Br. J. Pharmac. (1981), **73**, 749–754

AN INVESTIGATION INTO THE TYPE OF β-ADRENOCEPTOR MEDIATING SYMPATHETICALLY ACTIVATED RENIN RELEASE IN THE CAT

EDWARD J. JOHNS

Department of Physiology, The Medical School, Birmingham B15 2TJ

1 Stimulation of the renal nerves in the cat was previously shown to cause renin release which could be blocked by propranolol. An attempt was made in this study to determine the type of β-adrenoceptor mediating this response.

2 In anaesthetized, unilaterally nephrectomized cats, a comparison was made of the ability of two selective β-adrenoceptor antagonists to block the tachycardia and hypotension caused by isoprenaline (mediated respectively by β_1- and β_2-adrenoceptors) and the release of renin caused by renal nerve stimulation.

3 Isoprenaline (mean dose of 0.224 ± 0.022 nmol/kg), increased heart rate by approximately 43 beats/min and decreased mean blood pressure by 47 mmHg. Stimulation of the distal cut ends of the renal nerves, at a rate sufficient to reduce renal blood flow by 30%, resulted in an approximately 150% increase in plasma renin activity.

4 Administration of the selective β_1-adrenoceptor antagonist, atenolol (0.38 to 11.28 μmol/kg), caused a dose-related inhibition of nerve stimulated renin release and of isoprenaline-induced tachycardia, with no diminution of the vasodepressor response to isoprenaline; in contrast, the selective β_2-adrenoceptor antagonist, erythro-DL-(7-methylindan-4-yloxy)-3-isopropylamino-butan-2-ol (ICI 118, 551, 0.03 to 2.86 μmol/kg), caused a dose-related inhibition of the isoprenaline-induced vasodepression without altering the increase in plasma renin activity caused by renal nerve stimulation. Only at the highest dose of ICI 118, 551 was there a reduction of isoprenaline-induced tachycardia, by about 40%.

5 The selective inhibition of neurally activated renin release by atenolol but not by ICI, 118, 551 is consistent with the suggestion that the β-adrenoceptors mediating renin release resemble those in the heart more closely than those in peripheral blood vessels.

Introduction

Activation of the sympathetic nervous system can cause renin release which may occur independently of changes in renal haemodynamics (LaGrange, Sloop & Schmid, 1973). In an early study (Coote, Johns, MacLeod & Singer, 1972) it was shown that direct electrical stimulation of the renal nerves of the cat caused the release of renin which was almost entirely blocked by propranolol even though the changes in renal blood flow were unaffected. Since that time, a variety of pharmacological and physiological studies have led to the view that β-adrenoceptors are present on the juxtaglomerular cells of the afferent arteriole and that they mediate the release of renin induced by adrenergic nerve stimulation (Reid, Morris & Ganong, 1978). Attempts have been made to determine whether these receptors are more like those of the heart (β_1-adrenoceptors) or more like those of the peripheral vasculature (β_2-adrenoceptors) but a clear concensus has not been reached.

In previous investigations using cats, Johns & Singer (1974a) showed that propranolol (a non-selective β-adrenoceptor antagonist) was more potent than atenolol (a selective β_1-adrenoceptor antagonist) in blocking renin release caused by stimulation of the renal nerves and concluded that the β-adrenoceptors involved were distinctly different from those of the heart. This was supported by an *in vitro* study (Johns, Richards & Singer, 1975) which showed that both selective and non-selective β-adrenoceptor agonists could stimulate renin release from cat isolated renal cortical cells. In another study Weber, Stokes & Gain (1974) demonstrated that, in conscious rabbits, isoprenaline-induced renin release was blocked more effectively by β_2- than β_1-adrenoceptor antagonists and suggested that β_2-adrenoceptors mediated this renin release.

In contrast, Oates, Stoker, Monaghan & Stokes

0007-1188/81/070749-06 $01.00

(1978) and Desaulles, Meisch & Schwartz (1978) concluded that, in rats β_1-adrenoceptors were involved in sympathetically-mediated renin release from the kidney. Similarly, studies both in conscious dogs (Himori, Izumi & Ishimori, 1980) and in anaesthetized dogs (Kopp, Aurell, Nilsson & Ablad, 1980) demonstrated that adrenergic renin release was probably mediated by β_1-adrenoceptors. Finally, studies in man of either basal (Åberg, 1974; Gavras, Gavras, Brunner & Liang, 1979) or adrenergically stimulated renin levels (Esler & Nestel, 1973; Davies, Wiggins, Slater & Geddes, 1978) are consistent with the involvement of β_1-adrenoceptors.

In this present investigation an attempt has been made to examine more fully the type of β-adrenoceptor mediating renin release in response to renal nerve stimulation in the cat. Advantage has been taken of the availability of a new, highly selective, β_2-adrenoceptor antagonist, erythro-DL-methylindan-4-yloxy-3-isopropylaminobutan-2-ol (ICI 118, 551) which is devoid of partial agonist activity but possesses membrane stabilizing properties (Bilski, Dorries, Fitzgerald, Jessup, Tucker & Wale, 1980). We studied the effect of either this drug or atenolol on three different responses mediated by β-adrenoceptors; firstly, increases in renin release induced by renal nerve stimulation; secondly, isoprenaline-induced tachycardia (a β_1-adrenoceptor response); thirdly, isoprenaline-induced hypotension (a β_2-adrenoceptor response).

Methods

Male cats, weighing 2.9 to 4.7 kg, were anaesthetized with sodium pentobarbitone (168 μmol/kg i.p.) and maintained with further small intravenous doses as necessary. Cannulae were inserted into the left carotid artery, for measurement of blood pressure (Statham P23Dc transducer and Grass Polygraph, Model 7) and removal of blood samples, and into the right jugular vein for infusion of saline (150 mmol/l NaCl) and drugs. Heart rate was measured with a tachygraph (Grass) triggered by the arterial pulse wave. Loose ligatures were placed around both vagi in the neck.

The right kidney was removed and the left kidney was exposed retroperitoneally. A non-cannulating flow probe (Biotronix) was placed on the renal artery for measurement of renal blood flow (S.E. Labs. M275 flowmeter). The renal nerves were sectioned and dissected from their junction at the coeliac ganglion to give sufficient length to place over bipolar silver wire stimulating electrodes for stimulation. At the end of all surgical preparations both vagi were sectioned and the animals allowed at least 1 h to stabilize.

Renal nerve stimulation

Electrical stimulation was for periods of 10 min at 15 V, 0.2 ms (Grass S8 stimulator) at a frequency sufficient to reduce renal blood flow by 30% (usually between 2.0 and 7.0 Hz). Only small changes in frequency were necessary to maintain renal blood flow at the lower level during the period of stimulation.

Isoprenaline test

Five min after renal nerve stimulation had ceased, a bolus intravenous injection of isoprenaline was given at a dose sufficient to increase heart rate by between 40 to 50 beats/min and was associated with a fall in mean blood pressure of between 40 and 70 mmHg. This dose was determined before an antagonist was given. The peaks of the isoprenaline-induced responses were measured.

Administration of drugs

Saline (0.9% w/v NaCl solution) was infused at 12 ml/h on completion of the jugular vein cannulation. Each of the β-adrenoceptor antagonists was dissolved in saline and appropriate doses were given in volumes between 1.0 and 2.0 ml by intravenous injection over 90s. The drugs were injected not less than 5 min after the isoprenaline test; and at least 15 min elapsed before the baseline measurements were begun. Atenolol was given to achieve cumulative dose levels of 0.38, 1.13, 3.76 and 11.28 μmol/kg (equivalent to 0.1, 0.3, 1.0 and 3.0 mg/kg) and ICI 118, 551 of 0.03, 0.09, 0.29, 0.86 and 2.86 μmol/kg (equivalent to 0.01, 0.03, 0.1, 0.3 and 1.1 mg/kg).

Blood sampling

Arterial blood samples (0.6 ml) were collected into cooled syringes previously rinsed with disodium edetate (160 mmol/l). They were immediately centrifuged at 4°C, the plasma removed and stored in the deep-freeze until assayed. Erythrocytes were resuspended in saline and returned to the animal as soon as possible.

Renin estimation

Plasma was incubated at 37°C for 1 h in the presence of enzymatic inhibitors (2,3-dimercapto-propanol and 8-hydroxyquinoline) to generate angiotensin I (Haber, Koerner, Page, Kliman & Purnode, 1969) which was estimated by radioimmunoassay (C.I.S. (U.K.) Ltd.). Plasma renin activity (PRA) was expressed as pmol angiotensin I generated per ml plasma per hour (pmol ml^{-1} h^{-1}). Changes in circulating

levels of PRA were taken as reflecting changes in secretion of renin from the remaining kidney.

Experimental protocol

A blood sample was taken to estimate the basal value of PRA 2 min before the start of a 10 min period of renal nerve stimulation. During the ninth min of stimulation a second blood sample was taken for the experimental value of PRA. Five min after the end of stimulation an injection of isoprenaline was given. After a further 5 min the antagonist was injected and the animals were left for 15 min to stabilize and to ensure that PRA levels had returned to basal values following the isoprenaline tests before a further sequence of sampling and stimulation was carried out.

Statistics

All data are expressed as means ± s.e. Student's *t* test was used for analysis of paired data within each group and unpaired data between groups. Differences were considered to be statistically significant when $P < 0.05$.

Drugs

The drugs used were (−)-isoprenaline sulphate (Macarthys); atenolol (ICI) and ICI 118, 551 (erythro-DL-(7 methylindan-4-yloxy)-3-isopropylaminobutan-2-ol, ICI).

Results

Eleven cats were used in total; five were used to study atenolol and four ICI 118, 551. In 11 cats, isoprenaline (mean dose 0.24 ± 0.02 nmol/kg) caused a peak rise in heart rate of 42.6 ± 4.0 beats/min (from 173.0 ± 13.6 to 215.0 ± 14.0 beats/min) and a maximum fall in blood pressure of 47.0 ± 3.8 mmHg (from 132.0 ± 6.0 to 85.0 ± 4.3 mmHg). Renal nerve stimulation in these 11 animals, sufficient to cause renal blood flow to decrease by 30% and in the absence of drug, produced a rise in PRA of 2.74 ± 0.57 pmol $ml^{-1}h^{-1}$ (from 1.71 ± 0.38 to 4.45 ± 0.80 pmol $ml^{-1}h^{-1}$; $P < 0.001$). Considerable variation between animals occurred in all these responses.

Effects of atenolol

The cumulative administration of atenolol resulted in a slight fall in PRA, from a level of 2.27 ± 0.79 pmol $ml^{-1}h^{-1}$ in the absence of drug, to 1.84 ± 0.27 pmol $ml^{-1}h^{-1}$ after the final dose, totalling 11.28 μmol/kg, but this was not statistically significant ($P > 0.5$). Mean blood pressure was not significantly changed, being 126.0 ± 7.3 mmHg before and 135.0 ± 11.1 mmHg after the highest dose of atenolol ($P > 0.5$); however, there was a significant fall in heart rate from 187.0 ± 20.3 to 146.0 ± 13.4 beats/min within this same period ($P < 0.01$).

The interaction between atenolol and isoprenaline or renal nerve stimulation was assessed by expressing as a percentage of the control values the evoked changes in heart rate, blood pressure and PRA at each successive dose level of the antagonist. The effects of atenolol on these parameters are shown in Figure 1. Atenolol had no effect on the hypotensive response to the test dose of isoprenaline; in contrast, there was a dose-related inhibition of the tachycardia caused by isoprenaline, reaching 65% inhibition after the cumulative administration of 11.28 μmol/kg atenolol. Similarly, the rise in PRA resulting from renal nerve stimulation was progressively inhibited with increasing dosage of atenolol which caused about 80% inhibition at its highest dose. Clearly, the effect on renin release resembles closely that on the cardiac rather than on the vascular response.

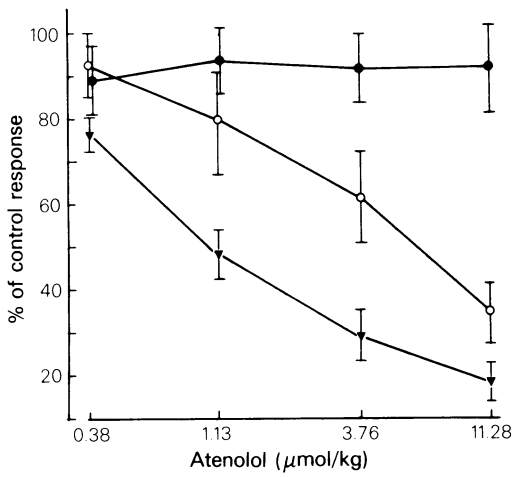

Figure 1 The percentage of the control response obtained in the absence of the drug is plotted against each dose of atenolol used. The changes in plasma renin activity due to renal nerve stimulation is represented by (▼), the changes in blood pressure to isoprenaline by (●) and the heart rate responses to isoprenaline by (○).

Effects of ICI 118, 551

ICI 118, 551 had little effect on the basal values of PRA, a slight rise from 1.23 ± 0.25 pmol $ml^{-1}h^{-1}$ in the absence of drug to 1.96 ± 0.40 pmol $ml^{-1}h^{-1}$ after 2.86 μmol/kg not being statistically significant

($P > 0.1$). Basal mean blood pressure was unaffected by ICI 118, 551, being 136.7 ± 9.3 mmHg in the absence of drug and 138.3 ± 4.4 mmHg after the highest dose but a significant fall in heart rate occurred, from 160.8 ± 18.5 beats/min before and 130.0 ± 17.0 beats/min after 2.86 μmol/kg ICI 118, 551 ($P < 0.001$).

The ways in which the responses to isoprenaline and renal nerve stimulation were modified by ICI 118, 551 are shown in Figure 2. There was a clear dose-related inhibition of the isoprenaline-induced hypotension, virtually complete abolition resulting from the highest dose. However, the rise in heart rate caused by isoprenaline was little affected by 0.86 μmol/kg of ICI 118, 551 (20% inhibition) although it was significantly inhibited by over 40% by 2.86 μmol/kg ($P < 0.02$). The rise in PRA induced by renal nerve stimulation was clearly unaffected by increasing the dose of ICI 118, 551 up to 0.86 μmol/kg although at the highest dose used there was a slight but insignificant decrease by 13% ($P > 0.8$). Clearly, over a dose range of this drug that completely blocked vascular responses to a chosen dose of isoprenaline, there was merely a small reduction of cardiac responses (particularly obvious at the highest doses of ICI 118, 551), and virtually no effect on the PRA responses.

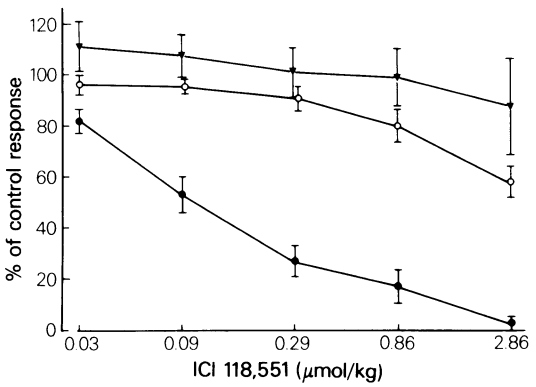

Figure 2 The percentage of the control response obtained in the absence of drug is plotted against each dose of ICI 118, 551. The change in plasma renin activity due to renal nerve stimulation is represented by (▼) and the heart rate response to isoprenaline by (○), and that of the blood pressure response to isoprenaline by (●).

Discussion

The present investigation provides evidence that nerve-mediated renin release in the cat occurs via activation of β-adrenoceptors which are more like those of the heart than those of the peripheral vasculature. This supports studies in other species such as man (Esler & Nestel, 1973; Åberg 1974; Gavras *et al.*, 1979), rat (Desaulles, *et al.*, 1978; Oates *et al.*, 1978) and dog (Himori *et al.*, 1980; Kopp *et al.*, 1980) all of which suggested that adrenergically-mediated renin release relied on β_1-adrenoceptors.

In the present experiments, administration of the selective β_1-adrenoceptor antagonist, atenolol (up to 11.28 μmol/kg), caused the renin release resulting from 10 min of renal nerve stimulation to be inhibited in a dose-related fashion which was very similar to the inhibition of the isoprenaline-induced rises in heart rate. However, the falls in blood pressure in response to isoprenaline were unaffected over this dose range of atenolol. Twenty min elapsed between the isoprenaline test and the removal of the next blood sample for PRA. We had previously found (Johns & Singer, 1974b) that such a time period was sufficient for PRA values to return to basal values after a stimulus. In the present study, basal values of PRA were slightly, but not significantly, lower when the dose of atenolol was increased which was comparable to results obtained previously (Johns & Singer, 1974a).

Cumulative doses of the β_2-selective antagonist, ICI 118, 551, up to 2.86 μmol/kg, had virtually no effect on the ability of renal nerve stimulation to cause renin release although at the highest dose of ICI 118, 551 there was a significant attenuation of the heart rate response to isoprenaline. Bilski *et al.* (1980) showed that this drug had a 'selectivity index' of 2.09 (i.e. the difference between pA_2 values on guinea-pig uterus and atrium); this means that an increase in dose of more than 100 times that required to cause β_2-blockade would be required to produce a similar blockade of β_1-adrenoceptors. Thus, at the highest doses used in this study there may have been some degree of blockade of β_1-adrenoceptors. However, it is clear that, over this dose range, ICI 118, 551 was extremely effective in reducing the blood pressure responses to isoprenaline. The similarity of inhibition by atenolol, and the lack of inhibition by ICI 118, 551, of the heart rate responses to isoprenaline and the nerve-mediated renin release is supportive evidence that the β-adrenoceptors involved are more like those of the heart than the peripheral vasculature.

In a previous study using the cat, in which the effect of renal nerve stimulation on renin release was examined, Johns & Singer (1974a) showed that the amount of propranolol required to block the neural renin release was some five times less than atenolol and this was taken to indicate that the kidney β-adrenoceptors were different from those of the heart. However, a difference in doses of between 40 and 100 times should be the basis for distinguishing β_1- from β_2-adrenoceptors as Harms & Spoelstra (1978)

and Barrett (1977) have obtained a cardioselectivity index for atenolol of 1.64 and 2.04 respectively. Further, it has now been found that in other tissues atenolol is less potent than propranolol (Barrett, 1977) and such a difference in potency may well have accounted for our earlier observations.

In a study using isolated renal cortical cells of the cat, Johns *et al.* (1975) demonstrated that salbutamol, a β_2-adrenoceptor agonist, stimulated renin release in a very similar manner to isoprenaline, a non-selective β-adrenoceptor agonist. These catecholamines were also shown to stimulate renin release from the isolated perfused kidney of the rat (Nakane, Nakane, Roux, Corvol & Menard, 1980). It is entirely possible that during the preparation of the renal cortical cells (Johns *et al.*, 1975) the pretreatment could have in some way modified receptors on the surface membranes of the cells. Such a suggestion is supported by the fact that relatively high doses of catecholamines were used as compared to other *in vitro* studies which employed kidney slices (Desaulles *et al.*, 1978; Weinberger, Aoi & Henry, 1975) in which the use of collagenase-like enzymes had been avoided.

Weber *et al.* (1974) using the conscious rabbit, indicated that β_2-adrenoceptors were involved in mediating renin release. They demonstrated that blocking agents having β_2-adrenoceptor antagonist properties were much more effective than non-selective or β_1-adrenoceptor antagonists in inhibiting isoprenaline-induced increases in renin release. However, it is important to recognise that isoprenaline can act not only on the β-adrenoceptors of the juxtaglomerular cells but also on the β-adrenoceptors of the renal vasculature to cause vasodilatation.

One of the consequences of such renal vasodilatation is to cause renin release by activation of the vascular receptor within the kidney sensitive to pressure (Davis & Freeman, 1976). In an earlier study (Johns & Singer, 1974b) we demonstrated that mechanical reduction in renal blood flow or reduction of renal perfusion pressure (situations in which the kidney vascular receptor would be activated) caused renin release which could not be blocked by propranolol. In other experiments stimulation of the renal nerves at a rate which caused almost identical changes in renal blood flow (Coote *et al.*, 1972; Johns & Singer, 1974a) resulted in renin release, almost all of which could be blocked by propranolol. Thus, in the study of Weber *et al.* (1974) blockade of these indirect actions of isoprenaline by the β_2-adrenoceptor antagonists could well have accounted for their greater effectiveness in blocking renin release and therefore led to the suggestion of β_2-adrenoceptors mediating renin release.

The present study was an attempt to examine more fully the β-adrenoceptor involved in mediating renin release from the kidney; the conclusion is that the β-adrenoceptors causing renin release in the cat are more like those of the heart than those of the peripheral vasculature.

I wish to thank ICI (Pharmaceuticals Division, Macclesfield) for financial assistance and supply of atenolol and ICI 118, 551.

References

ÅBERG, H. (1974). Plasma renin activity after the use of a new beta-adrenergic blocking agent (ICI 66, 082). *Int. J. Pharmac.*, **9**, 98–100.

BARRETT, A.M. (1977). The pharmacology of atenolol. *Postgrad. Med. J.*, **53** (Suppl. 3), 58–64.

BILSKI, A., DORRIES, S., FITZGERALD, J.D., JESSUP, R., TUCKER, H. & WALE, J. (1980). ICI 118, 551, a potent β_2-adrenoceptor antagonist. *Br. J. Pharmac.*, **69**, 292–293P.

COOTE, J.H., JOHNS, E.J., MACLEOD, V.H. & SINGER, B. (1972). Effect of renal nerve stimulation, renal blood flow and adrenergic blockade on plasma renin activity in the cat. *J. Physiol.*, **226**, 15–36.

DAVIES, R., WIGGINS, R., SLATER, J.D.H. & GEDDES, D. (1978). Nature of the adrenoceptor that mediates renin release in man. *Cardiovascular Medicine*, **3**, 571–575.

DAVIS, J.O. & FREEMAN, R.H. (1976). Mechanisms regulating renin release. *Physiol. Rev.*, **56**, 1–56.

DESAULLES, E., MIESCH, F. & SCHWARTZ, J. (1978). Evidence for the participation of β_1-adrenoceptors in isoprenaline-induced renin release from rat kidney slices *in vitro*. *Br. J. Pharmac.*, **63**, 421–425.

ESLER, M.D. & NESTEL, P.J. (1973). Evaluation of practolol in hypertension. Effects on sympathetic nervous system and renin responsiveness. *Br. Heart. J.*, **35**, 469–472.

GAVRAS, I., GAVRAS, H., BRUNNER, H.H. & LIANG, C.S. (1979). Antihypertensive action of β-adrenoceptor blockade and the renin-angiotensin system. *Br. J. clin. Pharmac.*, Suppl. 2, **7**, 213–217S.

HABER, E., KOERNER, T., PAGE, L.B., KLIMAN, B. & PURNODE, A. (1969). Application of a radioimmunoassay for angiotensin I to physiologic measurements of plasma renin activity in normal human subjects. *J. clin. Endocr.*, **29**, 1349–1355.

HARMS, H.H. & SPOELSTRA, A.J.G. (1978). Cardiac and bronchial β-adrenoceptor antagonistic potencies of atenolol, metoprolol acebutolol, practolol, propranolol and pindolol in the anaesthetized dog. *Clin. exp. Pharmac. Physiol.*, **5**, 53–59.

HIMORI, N., IZUMI, A. & ISHIMORI, T. (1980). Analysis of β-adrenoceptors mediating renin release by isoproterenol in conscious dogs. *Am. J. Physiol.*, **238**, F387–F397.

JOHNS, E.J., RICHARDS, H.K. & SINGER, B. (1975). Effects

of adrenaline, noradrenaline, isoprenaline and salbutamol on the production and release of renin by isolated renal cortical cells of the cat. *Br. J. Pharmac.*, **53,** 67–73.

JOHNS, E.J. & SINGER, B. (1974a). Comparison of the effects of propranolol and ICI 66082 in blocking the renin releasing effect of renal nerve stimulation in the cat. *Br. J. Pharmac.*, **52,** 315–318.

JOHNS, E.J. & SINGER, B. (1974b). Specificity of blockade of renal renin release by propranolol in the cat. *Clin Sci. mol. Med.*, **47,** 331–343.

KOPP, U., AURELL, M., NILSSON, I.M. & ABLAD, B. (1980). The role of β_1-adrenoceptors in the renin release response to graded renal sympathetic nerve stimulation. *Pflugers Arch.*, **387,** 107–113.

LAGRANGE, R.G., SLOOP, C.H. & SCHMID, H.E. (1973). Selective stimulation of renal nerves in the anaesthetized dog. *Circulation Res.*, **33,** 704–712.

NAKANE, H., NAKANE, Y., ROUX, A., CORVOL, P. & MENARD, J. (1980). Effects of selective and nonselective beta adrenergic agents on renin secretion in isolated perfused rat kidney. *J. Pharmac. exp. Ther.*, **212,** 34–38.

OATES, H.F., STOKER, L.M., MONAGHAN, J.C. & STOKES, G.S. (1978). The beta-adrenoceptor controlling renin release. *Archs. int. Pharmacodyn.*, **234,** 205–213.

REID, I.A., MORRIS, B.J. & GANONG, W.F. (1978). The renin angiotensin system. *A. Rev. Physiol.*, **40,** 377–410.

WEBER, M.A., STOKES, G.S. & GAIN, J.M. (1974). Comparison of the effects on renin release of beta adrenergic antagonists with differing properties. *J. clin. Invest.*, **54,** 1413–1419.

WEINBERGER, M.H., AOI, W. & HENRY, D.P. (1975). Direct effect of beta-adrenergic stimulation on renin release by the rat kidney slice *in vitro*. *Circulation Res.*, **37,** 318–324.

(Received February 2, 1981.
Revised March 10, 1981.)