EFFECTS OF ALPHA BLOCKERS ON BLOOD PRESSURE
AND ON THE Ca-CONTRACTURE OF CAT AORTIC STRIPS

Katsuji HOSHI and Sumiko FUJINO
Department of Pharmacology, Hokkaido Institute of Pharmaceutical Sciences,
Katsuraoka 7-1, Otaru 047-02, Japan
Accepted January 23, 1980

Abstract—To further clarify the hypotensive mechanism of adrenergic alpha blockers,
effects of several alpha blockers on systemic blood pressure and on Ca-contracture of
isolated, cat aortic strips were studied. For this purpose, the effects of known adrenergic
alpha blockers, phentolamine, phenoxybenzamine, and a newly synthesized adrenergic
alpha blocker (2-(N-(n-Butyloyl)homopiperazine-N'-yl)-4-amino-6,7-dimethoxy quinazoline;
E-643) were compared with those of nitroglycerin and verapamil. Systemic
blood pressure was decreased by administration (2 \times 10^{-8} \text{ moles/kg i.v.}) of all drugs
except phenoxybenzamine. The order of maximal fall of diastolic blood pressure
after the injection was: nitroglycerin > E-643 > phentolamine > verapamil > phenoxy-
benzamine. Although “adrenaline reversal” was observed after 2 \times 10^{-7} \text{ moles/kg of}
phenoxybenzamine, i.e. a 10-fold increase in the dose of phenoxybenzamine, there was
no decrease in systemic blood pressure with this dose. All these drugs in a concentration
of 2 \times 10^{-8} \text{ M} inhibited the Ca-contracture (phasic and tonic) of the depolarized aortic
strips. The order of inhibition of phasic and tonic contracture was: nitroglycerin >
E-643, verapamil > phentolamine > phenoxybenzamine. The pA2 values for phentol
amine and E-643 in antagonizing contractions produced by noradrenaline of cat aortic
strips were 7.8 and 8.2, respectively. Hypotensive effects of these drugs (except phenoxy-
benzamine), paralleled the inhibitory effects on the Ca-contracture of the aortic strips.
These results suggest that alpha blockers such as phentolamine and E-643 exert a
systemic hypotensive effect not through their alpha blocking action but by an inhibitory
action on the contractile Ca-mechanism.

Considerable evidence is available showing that adrenergic alpha blockers have a
typical antagonistic effect on the vasoconstrictor response produced by adrenergic nerve
stimulation or injection of catecholamines (1–6). It is unclear, however, whether or not
adrenergic alpha blockers have a direct hypotensive action, independent of the blockade
of adrenergic alpha receptors in vascular smooth muscle. To clarify the hypotensive
mechanism of adrenergic alpha blockers, the effects on systemic blood pressure and on the
Ca-induced contracture of isolated, cat aortic strips were studied. The effects of known
adrenergic alpha blockers, i.e. phentolamine and phenoxybenzamine, a new adrenergic
alpha blocker, 2-(N-(n-Butyloyl)homopiperazine-N'-yl)-4-amino-6,7-dimethoxy quinazoline
(E-643) (7), were compared with those of nitroglycerin and verapamil, a Ca-antagonist.

MATERIALS AND METHODS

Experiments on systemic blood pressure, blood flow and ECG: Fifty-five healthy cats
of both sexes, weighing 2–4 kg, were anesthetized with sodium pentobarbital (30 mg/kg
i.p.). The level of anesthesia was maintained constant throughout the experiment by giving
small additional doses of the anesthetic as required. Systemic blood pressure was recorded from the right common carotid artery via a pressure transducer (MPU-0.5, Nihon Kohden) and blood flow through the left common carotid artery was recorded via a flow probe (Cuff type, lumen size 1 mm, FA010T, Nihon Kohden) on a multiple purpose polygraph (Model VC-45, Nihon Kohden). The electrocardiogram (Lead II) was recorded simultaneously on the same polygraph. To observe the effect of drugs on systemic blood pressure, adrenergic alpha blockers, nitroglycerin and verapamil in a dose of $2 \times 10^{-8}$ moles/kg were administered into the femoral vein in a single injection. Phenoxybenzamine ($2 \times 10^{-7}$ moles/kg) was also injected. To determine the degree of reversal seen with adrenaline, phenoxybenzamine of $2 \times 10^{-7}$ moles/kg and adrenaline of 10 µg/kg were injected concomitantly.

**Experiments on the Ca-contracture of cat aortic strips:** Spiral aortic strips (about 2 mm in width and 20 mm in length) were isolated from the aorta of anesthetized cats and fixed under a resting tension of 100 mg to a mechanoelectronic transducer system isometrically. Thereafter, the strip was suspended in an organ bath containing 10 ml of Tyrode solution (NaCl 137 mM, KCl 2.7 mM, CaCl$_2$ 1.8 mM, MgCl$_2$ 1.0 mM, glucose 5.0 mM, NaH$_2$PO$_4$ 0.4 mM, NaHCO$_3$ 12.0 mM) bubbled with CO$_2$(5%) + O$_2$(95%) gas at 20°C. After equilibration with the solution for about one hour, the bathing solution was replaced with fresh normal Tyrode solution. Ten minutes later this bathing solution was replaced with a Ca-free Tyrode solution for 3 min, after which this solution was replaced with a Ca-free K-solution (KCl; 139.7 mM, KH$_2$PO$_4$; 0.4 mM, MgCl$_2$; 1.0 mM, KHC0$_3$; 12.0 mM, glucose; 5.0 mM) for 6 min (during which time the solution was replaced twice). At the time of this exchange, the strips transiently contracted due to depolarization by potassium ions, however, soon relaxed to the initial resting state. Finally, when a K-solution containing Ca of 1.8 mM was added, both phasic and tonic contractures were observed. Maximal developed tension of both phasic and tonic contractures was measured, and values obtained in the presence a drug were compared with those obtained in the absence of the drug (the control value). The strips were treated with drugs for 19 min (normal Tyrode solution; 10 min, Ca-free Tyrode solution; 3 min, Ca-free K-solution; 6 min).

**Determination of pA$_2$ of adrenergic alpha blockers on noradrenaline-induced contraction in aortic strips:** Spiral aortic strips were isolated by the same techniques as those for Ca-contracture. The other procedures were similar to those of Ca-contracture with the exception of temperature and bathing solution. Temperature of the bathing solution was kept at 32°C, and the solution had the following composition (mM); NaCl 118, KCl 4.8, CaCl$_2$ 2.5, KH$_2$PO$_4$ 1.19, MgSO$_4$ 1.19, NaHCO$_3$ 12.5 and glucose 10.0. pA$_2$ value for phentolamine or E-643 was calculated by means of Van Rossum's method (8).

**Drugs:** The following drugs were used: phentolamine mesylate (CIBA-Geigy), nitroglycerin (Nihon-Kayaku), phenoxybenzamine hydrochloride (Tokyo-Kasei-Kogyo), dl-verapamil and E-643 (Eisai), dl-adrenaline hydrochloride and dl-noradrenaline hydrochloride (Sankyo-Yakuhin).
RESULTS

Effect of drugs on systemic blood pressure, blood flow and ECG: As shown in Fig. 1, adrenergic alpha blockers (except phenoxybenzamine) \((2 \times 10^{-8} \text{ moles/kg})\) produced a fall of systemic blood pressure, to different degrees in cats. The decrease seen with phentolamine was accompanied by an increase in blood flow through the left common carotid artery. Nitroglycerin and verapamil \((2 \times 10^{-8} \text{ moles/kg})\) also produced a fall of systemic blood pressure and a marked increase in carotid arterial blood flow immediately after injection (Fig. 2). Table 1 shows responses of systemic blood pressure to adrenergic alpha blockers, nitroglycerin and verapamil. The order of the extent of maximal fall of systolic blood pressure (% of that immediately prior to injection) by each drug was as follows; E-643 >
| Drugs               | Initial systemic blood pressure (mm Hg) | Onset of fall (min) | Time of maximal fall (min) | Return to initial pressure (min) | % of maximal fall |
|---------------------|----------------------------------------|---------------------|---------------------------|---------------------------------|-------------------|
|                     | Systolic pressure                        | Diastolic pressure  |                           |                                 |                   |
| phenolamine (5)     | 187±18                                  | 118±14              | 0.74±0.11                 | 1.47±0.12                       | 4.39±0.18         | 26.9±2.09        | 28.9±7.3         |
| phenoxybenzamine* (4) | 162±14                                  | 106±10              | —                         | —                               | —                 | —                | —                |
| E-643 (7)           | 174±12                                  | 113±9.4             | 0.88±0.40                 | 5.14±0.48                       | 90.92±5.08        | 35.3±0.16        | 31.3±5.1         |
| nitroglycerin (4)   | 183±8.7                                 | 116±2.3             | 0.59±0.04                 | 1.24±0.01                       | 8.59±0.09         | 19.1±2.90        | 40.0±5.8         |
| verapamil (12)      | 183±8.2                                 | 129±9.8             | 0.66±0.04                 | 1.15±0.05                       | 2.93±0.21         | 7.5±0.61         | 9.6±2.3          |

*2×10^{-7} moles/kg.

Values in parentheses indicate the number of animals.
As regards maximal fall of diastolic blood pressure, the order was: nitroglycerin > E-643 > phentolamine > verapamil. Phenoxymandelmine, even with 10-fold increase in doses (2 x 10^{-7} moles/kg), failed to produce a decrease in systemic blood pressure. As shown in Fig. 3, phenoxymandelmine (2 x 10^{-7} moles/kg) showed a typical “adrenaline reversal”. The order of the duration of the hypotensive effect, as shown in Table 1, was E-643 > nitroglycerin > phentolamine > verapamil, and the order of time required to reach the maximal fall was E-643 > phentolamine > nitroglycerin > verapamil. E-643 had the longest hypotensive action among the drugs used. There were no changes whatever in the ECG.

**Effect of drugs on Ca-contracture:** Figure 4 shows typical mechanograms of the effects of phentolamine, phenoxymandelmine, E-643, nitroglycerin, and verapamil on the Ca-contracture of depolarized aortic strips. When the Ca-free K-solution was replaced with a Ca containing K-solution (Ca: 1.8 mM), the strips showed both typical phasic (transient) and tonic contracture (control in Fig. 4). Figure 5 summarizes the effects of the drugs on

---

**Fig. 3.** A fall in systemic blood pressure by adrenaline (10 μg/kg i.v.) after administration of phenoxymandelmine (2 x 10^{-7} moles/kg i.v.).

**Fig. 4.** Typical effects of phentolamine, phenoxymandelmine, E-643, nitroglycerin and verapamil (2 x 10^{-6} M) on Ca-contractures of depolarized cat aortic strips. The arrow indicates the replacement of the Ca-free solution by a Ca-contained solution (1.8 mM).
the peak developed tension of both phasic and tonic Ca-contracture. The inhibitory effects of the drugs on the phasic contracture of the aortic strips were in the descending order of: nitroglycerin > verapamil > E-643 > phentolamine > phenoxybenzamine. The inhibitory effects on the tonic contracture were in the descending order of: nitroglycerin > E-643 > verapamil > phentolamine > phenoxybenzamine.

**Relationship between hypotensive effects, in vivo, and inhibitory effects of Ca-contracture in aortic strips:** Table 2 shows the relationship between hypotensive effects of phentolamine and E-643 and their inhibitory effects on Ca-contracture. The order of the hypotensive
The effects of the three adrenergic alpha blockers was much the same as that of the inhibitory effects on Ca-contracture of the strips. A similar relationship was observed with nitroglycerin and verapamil.

**Effect of phentolamine and E-643 on noradrenaline-induced contraction:** Figure 6 shows shifts in the dose-response curves with phentolamine ($3.6 \times 10^{-8}$ M) or E-643 ($3.6 \times 10^{-7}$–$10^{-8}$ M) with regard the contractile response to noradrenaline. The $pA_2$ values for phentolamine and E-643 in antagonizing contractions produced by noradrenaline in the muscle were 7.8 and 8.2.

**DISCUSSION**

Alpha blockers, phentolamine and E-643, but not phenoxybenzamine produced a hypotensive effect in cats, and all the alpha blockers examined inhibited Ca-contracture of depolarized cat aortic strips much to the same extent as seen with nitroglycerin and verapamil. The hypotensive effect of phentolamine was in good agreement with findings in cats reported by Das and Parratt (9). The lack of hypotensive effect in phenoxybenzamine seen in the present experiments is consistent with the findings of Cummins and Griffith in humans (10), but not with our previous findings in normotensive and spontaneously hypertensive rats (11). Both phenoxybenzamine and phentolamine have been regarded as blockers of adrenergic alpha receptors (1–6) as both drugs produce a concentration-dependent inhibition of the pressor responses to noradrenaline. Phenoxybenzamine is also more potent than phentolamine in inhibiting the neuronal re-uptake of catecholamines (12), therefore, the former is probably more effective than the latter in enhancing the overflow of circulating catecholamines (13). The complete adrenergic alpha blockade seen with phenoxybenzamine is interpreted as being a typical adrenaline reversal (Fig. 3). Nevertheless, as phenoxybenzamine showed no hypotensive action (Fig. 1), it is deduced that the concentration level...
of the endogenous circulating catecholamines is too low to induce a hypotension, under the
condition of alpha-blockade. Consequently, it follows that the hypotensive state observed
in the presence of phentolamine or another alpha blocker, E-643 was the result of an effect
other than an alpha adrenergic receptor blockade. The most likely explanation is that the
effect was one of vasodilation due to a direct inhibitory action of the contractile apparatus
of vascular smooth muscle. As described in the results, the phentolamine-induced hypo-
tension was accompanied by an increase in carotid arterial blood flow without changes in
the ECG. These results also suggest a vasodilation. The finding that E-643 did not increase
the carotid arterial flow (Fig. 1) would however, suggest that a vasodilation is not involved
here. Further studies should clarify this point. The direct inhibiting action of the con-
tractile mechanism of vascular smooth muscle as a mechanism of the hypotensive actions
of phentolamine and E-643 is supported by the results obtained from the experiments on
the Ca-contracture of aortic strips. Both phentolamine and E-643 at 2 × 10⁻⁶ M inhibited
the Ca-contracture (phasic and tonic) of depolarized the aortic strips. The order of inhibitory
effects on phasic and tonic components was: nitroglycerin > E-643, verapamil > phentolamine
> phenoxybenzamine. Hypotensive effects of these drugs were in fairly good parallel with
their inhibitory effects on the Ca-contracture of the aortic strips (Table 2).

pA₂ values for phentolamine and E-643 in antagonizing contractions produced by
noradrenaline in cat aortic strips are 7.8 and 8.2, respectively (Fig. 6). Inhibitory effects
of E-643 on both the Ca-contracture and noradrenaline-induced contraction were more
extensive than those seen with phentolamine. Nitroglycerin and verapamil have a direct
dilating action on the arteriole-venous vasculature and the former induces a relaxation of
vascular smooth muscle not only by inhibiting Ca-influx across the plasma membrane, but
also by promoting sequestration of the intracellular Ca (14), while verapamil relaxes smooth
muscle tissue by decreasing the Ca-influx (15, 16). All these results taken together suggest
that alpha adrenergic blockers such as phentolamine and E-643 also have an inhibitory
effect on the contractility mediated by Ca as do nitroglycerin and verapamil.

Our study also shows that alpha blockers such as phentolamine and E-643 induce a
hypotension not via an adrenergic alpha blockade but by a direct inhibitory action on the
contractile Ca mechanism in vascular smooth muscle.

A preliminary report of this study has already been documented (17).

REFERENCES
1) Green, H.D., Denison, A.B., Jr., Williams, W.O., Jr., Garvey, A.H. and Tabor, C.G.: Compar-
ison of the potency of dibenamine, Ilidar, phentolamine (regitine) and
tolazoline (priscoline) in blocking the vasoconstrictor responses in canine muscle to
lumbar sympathetic stimulation and to intra-arterial injection and 1-epinephrine and
of 1-norepinephrine. J. Pharmacol. exp. Ther. 112, 462-472 (1954)
2) Nickerson, M. and Hollenberg, N.K.: Blockade of α-adrenergic receptors. Physiological
Pharmacology. Vol. 4, The nervous system-part D: Autonomic nervous system drugs.
Edited by Root, W.S. and Hofmann, F.G., p. 243-305, Academic press, Inc., New
York (1967)
3) Levin, J.A. and Beck, L.: Selective reduction in neurogenically induced constriction by
phenoxybenzamine. J. Pharmacol. exp. Ther. 155, 31-41 (1967)
4) Goodman, L.S. and Gilman, A.: The Pharmacological Basis of Therapeutics, 4th ed., p. 552–554, The Macmillan Company, New York (1970)
5) Miranda, P.M.S. and Gomez, B.: Greater effectiveness of phenoxybenzamine in blocking vasoconstrictor responses to central sympathetic stimulation than to norepinephrine administration in the cat. J. Pharmacol. exp. Ther. 175, 600–608 (1970)
6) Wyse, G. and Beck, L.: Phenoxybenzamine blockade of neural and exogenous noradrenaline. J. Pharmacol. 24, 478–480 (1972)
7) Igarashi, T., Nakajima, Y. and Ohtake, S.: Comparison of effects of blocking agents on blood pressure and heart rate in unanaesthetized spontaneously hypertensive (SHR) and normotensive rats. Clinical and Experimental Pharmacology and Physiology, Supp. 3, 89–92 (1976)
8) van Rossum, J.M.: Cumulative dose-response curves II. Technique for the making of dose-response curves in isolated organs and the evaluation of drug parameters. Arch. int. Pharmacodyn. Thér. 143, 299–330 (1963)
9) Das, P.K. and Parratt, J.R.: Myocardial and haemodynamic effects of phentolamine. Brit. J. Pharmacol. 41, 437–444 (1971)
10) Cummins, B.H. and Griffith, H.B.: Intracarotid phenoxybenzamine for cerebral arterial spasm. Brit. med. J. 13, 382–383 (1971)
11) Hoshi, K. and Fujino, S.: Mechanism of the hypotensive action of antihypertensive agents in spontaneously hypertensive rats. Folia pharmacol. japon. 74, 66p. (1978) (Abs. in English)
12) Cubeddu, L.X., Barnes, E.M., Langer, S.Z. and Weiner, N.: Release of norepinephrine and dopamine-β-hydroxylase by nerve stimulation. 1. Role of neuronal and extraneuronal uptake and of alpha presynaptic receptors. J. Pharmacol. exp. Ther. 190, 431–450 (1974)
13) Thoenen, H., Hurlimann, A. and Haefely, W.: Wirkungen von Phenoxybenzamine, Phentolamine und Azapetin auf adrenerge Synapsen der Katzenmilz. Helv. Physiol. Acta 22, 148–161 (1964)
14) Grön, G. und Fleckenstein, A.: Die elektromechanische Entkoppelung der glatten Gefäßmuskulatur als Grundprinzip der Coronardilatation durch 4-(2'-Nitrophenyl)-2,6-dimethyl-1,4-dihydropyridin-3,5-dicarbonsäure-dimethylester (BAY a 1040, Nifedipine). Arzneim-Forsch. 22, 334–344 (1972)
15) Fleckenstein, A., Tritthart, H., Fleckenstein, B., Herbst, A. und Grön, G.: Selektive Hemmung der Myokard-Contractilität durch Kompetitive Ca++-Antagonisten (Iproveratril, D-600, Prenylamin). Naunyn-Schmiedebergs Arch. Pharmak. 264, 227–228 (1969)
16) Kaufmann, R., Tritthart, H., Post, B. und Fleckenstein, A.: Totale Entkoppelung der elektrischen und mechanischen Aktivität Kultivierter embryonaler Herzmuskelzellen von Hühnchen durch Isoptin (Verapamil, Iproverapamil). Pflügers Arch. Eur. J. Physiol. 316, R12 (1970)
17) Fujino, S. and Hoshi, K.: Hypotensive mechanism of adrenergic alpha blockers in cats. Experientia 35, 634 (1979)