

Increased Numbers of Alpha Receptors in Sympathetic Denervation Supersensitivity in Man

BLEDDYN DAVIES, DEEPAK SUDERA, GIUSEPPE SAGNELLA, EUGENIA MARCHESI-SAVIOTTI, CHRISTOPHER MATHIAS, ROGER BANNISTER, and PETER SEVER, *Departments of Clinical Pharmacology, Medicine, and Neurology, St. Mary's Hospital Medical School, London, W2 1PG, England*

ABSTRACT Cardiovascular responses to intravenous administration of norepinephrine and the properties of alpha receptors on platelets were compared in normal human subjects and subjects with multiple system atrophy (MSA) and sympathetic degeneration. All the subjects with MSA had low plasma norepinephrine concentrations (in the supine position) (0.42 ± 0.09 nM, normal 3.47 ± 0.58 nM), which did not increase on tilt. The pressor sensitivity of subjects with MSA to norepinephrine infusion was increased 10- to 20-fold, demonstrating denervation supersensitivity to adrenergic agonists. Analysis of alpha receptors was by binding of [3 H]dihydroergocryptine to platelets. Results are shown as mean \pm standard error of the mean. In the MSA subjects, the number of alpha receptors ($1,712 \pm 699$ fmol/ 10^8 platelets) was about sevenfold greater than in normal subjects (224 ± 21 fmol/ 10^8 platelets), and the affinity, as measured by the equilibrium dissociation constant (K_d), was similar in both groups (MSA subjects, 9.6 ± 4.3 nM; normal subjects, 4 ± 0.5 nM).

These observations suggest that an increase in alpha-adrenergic receptor numbers may account for the denervation supersensitivity to infused norepinephrine in patients with sympathetic degeneration. All the subjects with MSA had low levels of the endogenous adrenergic transmitter norepinephrine: the simultaneous increase in alpha adrenergic receptors supports the theory of agonist regulation of receptor numbers.

INTRODUCTION

Multiple system atrophy (MSA)¹ in man is frequently associated with degeneration of sympathetic nervous

Address correspondence to Dr. Davies. Dr. Sagnella's current address is Department of Medicine, Charing Cross Hospital, London, W.6, England.

Received for publication 15 July 1980 and in revised form 23 November 1981.

¹Abbreviations used in this paper: [3 H]DHE, [3 H]dihydroergocryptine; K_d , equilibrium dissociation constant, MSA,

system (Shy-Drager syndrome) (1, 2), which is reflected by low endogenous concentrations of norepinephrine (3, 4). This degeneration results in denervation supersensitivity in the form of increased pharmacological responses to exogenous adrenergic agonists (4, 5). The supersensitivity results, in part, from defective baroreflexes (4), but impaired catecholamine clearance is probably important (4). It is unknown whether the increased responses to adrenergic agonists are also due to changes in the number or alterations in the nature of adrenergic receptors. This paper demonstrates an increase in the number of specific binding sites, presumably alpha receptors, for [3 H]dihydroergocryptine [3 H]DHE, a radiolabeled binding ligand, with alpha receptors on platelets isolated from patients with MSA who had denervation supersensitivity shown by increased pressor responses to the alpha agonist norepinephrine.

METHODS

Subjects. Five normal male volunteers (age 23–65) were compared with eight subjects (age 39–72) (three female) with MSA and sympathetic nervous degeneration. In the MSA subjects, testing of autonomic function showed postural hypotension, loss of systolic overshoot in the Valsalva manoeuvre, and lack of a pressor response to stress indicating a sympathetic efferent lesion with baroreceptor reflex loss (4). All MSA subjects had low recumbent plasma norepinephrine concentrations (measured radioenzymatically [6], (0.42 ± 0.09 nM; normal [3] 3.47 ± 0.58 nM, $P < 0.0005$, unpaired t test), which did not increase after 60° tilt for 10 min. The diagnosis of MSA with autonomic failure was made by the criteria outlined in references (3) and (4). None of the normal or MSA subjects were receiving any drugs for at least 2 wk before the study.

Norepinephrine infusions. The pressor responses of the MSA subjects to intravenous norepinephrine were compared with those in seven of the normal subjects (age 28–65). The studies had the informed consent of all the MSA and normal subjects, all were free of recognizable cardiac disease and had normal electrocardiographs. The electrocardiograph and blood pressure (measured by sphygmometer) were

multiple system atrophy; Rt, platelets' alpha receptors concentration.

monitored continuously throughout the norepinephrine infusions and emergency facilities were at hand. All subjects were supine for 1 h before and during infusion studies carried out at 1400 h. (\pm)Norepinephrine bitartrate (Levophed, Winthrop Laboratories, Sterling-Winthrop House, Surbiton-on-Thames, Surrey, England), (in saline, 0.85%) was infused stepwise (each dose for 5 min) at 50, 100, and 150 ng/kg⁻¹ per min⁻¹ in normal and 1.5, 3, and 6 ng/kg⁻¹ per min⁻¹ in MSA subjects via a brachial venous cannula (Abbocath 16 G, Abbott Diagnostics, Diagnostic Products, Abbott Labs Ltd., Queenborough Kent, ME 11 SEL, England) using a Meltech constant infusion pump. In each case, the responses were compared with infusion of 0.85% saline placebo, which caused no changes in blood pressure or heart rate. In normal subjects, blood pressure and heart rate were not changed by norepinephrine at 1.5–6 ng/kg⁻¹ per min⁻¹.

Alpha receptor binding studies. Receptor binding studies were carried out using platelets isolated from each of the 15 normal subjects and each of the 8 MSA subjects. Platelets were isolated (7) from venous blood samples (1% EDTA anticoagulant, 1 ml/9 ml blood), taken after 1 h recumbency in normal and MSA subjects at the same time of the day (1400 h), 1 wk after the infusion studies. Platelet counts were similar in normal and MSA subjects. Platelet alpha receptor binding was studied by incubation (37°C) with [³H]DHE (sp act 31 Ci mmol⁻¹, New England Nuclear, NEN, 11 Chaucer Close, South Wonston, Winchester, Hants SO213HQ, England) using the method of Peters, Elliot, and Grahame-Smith (7). The final concentrations (nanomolar) of [³H]DHE in the incubation mixture volume, 1.1 ml) were 1, 2, 4, 8, 16, and 32. Platelets (0.8×10^9) from each subject were incubated in duplicate at each of the final [³H]DHE concentrations. [³H]DHE binding was saturable and inhibited by (\pm)phentolamine mesylate (Ciba Pharmaceutical Company, Div. of Ciba-Geigy Corporation, Horsham, West Sussex, RH12 4AB, England) or (\pm)epinephrine bitartrate. Specific binding was estimated by incubation with phentolamine (5 μ M) (7), the specific binding was determined by subtracting the counts per minute bound in the presence of phentolamine from the counts per minute bound in the absence

of this drug. Nonspecific binding refers to the counts per minute bound to platelets in the presence of phentolamine (5 μ M). The degree of specific binding and the raw counts per minute corresponding to this are shown, for each concentration of [³H]DHE, in Table I. Interassay binding coefficients of variation [for incubating a single normal or MSA subject's platelets freshly after isolation and then again after overnight storage in incubation buffer (7) at 4°C] were <5%. Intraassay binding variation between duplicate samples of platelets from the same individual (MSA or normal) subject was <4%. All platelet binding assays were carried out immediately after platelet isolation or after storage in incubation buffer overnight (storage before incubation was carried out with platelets from four MSA and four normal subjects). Preliminary experiments showed that [³H]DHE binding in normal but not MSA platelets was markedly decreased (50–80%) by freezing and storage (48 h–1 wk) at –80°C.

Evaluation of results of receptor binding studies. The platelet alpha receptor concentration (Rt) and equilibrium dissociation constant (K_d) values for [³H]DHE binding in each subject were calculated by nonlinear regression analysis (8) of the [³H]DHE binding curves obtained with platelets from each individual. The analysis was done by using a CBM Commodore model 3032 computer (Commodore Business Machines Ltd., London NW1 3BL, England) with the program devised by Duggleby (8). This program is for a model of a single ligand binding site with a simple reversible unimolecular interaction with the [³H]DHE ligand without positive or negative cooperativity. Therefore, preliminary analysis of each normal and MSA individual binding curve was done by the method of Scatchard (9). In each case, Scatchard plots (not shown) were linear. Also, Hill plots (not shown) (9) of the binding data gave slopes of 1 for platelets from normal and MSA subjects. The linear Scatchard plots and Hill slopes of 1 indicated (9) that the [³H]DHE binding reaction was of the simple, reversible, unimolecular non-cooperative type. Scatchard plots may be used to calculate Rt and K_d values, but, because the plots are linear transformations of ligand binding curves they tend to increase the

TABLE I
Specific Binding of [³H]DHE to Platelets

Final [³ H]DHE concentration	Normal		MSA	
	cpm	fmol	cpm	fmol
nM				
1	1,666 \pm 166	51.6 \pm 5.1	10,803 \pm 1,380	335 \pm 43
2	2,698 \pm 272	83.6 \pm 8.4	18,084 \pm 1,794	561 \pm 56
4	3,812 \pm 292	118 \pm 9	26,916 \pm 2,014	834 \pm 62
8	5,140 \pm 393	159 \pm 12	30,311 \pm 2,667	940 \pm 83
16	5,933 \pm 527	184 \pm 16	35,246 \pm 2,143	1093 \pm 66
32	5,548 \pm 695	172 \pm 22	33,521 \pm 1,334	1039 \pm 41

Specific [³H]DHE binding to normal ($n = 15$) and MSA ($n = 8$) platelets. Values are mean \pm SEM for interindividual variation (intraindividual variation was <4%) for specific binding to platelets expressed as counts per minute and femtomoles of [³H]DHE bound at each concentration of [³H]DHE used in the assay (1–32 nM). Counts per minute were obtained using a Packard Model 6880 liquid scintillation spectrophotometer with an efficiency of 47% for ³H under the assay conditions. Specific binding was defined as counts per minute bound in the absence of phentolamine minus counts per minute in the presence of phentolamine (5 μ M). No binding occurred to the incubation test tubes at any [³H]DHE concentration in the presence or absence of platelets.

degree of error in the calculation of R_t and K_d values from the binding data. The direct analysis of the binding curves by nonlinear regression refines the calculation of R_t and K_d values by circumventing this source of error derived from Scatchard linearization of the data (8).

RESULTS

Norepinephrine pressor responses. The increase in systolic blood pressure was used as a measure of in vivo "alpha receptor" responses and the dose-response curve for normal and MSA subjects is shown in Fig. 1. MSA subjects had marked increases in pressure with norepinephrine doses that did not cause a pressor response in normal subjects; the norepinephrine dose-response curve in MSA lay far to the left of that in normal subjects.

Specific platelet [^3H]DHE binding. The degree of specific [^3H]DHE binding to platelets from normal or MSA subjects is shown in Table I. Specific binding was much greater in MSA platelets (~80% of total counts per minute bound) than in normal platelets (~55–60% of total counts per minute bound). [^3H]DHE binding was saturable in platelets of individuals from both groups. Typical [^3H]DHE binding curves are shown in Fig. 2 for one normal and one MSA subject; the pattern was similar for all normal and MSA subjects. The binding curves of platelets from the individual subjects, in Fig. 2, show that much more [^3H]DHE was bound to the MSA than to the normal platelets. Nonlinear regression analysis (8) of the individual binding curves shown in Fig. 2 gave values of R_t (fmol/ 10^8 platelets) and K_d (nanomolar), respectively, for the normal individual of 312 and 3.8 and for the MSA individual

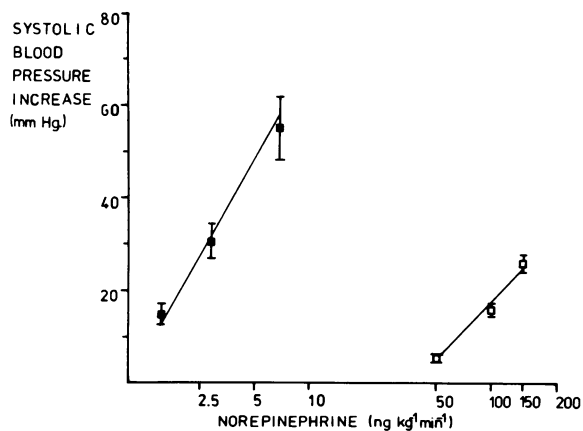


FIGURE 1 Norepinephrine dose-response curve (fitted by linear regression analysis) for normal (□) and MSA subjects (■). The mean \pm SEM are shown for the increase in systolic blood pressure in response to intravenous norepinephrine given stepwise for 5 min, at each dose shown. No increase in pressure occurred with 0.85% saline placebo or, in normal subjects, at norepinephrine rates of 1–6 ng/kg⁻¹ per min⁻¹.

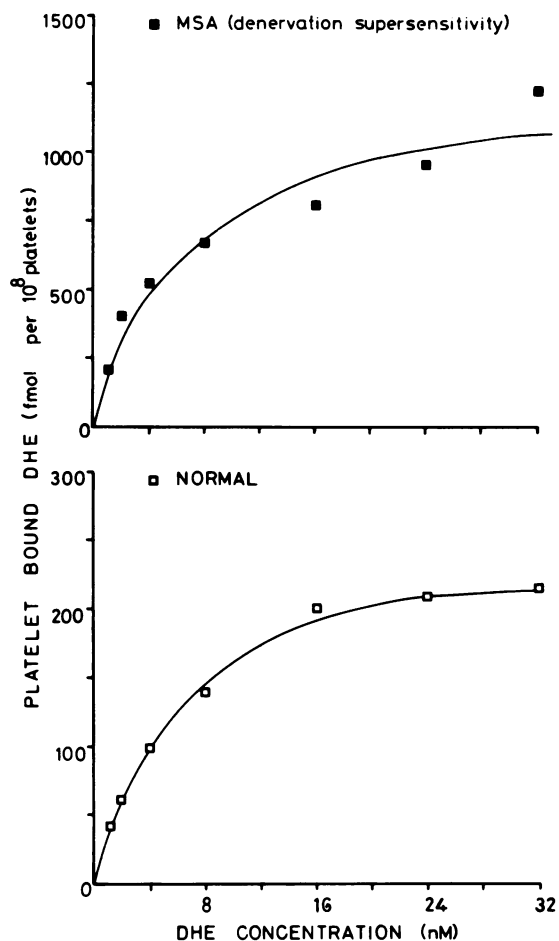


FIGURE 2 Typical binding curves of [^3H]DHE binding to platelets from one normal (□) and one MSA (■) subject. Platelets were incubated in duplicate at each [^3H]DHE concentration shown in the figure (1–32 nM) and the total binding determined. Specific binding was calculated by subtracting from the total the value of [^3H]DHE binding obtained by incubation of platelets with phentolamine (5 μM), in duplicate, at each of the [^3H]DHE concentrations shown. Inter- and intra-assay coefficients of variation were <5% and <4%, respectively. Nonlinear regression analysis (see text and reference 8) of these individual curves gave values of R_t (femtomoles 10^8 platelets) and K_d (nanomolar), respectively for the normal individual of 312 and 3.8 and for the MSA subject, of 1,326 and 5.8. The binding curves from all the normal and all the MSA subjects were of a similar pattern to the typical examples shown here.

of 1,326 and 5.8. The mean R_t and K_d values with standard error of the mean for interindividual variation, calculated by nonlinear regression (8) from all the individual binding curves in the normal and the MSA group are shown in Table II. R_t was significantly greater for the MSA platelets. However, K_d values, although showing a tendency to be greater for MSA platelets, were not statistically different between the

TABLE II
Rt and K_d for Platelet [3 H]DHE Binding

[3 H]DHE binding parameter	Method of analysis	Normal control group	Denervation supersensitivity group (MSA)	P*
Rt, $fmol/10^8$ platelets	Nonlinear regression	224±21	1,712±699	<0.01
	Scatchard	250±21	1,678±264	<0.01
K_d , nM	Nonlinear regression	4±0.5	9.6±4.3	NS
	Scatchard	4±0.4	9.5±6.3	NS

Values represent mean±SEM.

* Wilcoxon rank sum test.

Parameters of [3 H]DHE binding to platelet alpha receptors in normal subjects ($n = 15$) and MSA subjects with alpha receptor denervation supersensitivity ($n = 8$). Rt and K_d for [3 H]DHE binding were calculated by nonlinear regression or Scatchard analysis of the [3 H]DHE binding curve for platelets from each individual: values shown are mean±SEM for interindividual variation in each group.

two groups, thus indicating similar affinities of the platelet [3 H]DHE receptors in the two groups.

Nonspecific platelet [3 H]DHE binding. The amount of nonspecific binding (Table III) was approximately the same as the amount of specific binding (Table I) with normal platelets. However, for MSA platelets, the amount of nonspecific binding of [3 H]DHE (that is, counts per minute bound in the presence of phentolamine) was much less than the amount of specific [3 H]DHE binding (that is, binding prevented by incubation with phentolamine) to platelet alpha receptor sites (Tables I and III). Nevertheless, nonspecific binding was quantitatively greater in MSA compared with normal platelets (Table III). For both normal and MSA platelets, the degree of nonspecific binding was saturable. Scatchard analysis of the nonspecific binding

curves (Table IV) gave values for the number of nonspecific binding sites, which were greater in MSA than normal platelets ($P < 0.01$, Wilcoxon rank sum test); however, K_d values for [3 H]DHE binding were similar in normal and MSA platelets (Table IV). Comparison of the number of specific with nonspecific [3 H]DHE binding sites between normal and MSA platelets showed, in each case, that the number of specific and nonspecific binding sites and K_d values were similar (Tables III and IV).

DISCUSSION

All the subjects with MSA and sympathetic degeneration showed denervation supersensitivity of the pressor response to the alpha agonist, norepinephrine,

TABLE III
Nonspecific Binding of [3 H]DHE to Platelets

Final [3 H]DHE concentration	Normal		MSA	
	nM	cpm	fmol	cpm
1		1,242±235	39±7.3	2,700±1,380
2		2,000±435	62±14	5,400±1,800
4		2,490±430	77.2±13	5,900±2,000
8		2,920±501	90±16	6,650±2,700
16		4,000±697	124±22	8,810±2,100
32		5,005±790	157±25	10,600±1,300
				83±43
				56±56
				183±62
				206±84
				273±62
				329±40

Nonspecific [3 H]DHE binding to normal ($n = 15$) and MSA ($n = 8$) platelets. Values are mean±SEM for interindividual variation and are given as raw counts per minute (r , 0.95 and 0.92, respectively) and femtomoles [3 H]DHE bound corresponding to each final [3 H]DHE concentration used. Nonspecific binding was defined as counts per minute bound in the presence of phentolamine (5 μ M).

TABLE IV
Scatchard Analysis of Nonspecific [³H]DHE Binding to Platelets

Parameter	Normal	MSA	P*
Rt, fmol/10 ⁸ platelets	292±67	1,111±627	<0.01
K _d	4.3±0.84	18±9.5	NS

Individual nonspecific [³H]DHE binding "curves" were analyzed by the method of Scatchard. Values are mean±SEM.

* Wilcoxon rank sum test, NS.

because the dose-response curve for the increase in systolic blood pressure lay to the left of that in normal subjects (Fig. 1). In terms of the increase in systolic blood pressure, the MSA subjects were ~10 times more sensitive to intravenous norepinephrine than normal subjects, confirming the findings of previous studies (4). This in part represents alpha receptor denervation supersensitivity, but the pressor effect of norepinephrine also results from baroreflex loss and possibly from decreased clearance of norepinephrine, in addition to the supersensitivity resulting directly from denervation of vascular adrenergic receptors (4).

The importance of changes in adrenergic receptors in the supersensitivity to norepinephrine was shown by the greater binding of [³H]DHE to platelets isolated from MSA subjects (Fig. 2, Tables I and II). Analysis of [³H]DHE binding showed that platelets from MSA subjects had about seven times as many alpha receptors as normal platelets (Table II). However, K_d values were not significantly different in MSA subjects, thus indicating a similar affinity (9) of the alpha receptors in MSA. In animal experiments, chemical sympathetic denervation with 6-hydroxydopamine (10) or depletion of endogenous adrenergic transmitters with reserpine (11) increased alpha receptor numbers in various tissues without alteration in the affinity of the receptors, as measured by association constant values. It was not possible in this study to determine whether the increased alpha receptor number in the MSA group is due to classical alpha₁ or to alpha₂ receptors (12) or both. Alpha₁ and alpha₂ receptors both occur on platelets (13, 14), and [³H]DHE does not distinguish between these subtypes (13, 14). This point is being further investigated.

The significance of the greater degree of nonspecific binding of [³H]DHE to the MSA compared with the normal platelets is unknown. In both normal and MSA platelets, the amount of nonspecific binding was saturable. Scatchard analysis of nonspecific binding curves showed the concentration of the nonspecific sites to be similar to that of alpha receptor sites on both normal and MSA platelets; K_d values for nonspecific binding were also similar to those for specific [³H]DHE binding.

The greater number of nonspecific sites may reflect an increase in the membrane surface area of the MSA as compared with the normal platelets, or may result from changes in the physico-chemical properties of the platelet membranes. However, more work is needed to clarify satisfactorily the matter of nonspecific binding.

The MSA subjects were older (36–72 yr) than the control group (23–65 yr). It was not possible to study the effect of age on alpha receptor concentration in this study because of insufficient numbers. However, the two oldest normal subjects (62 and 65 yr) had similar alpha receptor concentrations (148 and 263 fmol/10⁸ platelets, respectively) to the rest of the group (aged 28–36 yr, alpha receptor concentrations 105–362 fmol/10⁸ platelets). It is well documented that beta receptor concentrations on lymphocytes (15) and the chronotropic response to isoproterenol (16) both decrease with age in man, and in isolated rat heart the inotropic effect of norepinephrine decreases with ageing (17).

The changes in alpha receptor numbers in the MSA group may be of wider biological significance. Patients with MSA and sympathetic nervous degeneration usually have low concentrations of endogenous norepinephrine in blood (this applies to all MSA subjects in the present study), and the concentration fails to increase with maneuvers such as tilt, which in normal subjects stimulates the sympathetic nervous system with greater spill-over of norepinephrine into the blood (3, 4). It has been widely suggested that adrenergic receptor number may be regulated in part by the amount of agonist to which a tissue receptor population is exposed: increased amounts of agonist lead to an apparently smaller number of receptors ("desensitization") and decreased amounts of agonist result in a larger number of receptors (18, 19). Our findings appear to support this view.

In conclusion, this paper shows that denervation supersensitivity in the form of increased in vivo pressor responses to the alpha agonist norepinephrine in subjects with MSA is probably due to the increased alpha receptor numbers.

ACKNOWLEDGMENTS

The authors wish to thank Ciba Pharmaceutical Company, Div. of Ciba Laboratories, Horsham, West Sussex RH12 4AB, London, for providing the phentolamine mesylate used in these studies.

This work was supported by trust grants from Ciba-Geigy Corporation and Wellcome Foundation, Ltd., The Wellcome Research Laboratories, Kent, England.

REFERENCES

1. Shy, G. M., and G. A. Drager. 1960. A neurological syndrome associated with orthostatic hypotension. *Arch. Neurol.* **2**: 511-527.
2. Bannister, R., and D. R. Oppenheimer. 1972. Degenerative diseases of the nervous system associated with autonomic failure. *Brain.* **95**: 457-474.
3. Bannister, R., P. Sever, and M. Gross, 1977. Cardiovascular reflexes and biochemical responses in progressive autonomic failure. *Brain.* **100**: 327-344.
4. Bannister, R., B. Davies, E. Holly, T. Rosenthal, and P. Sever, 1979. Defective cardiovascular reflexes and supersensitivity to sympathicomimetic drugs in autonomic failure. *Brain.* **102**: 163-176.
5. Davies, B., R. Bannister, and P. Sever. 1978. Pressor amines and monoamine oxidase inhibitors for treatment of postural hypotension in autonomic failure. *Lancet.* **I**: 172-175.
6. Henry, D. P., B. J. Starman, D. G. Johnson, and R. H. Williams. 1975. A sensitive radioenzymatic assay for norepinephrine in tissues and plasma. *Life Sci.* **16**: 375-384.
7. Peters, J. R., J. M. Elliot, and D. G. Grahame-Smith. 1979. Effect of oral contraceptives on platelet noradrenaline and 5-hydroxytryptamine receptors. *Lancet.* **II**: 933-936.
8. Duggleby, R. G. 1981. A nonlinear regression program for small computers. *Anal. Biochem.* **110**: 9-18.
9. Boeynaems, J. M., and J. E. Dumont. 1975. Quantitative analysis of the binding of ligands to their receptors. *J. Cyclic Nucleotide Res.* **1**: 123-142.
10. U'Pritchard, D. C., W. D. Bechtel, B. M. Roust, and S. H. Snyder. 1979. Multiple apparent alpha-noradrenergic binding sites in rat brain: effect of 6-hydroxydopamine. *Mol. Pharmacol.* **16**: 47-60.
11. U'Pritchard, D. C., and S. H. Snyder. 1978. Increase in alpha-receptor number in reserpine sensitivity in rats. *Eur. J. Pharmacol.* **51**: 145-155.
12. Drew, G. M., and S. B. Whiting. 1979. Evidence for 2 distinct types of post-synaptic alpha-adrenoceptors in vascular smooth muscle in vivo. *Br. J. Pharmacol.* **67**: 207-215.
13. Grant, J. A., and M. C. Scrutton. 1979. Novel alpha₂ adrenoceptors primarily responsible for inducing human platelet aggregation. *Nature (Lond.)* **277**: 659-661.
14. Scrutton, M. C., and J. A. Grant. 1979. Dihydroergocryptine is a nonselective antagonist for human platelet alpha-adrenoceptors. *Nature (Lond.)* **280**: 700.
15. Schocken, D. D., and Roth, G. S. 1977. Reduced beta-adrenergic receptor concentrations in ageing man. *Nature (Lond.)* **267**: 856-858.
16. Vestal, R. E., A. J. J. Wood, and D. G. Shand. 1979. *Clin. Pharmacol. Ther.* **26**: 181-186.
17. Lakatta, E. G., G. Gerstenblith, C. S. Angell, N. W. Shock, and M. L. Weisfeldt. 1975. *Circ. Res.* **36**: 262-269.
18. Williams, L. T., and R. J. Lefkowitz. 1978. *In Receptor Binding Studies in Adrenergic Pharmacology.* Raven Press, New York. 127-153.
19. Catt, K. J., J. P. Harwood, G. Aguilera, and M. L. Dufau. 1979. Hormonal regulation of peptide receptors and target cell responses. *Nature (Lond.)* **280**: 109-116.