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Short communication

Vascular α_{1D}-adrenoceptors have a role in the pressor response to phenylephrine in the pithed rat

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Abstract

In rats, the pressor response to intravenous (i.v.) phenylephrine is mediated by vascular α_{1A}- and α_{1B}-adrenoceptors, but the role of α_{1D}-adrenoceptors is uncertain. These studies evaluated the effect of a selective α_{1D}-adrenoceptor antagonist, BMY 7378 (8-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-8-azaspirol[4,5]decane-7,9-dione 2HCl), on the pressor effect to i.v. phenylephrine ($\alpha_{1A/B/D}$-adrenoceptor agonist) and (*R*)A-61603 (α_{1A}-adrenoceptor agonist; *N*-[5-(4,5-dihydro-1*H*-imidazol-2yl)-2-hydroxy-5,6,7,8-tetrahydronaphthalen-1-yl] methanesulfonamide HBr) in pithed rats. Pretreatment with BMY 7378 (0.1–1 mg/kg) competitively antagonized the phenylephrine pressor response, but not the (*R*)A-61603 pressor curve. At 10 mg/kg, BMY 7378 antagonized the (*R*)A-61603 response, indicating the non-selective blockade of α_{1A}-adrenoceptors. These findings demonstrate that i.v. phenylephrine can activate vascular α_{1D}-adrenoceptors in the pithed rat.

Keywords: BMY 7378; 5-Methylurapidil; A-61603; α_1-Adrenoceptor; Arterial pressure

1. Introduction

Pharmacological studies in isolated blood vessels have demonstrated the existence of multiple α_1-adrenoceptor subtypes in vascular tissue (Vargas and Gorman, 1995). These receptors have been labelled the α_{1A}, α_{1B} and α_{1D} subtypes based on the differential affinity of these receptors for subtype-selective antagonists (Ford et al., 1994; Vargas and Gorman, 1995). This subdivision is supported by molecular evidence of three distinct genes that correspond to each of the native α_1-adrenoceptors (Ford et al., 1994; Minneman and Esbenshade, 1994; Hieble et al., 1995). While these subtypes have been identified in a variety of isolated vascular tissues, elucidation of the physiological role of each α_1-adrenoceptor subtype in the regulation of peripheral vascular tone in whole animals has progressed slowly, a key limitation being the availability of agents that clearly discriminate the three subtypes (Vargas and Gorman, 1995).

Recently, radioligand binding and functional assays have shown BMY 7378 (8-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-8-azaspirol[4,5]decane-7,9-dione 2HCl) to be a selective antagonist for the α_{1D}-adrenoceptor (Goetz et al., 1995; Piascik et al., 1995). In general, these reports show that BMY 7378 displays approximately 126- and 100-fold greater affinity for the α_{1D}-adrenoceptor than the α_{1A} and α_{1B} subtypes, respectively. Therefore, based upon its relatively high selectivity for the α_{1D} subtype, BMY 7378 is a new pharmacological probe to study vascular α_{1D}-adrenoceptor function in vitro and in vivo.

Previous studies in the rat indicate that both vascular α_{1A}- and α_{1B}-adrenoceptors mediate the pressor response to intravenous (i.v.) α_1-adrenoceptor agonists, like cirazoline and phenylephrine (Piascik et al., 1990; Schwietert et al., 1992; Vargas et al., 1993). The contribution of vascular α_{1D}-adrenoceptors to the pressor response, however, was not addressed in those early studies because recognition of the α_{1D}-adrenoceptor subtype has gained acceptance only recently and α_{1D}-selective antagonists have just emerged for experimental use (Hieble et al., 1995; Goetz et al., 1995). Therefore, we assessed the role of vascular α_{1D}-adrenoceptors in the vasocontractile response to two α_1-adrenoceptor agonists in vivo. Specifically, our experiments compared the inhibitory effects of various doses of BMY 7378 on the vasopressor responses to phenylephrine

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and (*R*)A-61603 (*N*-[5-(4,5-dihydro-1*H*-imidazol-2yl)-2-hydroxy-5,6,7,8-tetrahydronaphthalen-1-yl] methanesulfonamide HBr) in the pithed rat. Initially, we evaluated whether BMY 7378 would non-selectively shift the pressor response curve for (*R*)A-61603, a potent and highly selective α_{1A}-adrenoceptor agonist (Knepper et al., 1995). In addition, we evaluated the effect of BMY 7378 on the pressor response to phenylephrine, an agonist which stimulates all α_1-adrenoceptors (Knepper et al., 1995).

2. Materials and methods

Male Long-Evans rats (350–450 g; Charles River, Wilmington, MA) were used in all studies. Rats were anesthetized, pithed and instrumented for diastolic blood pressure recording as described previously (Vargas et al., 1994) and all test compounds were given in saline for i.v. injection (1 ml/kg body weight). To assess the inhibitory effect of BMY 7378 on the ability of phenylephrine ($\alpha_{1A/B/D}$-adrenoceptor agonist) and (*R*)A-61603 (α_{1A}-adrenoceptor agonist) to elevate diastolic pressure, a range of BMY 7378 doses (0.1, 0.3, 1 and 10 mg/kg) were administered to separate groups of pithed rats. 15 min later, the agonist dose-response curve was generated. Pressor response curves obtained from BMY 7378-treated rats were compared to control curves obtained in a separate group of saline-treated rats. For reference purposes, we evaluated the inhibitory effect of the selective α_{1A}-adrenoceptor antagonist 5-methylurapidil (Gross et al., 1988) on the i.v. (*R*)A-61603 response. In this study, separate groups of rats were pretreated with various doses of 5-methylurapidil (0, 0.5, 1 and 5 mg/kg) 15 min before (*R*)A-61603 administration. For each rat, an agonist ED_{50} value was calculated by nonlinear regression (Vargas et al., 1993) and group data are expressed as the mean ± S.E.M. Group comparisons were made (unpaired Student's *t*-test) with statistical significance defined at the $P < 0.05$ level.

BMY 7378 dihydrochloride and 5-methylurapidil were obtained from Research Biochemicals (Natick, MA) and phenylephrine hydrochloride from Sigma (St. Louis, MO). (*R*)A-61603 was supplied by Dr. A.A. Hancock (Abbott Laboratories, Abbott Park, IL). Stock solutions of 5-methylurapidil and BMY 7378 were prepared in 1% acetic acid and (*R*)A-61603 was prepared in absolute ethanol prior to dilution in saline.

3. Results

(*R*)A-61603 is a potent agonist with maximal pressor activity similar to that for phenylephrine (Fig. 1; Table 1). Dose-response analysis indicated that (*R*)A-61603 was 55 times more potent than phenylephrine in its ability to elevate diastolic pressure in the pithed rat. Intravenous

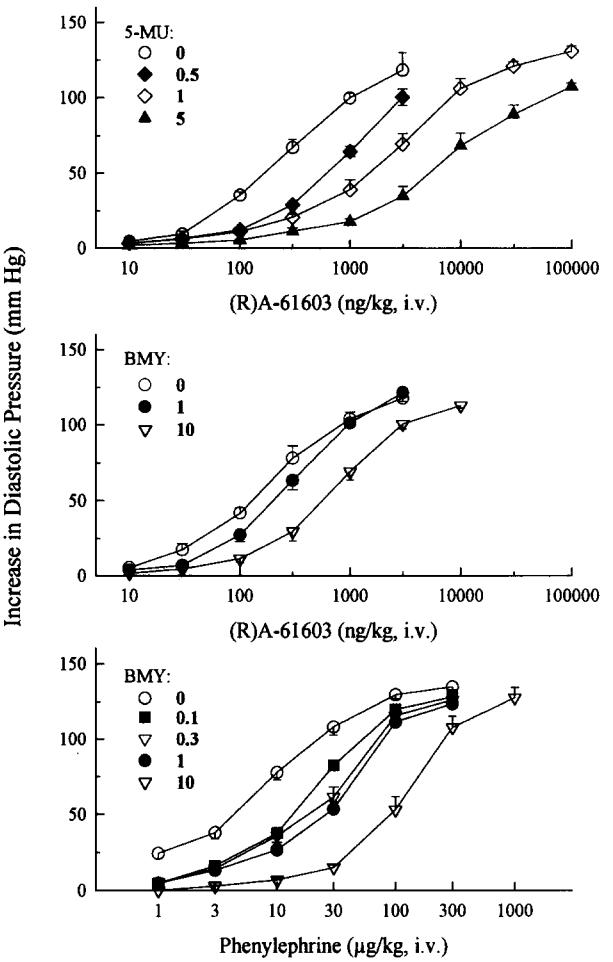

Fig. 1. Inhibitory effect of 5-methylurapidil (5-MU; mg/kg) on the increase in diastolic pressure (mm Hg) induced by (*R*)A-61603 (upper) and the inhibitory effect of BMY 7378 (BMY; mg/kg) on the pressor response to (*R*)A-61603 (middle) and phenylephrine (lower) in pithed rats. All compounds were given intravenously and antagonists were administered 15 min before each agonist. Each point and error bar represent the mean and S.E.M. of 4–13 rats per group.

administration of 0.5, 1 and 5 mg/kg 5-methylurapidil produced 4-, 16- and 43-fold shifts in the pressor response curve for the α_{1A}-adrenoceptor agonist (*R*)A-61603. 5-Methylurapidil behaved as a competitive antagonist in the pithed rat since E_{max} pressor values for (*R*)A-61603 were comparable in the 5-methylurapidil-treated and saline control groups (Fig. 1). In contrast with 5-methylurapidil, the 0.3 and 1 mg/kg (i.v.) doses of BMY 7378 did not affect the (*R*)A-61603 pressor response since the ED_{50} dose ratios were near unity (Fig. 1; Table 1). However, the highest dose of BMY 7378 (10 mg/kg) produced a competitive 4-fold shift in the (*R*)A-61603 dose-response curve (Fig. 1).

In contrast with its negligible effect on (*R*)A-61603 responses, BMY 7378 (0.3 and 1 mg/kg) did antagonize the pressor response to i.v. phenylephrine in pithed rats (Fig. 1; Table 1). These doses shifted significantly the phenylephrine curve 3- and 4-fold, respectively. The com-

Table 1
Effect of BMY 7378 (BMY) on the parameters of the dose-pressor response curves for the α_1-adrenergic agonists (*R*)A-61603 and phenylephrine in the pithed rat

Group (antagonist dose; mg/kg, i.v.)	Agonist ED_{50} (μg/kg, i.v.)	Dose ratio [a]	E_{max} (mm Hg)	*n*
(*R*)A-61603, control	0.16±0.03	–	118±7	7
+ BMY (0.3)	0.17±0.01	1.0	119±7	4
+ BMY (1)	0.26±0.04	1.6	122±6	4
+ BMY (10)	0.62±0.11 [b]	3.8	113±2	4
Phenylephrine, control	9±2	–	135±3	13
+ BMY (0.1)	19±3 [c]	2.2	128±5	4
+ BMY (0.3)	26±4 [c]	3.0	126±3	4
+ BMY (1)	38±5 [c]	4.3	128±4	4
+ BMY (10)	137±31 [c]	15.5	128±7	4

Agonists were administered 15 min after BMY 7378. All values are the mean±S.E.M and *n* is the number of rats per treatment. [a] Ratio of $ED_{50\text{-BMY}}/ED_{50\text{-CONTROL}}$ values. [b] Significantly different than (*R*)A-61603 control obtained in a separate group ($P < 0.05$). [c] Significantly different than phenylephrine control obtained in a separate group ($P < 0.05$).

petitive antagonist activity of BMY 7378 was also evident at 0.1 mg/kg, the lowest dose tested (Table 1). The 10 mg/kg dose of BMY 7378 produced a 16-fold shift in the phenylephrine pressor response curve.

4. Discussion

The results of this study indicate that the α_{1D}-adrenoceptor antagonist BMY 7378 did not affect the pressor response to i.v. (*R*)A-61603 in the pithed rat, but the same doses of BMY 7378 did significantly antagonize the pressor effects of phenylephrine under identical experimental conditions. These observations suggest that low doses of BMY 7378 (0.1 to 1 mg/kg, i.v.) induce the relatively selective blockade of vascular α_{1D}-adrenoceptors in the pithed rat. In addition, the ability of BMY 7378 to antagonize the pressor effects of phenylephrine implies that vascular α_{1D}-adrenoceptors are activated by the exogenous administration of this α_1-adrenoceptor agonist. This latter finding is the first demonstration that α_{1D}-adrenoceptors mediate vascular smooth muscle contraction in vivo.

It was recently reported that racemic A-61603 and its (*R*)-isomer are potent and selective full agonists for native and cloned α_{1A}-adrenoceptors (Knepper et al., 1995). In the current study i.v. administration of (*R*)A-61603 in the pithed rat was used as a model of vascular α_{1A}-adrenoceptor stimulation in vivo to assess whether BMY 7378 interacts with this adrenoceptor subtype. In the pithed rat, the α_{1D}-adrenoceptor antagonist BMY 7378 (0.3 and 1 mg/kg) did not alter the pressor response curve to i.v. (*R*)A-61603, an observation which demonstrates that vascular α_{1A}-adrenoceptor blockade does not occur at these two doses. However, the (*R*)A-61603 pressor response curve was very sensitive to α_{1A}-adrenoceptor blockade with 5-methylurapidil (Gross et al., 1988). The doses of 5-methylurapidil that shifted the (*R*)A-61603 pressor response curve have been shown previously to antagonize vascular α_{1A}-adrenoceptors in the pithed rat (Vargas et al., 1994). The 10 mg/kg dose of BMY 7378 did antagonize the (*R*)A-61603 dose-pressor response curve like 5-methylurapidil, a finding which demonstrates that BMY 7378 does not maintain its α_{1D}-adrenoceptor selectivity at this high dose.

To investigate whether vascular α_{1D}-adrenoceptors contribute to the pressor effects of phenylephrine, we evaluated the inhibitory effect of BMY 7378 on the phenylephrine dose-response curve in pithed rats. The pressor response to phenylephrine was sensitive to inhibition by 0.1–1 mg/kg BMY 7378 (i.v.). This observation led us to conclude that BMY 7378 inhibited the phenylephrine pressor curve due to the selective blockade of vascular α_{1D}-adrenoceptors, especially since these relatively low doses did not directly antagonize vascular α_{1A}- and, by inference, α_{1B}-adrenoceptors (see below). Therefore, it appears that exogenous administration of phenylephrine, but not (*R*)A-61603, produces vasocontraction that is mediated partly by the activation of vascular α_{1D}-adrenoceptors in the pithed rat. It should be stressed that α_{1A}- and α_{1B}-adrenoceptors may also be involved because 5-methylurapidil and chloroethylclonidine (alkylating $\alpha_{1B/D}$-adrenoceptor antagonist (Perez et al., 1991)), respectively, have been shown to inhibit partially the increase in diastolic pressure caused by phenylephrine and cirazoline in the rat (Piascik et al., 1990; Schwietert et al., 1992; Vargas et al., 1993).

Goetz et al. (1995) showed BMY 7378 to be a selective antagonist for α_{1D}-adrenoceptors (pK_i 8.2 ± 0.11), but the compound also displays affinity for α_{1A}- (pK_i 6.1 ± 0.03) and α_{1B}- (pK_i 6.2 ± 0.01) adrenoceptors, though with much lower affinity. While the interaction of BMY 7378 at vascular α_{1B}-adrenoceptors was not directly evaluated in the pithed rat, deduction indicates that this antagonist may bind non-selectively with this subtype in vivo, but only after high dose administration, i.e., 10 mg/kg. This possibility is inferred from the equipotency of BMY 7378 for both α_{1A}- and α_{1B}-adrenoceptors in vitro and the ability of the 10 mg/kg dose to cause the non-selective blockade of α_{1A}-adrenoceptors in the pithed rat. Obviously, a limitation of this work is the lack of selective α_{1B}- and α_{1D}-adrenoceptor agonists to directly evaluate the interaction of BMY 7378 with these α_1-adrenoceptor subtypes in vivo.

In summary, it is concluded that low doses of BMY 7378 selectively antagonize α_{1D}-adrenoceptors in vivo, as manifested by the ability of this compound to block the pressor effects to i.v. phenylephrine ($\alpha_{1A/B/D}$-adrenoceptor agonist) without affecting the pressor effect induced by α_{1A}-adrenoceptor stimulation, i.e., (*R*)A-61603. Therefore, certain doses of BMY 7378 may be used to distinguish vascular α_{1D}-adrenoceptors in vivo. In addition, the sensi-

tivity of the phenylephrine pressor response to BMY 7378 implies that α_{1D}-adrenoceptors are functionally expressed by vascular smooth muscle cells and may have a physiological role in the regulation of vascular resistance in the rat. This pharmacological evidence obtained from the pithed rat substantiates recent in situ hybridization and mRNA analysis which found α_{1D}-adrenoceptors localized at various sites in the vascular system (Piascik et al., 1994, 1995).

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