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Alteration of Sensitivity of Adrenergic Vascular Responses after Prolonged Exposure to Agonists via Osmotic Minipump (41584)

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Abstract. A study was performed to determine whether a constant 1-week exposure to either alpha or beta agonists *in vivo* would allow alteration or manipulation of the responses of rat aortic alpha- and beta-adrenergic receptors. Osmotic minipumps delivering either phenylephrine, isoproterenol, or propranolol for 7 days at a dose of 3.2, 4.2, or 5.2 mg/kg/day, respectively, were implanted in male Holtzman rats under halothane anesthesia. Seven days later, rats were killed and aortic ring preparations were used to measure alpha- and beta-adrenergic responses. In phenylephrine-pretreated rats, alpha-adrenergic responses, as measured by contractions induced by phenylephrine, were markedly reduced ($P < 0.05$) across a dose range of 10^{-9} to 10^{-6} *M*. In contrast, in these same phenylephrine-pretreated preparations, the beta-adrenergic responses involving isoproterenol-induced relaxation were significantly increased ($P < 0.05$) across a dose range of 10^{-7} to 10^{-5} *M*. Isoproterenol pretreatment for 7 days resulted in a statistically significant reduction of beta-adrenergic aortic relaxation, whereas the alpha-adrenergic responses to phenylephrine remained unchanged compared with controls. Propranolol pretreatment had no effect on either alpha- or beta-adrenergic responses. These findings indicate that the alpha agonist-induced response after *in vivo* pretreatment induces reciprocal changes in the functionally related beta-adrenergic apparatus, and also suggest linkage between these two receptors. In contrast, the beta response appears to desensitize or downregulate in response to beta agonist exposure in a manner that seems to be independent of or to operate in the absence of an alteration of the alpha response.

The catecholamines appear to play an important role in regulating the function of their own receptors. A "desensitization" phenomenon (a decrease in responsiveness after an increase in the availability of a neurotransmitter to its receptor site) has been observed *in vitro* when isolated frog erythrocyte plasma membrane (1), aorta (2), or brain tissues (3, 4) were incubated with catecholamine agonists or with antidepressants (5). A radioligand binding technique was used in these investigations of the time-dependent decrease in receptor number. A loss of physiological or pharmacological responses can be detected by measuring a decrease in catecholamine-sensitive adenylate cylcase or a decrease in the vascular response to adrenergic agonists. Long term *in vivo* administration of desmethylimipramine (DMI) (6), a tricyclic antidepressant, or monoamine oxidase inhibitors (7) that are thought to indirectly increase the effective concentration of neurotransmitter at the synapse also attenuated responsiveness to catecholamines. This loss of responsiveness was correlated with a decreased density of receptors (8).

Because of the availability of the Alzet osmotic minipump, which is capable of maintaining a steady-state level of drug for a long period of time, we investigated this desensitization phenomenon in both alpha- and beta-adrenergic vascular responses in an *in vivo* study.

The results support the hypothesis that manipulation which alters the amount of catecholamine having access to adrenergic postsynaptic receptors elicits compensatory changes in adrenergic responses.

Materials and Methods. The following compounds were studied: l-phenylephrine hydrochoride and isoproterenol hydrochloride (Sterling–Winthrop Research Institute, Rensselaer, N.Y.), and propranolol hydrochloride (Ayerst Laboratories Inc., New York, N.Y.).

Male Holtzman rats (Holtzman Laboratories, Madison, Wisc.) weighing 250–400 g were used. Rats were housed four per cage at 24°–25°C with controlled lighting (0600–1800 light) and free access to food and water. An Alzet osmotic minipump (Lot No. 05907, Model 2001, Alza Corp., Palo Alto, Calif.)

0037-9727/83 $1.50

was implanted sc caudal to the last rib of the left side while the rats were under halothane anesthesia. The minipumps of this lot consist of a 227-μl collapsible reservoir that releases its contents continuously at a rate of 1.05 $\pm$ 0.04 μl/hr by a driving force exerted by the swelling of the osmotic substance surrounding the reservoir (9, 10). The release rate remains constant for at least 7 days and produces a steady-state distribution of drugs in the rats. In our experiment, control animals received minipumps containing 0.1% ascorbic acid, and animals of three other groups received minipumps containing l-phenylephrine hydrochloride, isoproterenol hydrochloride, or propranolol hydrochloride dissolved in 0.1% ascorbic acid solution at concentrations corresponding to a daily release of 3.2, 4.2, or 5.2 mg/kg/day, respectively.

One and 7 days after implantations of the minipumps, mean blood pressure and heart rate of the unanesthetized rats were monitored repeatedly by the tail-cuff technique. Blood pressure was measured by a programmed electrosphygmomanometer (PE 300, Narco Bio-Systems, Inc., Houston, Tex.), and pulse signals then triggered the Tachgraph preamplifier (Grass Instrument Co., Quincy, Mass.) for heart-rate recording. Both blood pressure and heart rate were displayed with a Grass Model 7 polygraph.

Seven days after the minipump implantation, rats were killed by a blow to the head and the thoracic aorta between the aortic arch and the diaphragm was removed immediately. The aortas were carefully dissected free of fat and connective tissue, and aortic rings (3–4 mm wide) were prepared. Ligatures were constructed from two 30-gauge disposable stainless-steel hypodermic needles. They were then slipped into the ring and used to attach the ring to a holder at one end and to a force-displacement transducer (FT 03C, Grass Instrument Co.) at the other according to the method of Hooker *et al.* (11). Contractile responses (in milligrams of tension) were recorded with a Grass Model 7 polygraph. The preparations were suspended vertically in a 5-ml tissue bath of the following composition (m*M*): NaCl, 118.41; KCl, 4.56; $CaCl_2 \cdot 2H_2O$, 1.28; KH_2PO_4, 1.19; $MgSO_4 \cdot 7H_2O$, 1.19; $NaHCO_3$, 24.99; and dextrose, 11.10. The preparations were equilibrated with 95% O_2/5% CO_2 at 37°C under a resting tension of 2.0 g. The preparations were allowed to equilibrate for 2 hr in the tissue bath before the experiment was initiated.

Phenylephrine hydrochloride, propranolol hydrochloride, and isoproterenol hydrochloride were dissolved in saline just before introduction in the appropriate volume into the muscle bath, thereby attaining the final concentration on a salt-free basis.

A dose-response curve for phenylephrine was obtained by applying increasing single doses consecutively every 5 min. After application of each of the chemicals, the tissue was permitted to rest for 20 min with repeated exchange of the bath medium. Relaxation with isoproterenol was studied by initially contracting the aortic ring with phenylephrine (10^{-7} *M*) and then adding increasing doses of isoproterenol every 5 min. Relaxation was expressed as the percentage decrease in contraction.

Results are given as mean values $\pm$ standard error of means. The data obtained were subjected to statistical analysis, using the

TABLE I. INFLUENCE OF PROLONGED INFUSION OF ADRENERGIC AGENTS ON BLOOD PRESSURE AND HEART RATE[a]

Group	Day 1		Day 7	
	Blood pressure (mm Hg)	Heart rate (beats/min)	Blood pressure (mm Hg)	Heart rate (beats/min)
Control	120 ± 5 (5)	366 ± 7 (5)	128 ± 4 (5)	349 ± 6 (5)
Phenylephrine	131 ± 6 (4)	352 ± 6 (4)	124 ± 4 (5)	334 ± 11 (5)
Isoproterenol	118 ± 5 (5)	487 ± 13[b] (5)	130 ± 5 (4)	456 ± 14[b] (4)
Propranolol	124 ± 5 (3)	350 ± 8 (3)	114 ± 5 (5)	331 ± 8 (5)

[a] Numbers in parentheses indicate numbers of experiments.
[b] Significantly different from control value, $P < 0.05$.

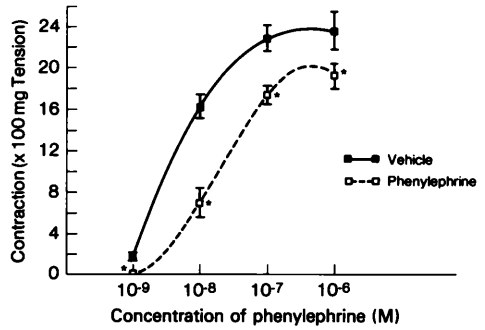

FIG. 1. Effect of pretreatment with phenylephrine (3.2 mg/kg/day) for 7 days on vascular contraction. Values are means ± SEM; $n = 6$. An asterisk indicates that the value is significantly different from the control values ($P < 0.05$).

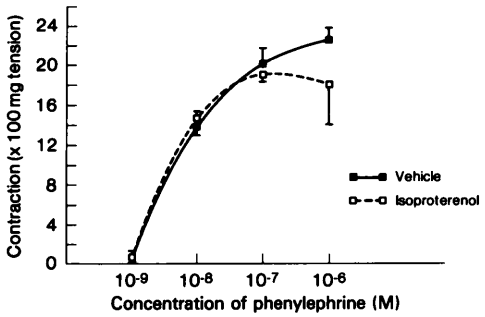

FIG. 3. Effect of pretreatment with isoproterenol (4.2 mg/kg/day) for 7 days on vascular contraction. Values are means ± SEM; $n = 5$.

Student's t test. Differences were considered to be statistically significant at $P < 0.05$.

Results. Table I summarizes the effects of chronic infusion of the adrenergic agents on blood pressure and heart rate. Blood pressure was not changed in any of the treated groups; it was slightly elevated in the phenylephrine-treated group on Day 1 and slightly reduced in the propranolol-treated group on Day 7, but the changes were not statistically significant. However, the heart rate was markedly increased in the isoproterenol-treated group on Day 1 and the increase remained statistically significant after 7 days of infusion.

The effects of pretreatment with phenylephrine (3.2 mg/kg/day for 7 days) on alpha- and beta-adrenergic responses are shown in Figs. 1 and 2. Phenylephrine produced a dose-dependent response in both vehicle- and phenylephrine-pretreated aortic rings at a range of 10^{-9} to 10^{-6} M (Fig. 1). Pretreatment with phenylephrine significantly lowered the contraction induced by phenylephrine ($P < 0.05$) at all doses. In contrast to this alpha-adrenergic response, pretreatment with phenylephrine moved the dose-response curve for the beta agonist isoproterenol to the left (Fig. 2); the beta-adrenergic response (relaxation) was significantly enhanced ($P < 0.05$) at all doses.

Figures 3 and 4 demonstrate the effects of pretreatment with isoproterenol (4.2 mg/kg/day for 7 days) on alpha- and beta-adrenergic responses in the aortic rings. As shown in Fig. 3, there was no significant difference in the alpha-adrenergic response between the vehicle- and isoproterenol-pretreated groups, i.e.,

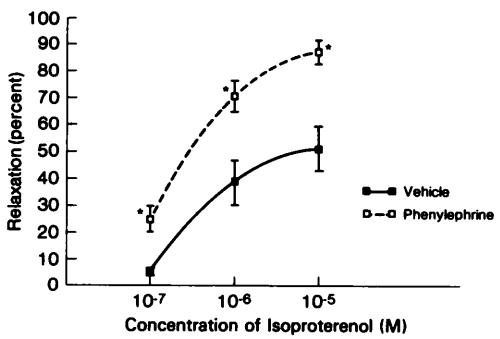

FIG. 2. Effect of pretreatment with phenylephrine (3.2 mg/kg/day) for 7 days on vascular relaxation. Values are means ± SEM; $n = 6$. An asterisk indicates that the value is significantly different from the control values ($P < 0.05$).

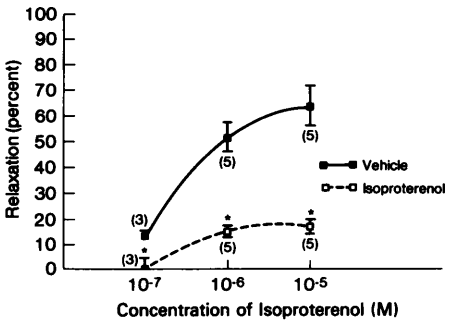

FIG. 4. Effect of pretreatment with isoproterenol (4.2 mg/kg/day) for 7 days on vascular relaxation. Values are means ± SEM; n is given in parentheses. An asterisk indicates that the value is significantly different from the control value ($P < 0.05$).

pretreatment with isoproterenol did not significantly alter contractility with phenylephrine. However, pretreatment with isoproterenol significantly decreased the relaxation response at all dose levels ($P < 0.05$), as shown in Fig. 4.

Pretreatment with propranolol (5.2 mg/kg/day for 7 days) had no effect on the alpha- and beta-adrenergic responses in the aortic rings. The phenylephrine dose-response curve of the propranolol-pretreated group was not significantly different from that of the vehicle-pretreated group (Fig. 5). Similarly, there was no difference in the beta-adrenergic response between the two groups (Fig. 6).

Discussion. Our results demonstrate that continuous *in vivo* sc infusion of phenylephrine, using an Alzet minipump, can induce a decrease in the alpha-adrenergic vascular reactivity response to phenylephrine. This decrease takes several days and is generally irreversible, as suggested by Dibner and Molinoff (3), in contrast to the effects of *in vitro* agonist treatment where onset is rapid and reversible.

The phenomenon of desensitization or downregulation of the beta receptor has been reported by other investigators, who have suggested that it is due to the net decrease in beta receptor number (5) or the formation of a high affinity, slowly dissociating form of the receptor (12).

Our data indicate that aortic rings of some groups of rats pretreated with phenylephrine undergo a different phenomenon, that of supersensitivity of the beta receptor. Whether this supersensitivity is the net consequence of

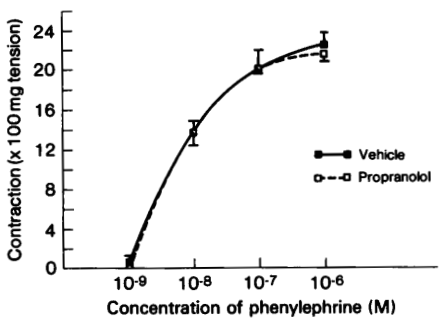

FIG. 5. Effect of pretreatment with propranolol (5.2 mg/kg/day) for 7 days on vascular contraction. Values are means ± SEM; $n = 5$.

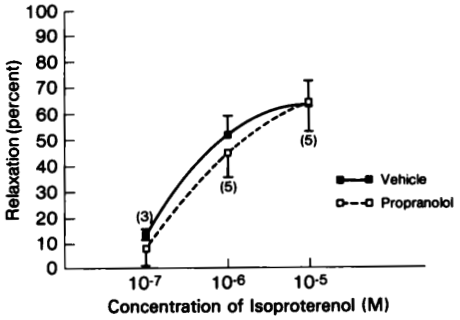

FIG. 6. Effect of pretreatment with propranolol (5.2 mg/kg/day) for 7 days on vascular relaxation. Values are means ± SEM; n is given in parentheses.

the dynamic change in receptors (i.e., receptor number and affinity) needs to be proven. An opposite reciprocal relationship in rat aorta $alpha_2$- and beta-adrenergic responses after week-long administration of isoproterenol has been reported (13). However, that study indicated that direct agonist-induced beta receptor desensitization *in vivo* induces reciprocal changes in functionally related cortical $alpha_2$ receptors, which suggests that there might be a linkage between these two receptors. Other reports, on the other hand, indicate that no change in $alpha_1$ receptors was found even though desensitization of cardiac beta receptors occurred (14).

In our experiments, rats pretreated with the beta agonist isoproterenol showed a decrease in beta-adrenergic responses (desensitization or downregulation), whereas their alpha-adrenergic response remained intact. This discrepancy between our study and that of Wang and U'Prichard (13) may be due to the fact that the tissue and preparation were different. Finally, the week-long infusion of l-propranolol in our experiments did not cause any significant effect on either alpha- or beta-adrenergic responses. Propranolol infusion has been reported to cause a significant increase in both the number of receptors and in the maximal effect of isoproterenol on cAMP accumulation in rats (6). Myers *et al.* (15) obtained the opposite result in mice; in their experiments they showed that propranolol, at a dose of 16 mg/kg/day for 5 days, caused a decrease in left ventricular cAMP when challenged by isoproterenol.

Our results demonstrate the phenomenon

of agonist-induced desensitization or supersensitization of adrenergic responses as expressed by changes in vascular reactivities. Whether these alterations of responses correlate with any real change in receptor numbers and kinetics needs further clarification by direct-measurement assays of radioligand binding.

In conclusion, the present study shows that week-long administration of either an alpha or beta agonist by a process (minipump) that increases access of the substance to alpha or beta receptors leads to a decrease in that specific adrenergic response. These results support the hypothesis of desensitization or downregulation. In addition, there seems to be a linkage between alterations in alpha- and beta-adrenergic responses when alpha agonists are used for pretreatment. None of these changes appear to occur after pretreatment with a beta blocker (propranolol). It seems possible that changes in receptor response due to an excess of neurotransmitters in the system may be involved in the etiology of a disease such as hypertension.

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