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Characterization of Inhibition by Nifedipine and Nitroprusside of the Pressor Responses to α_1-Adrenoceptor Agonists Cirazoline and Sgd 101/75 in Pithed Rats

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In this study the effects of two unrelated vasodilators, nifedipine and nitroprusside, on the pressor responsiveness to the α_1-adrenoceptor full agonist cirazoline and partial agonist Sgd 101/75 in pithed rats were examined. The experiments were performed on the vasoconstriction which was mediated by newly synthetized α_1-adrenoceptors after removal of existing α_1-adrenoceptors by phenoxybenzamine treatment (5 mg/kg, i. p.). The $t_{1/2}$ for recovery of the maximum response and ED_{50} of cirazoline were 23.1±5.5 and 26.9±7.4 hours, respectively, while that for recovery of the maximum response of Sgd 101/75 was 59.2±18.9 hours. The relationship between the pressor response and the fractional receptor occupancy for cirazoline showed a rectangular hyperbola. This occupancy-response curve markedly shifted to the right one day after phenoxybenzamine and subsequently returned to the control, indicative of a large receptor reserve. However, for Sgd 101/75 the occupancy-response curve exerted less of a hyperbola and shifted little after phenoxybenzamine. While the maximum response to cirazoline in the control rats was resistant to inhibition by the calcium entry blocker nifedipine, this resistance was significantly reduced one and 3 days after phenoxybenzamine, just as the maximum response to Sgd 101/75 was sensitive to nifedipine in the control rats. Likewise, when nitroprusside was used instead, the results were similar for the cirazoline and Sgd 101/75 effects. In summary, it seems unlikely that the resistance to the calcium entry blocker of the full agonist effect can be wholly ascribed either to the receptor reserves or to the differential calcium utilization itself. Alternatively, it is suggested that the differential resistance to calcium antagonists can result from the magnitude of the variables involved in the activation of α_1-adrenoceptor coupling processes depending on the full or partial agonist.

Key Words: Calcim antagonists, α_1-Adrenoceptors

In the vasculature of many mammalian species including humans, the α_1- and α_2-adrenoceptors coexist at postsynaptic sites mediating vasoconstriction (Drew and Whiting, 1979; Docherty and McGrath, 1980; Kobinger and Pichler, 1980). From numerous studies, the vasoconstrictor responses to the α_1-adrenoceptor agonists have been known to be readily antagonized by calcium channel blockers; whereas the pressor responses elicited by α_2-adrenoceptor agonists are resistant to them (Van Meel *et al.* 1983; Cavero *et al.* 1983). Then, α_1-adrenoceptor-mediated vasoconstriction is further suggested to have two distinct processes of calcium utilization based on the differential influence of calcium entry blockers (Timmermans *et al.* 1983a; b; 1985). The vasoconstriction induced by the full agonist such as phenylephrine or methoxamine is resistant to calcium entry blockers and the vasoconstriction by the partial agonists Sgd 101/75 or St 587 seems to be wholly governed by the entry of extracellular calcium ions. The resistance has been reportedly controversial, either ascribing to a large receptor reserve of the α_1-adrenoceptors for the full agonists (Matthews *et al.* 1984; Ruffolo *et al.* 1984) or to different α_1-adrenoceptor recognition sites which are linked to calcium influx-dependent and independent processes (Timmermans *et al.* 1985).

In the present study, we examined the effects of two unrelated vasodilators, nifedipine and nitroprusside, on the pressor responsiveness to the

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full agonist cirazoline and partial agonist Sgd 101/75 in pithed rats. The rats were pretreated with 5 mg/kg phenoxybenzamine to remove the existing α_1-adrenoceptors. Thus, the vasoconstriction by these agonists was considered to be mediated by newly synthetized α_1-adrenoceptors. In this manner, it was possible to assess the relationship of the vasoconstriction induced by α_1-adrenoceptor agonists with the antagonism of the calcium antagonists.

MATERIALS AND METHODS

Male Sprague-Dawley rats weighing 220-280 g were given 5.0 mg/kg phenoxybenzamine, i. p. The control animals received saline, The Rats were anesthetized with sodium secobarbital (30 mg/kg, i. p.). Following bilateral vagotomy they were pretreated with atropine and *d*-tubocurarine (1.0 mg/kg, i. p., each). The arterial pressure was measured through the carotid artery with a pressure transducer (P-1000B, Narco Bio-Systems) and recorded on a physiograph (MK IV, Narco Bio-Systems). The animals were artificially ventilated at a frequency of 40 cycles/min with a volume of 20 ml/kg by respirator (Model V5KG, Narco Bio-Systems). Rats were pithed by insertion of a steel rod (1.5 mm in diameter) through the right orbit and foramen magnum down into the spinal canal by the method described by Gillespie *et al.*, (1970). They were allowed to equilibrate for 30 min. The i. v. dose of pressor agent was increased approximately 3-fold with each successive administration. One complete dose-response curve was performed for one animal. To block the activation of the α_2-adrenoceptors and β-adrenoceptors, yohimbine (1.0 mg/kg) and propranolol (0.5 mg/kg) were injected i.p., respectively.

The recovery rate ($t_{1/2}$) of the maximum response and ED_{50} determined after phenoxybenzamine treatment was calculated according to the simplest model of exponential recovery (Riggs 1963; Hamilton *et al.* 1984). In in vivo studies it is impossible to estimate the K_A (dissociation constant of agonist-receptor complex) value. However, to evaluate the capacity of spare receptors for the α_1-adrenoceptors which are assumed to be newly synthetized after phenoxybenzamine (5.0 mg/kg), both the q value (the fraction of receptors remaining intact, not alkylated, following treatment with a miligram level of phenoxybenzamine) and $\frac{[R_A]}{[R_T]}$ (the fractional roceptor occupancy) were calculated by using the apparent K_A calculated according to the equations described by Furchgott (1966) and Furchgott and Bursztyn (1967).

$$\frac{1}{[A]} = \frac{1-q}{q \cdot K_A} + \frac{1}{q \cdot [A']}$$

where [A] and [A'] are the corresponding equieffective concentrations of agonist before and after partial receptor inactivation by a microgram level of phenoxybenzamine (for cirazoline 200 μg/kg and for Sgd 101/75 20 μg/kg).

The reciprocals of the concentrations of agonists before receptor inactivation (1/[A]) are plotted against the reciprocals of the equieffective concentration of the agonist after partial inactivaltion (1/[A']). Thereafter, the apparent K_A and q value were determined (K_A=(slope-1)/intercept, q=1/slope). Further, the fractional occupancy of each concentration of agonist was calculated and the pressor response at each point was plotted as a function of the fractional receptor occupancy. $\frac{[R_A]}{[R_T]} = [A]/(K_A+[A])$, where $[R_A]$ is the concentration of receptor-agonist complex and $[R_T]$ the total receptor concentration.

The drugs used were cirazoline HCl (Synthelabo), nifedipine HCl (Miles Lab.), phenoxybenzamine HCl (Smith Kline & French Labs.), Sgd 101/75 HCl (Siegfried), sodium nitroprusside (Roche), atropine sulfate (Sigma) and *d*-tubocurarine chloride (Sigma).

Nifedipine was dissolved in a mixed solution of 10 % ethanol, 10 % Tween 80 and 5 % glucose. Nifedipine and nitroprusside were protected from light and prepared immediately before use. All drugs were diluted with fresh saline when necessary.

Statistical analyses were done with Student's t-test and a level of $p<0.05$ was judged as significant.

RESULTS

In the intact control rats, administration of phenoxy-benzamine (5.0 mg/kg, i.p.) caused a significant fall in mean arterial pressure from 138.0±2.1 mmHg to 101.2±3.4 mmHg($p<0.001$) when determined 3 hours after treatment. It recovered to control level after 24 hours. The mean diastolic arterial pressure was 38.4±0.8 mmHg (n=80) in the pithed control rats.

Turnover of the α_1-adrenoceptors

As shown in Fig. 1 and Table 1, the dose-response curves to cirazoline and Sgd 101/75 shifted slightly to the right with a profound depression of the maximum response 3 hours after phenoxybenzamine treatment, and at subsequent times they slowly returned toward

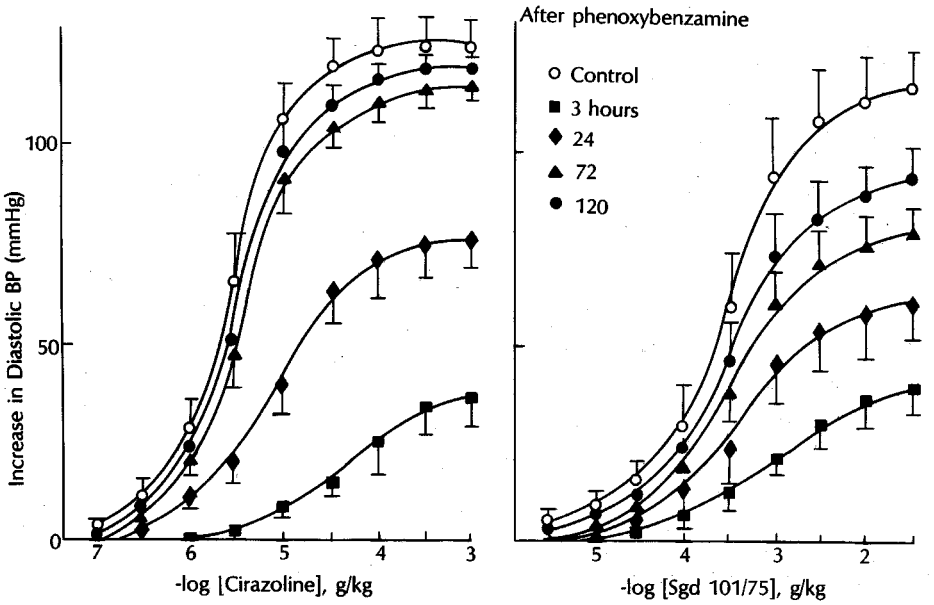

Fig. 1. *Recovery of the diastolic blood pressure (BP) responses to cirazoline (left) and Sgd 101/75 (right) following irreversible alkylation of the α_1-adrenoceptors with phenoxybenzamine was determined at various intervals in pithed rats. Each point represents the mean ±S.E.M. of 6–9 experiments.*

Table 1. Recovery of the maximum pressor increase and ED_{50} values of α_1-adrenceptor agonists after treatment with phenoxybenzamine (5.0 mg/kg) in pithed rats

Time after phenoxybenzamine, hrs	Cirazoline		Sgd 101/75	
	Max BP, ΔmmHg	ED_{50}, μg/kg	Max BP, ΔmmHg	ED_{50}, μg/kg
Control	130.5±6.4	2.7±0.4	115.1±8.0	280.0± 80.3
3	37.7±9.8[b]	78.6±8.2[b]	50.0±5.2[b]	602.7±158.7
24	80.3±9.5[a]	12.7±2.5[b]	71.0±5.9[b]	393.0±146.3
72	117.1±4.9	5.3±1.1[a]	83.2±6.5[a]	298.8± 68.4
120	122.7±4.3	4.8±0.5[a]	95.2±7.0	320.1±133.9
240	125.0±3.3	3.3±0.4	ND	ND

Each result represents the mean ±S.E.M. of 6-9 experiments.
a, $p<0.05$; b, $p<0.01$: Significantly different from the control.
Maximum pressor increase (Max BP) was assessed as a pressor response to 1 mg/kg cirazoline and 30 mg/kg Sgd 101/75, respectively.
ED_{50}: Dose of agonist required 50% of the maximum pressure response.
ND

their respective control values. However, the recovery of Sgd 101/75 response was much delayed. The $t_{1/2}$ for recovery of the maximum response and ED_{50} of cirazoline were 23.1±5.5 and 26.9±7.4 hours, respectively. The $t_{1/2}$ for recovery of the maximum response to Sgd 101/75 was 59.2±18.9 hours, but that of ED_{50} was undetectable

Assessment of the receptor reserves

The fraction of receptors remaining intact(q) which were not alkylated was compared between the

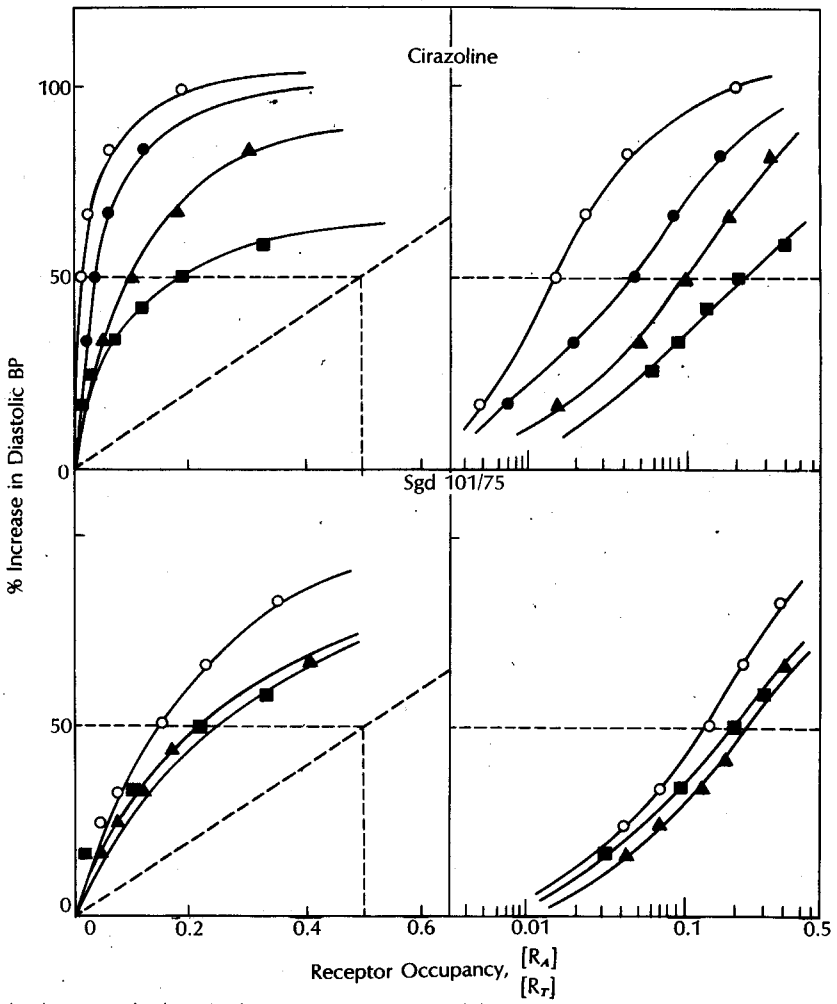

Fig. 2. *A relationship between the fractional receptor occupancy and the pressor response to cirazoline (upper) and Sgd 101/75 (lower) was plotted for control pithed rats and the changes in their relationships were determined according to the time courses after phenoxybenzamine treatment. The diastolic pressor effects (BP) were depicted on the linear axis in the left and on the logarithmic scale in the right.*

responses to cirazoline and Sgd 101/75. In this connection, the doses of phenoxybenzamine; 200 μg/kg for cirazoline-sensitive site and 20 μg/kg for Sgd 101/75-sensitive site were chosen so as to cause reduction of approximately the same amount of the pressor response to both agonists. Treatment with these doses yielded approximately 38 % inhibition of the maximum response to both agonists, respectively. Therefore, 62 % of the maximum response to cirazoline occurred upon alkylation of 98.7 % of the α_1-adrenoceptors as illustrated with the q value in Table 2, whereas the same amount of Sgd 101/75 response was manifested upon alkylation of 70.5% of the α_1-adrenoceptors, and $\frac{[R_A]}{[R_T]}$ 50% of Sgd 101/75 was 11-fold higher than that of cirazoline, this evidence suggesting an involvement of a large receptor reserve for cirazoline response.

When the relationship between the fractional receptor occupancy and the pressor response was plotted on linear occupancy scale (Fig. 2), a rectangular hyperbolic curve was shown for cirazoline whereas for Sgd 101/75 less hyperbola was manifested. One day after phenoxybenzamine treatment, the curve of

Table 2. The *q* values and relative occupancy of cirazoline and Sgd 101/75 in pithed rats

Agonist	Days after phenoxybenzamine	*q*	$\frac{[R_A]}{[R_T]}$ 50%
Cirazoline	Control (6)	0.013	0.013±0.004
	1 (6)	0.186[b]	0.180±0.044[b]
	3 (6)	0.101[a]	0.103±0.023[a]
	8 (4)	0.038	0.044±0.020
Sgd 101/75	Control (6)	0.295	0.142±0.029
	1 (4)	0.327	0.199±0.061
	3 (4)	0.251	0.301±0.116

Values are mean ±S.E.M.

q: See text.

$\frac{[R_A]}{[R_T]}$ 50% was caculated as a relative response of 50% of the maximum response to each agonist in control rats. Numbers in parentheses represent the number of experiments.

a, $p<0.05$; b. $p<0.01$: Significantly different from the control.

cirazoline effect markedly shifted to the right in a diminished hyperbola shape indicative of a reduced receptor reserve.

Accordingly, the $\frac{[R_A]}{[R_T]}$ 50% increased by 14-fold one day after phenoxybenzamine and thereafter, it slowly returned to the control level. These alterations were paralleled by changes in the *q* value. However, in the relationship between the pressor response to Sgd 101/75 and the receptor occupancy, the curve was not significantly shifted after phenoxybenzamine and $\frac{[R_A]}{[R_T]}$ 50% was little changed throughout the receptor recovery.

Effect of calcium antagonists

The effects of the calcium channel blocker nifedipine and nitrovasodilator nitroprusside on the pressor responses to cirazoline and Sgd 101/75 were evaluated. As illustrated in Fig. 3 and Table 3, two dif-

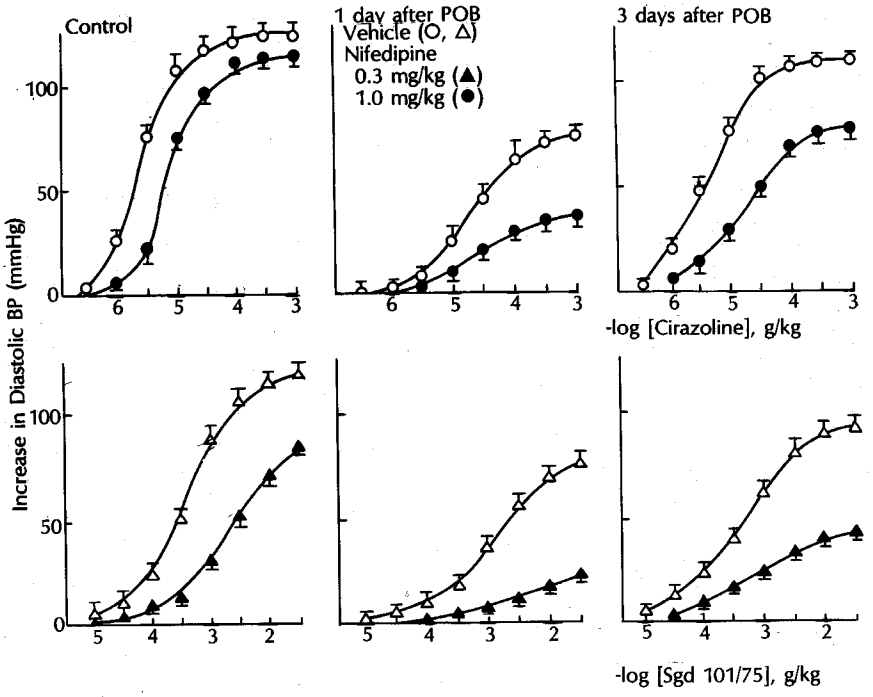

Fig. 3. The antagonism by nifedipine of the pressor responses to cirazoline (A) and Sgd 101/75 (B) in the control rats, and in the rats one and 3 days after treatment with phenoxybenzamine (POB). Each point represents the mean ± S.E.M. of 4–6 experiments.

ferent doses of nifedipine were chosen and administered (i. a.): 1.0 mg/kg for cirazoline and 0.3 mg/kg for Sgd 101/75 so as to compare the antagonism. The maximum pressor responses to cirazoline in the control rats showed much more resistance to inhibition by nifedipine than did those to Sgd 101/75 (9.1±2.5 vs. 22.7±4.0 % inhibition). However, one and 3 days after phenoxybenzamine, the resistance to inhibition by nifedipine of the cirazoline response was profoundly reduced (48.2±7.4 %, one day and 31.9 ±8.0 %, 3 days). Parallelly, the sensitivity to inhibition by nifedipine of the Sgd 101/75

Table 3. Percent inhibition by nifedipine and nitroprusside of the maximum pressor responses to cirazoline and Sgd 101/75

Days after phenoxybenzamine	Inhibition by Nifedipine, %		Inhibition by Nitroprusside, %	
	Cirazoline	Sgd 101/75	Cirazoline	Sgd 101/75
Control	9.1±2.5 (6)	22.7± 4.0(6)	0 (5)	6.8± 3.0 (4)
1	48.2±7.4 (7)[b]	69.1± 4.0 (7)[b]	66.7±9.0 (5)[b]	48.3±15.8 (5)[b]
3	31.9±8.0 (5)[a]	54.3±13.9 (4)[a]	21.2±6.8 (5)[a]	22.0± 9.8 (4)[a]
8	9.9±3.3 (4)	–	0 (4)	–

Pressor responses to 1.0 mg/kg cirazoline and 10.0 mg/kg Sgd 101/75 were considered as 100% and % inhibition was calculated. Two different doses of nifedipine and nitroprusside were administered (i.a.): for cirazoline effect, 1.0 mg/kg nifedipine and 66.7 μg/kg/min nitroprusside were used, respectively and for Sgd 101/75 effect, 0.3 mg/kg nifdeipine and 6.7 μg/kg/min nitroprusside used, respectively.

a, $p<0.05$; b, $p<0.01$: Significantly different from the control.

Numbers in parentheses represent the number of experiments.

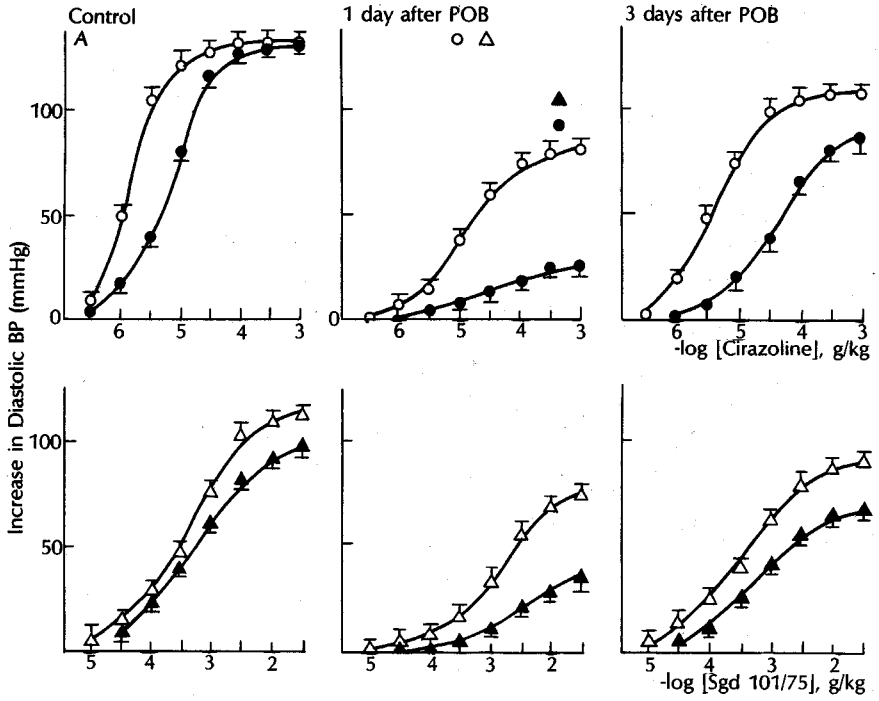

Fig. 4. The antagonism by nitroprusside of the pressor responses to cirazoline (A) and Sgd 101/75 (B) in the control rats, and in the rats after phenoxybenzamine treatment (POB). Each point represents the mean ± S.E.M. of 4–6 experiments.

response was greatly increased (69.1±4.0 %, one day and 54.3±13.9 %, 3 days).

Likewise, when nitroprusside was used (infusion with a dose of 66.7 μg/kg/min for cirazoline effect and 6.7 μg/kg/min for Sgd 101/75 for 15 min), the results were very similar to the results yielded by nifedipine on the responses to cirazoline and Sgd 101/75. Interestingly, the inhibition by nitroprusside of the maximum responses to both cirazoline and Sgd 101/75 was similarly enhanced one and 3 days after phenoxybenzamine treatment as shown in Fig. 4 and Table 3.

DISCUSSION

In the preliminary study, treatment with cycloheximide caused a prolongation of the recovery of the pressor response to cirazoline which was previously delayed after phenoxybenzamine. It appears likely that the rate of recovery of the α_1-adrenoceptor-mediated vasoconstriction reflects the degree of the activation of newly synthetized recepror protein as suggested by Hamiliton *et al.* (1982; 1984).

In the present study using pithed rats the changes in the antagonism by unrelated organic vasodilators (nifedipine and nitroprusside) were assessed comparing the changes in receptor reserves at various intervals after phenoxybenzamine treatment. The half-lives for recovery of the maximum response and ED_{50} of cirazoline, α_1-adrenoceptor full agonist (Matthews *et al.* 1984; Ruffolo *et al.* 1984) were 23.1 and 26.9 hours, while Sgd 101/75 despite α_1-adrenoceptor selective agonist (Timmermans *et al.* 1983; Mathy *et al.* 1984) exhibited 59.2 hours. The recovery rate of the cirazoline-induced response is consistent with that of the maximum pressor response to phenylephrine in rabbits (21.6 hours) which was demonstrated by Hamilton *et al.* (1984). Taken together, the half-life of Sgd 101/75 lies in the midst between the recovery rate of the in vivo response to cirazoline and that of specific [^{3}H] prazosin binding (86.4 hours; Hamilton *et al.* 1984). Thus it is assumed that the discrepancy between the recovery rate of cirazoline and that of Sgd 101/75 could be accounted for by the spare receptor concepts (Stephenson 1956; Ruffolo and Waddell 1982), that is, a substantial portion of the α_1-adrenoceptors for the cirazoline effect being necessary in a lesser degree than that for Sgd 101/75 in eliciting full responses.

In the control rats, the pressor responses induced by cirazoline showed a rectangular hyperbolic curve when plotted as a function of the fractional α_1-adrenoceptor occupancy. whereas the response by Sgd 101/75 produced less of a hyperbolic shape. Interestingly, the receptor occupancy-response curve of cirazoline one day after phenoxybenzamine shifted to the right nearly overlapping the control curve of Sgd 101/75. The curve for cirazoline subsequently returned toward the control curve with time, while the curve for Sgd 101/75 changed little throughout.

Recently, there has been a growing interest in the functional antagonism of α_1- and α_2-adrenoceptor-mediated responses by vasodilators (Van Meel *et al.* 1983; Pedrinelli and Tarazi 1985; Eskinder and Gross 1986). Likewise, the pressor response to Sgd 101/75 in control rats was sensitively inhibited by nifedipine (0.3 mg/kg). However, that to cirazoline consistently showed higher resistance to nifedipine (1.0 mg/kg) as previously demonstrated elsewhere (Mathy *et al.* 1984; Ruffolo *et al.* 1984; Timmermans *et al.* 1983a; 1985). Nevertheless, one and 3 days after phenoxybenzamine, the reduction of inhibition by nifedipine was evident not only for cirazoline but also for Sgd 101/75 induced responses. These results were also manifested very similarly in the antagonism by nitroprusside. Nitroprusside relaxes the vascular smooth muscle independent of extracellular calcium (Verhaege and Shepherd 1976; Kreye 1980; Rapoport *et al.* 1982), and this action has been reported to be mediated by cyclic GMP-dependent protein phosphorylation (Ignarro and Kadowitz 1985) or inhibition of receptor-linked calcium channels (Karaki and Weiss 1980) or inhibition of phosphoinositide turnover (Rapoport 1986). These mechanisms have explained a decrease in intracellular levels of calcium by the mechanisms: increased bindings, sequestration or efflux of calcium (Rapoport *et al.* 1982; Lincoln and Johnson 1984).

In this regard, there appears some contradiction that the resistance to calcium entry blockers of the full agonist effect can be wholly ascribed either to the receptor reserves or to calcium utilization itself due to different α_1-adrenoceptor recognition sites, since Sgd 101/75 lacks some properties to release intracellular calcium (Bou and Massingham 1986; Chiu *et al.* 1986) and further in the present study. two unrelated vasodilators exert a similarity to inhibit the vasoconstriction by cirazoline and Sgd 101/75 one and 3 days after phenoxybenzamine.

The vasoconstriction by α_1-adrenoceptor agonists can be the net result of a series of coupling events from agonist-receptor binding to tension development by utilizing the free calcium ions. The current hypothesis on the α_1-adrenoceptor coupling mechanism (from activation to contraction) has described the agonist-receptor binding, activation of

phospholipase C and cleavage of polyphosphoinositides (Berridge 1983; Nishizuka 1984). These coupling steps have explained the processes of calcium mobilization regarding intracellular calcium release and the entry of extracellular calcium of vasoconstriction (Danthuluri and Deth 1984; Forder *et al.* 1985). Thus, it is indicated that the higher resistance to calcium antagonists can be ascribed largely to the full activation of the α_1-adrencoeptor coupling systems in response to the full agonists, in which they modulate both intracellular calcium release and the influx of extracelluar calcium. Otherwise, the decreased resistance to a calcium antagonist can result from poor activation of the α_1-adrenocptor coupling processes in response to the partial agonist. However, this in vivo experiment may limit further illustrations.

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