peripheral tissues and on many neuronal populations within the central nervous system. Agonists and antagonists interacting with adrenoceptors have proven useful in the treatment of a variety of diseases, including hypertension, angina pectoris, congestive heart failure, asthma, depression, benign prostatic hypertrophy and granuloma. Based on both pharmacological and molecular evidence, it is now clear that a more useful classification scheme can be based on three major types, i.e. $\alpha_1$, $\alpha_2$ and β, each of which is further divided into at least three subtypes (Bylund et al., 1998). Currently, three distinct $\alpha_1$-adrenoceptor family members —$\alpha_{1A}$, $\alpha_{1B}$ and $\alpha_{1D}$—have been demonstrated, and their cDNA have been identified (Bylund et al., 1998; Ford et al., 1994; Hieble et al., 1995).

In addition, Muramatsu et al. (1990) have subclassified $\alpha_1$-adrenoceptors of blood vessels into two subtypes, $\alpha_{1H}$ and $\alpha_{1L}$, according to different affinities for prazosin. The $\alpha_{1H}$ subtype has a high affinity for prazosin ($pA_2 > 9.0$) and the $\alpha_{1L}$ subtype has a low affinity for prazosin ($pA_2 < 9.0$). A separate gene encoding for $\alpha_{1L}$-adrenoceptor has not been identified, but there is now evidence to support the idea that the $\alpha_{1L}$-adrenoceptor is a phenotype of the cloned $\alpha_{1a}$-adrenoceptor (Argyle and McGrath, 2000; Chang et al., 1998). On the other hand, Muramatsu et al. (1996) demonstrated that JTH-601 has approximately 10 times higher affinity than prazosin for the $\alpha_{1L}$ subtype, whereas both compounds displayed equal binding affinities for the $\alpha_{1A}$ subtype. Since then, JTH-601 has been used as an $\alpha_{1L}$-adrenoceptor antagonist in a number of analyses of $\alpha_1$-adrenoceptor pharmacology (Stam et al., 1999; Suzuki et al., 1999).

In the field of rhinology, it has been reported that the vasoconstricting effect of adrenoceptor agonists on the nasal mucosa vasculature is mainly mediated by $\alpha_1$-adrenoceptors (Jackson, 1979). We previously reported that the noradrenaline-induced contractile effect on the guinea pig nasal mucosa vasculature is mainly mediated by the $\alpha_{1A}$-adrenoceptor. The $pA_2$ value for prazosin in the tissue was $8.04 \pm 0.10$ (means ± S.D., $n = 6$), significantly less than 9.0 (Tanimitsu et al., 2000). Thus, according to the previously described classification, $\alpha_{1L}$-adrenoceptors are functionally dominant in the nasal mucosa. Therefore, in this

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study, we attempted to investigate the effect of JTH-601, a putative $\alpha_{1L}$-adrenoceptor antagonist, on the guinea pig nasal mucosa vasculature by using an in vitro bioassay method.

## 2. Materials and methods

This study was approved by the Animal Welfare Committee of Hiroshima University School of Medicine. Male Hartley guinea pigs, weighing 400–600 g, were anesthetized with pentobarbital sodium (35 mg/kg, i.p.) and 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 $15 \times 5$ mm were vertically fixed by a hook in an organ bath containing Krebs' bicarbonate solution, aerated with a mixture of 95% $O_2$ + 5% $CO_2$ and maintained at 37°C. Constituents of the solution were as follows (in 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.

We used an in vitro method for detecting changes in the muscle tension of the nasal mucosa vasculature (Jackson, 1979). Because vascular smooth muscle is the only tissue that possesses contractility in the nasal mucosa layer (Ichimura and Richard, 1984), it is reasonable to assume that the contractile response is mediated through adrenoceptors in the vascular smooth muscle (Tanimitsu et al., 2000).

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, Japan). Resting tension was adjusted to an optimal tension of 0.5 g. Isometric contractions and relaxations were displayed on a pen-writing oscillograph (model SR 6211, Graphtec, Tokyo, Japan). Before the start of the experiment, the strips were left to equilibrate for 60 min. During this period, the bathing medium was replaced approximately every 15 min.

Noradrenaline was cumulatively (0.1 μM–1 mM) added to the organ bath in the presence of neuronal and extraneuronal uptake inhibitors and 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 these 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. After recording the control response to noradrenaline, preparations were washed and equilibrated as described above and treated with the appropriate concentration of JTH-601 for 30 min. Then, a concentration–response curve for noradrenaline in the presence of JTH-601 was obtained. This process was repeated at 0.01 and 0.3 μM JTH-601. The $pA_2$ value for the JTH-601 was estimated according to the Schild method (Arunlakshana and Schild, 1959).

The drugs used in the present experiment were as follows: (−)-noradrenaline bitartrate, desmethylim-

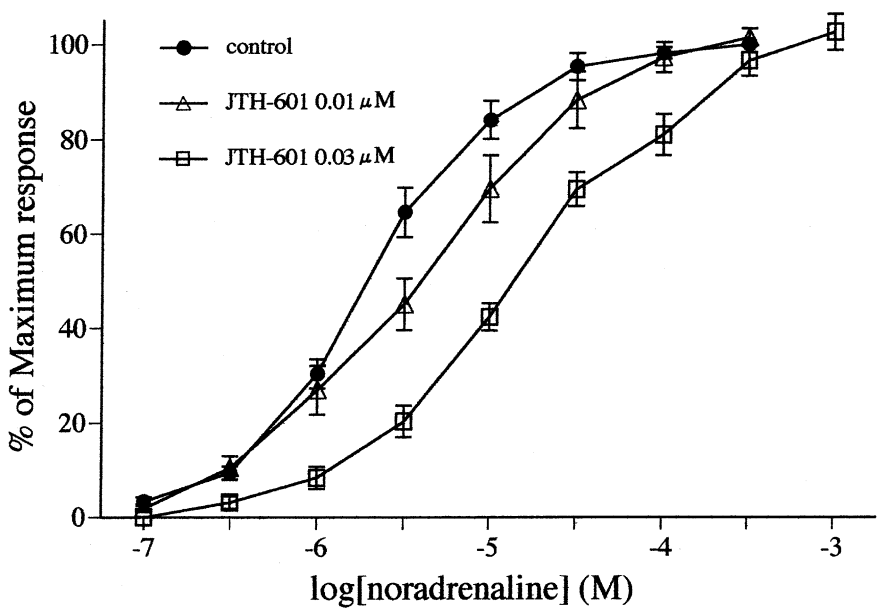


Fig. 1. Effect of JTH-601 on the concentration–response curve for noradrenaline in the guinea pig nasal septum mucosa. Experiments were carried out in the absence and presence of the indicated concentrations of JTH-601. Maximum contraction in the controls was designated as 100%. The maximum noradrenaline-induced contraction ranged between 0.05 and 0.15 g, averaging 0.1 g, and the noradrenaline $pD_2$ ($= pEC_{50}$) value in the absence of antagonist was 5.69 ± 0.11. Each symbol and bar indicates the means ± SEM of six experiments.

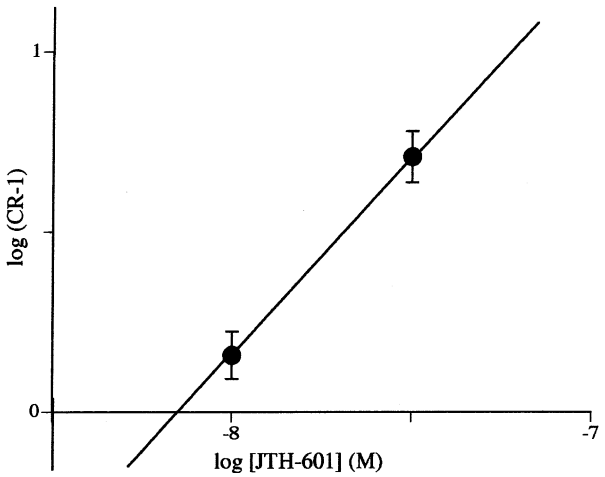


Fig. 2. Schild plot for inhibition of noradrenaline-induced contraction by antagonists in guinea pig nasal septum mucosa. Each point and bar indicates the means ± SEM of six experiments. The slope of the Schild plot was 0.92 (0.79–1.06), which was not significantly different from unity. The p$A_2$ value for JTH-601 was 8.14 ± 0.04.

ipramine hydrochloride (Sigma, St. Louis, USA), (±)—propranolol hydrochloride (Research Biochemicals, Natic, USA), hydrocortisone (Wako, Osaka, Japan) and JTH-601 (3-*N*-[2-(4-hydroxy-2-isopropyl-5-methylphenoxy)ethyl]-*N*-methylamino-methyl)-4-methoxy-2,5,6-trimethylphenol hemifumarate (Japan Tobacco, Takatsuki, Japan).

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

## 3. Results

JTH-601 had no influence on the resting tension of the guinea pig nasal mucosa vasculature (data not shown, $n = 6$). Noradrenaline induced a contractile response to the preparations depending on concentration. JTH-601 shifted the contractile response curves to the right, resulting in a parallel shift to the right of the control concentration–response curve (Fig. 1). The slope of the Schild plot (Fig. 2) for JTH-601 was 0.92 (0.79–1.06, 95% confidence limit, $n = 6$), which was not significantly different from unity. Therefore, it seems likely that JTH-601 competitively antagonizes the contractile response to noradrenaline. The p$A_2$ value determined by this Schild plot analysis was 8.14 ± 0.04 (means ± SEM, $n = 6$).

## 4. Discussion

Currently, the $\alpha_1$-adrenoceptor is classified into three native subtypes: $\alpha_{1A}$, $\alpha_{1B}$ and $\alpha_{1D}$, the clones of which are designated as $\alpha_{1a}$, $\alpha_{1b}$ and $\alpha_{1d}$, respectively (Bylund et al., 1998; Ford et al., 1994; Hieble et al., 1995). In addition, Muramatsu et al. (1990) have subclassified $\alpha_1$-adrenoceptors of blood vessels into two subtypes, $\alpha_{1H}$ and $\alpha_{1L}$, according to different affinities for prazosin. The $\alpha_{1H}$ subtype has a high affinity for prazosin (p$A_2 > 9.0$) and the $\alpha_{1L}$ subtype has a low affinity for prazosin (p$A_2 < 9.0$). Evidence for the existence of $\alpha_{1L}$ is based only on pharmacological data. However, there is now accumulating evidence that the $\alpha_{1A}$-adrenoceptor comprises at least four isoforms and that the $\alpha_{1L}$-adrenoceptor may be a phenotype of the cloned $\alpha_{1a}$-adrenoceptors (Chang et al., 1998).

It is not known which adrenoceptor subtype exists in the nasal mucosa vasculature. Jackson (1979) reported that the vasoconstricting effect of adrenoceptor agonists on the nasal vasculature is mainly mediated by $\alpha_1$-adrenoceptors, while Ichimura and Jackson (1984) found evidence of $\alpha_2$-adrenoceptors in the nasal blood vessels of the dog. We have previously reported that prazosin and the putative $\alpha_{1A}$-adrenoceptor antagonists, WB4101 (2-(2,6-dimethoxyphen-oxyethyl)amino-methyl-1,4-benzodioxane) and 5-methylurapidil competitively antagonized the response to noradrenaline. The p$A_2$ value for prazosin in the guinea pig nasal mucosa vasculature was 8.04 ± 0.10, significantly less than 9.0 (Tanimitsu et al., 2000). Thus, according to the previously described classification (Muramatsu et al., 1990), $\alpha_{1L}$-adrenoceptors are functionally dominant in the tissue. Conversely, the putative $\alpha_{1B}$ and $\alpha_{1D}$-adrenoceptor antagonists (spiperone and BMY7378 (8-[2-[4-(2-methoxyphenyl)-1-piperazinyl]-ethyl]-8-azaspiro[4,5]decane-7,9-dione)) did not antagonize competitively. Therefore, it seems likely that the contractile response to noradrenaline in guinea pig nasal mucosa is mainly mediated through $\alpha_{1A}$- and/or $\alpha_{1L}$-adrenoceptors, and that $\alpha_{1B}$-, $\alpha_{1D}$- and $\alpha_2$-adrenoceptors do not play a significant role (Tanimitsu et al., 2000).

It has been reported that JTH-601 has a higher affinity for $\alpha_{1L}$-adrenoceptor and a lower affinity for $\alpha_{1H}$-adrenoceptor (except $\alpha_{1A}$-adrenoceptors) than prazosin (Muramatsu et al., 1990; Stam et al., 1999; Suzuki et al., 1999). Suzuki et al. (1999) reported that JTH-601 has a higher selectivity for the prostate than the artery in dogs and in humans. Stam et al. (1999), however, showed that JTH-601 produced rightward shifts of the noradrenaline concentration–response curves in the isolated rat small mesenteric artery, although the shift did not occur in a concentration-dependent manner. They estimated the p$A_2$ value of JTH-601 as 8.34 ± 0.16 ($n = 5$) for the high affinity component. The value is very close to that obtained in the present study. Van der Graaf et al. (1997) and Argyle and McGrath (2000) also demonstrated that the functional $\alpha_1$-adrenoceptor in the rabbit urethra and the canine subcutaneous resistance arteries is the $\alpha_{1L}$-adrenoceptor or $\alpha_{1A}$/$\alpha_{1L}$-adrenoceptor subtype, respectively. All these data indicate that the $\alpha_1$-adrenoceptor mediating noradrenaline-induced contraction of the tissues displays a distinct $\alpha_{1L}$-adrenoceptor pharmacology.

In conclusion, JTH-601 competitively antagonized a noradrenaline-induced contractile response. The p$A_2$ value of JTH-601 was $8.14 \pm 0.04$, nearly identical to that of prazosin ($8.04 \pm 0.10$; Tanimitsu et al., 2000). Therefore, the $\alpha_{1L}$-adrenoceptor may be functionally important in the guinea pig nasal mucosa vasculature.

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