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Short communication

Evidence for an age-dependent functional expression of α_{1D}-adrenoceptors in the rat vasculature

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Abstract

The role of the α_1-adrenoceptor subtypes, and their possible change with maturation, in α_1-adrenoceptor-induced pressor responses in the rat has not been established. Thus, the effects of the α_{1D}-, $\alpha_{1A/1D}$- and $\alpha_{1B/1D}$-adrenoceptor antagonists, BMY 7378 (8-(2-(4-(2-methoxyphenyl)-1-piperazinyl)ethyl) 8-azaspiro (4.5) decane-7,9-dione 2HCl), 5-methyl-urapidil and chloroethylclonidine, respectively, on the pressor responses induced by phenylephrine in 1- and 5-month-old pithed rats were investigated. The pressor responses induced by phenylephrine were competitively antagonized by both BMY 7378 and chloroethylclonidine in 5-month-old, but not in young immature animals; in marked contrast, 5-methylurapidil antagonized with similar potency the phenylephrine-induced pressor responses in animals of both ages. The present pharmacological data suggest that functional expression of α_{1D}-adrenoceptors in the rat resistance vessels increases with age; α_{1A}-, but not α_{1B}- or α_{1D}-adrenoceptors, seem to predominate in immature animals. These findings represent the first evidence that age-related changes in functional α_1-adrenoceptor subtypes occur in the systemic vasculature in vivo. © 1997 Elsevier Science B.V.

Keywords: α_1-Adrenoceptor, subtype; Aging; Blood pressure; BMY 7378; Pithed rat

1. Introduction

It is well documented that the responsiveness of vascular smooth muscle to α_1-adrenoceptor stimulation changes with age though the precise mechanisms have not been elucidated (Docherty, 1990). In this regard, it has been recently suggested that the age-related alterations in α_1-adrenoceptor-mediated vasoconstrictor responses in isolated blood vessels may result from changes in the expression of the α_1-adrenoceptor subtypes; thus, functional, radioligand binding and molecular biology studies using rat aortic tissue have demonstrated that with age the expression of the α_{1A} subtype is increased whereas that of the α_{1B} subtype is decreased; the expression of the α_{1D} subtype, however, does not change (Gurdal et al., 1995a,b). Though interesting, these results in isolated conduit blood vessels are far from being conclusive as to the role of the α_1-adrenoceptor subtypes, and their possible change with maturation, in the responsiveness of the cardiovascular system to α_1-adrenoceptor stimulation. On these bases, we investigated the relative contribution of the different α_1-adrenoceptor subtypes in the pressor responses induced by phenylephrine in pithed rats, and the role of each subtype with maturation. The results suggest that the functional expression of α_{1D}-adrenoceptors in the resistance vessels of the rat increases with age whilst that of the α_{1A}-adrenoceptor subtype predominates in young immature animals.

2. Materials and methods

Male Wistar rats of 1 and 5 months of age ($n = 14$ each) were used. After anaesthesia with ether and cannulation of the trachea, the rats were pithed, artificially respired using an Ideal Palmer pump (56 cycles/min; volume: 20 ml/kg), and bilaterally vagotomized. Catheters were placed in the right femoral vein and the left carotid artery for drug administration and recording of diastolic blood pressure and heart rate, respectively; the carotid cannula was connected to a TXD-300 pressure transducer (Digi-Med, Lexington, KY, USA). Both diastolic blood pressure and heart rate were recorded simultaneously by a BPA-190b blood pressure analyzer (Digi-Med) and the body temperature of the animals was kept constant at 37°C.

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After the animals had been in a stable haemodynamic condition for at least 15 min, baseline values of diastolic blood pressure and heart rate were determined. After collection of these data, the animals from both ages received either physiological saline (1 ml/kg), BMY 7378 (8-(2-(4-(2-methoxyphenyl)-1-piperazinyl)ethyl) 8-azaspiro (4.5) decane-7,9-dione 2HCl) (1 mg/kg), 5-methyl-urapidil (1 mg/kg) or chloroethylclonidine (10 mg/kg); after 15 min (for saline, BMY 7378 and 5-methylurapidil) or 60 min (for chloroethylclonidine), dose-response curves for phenylephrine (1–1000 μg/kg; spaced by a factor of $10^{1/2}$), were elicited. Only one agonist dose-response curve was obtained per animal; in all cases, the maximum doses that could be tolerated by the animals were used.

2.1. Drugs

Phenylephrine hydrochloride, BMY 7378, 5-methyl-urapidil and chlorethylclonidine were obtained from Research Biochemicals International (Natick, MA, USA). All compounds were dissolved in physiological saline and, when needed (5-methylurapidil), 2% ascorbic acid was added. Fresh solutions were prepared for each experiment and the doses refer to the free base of substances.

2.2. Data presentation and statistical analysis

All data are presented as means ± S.E.M. The peak changes in diastolic blood pressure produced by phenylephrine in saline- and antagonist-treated animals of 1 and 5 months of age were determined and compared using Newman-Keuls' test, once an analysis of variance (randomized block design) had revealed that the samples represented different populations (Steel and Torrie, 1980). Where appropriate, the parallelism test (Tallarida and Murray, 1981) or *t*-test were used. ED_{50} and E_{max} values were obtained by non-linear regression. A P value of 0.05 or less (two-tailed) was considered statistically significant.

3. Results

Baseline values of diastolic blood pressure and heart rate in 1- and 5-month-old pithed animals ($n = 14$ each) were 33 ± 2 mm Hg and 245 ± 7 beats/min and 36 ± 1 mm Hg and 292 ± 4 beats/min, respectively. Although these values remained unmodified 15 min after administration of either saline, BMY 7378 or 5-methylurapidil, and 60 min after chloroethylclonidine, all the antagonists produced immediate pressor responses. In the case of chloroethylclonidine, this response was particularly prominent and significantly higher in 5-month-old (53.2 ± 1.4 mm Hg; $n = 3$) than in 1-month-old (22.7 ± 5.0 mm Hg; $n = 3$) rats. The intravenous (i.v.) administration of phenylephrine resulted in dose-dependent increases in diastolic blood pressure in animals of both ages (Fig. 1). In accordance with previous observations in isolated blood vessels (Gurdal et al., 1995a), the estimated potency of phenylephrine to increase diastolic blood pressure was significantly lower in mature animals (Table 1).

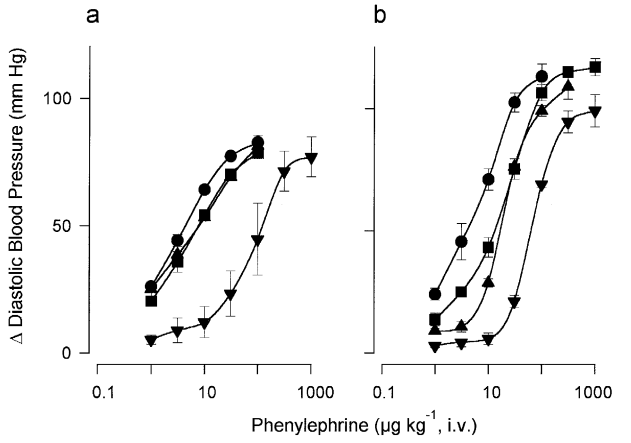

Fig. 1. The effects of i.v. bolus injections of saline (●, 1 ml/kg), BMY 7378 (▲, 1 mg/kg), 5-methylurapidil (▼, 1 mg/kg) or chloroethylclonidine (■, 10 mg/kg) on the pressor responses induced by phenylephrine in pithed rats of (a) 1 month and (b) 5 months of age. Points represent the mean ± S.E.M. of 3–4 experiments.

The i.v. treatment with either 5-methylurapidil (1 mg/kg), chloroethylclonidine (10 mg/kg) or BMY 7378 (1 mg/kg) to 5-month-old animals produced a significant parallel (parallelism test between curves was not significant) rightward displacement of the control dose-response curve for phenylephrine; interestingly, 5-methylurapidil was the most potent in this respect whereas chloroethylclonidine and BMY 7378 were equipotent (Fig. 1b; see also the corresponding ED_{50}, E_{max} and dose ratios in Table 1). In marked contrast, BMY 7378 and chloroethyl-

Table 1
Effects of i.v. bolus administration of BMY 7378 (1 mg/kg), 5-methylurapidil (1 mg/kg) and chloroethylclonidine (10 mg/kg) on the potency (ED_{50}) and efficacy (E_{max}) estimates for phenylephrine in 1- and 5-month-old pithed rats

Group	n	Agonist ED_{50} (μg/kg, i.v.)	E_{max} (mm Hg)	Dose ratio [a]
Control				
1 Month	4	2.7 ± 0.2	85.5 ± 1.0	–
5 Months	4	5.5 ± 0.9 [b]	119.9 ± 5.0 [b]	–
BMY 7378				
1 Month	4	4.72 ± 1.0	89.3 ± 5.0	1.7
5 Months	4	18.6 ± 2.0 [c]	109.0 ± 3.0	3.4
5-Methylurapidil				
1 Month	3	71.0 ± 16.0 [d]	82.0 ± 5.0	26.1
5 Months	4	63.9 ± 4.0 [e]	100.0 ± 2.0 [d]	11.7
Chloroethylclonidine				
1 Month	3	4.2 ± 0.4 [c]	82.6 ± 2.0	1.5
5 Months	3	17.3 ± 3.0 [d]	123.5 ± 5.0	3.2

Values are the mean ± S.E.M. of n experiments. [a] Ratio of ED_{50} with antagonist/ED_{50} of the control values; [b] $P < 0.05$ vs. 1 month and [c] $P < 0.05$; [d] $P < 0.005$; [e] $P < 0.001$ vs. control.

clonidine produced very weak blocking effects in 1-month-old rats; 5-methylurapidil antagonized, also with high potency, the phenylephrine-elicited effect in these animals (Fig. 1a; Table 1).

4. Discussion

The present results demonstrate, for the first time, that the age-dependent changes in vascular smooth muscle responsiveness to α_1-adrenoceptor stimulation do occur in the systemic vasculature in vivo. Consistent with previous investigations in both conscious (Piascik et al., 1990) and pithed (Schwietert et al., 1992) rats, the relatively selective α_{1A}-adrenoceptor antagonist, 5-methylurapidil (Hieble et al., 1995), which also shows relatively high affinity for the α_{1D}-adrenoceptor subtype (Forray et al., 1994; Buckner et al., 1996), behaved as a potent antagonist against the pressor responses induced by phenylephrine. In addition, the use of the selective α_{1D}-adrenoceptor antagonist, BMY 7378 (Goetz et al., 1995), has extended these findings thus providing evidence that the α_{1D} subtype does play a role in the α_1-adrenoceptor-mediated pressor responses in pithed rats. In fact, while preparing this work, another study showing the specific antagonism by BMY 7378 (0.1–1 mg/kg, i.v.) of phenylephrine-induced pressor effects in Long-Evans pithed rats was published (Zhou and Vargas, 1996). Thus, BMY 7378, along with 5-methylurapidil and chloroethylclonidine, allowed us to evaluate the relative contribution of the α_1-adrenoceptor subtypes in the pressor effects of phenylephrine in pithed rats and, most importantly, to test the hypothesis that the functional expression of the α_1-adrenoceptor subtypes involved in these responses changes with maturation.

In contrast to previous findings demonstrating that the expression of the α_{1D}-adrenoceptor in the rat aorta does not change with age (Gurdal et al., 1995b), the present data, showing a higher potency of BMY 7378 in mature animals, suggest that the functional expression of this α_1-adrenoceptor subtype in systemic resistance vessels increases with maturation. Furthermore, the expression of the aortic α_{1A}-adrenoceptor increased with age whereas that of the α_{1B}-adrenoceptor subtype decreased (Gurdal et al., 1995b). This seems not to be the case in the rat systemic vasculature as 5-methylurapidil, but not BMY 7378 or chloroethylclonidine, strongly antagonized the pressor effects of phenylephrine in 1-month-old rats thereby suggesting that the α_{1A}-adrenoceptor predominates in these animals.

That both α_{1A} and α_{1D} subtypes have a major role in 5-month-old pithed animals is suggested by: (i) the equipotent antagonist effects of 5-methylurapidil in animals from both ages; and (ii) the clear antagonist effects of BMY 7378 in mature – but not in young immature– animals. Since 5-methylurapidil displays high affinity for α_{1A}- and α_{1D}-adrenoceptors (Forray et al., 1994; Buckner et al., 1996), its higher antagonist potency, with respect to BMY 7378, in animals of 5 months of age (see the corresponding dose ratios in Table 1) may be explained by the blockade of both receptors in the systemic vasculature. Furthermore, since chloroethylclonidine has been shown to inactivate the α_{1B} and α_{1D} subtypes with similar efficacy (Perez et al., 1994; Schwinn et al., 1994), it might be suggested that in mature animals the blocking effects of chloroethylclonidine, which are comparable to those of BMY 7378 (Fig. 1b), are explained by interaction with α_{1D}-, but not with α_{1B}-adrenoceptors. In support of this possibility, the administration of BMY 7378 (1 mg/kg, i.v.) to 5-month-old pithed rats pretreated with chloroethylclonidine (10 mg/kg, i.v.) apparently failed to produce a further blockade of the phenylephrine-induced pressor response (not shown). Therefore, it is possible that α_{1B}-adrenoceptors have a minor role in mature animals, as inferred from previous results in conscious rats (Piascik et al., 1993).

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References

Buckner, S.A., Oheim, K.W., Morse, P.A., Knepper, S.M., Hancock, A.A., 1996. α_1-Adrenoceptor-induced contractility in rat aorta is mediated by the α_{1D} subtype. Eur. J. Pharmacol. 297, 241–248.

Docherty, J.R., 1990. Cardiovascular responses in ageing: a review. Pharmacol. Rev. 42, 103–125.

Forray, C., Bard, J.A., Wetzel, J.M., Chiu, G., Shapiro, E., Tang, R., Lepor, H., Hartig, P.R., Weinshank, R.L., Branchek, T.A., Gluchowski, C., 1994. The α_1-adrenergic receptor that mediates smooth muscle contraction in human prostate has the pharmacological properties of the cloned human α_{1c} subtype. Mol. Pharmacol. 45, 703–708.

Goetz, A.S., King, H.K., Ward, S.D.C., True, T.A., Rimele, T.J., Saussy Jr., D.L., 1995. BMY 7378 is a selective antagonist of the D subtype of α_1-adrenoceptors. Eur. J. Pharmacol. 272, R5–R6.

Gurdal, H., Cai, G., Johnson, M.D., 1995a. α_1-Adrenoceptor responsiveness in the aging aorta. Eur. J. Pharmacol. 274, 117–123.

Gurdal, H., Tilakaratne, N., Brown, R.D., Fonseca, M., Friedman E., Johnson, M.D., 1995b. The expression of α_1-adrenoceptor subtypes changes with age in the rat aorta. J. Pharmacol. Exp. Ther. 275, 1656–1662.

Hieble, J.P., Bylund, D.B., Clark, D.E., Eikenburg, D.C., Langer, S.Z., Lefkowitz, R.J., Minneman, K.P., Ruffolo Jr., R.R., 1995. International Union of Pharmacology X. recommendation for nomenclature of α_1-adrenoceptors: consensus update. Pharmacol. Rev. 47, 267–270.

Perez, D.M., Piascik, M.T., Malik, N., Gaivin, R., Graham, R.M., 1994. Cloning, expression and tissue distribution of the rat homolog of the bovine α_{1C}-adrenergic receptor provide evidence for its classification as the α_{1A} subtype. Mol. Pharmacol. 46, 823–831.

Piascik, M.T., Kusiak, J.W., Barron, K.W., 1990. α_1-Adrenoceptor subtypes and the regulation of peripheral hemodynamics in the conscious rat. Eur. J. Pharmacol. 186, 273–278.

Piascik, M.T., Smith, M.S., Barron, K.W., Soltis, E.E., 1993. The regulation of regional hemodynamics by alpha-1 adrenoceptor subtypes in the conscious rat. J. Pharmacol. Exp. Ther. 267, 1250–1255.

Schwietert, H.R., Mathy, M.-J., Wilhelm, D., Wilffert, B., Pfaffendorf, M., Van Zwieten, P.A., 1992. α_1-Adrenoceptor-mediated Ca^{2+}-entry from the extracellular fluid and Ca^{2+}-release from intracellular stores: no role for $\alpha_{1A,B}$-adrenoceptor subtypes in the pithed rat. J. Auton. Pharmacol. 12, 125–136.

Schwinn, D.A., Johnston, G.I., Page, S.O., Mosley, M.J., Wilson, K.H., Worman, N.P., Campbell, S., Fidock, M.D., Furness, L.M., Parry-Smith, D.J., Peter, B., Bailey, D.S., 1994. Cloning and pharmacological characterization of human alpha-1 adrenergic receptors: sequence corrections and direct comparison with other species homologues. J. Pharmacol. Exp. Ther. 272, 134–142.

Steel, R.G.D., Torrie, J.H., 1980. Principles and Procedures of Statistics, A Biomedical Approach. McGraw-Hill, Tokyo.

Tallarida, R.J., Murray, R.B., 1981. Manual of Pharmacological Calculations with Computer Programs. Springer, New York, NY.

Zhou, L., Vargas, H.M., 1996. Vascular α_{1D}-adrenoceptors have a role in the pressor response to phenylephrine in the pithed rat. Eur. J. Pharmacol. 305, 173–176.