Br. J. clin. Pharmac. (1981), **12**, 375–380

EFFECT OF PROPRANOLOL ON NORADRENALINE KINETICS IN PATIENTS WITH ESSENTIAL HYPERTENSION

M. ESLER, G. JACKMAN, P. LEONARD, HELEN SKEWS, A. BOBIK & G. JENNINGS
Baker Medical Research Institute, Commercial Road, Prahran, 3181, Victoria, Australia

1 The rates of noradrenaline spillover to, and removal from, plasma were measured in ten patients with essential hypertension treated with propranolol, to ascertain if long-term administration of this drug reduces sympathetic nervous system tone.

2 The plasma clearance of noradrenaline fell with propranolol, leading to a small rise in the mean plasma noradrenaline concentration. Sympathetic nervous activity in treated patients cannot be reliably gauged from plasma noradrenaline values because these are distorted by the reduction in noradrenaline clearance.

3 There was no consistent effect on noradrenaline spillover rates, which fell in six patients, but rose in the remaining four. The magnitude of the antihypertensive response was unrelated to these changes in noradrenaline release. During propranolol treatment, noradrenaline spillover rates were in every case within the normal range, much higher than in patients treated with the known sympathetic nervous systems suppressant, clonidine.

4 The principal mode of antihypertensive action of propranolol is something other than central suppression of sympathetic tone or pre-synaptic inhibition of noradrenaline release.

Introduction

Although β-adrenergic receptor blocking drugs are of proven efficacy as antihypertensive agents, their mechanism of action remains unknown after more than ten years of extensive investigation. Despite this lack of progress, interest in their mode of action continues, in part because this class of drugs is so well tolerated and efficacious the idea has grown that if their mode of action could be comprehended, better understanding of the pathogenesis of essential hypertension might follow.

Early reports of inhibition of sympathetic nervous system cardiovascular reflexes during β-adrenergic blockade, both in animals (Dunlop & Shanks, 1969) and patients with essential hypertension (Esler & Nestel, 1973), suggested that suppression of the sympathetic nervous system, perhaps at a site within the central nervous system (Kelliher & Buckley, 1970), might be an antihypertensive mechanism. This viewpoint was subsequently strengthened when it was found that administration of propranolol to rabbits reduced sympathetic nerve firing rates (Lewis & Haeusler, 1975). But confusion followed when sympathetic nervous system function was further assessed in patients with essential hypertension, using the plasma concentration of the sympathetic neurotransmitter, noradrenaline, as a measure of sympathetic activity. An early report of a reduction in plasma noradrenaline values with long-term β-adrenergic receptor blockade (Brecht *et al.*, 1976) was not confirmed in subsequent studies (Anavekar *et al.*, 1975; Esler *et al.*, 1977). β-adrenergic receptor blocking drugs may, in fact, elevate plasma noradrenaline values in hypertensive patients (Distler *et al.*, 1978; Jones *et al.*, 1980).

Although the plasma concentration of noradrenaline is a guide to overall sympathetic nervous system activity in untreated patients with essential hypertension (Esler *et al.*, 1977), this may not hold for patients treated with β-adrenergic receptor blockers. The plasma noradrenaline concentration is determined by the simultaneous rates of noradrenaline release to and removal from plasma. If clearance of noradrenaline from the circulation were altered by long-term administration of β-adrenoceptor blocking drugs, plasma noradrenaline values would misrepresent the existing level of sympathetic nervous tone. To assess better overall sympathetic nervous activity during chronic β-adrenoceptor blockade, we have

0306–5251/81/090375–06 $01.00

measured concurrently the rate of spillover of noradrenaline to plasma, clearance of noradrenaline from plasma, and the plasma concentration of noradrenaline in patients with essential hypertension treated with propranolol.

Methods

Propranolol drug trial

Twenty-two white patients with mild to moderately severe essential hypertension (WHO Stages I and II) participated. Mean age was 44 years (range 20–58 years). Thirteen were male and nine female; no female with child-bearing potential was included. The research was approved by the Alfred Hospital clinical research ethics committee, and was fully explained to all subjects who gave their informed consent.

In the dose-finding phase of the study, propranolol was commenced at 40 mg twice daily. The dose was increased second-weekly by 80 mg/day until blood pressure in the clinic had fallen below 150/90 mm Hg, or a propranolol dose of 320 mg daily had been reached. In the trial proper patients received, in randomized order, either this predetermined dose, or no treatment, each for one month. Catecholamine-testing was performed twice, at the end of these treatment- and drug-free phases, at which time blood pressures and heart rates at rest were measured.

Measurement of noradrenaline kinetics

In the first twelve patients entered into the study, plasma noradrenaline concentration alone was determined. In the final ten patients the rates of noradrenaline spillover to and removal from plasma, in addition to plasma noradrenaline values, were measured. For the measurement of noradrenaline kinetics, tritiated (–)-noradrenaline was infused intravenously until plateau concentration was reached in the central compartment, and plasma tritiated noradrenaline concentration and plasma noradrenaline specific activity determined at steady state (Esler *et al.*, 1979). The test was performed always at the same time of day (09.00–11.00 h), with subjects lying flat in a single-bed ward. All had eaten a light breakfast prior to attending, but coffee, tea, tobacco and alcohol were forbidden during the preceding 12 h. In propranolol-treated patients, the morning dose was taken at 07.00 h.

Tritiated (–)-noradrenaline of specific activity 24–28 Ci/mmol (New England Nuclear Corporation), and purity greater than 98%, was pharmaceutically prepared for administration to humans and infused into an antecubital vein at a rate of 0.35 μCi min^{-1} m^{-2} for 90 min. This was equivalent to an infusion rate of 0.012 nmol/min, insufficient to elevate plasma noradrenaline concentration or blood pressure (Esler *et al.*, 1979). The infusion was immediately preceded by an intravenous bolus injection of 15 μCi/m^2, to shorten the time to plateau concentration (Shipley & Clark, 1972), which was reached by 60 min in each case. Venous blood for assay of plasma tritiated noradrenaline and plasma noradrenaline concentration and specific activity, using methods previously described (Esler *et al.*, 1979), was withdrawn sequentially during the course of the infusion through an indwelling needle in an antecubital vein of the non-infused arm. The rate of spillover of noradrenaline (NA) to plasma and of clearance of noradrenaline from plasma at steady-state (plateau plasma concentration of tracer) were derived (Shipley & Clark, 1972; Esler *et al.*, 1979) from the following relationships:

$$\text{NA spillover rate} = \frac{\text{NA} - {}^3\text{H infusion rate}}{\text{Plasma NA specific activity}} \quad (1)$$

$$\text{Plasma clearance of NA} = \frac{\text{NA} - {}^3\text{H infusion rate}}{\text{Plasma NA} - {}^3\text{H concentration}} \quad (2)$$

The bulk of noradrenaline entering plasma under resting conditions appears to come from sympathetic nerves, with a small proportion only coming from the adrenal medulla (Esler *et al.*, 1979).

Establishing norms for noradrenaline kinetics

Noradrenaline spillover rate, plasma clearance and concentration were also measured, under identical conditions, in 21 untreated, healthy subjects of mean age 42 years (range 20–62 years). These normal subjects were recruited by advertisement from the general community to serve as a reference population. To provide clinical models of subnormal sympathetic nervous system activity, since it was of particular interest whether propranolol reduced sympathetic tone, two additional patient groups were tested. In the first of these, five patients with essential hypertension, none of whom participated in the propranolol phase of the study, sympathetic nervous activity was lowered by clonidine, a known suppressant of the sympathetic nervous system (Wing *et al.*, 1977). Clonidine, 0.15 mg three times daily, and placebo were given, each for one month in randomized order, and measurements of noradrenaline spillover rate made at the end of each phase. The placebo period commenced with a low dose of clonidine to prevent a clonidine withdrawal reaction. Seven patients with sympathetic nerve failure, idiopathic peripheral autonomic insufficiency (Zeigler, Lake & Kopin, 1977), served as the second clinical model of subnormal sympathetic nervous system activity.

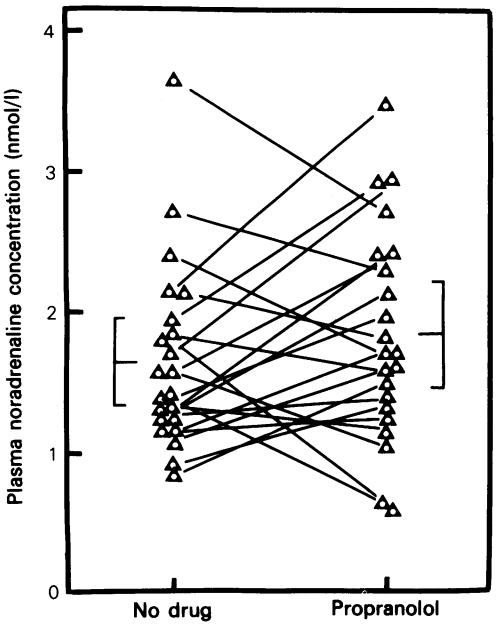

Figure 1 Plasma noradrenaline values in patients with essential hypertension, both untreated and during administration of propranolol. Means and s.d. are shown. There was no significant change in plasma noradrenaline concentration on the drug.

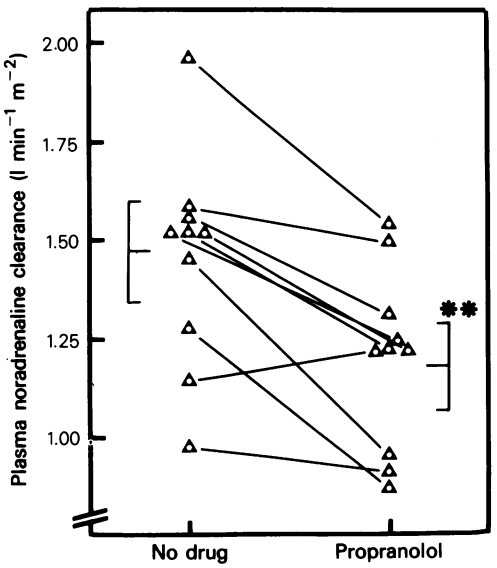

Figure 2 The effect of chronic administration of propranolol on plasma noradrenaline clearance in patients with essential hypertension. Noradrenaline clearance was reduced by propranolol; $P < 0.01$, paired t-test.

Results

The plasma concentration of noradrenaline rose marginally on propranolol, from 1.65 ± 0.65 nmol/l (mean ± s.d.) to 1.85 ± 0.75 nmol/l. (Figure 1; difference not significant). There was, however, no consistent pattern to the drug's effect, plasma noradrenaline values rising in thirteen patients, and falling in nine. The plasma clearance of noradrenaline was reduced by propranolol, falling in nine of ten patients; $P < 0.01$, paired t-test (Figure 2). The effect of propranolol on the noradrenaline spillover rate differed between patients, noradrenaline release falling on propranolol in six patients, but rising in four. Falls were noted particularly in patients in whom the spillover rate while untreated was high (Figure 3). The degree of any suppression of sympathetic tone produced by propranolol, gauged from the effect on noradrenaline spillover rate, was modest compared with that produced by clonidine. In clonidine-treated patients, noradrenaline release was much reduced, to values similar to those of patients with autonomic insufficiency. In no instance did propranolol lower the noradrenaline spillover rate to below the normal range (Figure 3).

Table 1 allows comparison among noradrenaline spillover rate values pre-treatement, changes in noradrenaline release during propranolol treatment, and the antihypertensive effect of the drug. The magnitude of the blood pressure fall noted was unrelated to either the pre-treatment noradrenaline release rate or the change in noradrenaline release during treatment.

Discussion

Most studies which have attempted to determine whether suppression of sympathetic nervous system activity is the mechanism by which β-adrenoceptor blocking drugs lower blood pressure in patients with essential hypertension have relied on the plasma concentration of the sympathetic neurotransmitter, noradrenaline, as the sole guide to sympathetic activity (Anavekar *et al.*, 1975; Brecht *et al.*, 1976; Jones *et al.*, 1980). But fallacies may arise if the plasma noradrenaline concentration is used uncritically as a measure of overall sympathetic nervous tone (Esler *et al.*, 1979) without some form of independent corroboration (Esler *et al.*, 1977). The plasma concentration is determined not only by the rate at which noradrenaline enters plasma, which is related to sympathetic nerve firing rates (Yamuguchi, De Champlain & Nardeau, 1977), but also by its rate of removal (Esler *et al.*, 1979). If the clearance of noradrenaline is lowered, the plasma concentration will

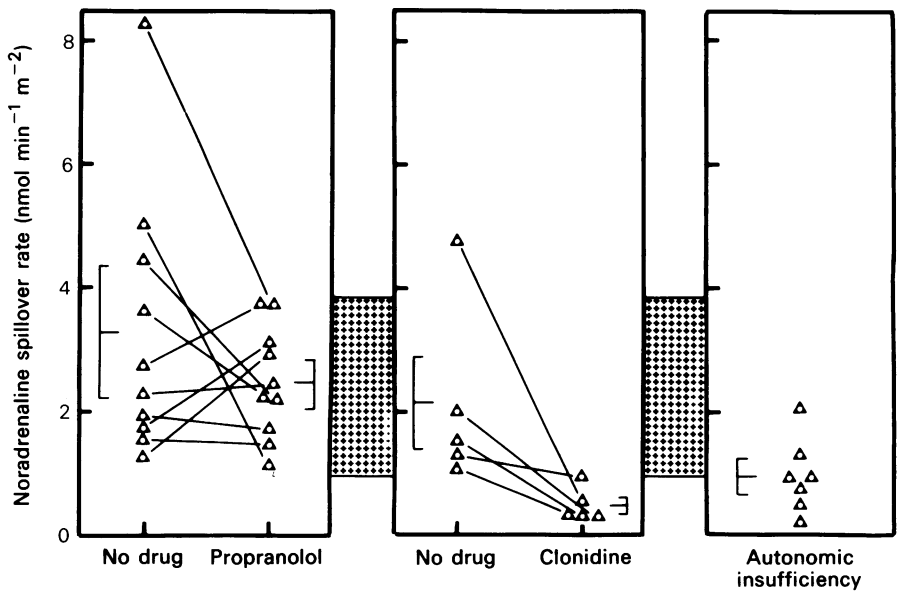

Figure 3 Effect of propranolol and clonidine on the rates of spillover of noradrenaline to plasma in patients with essential hypertension. The range of release rate values found in healthy subjects is indicated by the cross-hatching. Clonidine lowered the noradrenaline spillover rate to below normal in hypertensive patients, to levels similar to those found in patients with idiopathic peripheral autonomic insufficiency, while propranolol had no statistically significant effect overall.

Table 1 Values for noradrenaline spillover rate, mean blood pressure and heart rate during supine rest in patients with essential hypertension, both untreated and during chronic administration of propranolol in the doses shown.

			No treatment Values at rest				*Propranolol Values at rest*		
Subject	*Age (years)*	*Sex*	*Mean BP (mmHg)*	*Heart rate (beats/min)*	*Noradrenaline spillover rate (nmol min^{-1} m^{-2})*	*Propranolol dose (mg/day)*	*Mean BP (mmHg)*	*Heart rate (beats/min)*	*Noradrenaline spillover rate (nmol min^{-1} m^{-2})*
1	20	M	111	63	1.24	160	98	59	3.01
2	51	M	120	58	1.54	320	107	58	1.48
3	58	M	129	65	8.28	240	111	55	3.72
4	58	F	139	62	4.43	320	121	45	2.19
5	50	M	117	64	1.77	160	102	50	3.07
6	50	F	122	64	5.02	320	111	54	1.12
7	54	M	128	73	1.89	320	125	59	1.71
8	46	F	116	69	2.72	160	100	59	3.78
9	42	F	124	84	2.25	320	117	68	2.42
10	49	F	142	60	3.61	240	106	42	2.19
Mean	48		125	66	3.28	256	110	55	2.47
s.d.	11		10	8	1.51	74	9	8	0.91

be disproportionately high. We have found here that long-term administration of propranolol reduces plasma noradrenaline clearance by approximately 20%, perhaps explaining previous reports that β-adrenergic receptor blocking drugs often seem to elevate the plasma concentration of noradrenaline (Distler *et al.*, 1978; Jones *et al.*, 1980).

Removal of noradrenaline from plasma is achieved by neuronal uptake into sympathetic nerve endings, extraneuronal uptake by other tissues, such as vascular endothelium, and metabolic conversion by O-methylation, oxidative deamination, and conjugation (Kopin, 1979). The relative contribution to total clearance by each mechanism is uncertain, and which of these processes is altered by propranolol is not known. It is unlikely that the reduction in noradrenaline clearance results from impairment of neuronal uptake of noradrenaline. When neuronal uptake is reduced, a characteristic slowing of the rapid removal phase of noradrenaline removal from plasma, expressed in a lengthening of the $T_{1/2}$ of the disappearance curve after either injection of tritiated noradrenaline (Hertting, Axelrod & Whitby, 1961), or infusion of tritiated noradrenaline to plateau concentration (Esler *et al.*, 1981), is noted. We found that propranolol had no such effect (unpublished observations). Diminished hepatic extraction of noradrenaline, from reduced cardiac output and liver blood flow, is one possible mechanism of the reduced noradrenaline clearance, much as the metabolic clearance of aldosterone, for example, is lowered by propranolol (Pratt, Grim & Parkinson, 1980). Inhibition by propranolol of the enzymes responsible for the degradation of noradrenaline is another untested possibility.

The rate of release of noradrenaline fell during treatment with propranolol in six patients. Whether noradrenaline spillover rose or fell was not related to the order of testing, that is whether a patient received propranolol or no treatment first, so falls were unlikely to represent a regression towards the mean. Neither did they result from assay variability, which was small (Esler *et al.*, 1979). The noradrenaliane spillover rate tended to fall particularly in patients in whom values while untreated were elevated, suggesting that propranolol reduces sympathetic activity if it is increased, this reduction in resting sympathetic tone perhaps being responsible for the fall in blood pressure. But several compelling reasons exist for rejecting this notion. First, the magnitude of the fall in blood pressure with propranolol was related to neither the initial noradrenaline release rate nor to the change in noradrenaline release with treatment. In fact, some patients had a satisfactory antihypertensive response despite a rise in release rate on propranolol. In addition, noradrenaline spillover rate fell only to within the normal range, if in fact it fell at all. This contrasted with the effect of the known sympathetic nervous suppressant, clonidine, which at a dose producing a comparable antihypertensive response, reduced the noradrenaline spillover rate to very low values, similar to those found in patients with autonomic insufficiency. If propranolol does suppress resting sympathetic nervous activity, this reduction in sympathetic tone must be either incomplete, compared with clonidine, or selective, involving in particular, for antihypertensive effect, organs such as the heart and kidney which have a central role in blood pressure regulation.

We did not test the effect of propranolol on sympathetic nervous system reflexes. Our steady-state kinetics methodology is not appropriate for studying reflexes.

Evidence exists that reflex noradrenergic responses, to baroreceptor stimulation in rabbits (Korner *et al.*, 1980), and to upright posture in humans (Esler & Nestel, 1973; Fournier *et al.*, 1976; De Champlain, 1977), are reduced by β-adrenergic receptor blockade. It is possible, but unproven, that inhibition of sympathetic cardiovascular reflexes is one component of the antihypertensive action of β-adrenoceptor blockers. Standing in apparent contradiction to this evidence of inhibition of sympathetic reflexes is the effect of β-adrenoceptor blockers on the plasma noradrenaline response to dynamic exercise, the rise in noradrenaline levels with exercise being accentuated by β-adrenoceptor blockade (Distler *et al.*, 1978). But interpretation here is complicated by the reduction in noradrenaline clearance with beta-blockade we describe.

The hope has been expressed that indices of sympathetic nervous system activity might be used clinically to predict the response of patients with essential hypertension to antiadrenergic drugs (Esler & Nestel, 1973; Fourner *et al.*, 1976; De Champlain, 1977). The magnitude of the antihypertensive effect of β-adrenergic receptor blockers in individual patients, particularly at low doses, does appear to be related to biochemical measures of sympathetic nervous function, such as the plasma noradrenaline concentration at rest (Esler *et al.*, 1977; De Champlain, 1977), and the noradrenaline responses to upright posture (Esler & Nestel, 1973; Fourner *et al.*, 1976) and exercise (Distler *et al.*, 1978). At full doses of β-adrenergic blockers, however, these indices of sympathetic function either entirely fail to predict the therapeutic outcome, as we found here and have noted in other studies (Esler & Nestel, 1973; Esler, Zweifler, Randall & De Quattro, 1977), or, much as for plasma renin activity, have predictive power rather too low to be clinically useful.

This research was funded by a grant from the National Health and Medical Research Council of Australia.

References

ANAVEKAR, S.N., LOUIS, W.J., MORGAN, T.O., DOYLE, A.E. & JOHNSTON, C.I. (1975). The relationship of plasma levels of pindolol in hypertensive patients to effects on blood pressure, plasma renin and plasma noradrenaline levels. *Clin. exp. Pharmac. Physiol.*, **2**, 203–212.

BRECHT, H.M., BANTHIEN, F., ERNST, W. & SCHOEPPE, W. (1976). Increased plasma noradrenaline concentrations in essential hypertension and their decrease after long-term treatment with a β-receptor blocking agent (Pindolol). *Clin. Sci. mol. Med.*, **51**, suppl. 3, 485S–488S.

DE CHAMPLAIN, J. (1977). The sympathetic system in hypertension. *Clin. Endocrinol. Metab.*, **6**, 633–655.

DISTLER, A., KEIM, H.J., CORDES, U., PHILLIP, T. & WOLFF, H.P. (1978). Sympathetic responsiveness and antihypertensive effect of beta-receptor blockade in essential hypertension. *Am. J. Med.*, **64**, 446–451.

DUNLOP, D. & SHANKS, R.G. (1969). Inhibition of the carotid sinus reflex by the chronic administration of propranolol. *Br. J. Pharmac.*, **36**, 132–143.

ESLER, M., JACKMAN, G., BOBIK, A., KELLEHER, D., JENNINGS, G., LEONARD, P., SKEWS, H. & KORNER, P. (1979). Determination of norepinephrine apparent release rate and clearance in humans. *Life Sci.*, **25**, 1461–1470.

ESLER, M., JACKMAN, G., LEONARD, P., SKEWS, H., BOBIK, A. & KORNER, P. (1981). Effect of norepinephrine uptake-blockers on norepinephrine kinetics in humans. *Clin. Pharmac. Ther.*, **29**, 12–20.

ESLER, M., JULIUS, S., ZWEIFLER, A., RANDALL, O., HARBURG, E., GARDINER, H. & DE QUATTRO, V. (1977). Mild high-renin essential hypertension. A neurogenic human hypertension? *New Engl. J. Med.*, **296**, 405–411.

ESLER, M.D. & NESTEL, P.J. (1973). Evaluation of practolol in hypertension. Effects on sympathetic nervous system and renin responsiveness. *Br. Heart J.*, **35**, 469–474.

ESLER, M., ZWEIFLER, A., RANDALL, O. & DE QUATTRO, V. (1977). Pathophysiologic and pharmacokinetic determinants of the antihypertensive response to propranolol. *Clin. Pharmac. Ther.*, **22**, 299–308.

ESLER, M., ZWEIFLER, A., RANDALL, O., JULIUS, S. & DE QUATTRO, V. (1977). Agreement among three different indices of sympathetic nervous system activity in essential hypertension. *Mayo Clin. Proc.*, **52**, 379–382.

FOURNIER, A., HARDIN, J.M., ALEXANDRE, J.M., LOMBAERT, M., RONCO, G., BEZOC, J.F., DESMET, G. & QUICHARD, J. (1976). Antihypertensive effect of acebutolol: its relation to sympathetic nervous system responsiveness and to plasma renin and dopamine-β-hydroxylase activities. *Clin. Sci. mol. Med.*, **51**, suppl. 3, 477S–480S.

HERTTING, G., AXELROD, J. & WHITBY, L.G. (1961). Effect of drugs on the uptake and metabolism of H^3-norepinephrine. *J. Pharmac. exp. Ther.*, **134**, 146–153.

JONES, D.H., DANIEL, J., HAMILTON, C.A. & REID, J.L. (1980). Plasma noradrenaline concentration in essential hypertension during long-term β-adrenoceptor blockade with oxprenolol. *Br. J. clin. Pharmac.*, **9**, 27–31.

KELLIHER, G.J. & BUCKLEY, J.P. (1970). Central hypotensive action of dl- and d-propranolol. *J. pharm. Sci.*, **59**, 1276–1280.

KOPIN, I.J. (1979). Biochemical assessment of peripheral adrenergic activity. In *The Release of Catecholamines from Adrenergic Neurons*, ed Paton, D.M. Oxford: Pergamon Press.

KORNER, P.I., DORWARD, P.K., BLOMBERY, P.A. & FREAN, G.J. (1980). Central nervous β-adrenoceptors and their role in the cardiovascular action of propranolol in rabbits. *Circ. Res.*, **46**, suppl. 1, 26–32.

LEWIS, P.J. & HAEUSLER, G. (1975). Reduction in sympathetic nervous activity as a mechanism for hypotensive effect of propranolol. *Nature (Lond)*, **256**, 440–441.

PRATT, J.H., GRIM, C.E. & PARKINSON, C.A. (1980). Effects of propranolol on aldosterone plasma concentration and aldosterone metabolic clearance in hypertensive patients. *J. lab. clin. Med.*, **95**, 693–697.

SHIPLEY, R.A. & CLARK, R.E. (1972). *Tracer methods for* in vivo *kinetics*. New York: Academic Press.

WING, L.M.H., REID, J.L., HAMILTON, C.A., SEVER, P., DAVIES, D.S. & DOLLERY, C.T. (1977). Effects of clonidine on biochemical indices of sympathetic function and plasma renin activity in normotensive man. *Clin. Sci. mol. Med.*, **53**, 45–53.

YAMAGUCHI, N., DE CHAMPLAIN, J. & NARDEAU (1977). Regulation of norepinephrine release from cardiac sympathetic fibers in the dog by presynaptic α- and β-receptors. *Circ. Res.*, **41**, 108–117.

ZEIGLER, M.G., LAKE, C.R. & KOPIN, I.J. (1977). The sympathetic nervous system defect in primary orthostatic hypotension. *New Engl. J. Med.*, **296**, 293–297.

(Received September 19, 1980)