MEDICAL PRACTICE

*Occasional Review***Radioligand binding studies of adrenergic receptors and their clinical relevance**

PETER J BARNES

Responses to catecholamines are mediated by highly specific cell surface receptors (adrenoceptors) that bind the catecholamine with high affinity, and then relay the signal so that the characteristic cellular response follows.

Adrenoceptor classification

Ahlquist in 1948 first recognised that there were two distinct types of adrenoceptor, which he was able to differentiate by comparing the rank order of potency of several adrenergic agonists in various tissues.¹ Alpha-adrenoceptors which mediate smooth muscle contraction were characterised by the potency order adrenaline > noradrenaline > isoprenaline and beta-adrenoceptors, mediating relaxation, by the rank order isoprenaline > adrenaline > noradrenaline. Since then specific antagonists such as phentolamine, which blocks α -adrenoceptors, and propranolol, which blocks β -adrenoceptors, have been developed. Beta-adrenoceptors have been subclassified into β_1 -receptors, which mediate positive inotropic cardiac responses and lipolysis and for which adrenaline and noradrenaline are equipotent, and β_2 -receptors, which mediate relaxation in vascular, bronchial, and uterine smooth muscle for which adrenaline is more potent than noradrenaline.² Antagonists such as practolol, atenolol, and metoprolol are more potent at β_1 - than β_2 -receptors (and are therefore termed "cardioselective"), whereas agonists such as salbutamol and terbutaline are more potent at β_2 - than β_1 -receptors (and are therefore termed "selective" bronchodilators).

Some tissues show a response intermediate between β_1 and β_2 -receptors, which may be explained by the co-existence of both

types of β -receptor. More recently, the existence of α -receptor subtypes has been recognised, based on the development of selective agents.³ Alpha₁-receptors are the classical post-synaptic α -receptors which mediate smooth muscle contraction, whereas α_2 -receptors, located presynaptically, reduce the amount of noradrenaline released from the nerve ending—that is, an autoinhibitory effect. There is some evidence that α_2 -receptors may also be located post-synaptically and on platelets. Prazosin is a selective antagonist of α_1 -receptors and the plant alkaloid yohimbine a selective antagonist of α_2 -receptors, whereas phentolamine is equipotent at α_1 and α_2 -receptors. Clonidine is a more potent agonist at α_2 than α_1 -receptors.

Adrenoceptor function

Once activated by agonist, adrenoceptors bring about the appropriate cellular response, and there have recently been considerable advances in our understanding of the mechanisms. In the case of β -receptors binding of agonist activates the membrane-bound enzyme adenylate cyclase.⁴ It is suggested that binding of catecholamine agonists to adrenoceptor changes the fluidity of the cell membrane so that the hormone-receptor complex diffuses laterally along the membrane surface until it couples with and thereby activates adenylate cyclase.⁵ This activation process concerns a guanine nucleotide regulatory protein that binds guanosine triphosphate.⁶ Activated adenylate cyclase then stimulates conversion of adenosine triphosphate to adenosine-3',5'-monophosphate (cyclic AMP), which serves as the "second messenger" for the hormone and activates intracellular protein kinases. These in turn cause phosphorylation of enzymes that lead to the characteristic cellular response, such as relaxation of smooth muscle cells. By contrast, α_2 -receptors appear to inhibit adenylate cyclase activity and therefore reduce cyclic AMP levels.⁷ Alpha₁-receptors do not affect the adenylate cyclase system and probably have a direct effect on calcium ion flux across the cell membrane.⁸

Radioligand and binding

Recently a new approach has been used in the study of adrenoceptors which takes advantage of the high affinity of the receptor for adrenergic agents and uses radioactively labelled agonists and antagonists to label receptors directly. Radioligand binding entails incubating a cell membrane preparation that contains the receptors with the radioactive adrenergic agonist or antagonist (radioligand), then separating and counting the receptor bound radioactivity. Initial studies were unsuccessful because a large amount of radioligand was bound to non-receptor proteins (non-specific binding), obscuring binding to the adrenergic receptors (specific binding). The development of high affinity ligands with a high specific radioactivity and technological advances that allow rapid separation of bound and free radioligand has made it possible to identify binding that has all the characteristics of adrenoceptors.⁹ Specific binding is then defined by the amount of binding that has been displaced by a high concentration of competing drug, such as phentolamine (for α -receptors) or propranolol (for β -receptors). By increasing concentrations of radioligand it is possible to measure the affinity of binding and also the number of binding sites or receptor density.

Beta-adrenoceptors have been successfully labelled with the antagonists tritiated-dihydroalprenolol (^3H -DHA) and ^{125}I -hydroxybenzylpindolol and with the agonist ^3H -hydroxybenzylisoprenaline, which label both β_1 and β_2 -receptors.⁹ Alpha-receptors have been identified using ^3H -dihydroergocryptine,¹⁰ which labels α_1 and α_2 -receptors, and ^3H -prazosin, which selectively labels α_1 -receptors.¹¹

Binding of these radioligands shows the expected characteristics of adrenoceptors. Binding is saturable, indicating that a finite number of receptors is present, and is of high affinity of the same order as determined by physiological experiments. In competition experiments with other adrenergic agonists and antagonists the rank order of potency in displacing specific binding is in agreement with the known pharmacological potency of these agents; thus for ^3H -DHA binding isoprenaline is much more potent than noradrenaline, whereas for ^3H prazosin binding noradrenaline is more potent than isoprenaline. Binding is stereospecific with the L-isomer much more potent than the D-isomer.

Changes in adrenergic responsiveness could be due to alteration in either adrenoceptor affinity, receptor number, or in the coupling of receptor to effector mechanisms. With radioligand binding it is possible for the first time to make direct measurements of both receptor affinity and number. These methods have been successful in various tissues and animals, but the application of this technique to man has been hampered by the difficulty in obtaining suitable tissue. The demonstration, however, that circulating human lymphocytes¹² and polymorphs¹³ bear β_2 -adrenoceptors (about 1500 receptors/cell) and platelets¹⁴ bear α_2 -receptors (about 500 receptors/cell) makes it possible to study adrenoceptors directly in man.

Molecular mechanisms

Direct binding techniques have been used in further elucidating the molecular properties of adrenoceptors. In competition studies with selective adrenergic antagonists the existence of β_1 and β_2 receptor subtypes has been confirmed and in several tissues both subtypes coexist.^{15 16} Similarly α_1 and α_2 -receptors have been clearly differentiated, and most tissues contain a mixture of the two subtypes.⁷ Now that quantification of receptors is possible it has become clear that only a percentage of available receptors need to be occupied by the agonist to elicit a maximal response, suggesting that there are "spare receptors." These spare receptors provide a means by which changes in receptor number can regulate cellular sensitivity to catecholamines without changing the maximal response.¹⁷

Mechanisms of receptor-effector coupling have also been

studied. Computer-modelling of competition curves indicate that β -receptors and α_2 -receptors exist in either low or high affinity forms. In the presence of an agonist the high affinity form of receptor is induced, resulting in activation of nucleotide regulatory protein, which then primes adenylate cyclase for activation by GTP.¹⁸ Receptor affinity is also modulated by cations: sodium ions reduce, whereas magnesium ions increase, binding affinity for agonists but not antagonists, although whether this has clinical relevance is uncertain.

Regulation of receptors

By enabling measurement of both receptor affinity and number radioligand binding methods have greatly advanced understanding of various factors that regulate adrenoceptors. It is now apparent that a wide variety of hormones, drugs, and physiological and pathological conditions may change adrenoceptor number and therefore sensitivity of tissues to catecholamines.¹⁹ Many of these findings have important clinical implications. Regulation of adrenoceptors by catecholamines themselves has been termed *homologous* regulation, and by other influences as *heterologous* regulation.

Homologous regulation

Catecholamines regulate the concentration of their own receptors. An increase in catecholamine concentrations reduces the number of adrenoceptors ("down regulation") and conversely a reduction in agonist concentration increases receptor density ("up regulation"). This provides a means by which cells may be protected from excessive stimulatory concentrations of catecholamines by turning down receptor number yet are able to retain sensitivity to catecholamines when levels fall. In man there is a good inverse correlation between lymphocyte β -receptor number and circulating catecholamine concentrations suggesting that physiological fluctuations in catecholamines determine β -receptor density.²⁰

DOWN REGULATION

High concentrations of adrenergic agonists reduce receptor numbers, leading to reduced tissue responsiveness to catecholamines.²¹ This appears to be the molecular basis for tachyphylaxis or desensitisation. The mechanism of this reduction in receptor number is uncertain but may entail a change in shape of the receptor molecule or sinking of the receptor into the cell (internalisation). Desensitisation may also include increased formation of the low affinity form of the receptor, resulting in reduced affinity of binding.¹⁸ Down regulation represents a generalised homeostatic mechanism whereby tissues are protected from excessive levels of specific hormones.

In phaeochromocytoma with high concentrations of circulating catecholamines there is an appreciable reduction in lymphocyte β -receptor number,²⁰ which underlies the protective reduction in adrenergic sensitivity in this condition. β -adrenergic agonist bronchodilators, such as salbutamol and terbutaline, cause a reduction in lymphocyte β -receptor density in both normal subjects and asthmatic patients, and the reduced responsiveness to β -agonists reported in asthma is probably due to previous adrenergic treatment.²²

Down regulation has also been shown with α -receptors. Adrenaline causes a reduction in human platelet α -receptors associated with a reduced ability of adrenaline to cause platelet aggregation.²³ Withdrawal of clonidine may cause rebound hypertension²⁴ and may be another clinical example of down regulation. Chronic treatment with clonidine, a α_2 -agonist, may reduce central and peripheral α_2 -receptors so that when suddenly stopped there is a relative paucity of feedback inhibitory α_2 -

receptors. This results in increased noradrenaline release from noradrenergic nerves giving rise to hypertension.

UP REGULATION

By contrast experimental sympathetic denervation (either surgically or pharmacologically with guanethidine or 6-hydroxydopamine) removes the tonic release of neurotransmitter noradrenaline, leaving adrenoceptors unoccupied. This results in an increase in adrenoceptor number leading to increased adrenergic responsiveness or denervation hypersensitivity.²⁵ Similarly, chronic blockade of adrenoceptors by specific antagonists causes an increase in receptor number. In man chronic administration of propranolol increases leucocyte β -receptor numbers.²⁶ After abrupt withdrawal of propranolol, plasma concentrations rapidly fall but the increased β -receptor density may persist for several days. This may explain the supersensitivity of β -receptor mediated responses that result in "rebound angina" when β -blockers are stopped.²⁷ Possibly β -antagonists with intrinsic sympathomimetic activity such as oxprenolol would not lead to such an increase in β -receptor numbers and would be less likely to be associated with rebound angina.

Heterologous regulation

Since radioligand binding methods have been available many hormones, drugs, and physiopathological states have been shown to influence adrenoceptor numbers. Several of these conditions may do this by changing catecholamine concentration at the receptor and are therefore really examples of homologous regulation. Thus tricyclic antidepressants reduce β -receptor numbers in rat brain by inhibiting reuptake of noradrenaline, which results in down regulation of receptor numbers.²⁸

ASTHMA

Asthmatic patients show various reduced β -adrenergic responses, and this reduced β -adrenergic responsiveness may underlie the bronchoconstriction of asthma.²⁹ Lymphocytes from asthmatic patients did indeed have fewer β -receptors compared with normal subjects,³⁰ but it soon became apparent that this was due to down regulation from previous β -adrenergic bronchodilator treatment.²² Nevertheless, in untreated asthmatic patients there was still a small but important reduction in lymphocyte β -receptor number compared with normal subjects, and the more severe the airways obstruction the lower the β -receptor number.³¹ Increased α -adrenergic responsiveness has been described in asthmatic patients³² but little is known about the role of α -receptors in human asthma. In an animal model of chronic asthma a small decrease in pulmonary β -receptor numbers but a pronounced increase in α -receptor numbers has been shown.³³ In human lung there is some evidence for increased α -receptors in lungs from patients with airways obstruction.³⁴ Possibly in asthma there is a shift in the balance between bronchoconstrictor α - and bronchodilator β -receptors in the airways.³⁵

STEROIDS

Corticosteroids have been shown to increase β -adrenergic responsiveness in vitro, and in normal subjects the reduced responsiveness to inhaled β -agonists after prolonged administration may be reversed by intravenous hydrocortisone.³⁶ Chronic administration of hydrocortisone caused a considerable increase in rat lung β -receptor density³⁷ and high dose oral steroids an increase in lymphocyte β -receptor density in normal and asthmatic subjects.³⁸ This suggests that one function, although

probably not the most important, of steroids in asthma may be to increase airway β -receptors.

HEART FAILURE

In the failing heart various compensatory mechanisms operate to maintain normal output. In an animal model of chronic heart failure there was a considerable increase in both α - and β -receptors in the heart, and these changes were associated with a depletion of myocardial noradrenaline content.³⁹ This increase in adrenoceptors would lead to an increase in adrenergic responsiveness to circulating catecholamines and lead to an increased inotropic response. These findings emphasise the deleterious effects that β -blockers may have in heart failure.

THYROID DISEASE

Some of the features of hyperthyroidism resemble the effects of β -adrenergic stimulation, and since there is no increase in circulating catecholamines it was suggested that increased β -receptor number or affinity may occur in this condition. Indeed, in rats thyroid hormone administration resulted in increased cardiac β -receptor density.⁴⁰ Conversely, rats made hypothyroid had reduced cardiac β -receptor number whereas the number of α -receptors increased,⁴¹ suggesting that thyroid disorders may result in a shift in balance or interconversion between α - and β -receptors.⁴² In man, however, there is no evidence for any changes in β -receptor number on circulating leucocytes in thyroid disease.⁴³

MISCELLANEOUS

Reduced lymphocyte β -receptor number has been shown in aging man, which may explain the reduced sensitivity to catecholamines in the elderly.⁴⁴ This is probably related to an increase in circulating catecholamines in the elderly, however. In animals there is a change in adrenergic responsiveness of the uterus during the oestrus cycle; adrenaline exerts an α -adrenergic effect during oestrogen dominance but a β -adrenergic effect during progesterone dominance. Binding studies have shown that there is a pronounced increase in α -receptor density with oestrogen dominance, which then falls during progesterone dominance, while the β -receptor density does not change.⁴⁵ In human platelets oestrogens increase α_2 -receptor density which may be responsible for increased platelet aggregation, and therefore tendency to thrombosis associated with high oestrogen oral contraceptives.⁴⁶ In spontaneously hypertensive rats there are reduced numbers of β -receptors in arteries and myocardium. This may underlie the β -adrenergic hyporesponsiveness shown in hypertension and may be relevant to its pathogenesis since compensatory β -receptor mediated vasodilatation would be defective.⁴⁷

Administration of amphetamines⁴⁸ and withdrawal of alcohol⁴⁹ are associated with increased cardiac β -receptor density in rats. This may underlie the hyperadrenergic state in these conditions in man. Essential thrombocythaemia is a rare myeloproliferative disorder with increased numbers of platelets and complicated by thrombosis and haemorrhage. The platelets show reduced adrenaline-induced aggregation, which is associated with a reduction in platelet α_2 -receptors.⁵⁰ Abnormalities of platelet α -receptors may possibly underlie other disorders of platelet function.

Radioreceptor assays

Another area where radioligand binding studies may be clinically relevant is in their use to measure the concentration of adrenergic drugs in biological fluids using the same principles as

radioimmunoassay. By using a β -receptor preparation from bovine lung it is possible to measure the concentration of various β -blockers in plasma.⁵¹ Because of the extremely high affinity and specificity of the receptors it is possible to measure very low concentrations of β -blockers, which makes the assay very sensitive. Radioreceptor assays would be very useful in measuring the plasma concentrations of β -blockers for which no simple assay exists. Binding assays are also useful in the rapid screening of new pharmacological compounds for activity as α - and β -receptor agents, and also provide a simple means of studying the pharmacological selectivity of new drugs.

Conclusions

Direct radioligand binding studies of adrenoceptors have led to a more precise understanding of their function and particularly of the various factors that regulate function. Recent studies showing alterations in receptor density in animal models and in clinical disorders have given further information on the underlying mechanisms of disease and may lead the way to more rational treatment.

I thank Professor C T Dollery for helpful discussion and comments in preparing this manuscript.

References

- Ahlquist RP. A study of adrenergic receptors. *Am J Physiol* 1948;**153**: 586-600.
- Lands AM, Arnold A, McAuliff JP, Luduena FP, Brown TG. Differentiation of receptor systems activated by sympathomimetic amines. *Nature* 1967;**214**:597-8.
- Berthelson S, Pettinger WA. A functional basis for classification of α -adrenergic receptors. *Life Sci* 1977;**21**:596-606.
- Sutherland EW, Rall TW. The relation of adenosine -3',5'-phosphate and phosphorylase to the actions of catecholamines and other hormones. *Pharmacol Rev* 1960;**12**:365-99.
- Hirata F, Strittmatter WJ, Axelrod J. β -adrenergic receptor agonists increase phospholipid methylation, membrane fluidity and β -adrenergic receptor-adenylate cyclase coupling. *Proc Natl Acad Sci USA* 1979;**79**: 368-72.
- Pfeuffer T. GPT-binding proteins in membranes and the control of adenylyl cyclase activity. *J Biol Chem* 1977;**252**:7224-34.
- Hoffman BB, Lefkowitz RJ. Alpha-adrenergic receptor subtypes. *N Engl J Med* 1980;**302**:1390-5.
- Butcher FR. Calcium and cyclic nucleotides in the regulation of secretion from the rat parotid by autonomic agonists. *Adv Cyclic Nucleotide Res* 1978;**9**:707-21.
- Lefkowitz RJ. Identification and regulation of alpha- and beta-adrenergic receptors. *Fed Proc* 1978;**37**:123-9.
- Williams LT, Lefkowitz RJ. Alpha-adrenergic receptor identification by (³H) dihydroergocryptine binding. *Science* 1976;**192**:791-3.
- Barnes P, Karliner J, Hamilton CA, Dollery C. Demonstration of α_1 -adrenoceptors in guinea pig lung using (³H)-prazosin. *Life Sci* 1979;**25**: 1207-14.
- Williams LT, Snyderman R, Lefkowitz RJ. Identification of β -adrenergic receptors in human lymphocytes by (-) (³H)-alprenolol binding. *J Clin Invest* 1976;**57**:149-55.
- Galant SP, Underwood S, Duriseti L, Insel PA. Characterisation and high affinity of β_2 -adrenergic receptor binding of (-) (³H)-dihydroalprenolol to human polymorphonuclear cell particulates. *J Lab Clin Med* 1978;**92**: 613-8.
- Alexander RW, Cooper B, Handin RI. Characterisation of the human platelet α -adrenergic receptor: correlation of (³H)-dihydroergocryptine binding with aggregation of adenylate cyclase inhibition. *J Clin Invest* 1978;**61**:1136-44.
- Rugg EL, Barnett DB, Nahorski SR. Coexistence of β_1 and β_2 adrenoceptors in mammalian lung: evidence from direct binding studies. *Mol Pharmacol* 1978;**14**:996-1005.
- Minneman KP, Hegstrand LR, Molinoff PB. Simultaneous determination of β_1 and β_2 adrenergic receptors in tissues containing both receptor subtypes. *Mol Pharmacol* 1979;**16**:34-46.
- Pollet RJ, Levey GS. Principles of membrane receptor physiology and their application to clinical medicine. *Ann Intern Med* 1980;**92**:663-80.
- Kent RS, De Lean A, Lefkowitz RJ. A quantitative analysis of α -adrenergic receptor interactions: resolution of high and low affinity states of the receptor by computer modelling of ligand binding data. *Mol Pharmacol* 1980;**17**:14-23.
- Lefkowitz RJ. Direct binding studies of adrenergic receptors: biochemical, physiologic and clinical implications. *Ann Intern Med* 1979;**91**:450-8.
- Fraser J, Nadeau J, Robertson D, Wood AJJ. Regulation of human leucocyte beta-receptors by endogenous catecholamines: relationship of leucocyte beta-receptor density to the cardiac sensitivity to isoproterenol. *J Clin Invest* 1981 (in press).
- Mukherjee C, Caton MG, Lefkowitz RJ. Catecholamine-induced subsensitivity of adenylate cyclase associated with loss of β -adrenergic receptor binding sites. *Proc Natl Acad Sci* 1975;**72**:1945-9.
- Galant SP, Duriseti L, Underwood S, Insel PA. Decreased beta-adrenergic receptors on polymorphonuclear leucocytes after adrenergic therapy. *N Engl J Med* 1978;**299**:933-6.
- Cooper B, Handin RI, Young LH, Alexander RW. Agonist regulation of the human platelet β -adrenergic receptor. *Nature* 1978;**274**:703-6.
- Geyskes GG, Boer P, Dorhout Mees EJ. Clonidine withdrawal mechanisms and frequency of hypertension. *Br J Clin Pharmacol* 1978;**7**:55-62.
- Glaubiger G, Tsai BS, Lefkowitz RJ, Weiss B, Johnson EM. Chronic guanethidine treatment increases cardiac β -adrenergic receptors. *Nature* 1978;**273**:240-2.
- Aarons RD, Nies AS, Gal J, Hegstrand LR, Molinoff PB. Elevation of β -adrenergic receptor density in human lymphocytes after propranolol administration. *J Clin Invest* 1980;**65**:949-57.
- Nattel S, Ragno RE, VanLoon G. Mechanism of propranolol withdrawal phenomenon. *Circulation* 1979;**59**:1158-64.
- Wolfe BB, Harden TK, Sporn JR, Molinoff PB. Presynaptic modulation of β -adrenergic receptors in rat cerebral cortex after treatment with antidepressants. *J Pharmacol Exp Ther* 1978;**207**:446-57.
- Szentivanyi A. The beta-adrenergic theory of the atopic abnormality in bronchial asthma. *J Allergy* 1968;**42**:203-32.
- Kariman K, Lefkowitz RJ. Decreased beta-adrenergic receptor binding in lymphocytes from patients with bronchial asthma. *Clin Res* 1977;**25**: 503A.
- Brooks SM, McGowan K, Bernstein K, Atenau P, Peagler J. Relationship between numbers of beta-adrenergic receptors in lymphocytes and disease severity in asthma. *J Allergy Clin Immunol* 1979;**63**:401-6.
- Henderson WR, Shelhamer JH, Reingold DB, Smith LJ, Evans R, Kaliner M. Alpha-adrenergic hyper-responsiveness in asthma: analysis of vascular and papillary responses. *N Engl J Med* 1979;**300**:642-7.
- Barnes PJ, Dollery CT, MacDermot J. Increased pulmonary α -adrenergic and reduced β -adrenergic receptors in experimental asthma. *Nature* 1980;**285**:569-71.
- Barnes PJ, Karliner JS, Dollery CT. Human lung adrenoceptors studied by radioligand binding. *Clin Sci* 1980;**58**:457-61.
- Szentivanyi A. The radioligand binding approach in the study of lymphocytic adrenoceptors and the constitutional basis of atopy. *J Allergy* 1980;**65**:5-11.
- Holgate ST, Baldwin CJ, Tattersfield AE. β -adrenergic agonist resistance in normal human airways. *Lancet* 1977;**ii**:375-7.
- Mano K, Akborzadeh A, Townley RG. Effect of hydrocortisone on beta-adrenergic receptors in lung membranes. *Life Sci* 1979;**25**:1925-30.
- Sano Y, Ford L, Begley M, Watt G, Townley R. Effect of in vivo anti-asthma drugs on human leucocyte beta-adrenergic receptors. *Clin Res* 1980;**28**:431A.
- Karliner JS, Barnes P, Brown M, Dollery C. Chronic heart failure in the guinea pig increases cardiac α_1 - and β -adrenoceptors. *Eur J Pharmacol* 1980;**67**:115-8.
- Williams LT, Lefkowitz RJ, Watanabe AM, Hathaway DR, Besch HR. Thyroid hormone regulation of β -adrenergic receptor number. *J Biol Chem* 1977;**252**:2787-9.
- Ciaraldi T, Marinetti GV. Thyroxine and propylthiouracil effects in vivo on α - and β -adrenergic receptors in rat heart. *Biochem Biophys Res Commun* 1977;**74**:984-91.
- Kunos G. Thyroid hormone-dependent interconversion of myocardial α - and β -adrenoceptors in the rat. *Br J Pharmacol* 1977;**59**:177-89.
- Williams RS, Guthrow CE, Lefkowitz RJ. β -adrenergic receptors of human lymphocytes are unaltered by hypothyroidism. *J Clin Endocrinol Metab* 1979;**48**:503-5.
- Schocken DD, Roth GS. Reduced beta-adrenergic receptor concentrations in ageing man. *Nature* 1977;**267**:856-8.
- Roberts JM, Insel PA, Goldfien RD, Goldfien A. α -adrenoceptors but not β -adrenoceptors increase in rabbit uterus with oestrogen. *Nature* 1977;**270**:624-5.
- Peters JR, Elliott JM, Grahame-Smith DG. Effect of oral contraceptives on platelet noradrenaline and 5-hydroxytryptamine receptors and aggregation. *Lancet* 1979;**ii**:933-6.
- Limas CJ, Limas C. Decreased number of β -adrenergic receptors in hypertensive vessels. *Biochem Biophys Acta* 1979;**582**:533-6.
- Banerjee SP, Sharma UK, Kung LS, Chanda SK. Amphetamine induced β -adrenergic receptor subsensitivity. *Nature* 1978;**271**:380-1.
- Banerjee SP, Sharma UK, Khanna JM. Alterations in β -adrenergic receptor binding during ethanol withdrawal. *Nature* 1978;**276**:407-9.
- Kiaywin P, McDonough M, Insel PA, Shattell SJ. Platelet function in essential thrombocythemia: decreased epinephrine responsiveness associated with a deficiency of platelet α -adrenergic receptors. *N Engl J Med* 1978;**299**:505-9.
- Nahorski SR, Batta MI, Barnett DB. Measurement of β -adrenoceptor antagonists in biological fluids using a radioreceptor assay. *Eur J Pharmacol* 1978;**52**:393-6.

(Accepted 6 February 1981)