PHARMACODYNAMICS AND DRUG ACTION

Sensitization of human α_1- and α_2-adrenergic venous responses by guanadrel sulfate

The α_1- and α_2-adrenergic venoconstriction in dorsal hand veins of normal subjects was determined by infusion of phenylephrine or clonidine. Oral administration of prazosin reduced the constriction response to phenylephrine but not to clonidine. Subjects were treated for 3 weeks in a randomized crossover design with placebo or guanadrel sulfate. Guanadrel reduced sympathetic tone (i.e., plasma norepinephrine and norepinephrine release rate), whereas venous responses to phenylephrine and clonidine were both augmented during guanadrel treatment. The effect on phenylephrine responses was primarily attributable to a decrease in the median effective concentration with a small increase in maximum response. Clonidine showed a markedly increased maximum response with a small increase in the median effective concentration. Platelet α_2-adrenergic receptors increased slightly but there was no change in the amount of platelet pertussis toxin substrate during guanadrel treatment. Thus reduction in sympathetic tone in normal young men results in increased venous responses to both α_1- and α_2-agonists. (CLIN PHARMACOL THER 1990;48:537-43.)

Mohamed A. Sekkarie, MD, Brent M. Egan, MD, Richard R. Neubig, MD, PhD,[a] and Mark A. Supiano, MD[b] *Ann Arbor, Mich.*

The sympathetic nervous system has major pathophysiologic roles in disease states, including hypertension, and many antihypertensive drugs have their effects on the sympathetic nervous system. Vasoconstriction by sympathetic stimuli can be mediated by either α_1- or α_2-adrenergic receptors.[1,2] α_1-Receptors predominate in arterial responses, whereas veins have more prominent α_2-responses.[3]

Desensitization and supersensitivity of adrenergic re-

ceptors can occur in response to pharmacologic interventions that alter sympathetic nervous system activity. Regulation of β-adrenergic and α_2-adrenergic[4] receptor responses has been extensively studied. Less information is available about regulation of α_1-adrenergic receptors in humans; however, animal studies have shown that α_1-receptors in both vascular tissue[5] and salivary glands[6] are less susceptible to up-regulation than α_2- or β-receptors.

We have previously shown that reduction of sympathetic drive by the addition of guanadrel sulfate to the regimen of hypertensive patients treated with a diuretic agent caused an increase in the number of platelet α_2-receptors but did not increase norepinephrine-mediated vasoconstriction in the forearm or phenylephrine-induced mydriasis.[7] We proposed that this discrepancy was attributable to different subtypes of the α-adrenergic receptor; the forearm, vasoconstrictor and pupillary responses are primarily mediated by an α_1-receptor, whereas the platelet receptors are of the α_2 type. To directly test this hypothesis and to study the regulation of the α_1- and α_2-adrenergic receptors in a single human tissue, we tested the effect of guanadrel sulfate treatment of normal volunteers on venoconstrictor responses to the relatively selective α_1- and α_2-adrenergic agonists phenylephrine and clonidine, respectively.

Table I. Hemodynamic and biochemical data for placebo and guanadrel

	Placebo	Guanadrel	p Value	n
Systolic blood pressure (mm Hg)	118.3 ± 1.5	115.5 ± 3.4	NS	8
Diastolic blood pressure (mm Hg)	65.0 ± 4.3	62.8 ± 4.0	NS	8
Mean blood pressure (mm Hg)	84.6 ± 2.2	80.4 ± 3.4	NS	8
Heart rate (min^{-1})	66.2 ± 2.0	61.0 ± 3.0	<0.05	8
Plasma norepinephrine (pg/ml)	185 ± 24	116 ± 18	0.05-0.1	8
Plasma epinephrine (pg/ml)	71 ± 9	81 ± 15	NS	8
Platelet α_2-receptor (fmol/mg)	97 ± 18	138 ± 30	<0.05	8
Platelet G_i (pmol/mg)*	7.2 ± 1.4	5.4 ± 0.5	NS	6

Data are presented as values ± SE.
NS, $p > 0.10$.
The mean and SEM of hemodynamic and biochemical variables are shown for the placebo and guanadrel phases of the guanadrel study. Significance was determined by paired Student t tests. One-tailed tests were used for all variables except platelet pertussin toxin substrate (G_i) because we had previously shown significant decreases in all of those values.[7]
*A two-tailed test was used for platelet G_i because there was no a priori reason or previous data to determine the direction of changes.

Table II. Effect of guanadrel sulfate on [³H]norepinephrine kinetics

	Placebo	Guanadrel	p Value
NE_2 (μg/min/m²)	1.38 ± 0.33	0.51 ± 0.09	<0.05
Q_1 (μg/m²)	0.52 ± 0.07	0.21 ± 0.05	<0.05
Q_2 (μg/m²)	29 ± 7	11 ± 2	<0.05
V_C (L)	5.6 ± 0.7	5.0 ± 0.9	NS
R_{21} (μg/min/m²)	0.34 ± 0.06	0.13 ± 0.03	<0.05
C_{NE} (pg/ml)	182 ± 12	83 ± 14	<0.005

Data are from four subjects presented as mean values ± SEM.
The effect of guanadrel sulfate on [³H] norepinephrine kinetics was determined as previously described [15] (see Material and Methods section). The kinetic parameters were determined by nonlinear fitting by use of the SAAM29 program according to a two-compartment model.[15]
Significance was tested with p values (one-tailed) determined by paired Student t test. NS, $p > 0.10$.
NE_2, Rate of norepinephrine release into the extravascular compartment; Q_1 and Q_2, masses of norepinephrine in the vascular and extravascular compartments, respectively; R_{21}, mass flux rate for norepinephrine transfer from compartment 2 to compartment 1; V_C, apparent volume of distribution in the central compartment; C_{NE}, concentration of norepinephrine in the plasma compartment.

METHODS

Subjects. Normotensive white male volunteers, aged 18 to 30 years, were recruited through advertisement. They were all healthy and stated that they were not taking any medications. All subjects signed a written informed consent, which was approved by the Medical School Committee for Human Research (University of Michigan, Ann Arbor, Mich.).

Prazosin study design. A pilot study was performed to determine the effect of an α_1-blocker, prazosin, on phenylephrine- and clonidine-induced venoconstriction. Five normal subjects reported to the laboratory after fasting overnight. Catheters and the linear variable differential transducer were placed, and venous constriction induced by 640 ng/min phenylephrine and 1000 ng/min clonidine was measured. Subjects were given a single 1 mg oral dose of prazosin, and the study was repeated between 60 and 180 minutes after the dose. The order of phenylephrine and clonidine was randomized for the subjects, with two subjects receiving phenylephrine first and three receiving clonidine first.

Guanadrel study design. The study was a single-blind, randomized crossover comparison of 3 weeks of guanadrel or placebo. Guanadrel was given at a dose of 5 mg b.i.d. for 3 days, 10 mg b.i.d. for another 3 days, and 15 mg b.i.d. for the remainder of the 3 weeks. Home blood pressure measurements were done daily by the subjects, and a laboratory blood pressure was taken on day 21, when studies were performed. After a 2-week washout period in which no medications were given, the subject received 3 weeks of treatment with the alternate medication and the protocol was repeated.

Subjects reported to the laboratory at 8 AM of the study day after fasting overnight. Vital signs were measured, a 16-gauge teflon catheter was inserted into a large antecubital vein of each subject and, after 20 minutes of rest, blood was drawn for measurement of catecholamines and platelet α_2-receptor studies.

Dorsal hand vein constriction. The change in hand vein diameter was measured by use of the linear variable differential transducer technique of Aellig.[8] The linear

Table III. Regulation of venous responses to α_1- and α_2-adrenergic agonists

	Maximum response (% constriction)		Log EC_{50} (ng/min)	
	Placebo	Guanadrel	Placebo	Guanadrel
Clonidine	16 ± 1	63 ± 10	0.76 ± 0.11 (6)	1.23 ± 0.32 (17)
Phenylephrine	64 ± 6	88 ± 4	2.27 ± 0.15 (187)	1.82 ± 0.09 (65)

Response parameters (median effective concentration [EC_{50}] and maximum response) were obtained from nonlinear least-squares analysis of the data in Fig. 3. The log of the EC_{50} is shown because that parameter is estimated by the nonlinear least-squares analysis. The calculated EC_{50} (ng/min) is listed in parentheses. The data are shown as fitted parameter ± "estimated SE" provided by the analysis program (InPlot).

variable differential transducer is an electromechanical device that produces an electrical output proportional to the displacement of a movable magnetic core. The electrical output is then recorded on a potentiometric recorder. In summary, a 25-gauge three-eighths inch butterfly needle was placed in a dorsal vein of the hand. The line was secured and kept patent by infusing 5% dextrose in water at a rate of 0.1 ml/min by use of an infusion pump. The forearm was placed on an arm board making a 30° angle from the horizontal, the hand was flexed to a horizontal position and gently secured. A blood pressure cuff was placed on the upper arm to distend the hand vein by inflation of the cuff to 45 mm Hg. The linear variable differential transducer was placed on the study vein, 1 cm proximal to the needle tip. The blood pressure cuff was inflated to 45 mm Hg by use of a rapid cuff inflator (Hokanson E-10, D. E. Hokanson, Inc., Bellevue, Wash.), and the displacement of the rod by the congested vein was measured. After all the instruments were connected, the baseline hand vein diameter was measured twice, then a freshly prepared solution of clonidine (α_2-agonist) or phenylephrine (α_1-antagonist) in 5% dextrose in water was infused in stepwise increasing doses 1, 4, 16, 64, and 128 µg/min for clonidine and 10, 40, 160, 640, and 1280 µg/min for phenylephrine. Each dose was given for 4 minutes while the blood pressure cuff was deflated; the cuff was then inflated while the infusion continued and the hand vein diameter was measured. All infusions were given at a rate of 0.1 ml/min. After the maximal dose of one drug, 5% dextrose in water was infused and hand vein diameter measurements were repeated until they returned to the baseline value; at that time the second medication was infused in the same fashion. Four patients in each phase received clonidine first and the other four received phenylephrine first in both phases of the study. The clonidine and phenylephrine were given in the same sequence for each patient during the guanadrel and placebo period. Four of the eight subjects were continued on the placebo or guanadrel

for an additional 2 to 3 days, and norepinephrine kinetic studies were performed.

Plasma catecholamine levels were measured by a radioenzymatic method as previously described.[9]

Platelet α_2-receptor markers. Platelet α_2-adrenergic receptors were quantitated by measurements of 1 to 10 nmol/L [^{3}H]yohimbine binding to platelet membranes as described by Supiano et al.[10] Equilibrium dissociation constant (K_d) and maximal binding (B_{max}) values were determined by nonlinear least-squares fit of specific [^{3}H]yohimbine binding to a single-site model (InPlot, GraphPad Software, San Diego, Calif.). Pertussis toxin substrate in cholate/Lubrol extracts of platelet membrane was quantitated by pertussis toxin–induced [^{32}P]ADP ribosylation as described.[11-13] Platelet protein was measured by the method of Lowry et al.[14]

Norepinephrine kinetics were characterized as previously described.[15] In brief, tracer doses of [^{3}H]norepinephrine were infused intravenously for 1 hour to reach steady-state conditions. Tritium counts and plasma norephineprhine concentration were measured during the infusion and for 20 minutes after the infusion was discontinued. The data were fit to a two-compartment model of norepinephrine metabolism in humans and extravascular norepinephrine release rate (NE_2), and the quantity of norepinephrine in the two compartments was estimated by nonlinear least-squares analysis as described.[15]

Statistical analysis. Differences between placebo and guanadrel measurements were assessed by paired Student t tests. One-tailed tests were done for measurements for which we hypothesized the direction of change on the basis of previous information including the following: blood pressure, heart rate, plasma catecholamine levels, platelet α_2-receptor number (yohimbine B_{max}) and NE_2; two-tailed tests were done for platelet pertussis toxin substrate. A p value <0.05 was deemed significant. Differences between the placebo and guanadrel phases in the clonidine and phenylephrine dose response data was analyzed by repeated-measures
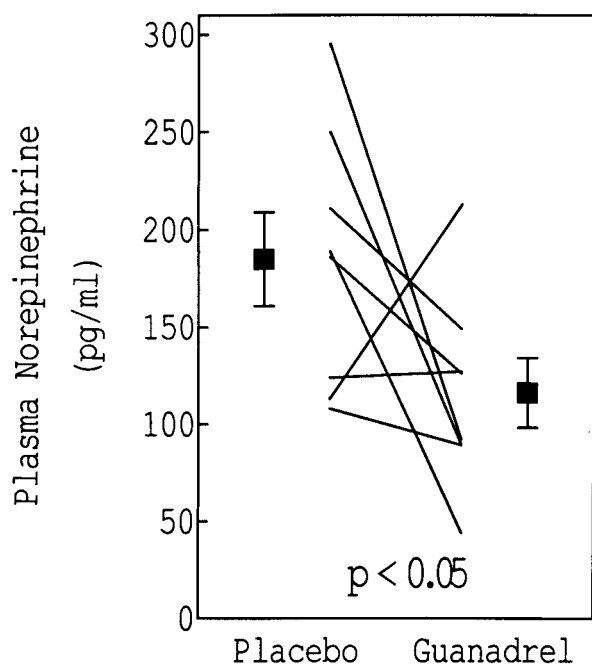
Fig. 1. Paired plasma norepinephrine level on placebo and guanadrel. *Symbols* show mean and SEM.

Fig. 2. Effect of α_1-blockade by prazosin on the hand vein responsiveness to phenylephrine given at 640 $\mu g/min$ and clonidine at 1000 $\mu g/min$.

ANOVA. The median effective concentration (EC_{50}) and maximum responses for phenylephrine and clonidine were determined by nonlinear least-squares fitting of the averaged data by use of InPlot (GraphPad Software).

RESULTS

In these normotensive subjects the reduction during guanadrel treatment of laboratory mean, systolic, and diastolic blood pressures was not statistically significant; however, heart rate was significantly reduced (Table I). Sympathetic nervous system tone, as assessed by both plasma norepinephrine concentration (Fig. 1 and Table I) and the rate of extravascular norepinephrine release (Table II), was decreased during the guanadrel phase. Plasma epinephrine was not affected.

As previously reported,[7] guanadrel treatment was associated with a 42% elevation (95% confidence interval -22% to 106%) in the B_{max} of platelet [^{3}H]yohimbine binding but this change was of borderline statistical significance ($p = 0.05$ to 0.10). The amount of platelet membrane pertussis toxin substrate, as determined by pertussis toxin substrate activity, was not altered by guanadrel treatment. The smaller number of subjects for pertussis toxin substrate measurements was attributable to technical difficulties in the preparation of samples for this assay.
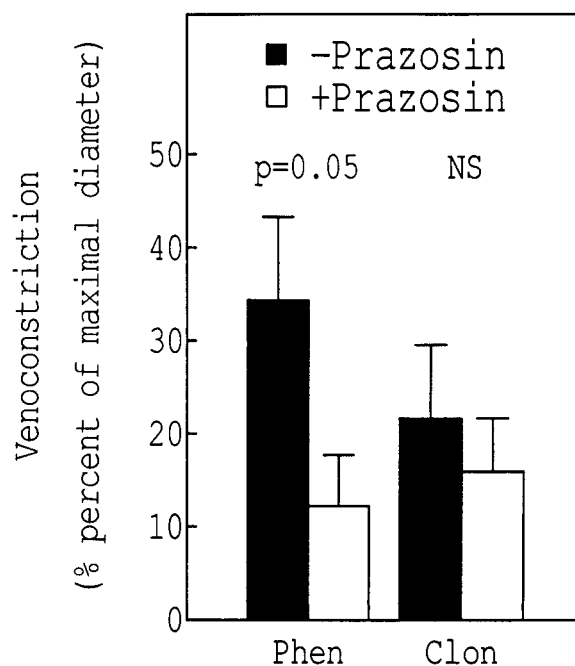
The pharmacologic specificity of effects of the two venoconstrictors was tested with the α_1-selective antagonist prazosin. The reduction in hand vein diameter caused by phenylephrine and clonidine is shown in Fig. 2 before and after oral administration of 1 mg prazosin. Prazosin reduced the venous responsiveness to phenylephrine ($p = 0.05$) but not to clonidine ($p > 0.25$).

Dose-response curves for phenylephrine and clonidine effects on hand vein diameter are shown in Fig. 3. Because of the variability inherent in these in vivo measurements and the small response to clonidine in the placebo phase, it was not possible to determine an EC_{50} and maximum response (R_{max}) for each individual. By averaging the responses we were able to obtain reliable estimates of the EC_{50} and R_{max} under all conditions. During the placebo phase, phenylephrine produced a much greater venoconstrictor response than did clonidine ($p < 0.02$, ANOVA) with calculated maximum responses of 64% and 16%, respectively (Table III). Guanadrel treatment enhanced the responses to both agonists but the phenylephrine response remained significantly greater than the clonidine response ($p < 0.005$, ANOVA). For phenylephrine, there was a significant ($p < 0.05$, ANOVA) left shift of the curve but there was no change in slope ($p > 0.1$). This was reflected in the nonlinear least-squares fitted parameters
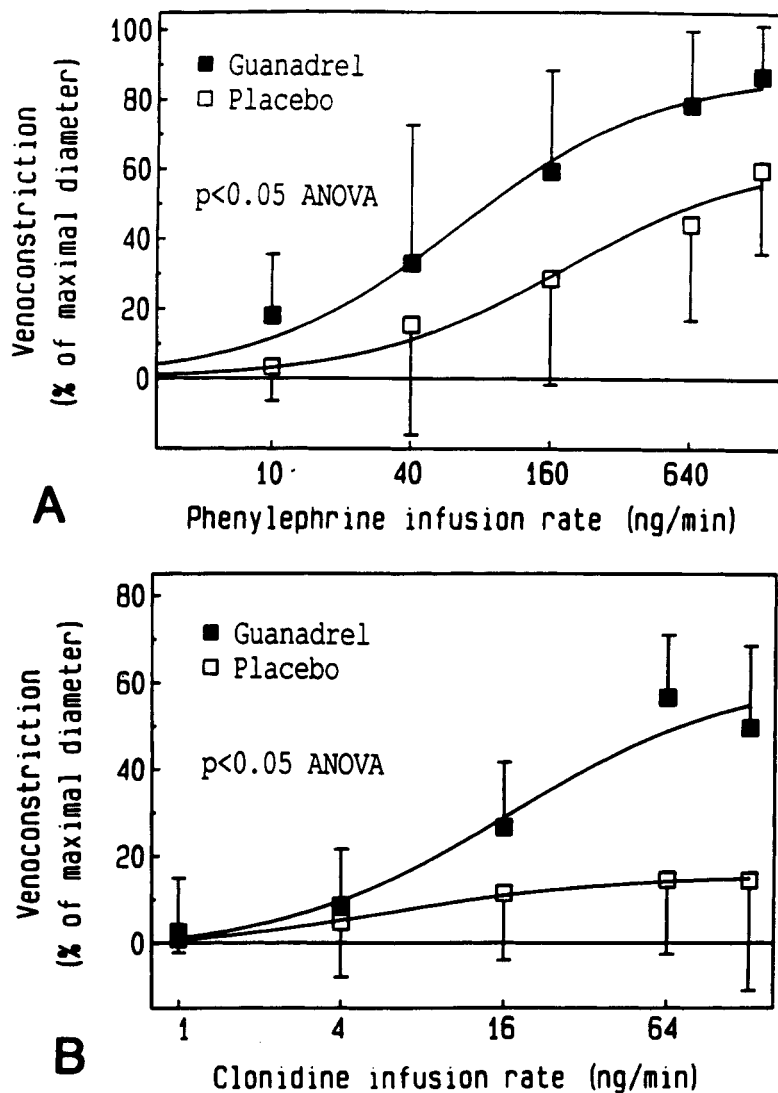
Fig. 3. Effect of guanadrel on the contractile effect of phenylephrine (**A**) and clonidine (**B**) on dorsal hand veins. Dose is given on the *abscissa* and venoconstriction expressed as a percentage of baseline vein diameter is shown on the *ordinate*. Data are presented as means ± standard deviation of eight determinations. *Curves* are nonlinear least-squares fits of the averaged data to a logistic function with a Hill slope of unity.

by a 2.9-fold lower EC_{50} with a minimal increase in the maximum response (Table III). In contrast, the maximum response to clonidine was greatly increased (Table III), whereas there was no decrease in the EC_{50}. When tested by ANOVA, clonidine also showed a significant change ($p < 0.05$) from placebo to guanadrel phases, and there was an increased slope of the dose-response curve ($p < 0.005$) that was consistent with the increased maximum response (Table III).

DISCUSSION

This is the first report of pharmacologic regulation of α_1- and α_2-adrenergic vascular responses in humans. Animal studies have shown more prominent up-regulation of α_2- than α_1-adrenergic receptor numbers in salivary glands[6] and responses in veins.[6] Our data in human dorsal hand veins show that both α_1- and α_2-venous responses up-regulate but that the pattern of up-regulation is different for the two receptor types.

The maximum response increases for the α_2-agonist, whereas the potency increases for the α_1-agonist.

The up-regulation of α_1- and α_2-adrenergic responses in human veins after treatment with guanadrel sulfate was associated, as expected, with reduced sympathetic drive. This was indicated by a moderate reduction in circulating norepinephrine and a greater reduction in norepinephrine release, as measured by [³H]norepinephrine tracer kinetics. The prominent effect on α-adrenergic vascular responses may be attributed to the significant reduction in norepinephrine release at the neurovascular junction, as estimated by the extravascular norepinephrine release rate, NE_2.

Identification of the α-receptor subtypes involved in the venous responses depends in part on the specificity of clonidine and phenylephrine as selective agonists. In studies on arterial vasoconstriction, the effects of clonidine were partially blocked by both yohimbine and prazosin, whereas those of methoxamine were blocked by prazosin alone.[1] In our study of clonidine responses, there was no significant blockade by prazosin, whereas the effects of phenylephrine were substantially reduced by prazosin (Fig. 1). The latter observation confirms that of Eichler et al.,[16] who showed that oral prazosin nearly completely eliminated the venous constriction response to phenylephrine. The increased importance of α_2-receptors in the venous responses to clonidine correlates with the observation that α_2-receptors play a more important functional role in veins than in arteries of humans and other species.[3,17,18]

The pattern of responses to phenylephrine and clonidine observed here is also consistent with those seen for α_1- and α_2-responses in in vitro systems. Generally, α_1-responses are characterized by substantial receptor reserve, whereas α_2-responses have less receptor reserve.[19,20] In a system with receptor reserve, an enhancement in responsiveness can only be expressed in an increased potency (decreased EC_{50}) with no increase in the maximal response. This was observed for the α_1-agonist phenylephrine, whereas a substantial increase in maximum response was seen for the α_2-selective agonist clonidine.

In this study, the increase in platelet α_2-adrenergic receptor numbers after guanadrel treatment (42%) did not reach the conventional criterion for statistical significance, whereas our previous study showed a definite increase (48% increase).[7] Three differences between the studies may explain this discrepancy. First, this study had fewer subjects (8 versus 11 subjects). Second, diuretic treatment or the fact that the subjects were hypertensive in the previous study may have resulted in higher basal catecholamine values (303 versus 185

pg/ml norepinephrine). The higher basal catecholamine levels could have partially down-regulated the α_2-receptors so that the up-regulation by guanadrel was more apparent in the hypertensive subjects. Finally, the mechanisms for up-regulation of α_2-receptors in hypertensive subjects may be enhanced.

To assess the role of effector system changes in the up-regulation of α_2-responses, we studied the effect of guanadrel on the inhibitory guanine nucleotide binding protein, pertussis toxin substrate in the platelet. No effect of guanadrel was seen on the content of pertussis toxin substrate as measured by the pertussis toxin substrate method. As we have reported previously, there is a substantial excess of pertussis toxin substrate over α_2-adrenergic receptors (50- 100-fold molar excess).[12] Thus, changes in the number of α_2-receptors of the magnitude observed here might not be expected to result in large fractional changes in the amount of pertussis toxin substrate present. Because the venous α_2-response increased threefold, whereas the platelet receptor number increased only 40% to 50%, there may still be changes in the distal effector coupling mechanisms. This could be occurring at a locus other than pertussis toxin substrate or could be attributable to enhanced efficiency of α_2-receptor–pertussis toxin substrate coupling without significant changes in cell content of the proteins. Finally, the greater increase in response than in receptor number could be attributable to the different tissues used for the two measurements.

In summary, reduction in sympathetic tone by guanadrel is accompanied by enhanced venous responsiveness to both α_1- and α_2-adrenergic stimuli. This up-regulation would be expected to maintain venous tone and reduce orthostatic hypotension in this setting of decreased sympathetic drive.

References

1. van Brummelen P, Jie K, Timmermans PB, Van Zwieten PA. Postjunctional alpha-adrenoceptors and the regulation of arteriolar tone in humans. J Cardiovasc Pharmacol 1985;7:S149-S152.
2. Jie K, van Brummelen P, Vermey P, Timmermans PB,

Van Zwieten PA. Identification of vascular postsynaptic alpha 1- and alpha 2-adrenoceptors in man. Circ Res 1984;54:447-52.

3. van Brummelen P, Jie K, Van Zwieten PA. Alpha-adrenergic receptors in human blood vessels. Br J Clin Pharmacol 1986;21:33S-39S.

4. Insel PA, Motulsky HJ. Regulation of alpha-2 adrenergic receptors. In: Limbird LE, ed. The alpha-2 adrenergic receptors. Clifton, New Jersey: Humana Press, 1988: 281-321.

5. Flavahan NA, Miller VM, Aarhus LL, Vanhoutte PM. Denervation augments alpha-2 but not alpha-1 adrenergic responses in canine saphenous veins. J Pharmacol Exp Ther 1987;240:589-93.

6. Bylund DB, Martinez JR, Pierce DL. Regulation of autonomic receptors in rat submandibular gland. Mol Pharmacol 1982;21:27-35.

7. Egan B, Neubig R, Julius S. Pharmacologic reduction of sympathetic drive increases platelet alpha-2-receptor number. CLIN PHARMACOL THER 1985;38:519-24.

8. Aellig WH. A new technique for recording compliance of human hand veins. Br J Clin Pharmacol 1981;11:237-43.

9. Peuler JD, Johnson GA, Simultaneous single isotope radioenzymatic assay of plasma norepinephrine, epinephrine and dopamine. Life Sci 1977;21:625-36.

10. Supiano MA, Neubig RR, Linares OA, Halter JB, Rosen SG. Effects of low sodium diet on regulation of platelet alpha 2-adrenergic receptors in young and elderly humans. Am J Physiol 1989;256(part I):E339-E344.

11. Bokoch GM, Katada T, Northup JK, Ui M, Gilman AG. Purification and properties of the inhibitory guanine nucleotide-binding regulatory component of adenylate cyclase. J Biol Chem 1984;259:3560-7.

12. Neubig RR, Gantzos RD, Brasier RS. Agonist and antagonist binding to alpha 2-adrenergic receptors in purified membranes from human platelets: implications of receptor-inhibitory nucleotide binding protein stoichiometry. Mol Pharmacol 1985;28:475-86.

13. Alradi AO, Carruthers SG. Evaluation and application of the linear variable differential transformer technique for assessment of human dorsal hand vein alpha-receptor activity. CLIN PHARMACOL THER 1985;38:495-502.

14. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem 1951;193:265-75.

15. Linares OA, Jacquez JA, Zech LA, et al. Norepinephrine metabolism in humans. Kinetic analysis and model. J Clin Invest 1987;80:1332-41.

16. Eichler HG, Ford GA, Blaschke TF, Swislocki A, Hoffman BB. Responsiveness of superficial hand veins to phenylephrine in essential hypertension. Alpha-adrenergic blockade during prazosin therapy. J Clin Invest 1989;83:108-112.

17. Tornebrandt K, Nobin A, Owman C. Pharmacological characterization of alpha-adrenergic receptor subtypes mediating contraction in human mesenteric arteries and veins. Blood Vessels 1985;22:179-95.

18. Docherty JR, Hyland L. Evidence for neuroeffector transmission through postjunctional alpha$_2$-receptors in human saphenous vein. Br J Pharmacol 1985;114:127-38.

19. Hamilton CA, Reid JL, Sumner DJ. Acute effects of phenoxybenzamine on α-adrenoceptor responses in vivo and in vitro: relation of in vivo pressor responses to the number of specific adrenoceptor binding sites. J Cardiovasc Pharmacol 1983;5:868-73.

20. Ruffolo RR Jr, Morgan EL, Messick K. Possible relationship between receptor reserve and the differential antagonism of alpha-1 and alpha-2 adrenoceptor-mediated pressor responses by calcium channel antagonists in the pithed rat. J Pharmacol Exp Ther 1984; 230:587-94.