Interference of calcium entry blockade in vivo with pressor responses to $\alpha$-adrenergic stimulation: effects of two unrelated blockers on responses to both exogenous and endogenously released norepinephrine

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ABSTRACT  To the extent that calcium availability is the final common mediator of vasoconstrictor responses, calcium entry blockade might interfere with physiologic responses to adrenergic stimulation. To test this hypothesis, we studied the effects of calcium entry blockade on pressor responses to norepinephrine in pithed, normal Sprague-Dawley rats in two different ways: (1) by evaluating the effects on pressor responsiveness to exogenous norepinephrine during differential blockade of $\alpha_1$- (prazosin, 0.3 mg/kg) and of $\alpha_2$- receptors (yohimbine, 0.3 mg/kg) and (2) by comparing the effects of calcium entry blockade with those of prazosin and those of rauwolscine (a specific $\alpha_2$-antagonist) on pressor responses to infusions of both endogenously released norepinephrine (electrical stimulation of the pithing rod) and exogenous norepinephrine. In the presence of $\alpha_1$-blockade, both nitrendipine (0.01 mg/kg) and verapamil (0.6 mg/kg) shifted the norepinephrine pressor dose-response curve to the right but were ineffective in $\alpha_2$-blocked animals. Furthermore, nitrendipine (range 0.01 to 0.3 mg/kg) proved to be more effective ($p < .001$) against exogenous norepinephrine than against electrical stimulation of the spinal cord, a behavior opposite that of selective $\alpha_1$-blockade (prazosin) and directionally comparable to that of selective $\alpha_2$-antagonism (rauwolscine). These data indicate that calcium entry blockade in vivo preferentially antagonizes the $\alpha_2$-pressor component of exogenous norepinephrine. In addition, both calcium entry blockers were consistently more active ($p < .01$) than rauwolscine (0.01 to 1 mg/kg) in antagonizing the pressor response to neural stimulation, suggesting that mechanisms different from "classical" $\alpha_2$-antagonism may also contribute to the overall effect of calcium entry blockade on the adrenergic control of peripheral vascular tone.


THE RELATIVELY recent discovery that a group of organic compounds generally labeled as "calcium entry blockers" had effects similar to those of reduction of extracellular calcium has led to a reevaluation of the role of calcium influx in the responses to receptor-mediated stimuli, particularly the vasoconstrictor responses to $\alpha$-adrenergic stimulation. Two concomitant and interrelated effects have been shown to occur as a consequence of binding of the adrenergic transmitter to $\alpha$-receptors: (1) opening of sarcoplasmic channels for intracellular entry of external calcium ions and (2) release of intracellular calcium from its storage sites. This suggests that pharmacologic interference with calcium influxes could therefore impair vasoconstrictor responses to $\alpha$-adrenergic stimulation in proportion to the relative dependence of adrenergic responsiveness on extracellular calcium concentrations.

This study was designed to test that inference by analyzing the effects of calcium entry blockade on pressor responses to norepinephrine. The pressor responses were induced by exogenous administration and endogenous release by electrical nerve stimulation; in both cases the relative $\alpha_1$- and $\alpha_2$-components of the responses were dissected out by the use of specific antagonists for each receptor subtype. Furthermore, because of the heterogeneity of calcium entry blockers, we chose two chemically unrelated cal-
cium antagonists, verapamil\(^5\) and nitrendipine,\(^6\) both of which were used in concentrations close to those used in therapeutic doses.\(^1,8\)

**Materials and methods**

**Animal preparation.** Female Sprague-Dawley rats (body weight 200 to 250 g), purchased from Hilltop Laboratory, Scottsdale, NJ, were allowed to stabilize for several days before the experiments. Under anesthesia with ether, the animals were pithed through the orbit by a blunt steel needle (2.0 mm in diameter) and immediately ventilated artificially (60 strokes/min, 1 ml/100 g body weight of room air). This procedure destroys the entire central nervous system but leaves intact the emerging nerve trunks.\(^5\) Both vagi were cut in the neck and the right carotid artery was cannulated for recording blood pressure and heart rate. The rats were kept warm by a lamp positioned about 30 cm above the chest and were allowed to recover for 45 to 60 min from the effects of spinal shock and anesthesia. All drugs were given in bolus form flushed with 0.05 ml saline; all were injected through the femoral vein except for verapamil, which had to be given intra-arterially (left carotid) because pilot studies showed that severe bradycardia and death followed its direct intravenous injection.

**Experimental procedure**

**Agonist dose-response curves.** Cumulative dose-response curves to noradrenaline were obtained as recently described by Cavero et al.\(^10\) Increasing doses of noradrenaline (increments of 0.5 log units) were given, each one after the preceding dose had produced its maximum effect. Pilot experiments confirmed that this method more rapidly produces results that are virtually identical to those of the single injection method.\(^10\) A standard dose of noradrenaline (0.2 \(\mu g/kg\)) was given before each dose-response curve to ensure that the responsiveness of all preparations were comparable with each other. When an antagonist had to be used in these studies, it was injected at least 15 min before determination of the dose-response curve.

**Antagonist dose-response curves.** The responsiveness of the preparation was first tested as follows: (1) For experiments with exogenous noradrenaline, the agonist was given at a dose (between 0.2 and 0.4 \(\mu g/kg\)) calculated from previous experiments in our laboratory to raise diastolic blood pressure by 45 to 55 mm Hg, about 50% of the maximum response. Pilot (response). (2) In experiments with electrical stimulation, another steel rod (indifferent electrode) was inserted under the skin and electrical shocks (monophasic square-wave pulses, 2 msec duration, supramaximal voltage) were applied at a frequency of 3 Hz for periods of 20 to 30 sec to obtain an increase in diastolic pressure comparable to that obtained by exogenous noradrenaline; muscular contractions were prevented by \(d\)-tubocurarine (1 mg/kg). For both stimuli, responses were obtained in duplicate or triplicate at 5 to 10 min intervals to ensure the consistency of the response; values were then averaged. Pressor responses to both stimuli remained constant for a period of at least 1 hr when tested at 10 min intervals.

The antagonist dose-response curve was then obtained by injecting increasing doses of the antagonist at 10 min intervals according to a cumulative dose schedule; at the end of each interval, the pressor responses to either exogenous noradrenaline or electrical neural stimulation were again recorded and compared with those obtained before the antagonist.

**Experimental design**

**Series 1.** These experiments were designed to dissect out the separate groups of animals (\(n = 5\)) by prazosin (0.3 mg/kg) or yohimbine (0.3 mg/kg), respectively.\(^11\) This dose of yohimbine displaced by a 9.8-fold factor the dose-response curve to the specific \(\alpha_2\)-agonist BHT-920\(^11\) (control mean effective dose \(ED_{50}\) 12.6 \(\mu g/kg\), range 9.1 to 20.9 vs 119.2 \(\mu g/kg\), range 76 to 174; \(p < .01, n = 5\) per group) without significantly altering the response to methoxamine, a selective \(\alpha_1\)-agonist\(^11\) (control ED\(_{50}\) 51.6 \(\mu g/kg\), range 35.4 to 62.3 vs ED\(_{50}\) after methoxamine 60.8 \(\mu g/kg\), range 47.0 to 77.4; \(p = NS, n = 4\) per group) To prevent any \(\beta\)-agonist activity of norepinephrine,\(^11\) all of these studies were performed in the presence of \(\beta\)-blockade (propranolol, 1 mg/kg). Thus the rats were left with either the \(\alpha_1\)- or the \(\alpha_2\)-receptors exposed (yohimbine blockade for \(\alpha_2\) and prazosin blockade for \(\alpha_1\)). As a control, a separate series of experiments were carried out in the presence of propranolol alone (\(n = 5\)).

**Series 2.** To study the relative importance of calcium influx in \(\alpha\)-adrenergic-mediated responses in vivo, nitrendipine (0.01 mg/kg) and verapamil (0.6 mg/kg) were given in separate experiments to animals pretreated with propranolol and prazosin (\(n = 5\)) and to those pretreated with propranolol and yohimbine (\(n = 5\)). This particular dose of verapamil was chosen because pilot experiments indicated that a lower dose of 0.3 mg/kg was ineffective in either experimental condition, while a higher one (1.0 mg/kg) caused a high mortality rate. In this setting the norepinephrine pressor dose-response curves represented, in the first case (prazosin pretreatment), the effects of calcium entry blockers on \(\alpha_2\)-mediated pressor responses and, in the second, the effects on \(\alpha_1\) responses. The results obtained were compared with those measured in the absence of calcium entry blockade (series 1).

**Series 3.** These experiments were planned to evaluate the relative efficacy of different types of blockade in interfering with pressor responses to exogenous norepinephrine and electrical neural stimulation; this included \(\alpha_1\)-blockade by prazosin (dose range 0.01 to 1.0 mg/kg), calcium entry blockade by nitrendipine (dose range 0.01 to 0.3 mg/kg), and \(\alpha_2\)-blockade by yohimbine (dose range 0.01 to 1.0 mg/kg). Yohimbine was chosen because it had been shown to maintain its selective antagonism even at the highest doses used.\(^13\) Six groups of rats were studied, two for each drug (\(n = 6\) for prazosin and for nitrendipine, \(n = 5\) for yohimbine).

**Series 4.** To further characterize the effect of calcium entry blockade on the responses to neural stimulation, verapamil (dose range 0.01 to 0.3 mg/kg was used. The results, along with those obtained with nitrendipine (series 3), were then compared with those obtained by rauwolscine administration against the same stimulus (series 3).

**Drugs.** \(dl\)-Propranolol HCl (Ioderal; Ayerst) was obtained from commercially available ampules. Solutions of yohimbine HCl (Aldrich), rauwolscine HCl (Accurate Chemicals), prazosin HCl (Pfizer, Inc.), nitrendipine (Miles Laboratories), verapamil HCl (Knoll, Inc.), and \(l\)-norepinephrine bitartrate (Levophed; Breon Laboratory) were prepared fresh each day. Nitrendipine was solubilized in propylene glycol and injected in boluses not exceeding 8 \(\mu l\); this volume of solvent did not induce any change in blood pressure or heart rate as documented in preliminary experiments. The vials containing the drug were wrapped in aluminum foil to avoid contact with light. With the exception of nitrendipine, all doses of drugs refer to their respective salts.

**Analysis of results**

**Agonist dose-response curves.** The absolute increments in diastolic blood pressure were computed and plotted on semilogarithmic scale. The individual dose-response curves were then interpolated by a logistic function\(^14\) to obtain the estimated ED\(_{50}\) values; the differences between groups were then tested by
the Mann-Whitney rank-sum test. The ratio between ED₅₀ values (expressed as geometric means) in absence and presence of the various antagonists was used to quantify shifts of the dose-response curves.

**Antagonist dose-response curves.** Absolute values and percent changes from basal values were used as the evaluation variables. Raw data so generated were analyzed by two-way analysis of variance, and the Neumann-Keul test was used for scanning differences between individual pairs of means. Results are expressed as mean ± SEM unless otherwise specified. Statistical significance was defined as p < .05.

**Results**

After pithing, basal systolic and diastolic blood pressures stabilized at 79.1 ± 1 and 42.3 ± 6 mm Hg, respectively (n = 76); heart rate averaged 298 ± 4.5 beats/min. Prazosin, yohimbine, and rauwolscine did not modify blood pressure or heart rate significantly in this preparation.

**Effect of prazosin and yohimbine on norepinephrine dose-response curve (in presence of propranolol, 1 mg/kg).** Yohimbine (0.3 mg/kg) shifted the norepinephrine dose-response curve to the right by about 10-fold (norepinephrine ED₅₀: control 0.28 μg/kg, range 0.27 to 0.41; after yohimbine 2.4 μg/kg, range 0.48 to 3.9, p < .001). In contrast, prazosin (0.3 mg/kg) did not significantly alter the norepinephrine ED₅₀ (control 0.28 μg/kg, range 0.27 to 0.41; after prazosin 0.84 μg/kg, range 0.24 to 1.05, p = NS), but it depressed its maximum pressor effect (control 108 ± 5.2 mm Hg; after prazosin 84.4 ± 4.6 mm Hg, p < .001) (figure 1).

**Effect of nitrendipine and verapamil on the norepinephrine dose-response curve in the presence of selective α₁- and α₂-blockade (in presence of propranolol, 1 mg/kg).** Nitrendipine (0.01 mg/kg iv) did not significantly change either diastolic blood pressure (40.3 ± 1.0 vs 37.9 ± 1.1 mm Hg, n = 10) or heart rate (284.0 ± 6.6 vs 286.8 ± 8.3 beats/min, n = 10). Verapamil (0.6 mg/kg iv) decreased both diastolic pressure (46.9 ± 1.0 vs 33.9 ± 1.0 mm Hg, n = 10, p < .05) and heart rate (336.2 ± 7.2 vs 301.3 ± 8.9 beats/min, n = 10, p < .001). No difference in either parameter was found between animals pretreated with prazosin and those given yohimbine.

Neither nitrendipine nor verapamil exerted any effect on the response to norepinephrine in rats pretreated with yohimbine (figure 2). In marked contrast with these results, both drugs at the same doses significantly displaced the norepinephrine dose-response curve in animals pretreated with prazosin (figure 2). The ED₅₀ for prazosin alone averaged 0.84 μg/kg (range 0.24 to 1.05); it averaged 8.45 μg/kg (range 4.16 to 16.2) when nitrendipine was added and 6.5 μg/kg (range 2.6 to 11.2) when verapamil was added (p < .001 for both as compared with prazosin alone).

**Comparison of α₁- (prazosin) and α₂-blockade (rauwolscine) with calcium entry blockade on the pressor responses elicited by exogenous norepinephrine and by electrical stimulation.** Prazosin (dose range 0.01 to 0.3 mg/kg) consistently antagonized the pressor responses to electrical stimulation to a greater extent (p < .01) than it interfered with responses to exogenous norepinephrine (dose range 0.01 to 1.0 mg/kg) (figure 2). In distinct contrast, α₂-blockade by rauwolscine (0.1 to 1.0 mg/kg) did not antagonize the pressor responses to neural stimulation except at the highest dose (−17.4 ± 4.9%; p < .05), although it did antagonize the effect of exogenous norepinephrine (figure 3).

Nitrendipine produced a dose-related decrease in diastolic blood pressure, reaching its nadir at 0.1 mg/kg (27.4 ± 1.2 mm Hg vs 41.3 ± 1.5; p < .001, n = 12). At this same dose level, heart rate was slightly but consistently decreased (254 ± 12.6 vs 281 ± 9.7; p < .01, n = 12). It depressed the response to both electrical stimulation and exogenous norepinephrine in a way directionally similar to rauwolscine (figure 3); the effect of exogenous norepinephrine was antagonized to a greater extent (p < .001) than the response to electrical neural stimulation. In contrast to rauwolscine, nitrendipine, even at a dose of 0.01 mg/kg, reduced the response to electrical stimulation (−18.5 ± 5.5%; p < .05) and this effect increased progressively with higher doses (figure 3). Under the same experimental
conditions, verapamil (figure 4) at lower doses (0.01 and 0.03 mg/kg) was ineffective against the same stimulation but exerted a significant antagonism at higher doses (−15.2 ± 4.8%; p < .05 at 0.1 mg/kg and −30.9 ± 9.7%; p < .01 at 0.3 mg/kg). The effects of rauwolscine on pressor responses to neural stimulation was quite different from those of verapamil and of nitrendipine (figure 4); this was observed whether the doses administered were expressed in milligrams or in molecular weight (figure 4).

Discussion

The results obtained in this series of studies indicate that calcium entry blockade by either nitrendipine or verapamil interfered effectively with pressor responses to infused norepinephrine when the α₁-adrenergic sites were left exposed (pretreatment with prazosin and propranolol); they did not significantly antagonize these responses in the group pretreated with propranolol and yohimbine, in which presumably only the α₁-adrenergic receptors were exposed. These effects were probably not due to binding of the drugs to the α₂-receptors. It has been shown that dihydropyridine calcium entry blockers do not bind to either α₁- or α₂-receptor sites, although verapamil can bind to both α₁- and α₂-receptors, the doses used in our study were probably too low to interact with these sites. Moreover, binding to both α₁- and α₂-receptors would leave unexplained the differential effect of verapamil on α₂-vs α₁-mediated vasoconstriction. Therefore our results suggest that calcium entry blockade, as obtained in vivo with two different drugs, antagonizes preferentially α₂-mediated adrenergic vasoconstriction.

These conclusions are supported by the results obtained in subsequent studies comparing the effects of prazosin and nitrendipine on pressor responses to exogenous and endogenously released norepinephrine. Nitrendipine was less potent against the pressor responses evoked by neural stimulation than against responses to exogenous norepinephrine — a pattern distinctly opposite that resulting from α₁-blockade but similar to the effects of rauwolscine, a specific α₂-antagonist (figure 3). These findings are consistent with preferential antagonism of α₂-mediated vasoconstriction by the calcium entry blocker because circulating norepinephrine also activates α₂-receptors while neural stimulation, at least in the rat,12, 21, 22 and probably in other species, uses this pathway to only a minor extent. In direct confirmation of this concept are
our findings with rauwolscine, which did not influence to any important degree (even at high doses) the neurally evoked pressor responses but which antagonized substantially the responses to exogenous norepinephrine (figure 3).

Our conclusions are in basic agreement with those of previous work with synthetic selective α-agonists.24-28 Our results extend the same conclusions to the physiologic neurotransmitter norepinephrine; calcium entry blockade by chemically different drugs led to preferential antagonism of α2-mediated vasoconstriction. In addition, they suggest that interference with adrenergic activity by calcium entry blockade might not be restricted to only functional postsynaptic α2-antagonism.

Our suggestion is based on the experiments comparing a "classical" α2-antagonist such as rauwolscine13 with either verapamil or nitrendipine. If a postsynaptic α2-antagonism could account for the whole spectrum of activity of calcium entry blockade on adrenergic responsiveness, then the effects of both calcium entry blockers should have been qualitatively similar to those of rauwolscine; however, the pattern of antagonism to neural stimuli was clearly different between the calcium entry blockade and rauwolscine (figure 4). The reason for this difference cannot be defined on the basis of our data. It is tempting to speculate that release of norepinephrine from neural endings was reduced by blockade of transneurolemmal calcium influx.29 However, this may not apply to all situations because results of experiments in vitro indicated that very high doses of calcium entry blockers were needed to decrease neurotransmitter release.30-32 These data were obtained from cardiac preparations30,31 and isolated vessels32 and might not necessarily hold for the whole vascular tree or situations in vivo. Further work is also needed to evaluate the effect of calcium entry blockade on the mechanisms of presynaptic α2-regulation as compared with its effect on postsynaptic responses and on ganglionic transmission.

In conclusion, our data show that two chemically unrelated calcium entry blockers, nitrendipine and verapamil, antagonized the pressor responses to exogenous norepinephrine by preferential α2-post synaptic antagonism. In addition, when tested against responses mediated by endogenously released norepinephrine, both drugs behaved differently from a specific α2-antagonist, rauwolscine. Thus other actions such as interference with neurotransmitter release could also take part in the complex effect of calcium entry blockade on neural control of peripheral vascular tone.

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References

10. Cavero I, Lefevre-Borg F, Roach AG, Gomeni R, Catton B: Functional and biochemical evidence for the lack of cardiac presynaptic α2 adrenoceptor stimulant property of cirazoline (LD 3098), a

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potent α₁ adrenoceptor agonist in dogs and rats. J Pharmacol Exp Ther 223: 241, 1982
17. Nayler WG, Thompson JE, Jarrot B: The interaction of calcium antagonists (slow channel blockers) with myocardial α adrenoceptors. J Mol Cell Cardiol 14: 185, 1982
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