Biochemical Pharmacology, Vol. 31, No. 4, pp. 467–484, 1982.
Printed in Great Britain.

0006-2952/82/040467-18 $03.00/0
Pergamon Press Ltd.

COMMENTARY

EVIDENCE FOR MORE THAN ONE TYPE OF POST-JUNCTIONAL α-ADRENOCEPTOR

J. C. McGRATH
Institute of Physiology, University of Glasgow, Glasgow G12 8QQ, U.K.

Existing sub-classifications of α-adrenoceptors

α- and β-adrenoceptors were conceived to explain quantitative differences between the effects of different catecholamines [1].

In the case of α-adrenoceptors, subdivision was postulated in an earlier commentary [61] on the basis of the anatomical location of the receptors and before a full profile of drug "selectivities" was established. α_1-Adrenoceptors were the "post-synaptic" α-adrenoceptors which mediate contraction of smooth muscle; they are assumed to be located on smooth muscle cells. α_2-Adrenoceptors were the "pre-synaptic" α-adrenoceptors which mediate a reduction of the action potential-induced output of transmitter from autonomic (particularly adrenergic) nerves; they are assumed to be located on the terminal regions of post-ganglionic neurones.

(In this commentary, the term "post-junctional" will be used rather than "post-synaptic", since the former covers more completely those receptors which are located on target organs and are accessible to circulating agonists, but may not be closely associated with the "synapse" between nerve and muscle or nerve and nerve. "Pre-junctional" can then be used for receptors which are believed to be located on nerve terminals. Where possible, use of this type of terminology, however, provides no more than an anatomical guide and should not imply a function. For example the receptors on nerve terminals may have as their physiological agonist the transmitter from a different nerve cell; are these pre- or post-junctional? It can be helpful to refer, in addition, to the supposed site of the receptors, e.g. nerve terminal, smooth muscle, ganglion cell body, etc.)

It was shown in various isolated preparations that some substances were relatively more potent as agonists at the pre-junctional (e.g. clonidine, xylazine) or post-junctional (e.g. phenylephrine) α-adrenoceptors. Although "selective" antagonists were harder to find, (1) all known α-adrenoceptor antagonists had post-junctional antagonism, (2) several, including yohimbine piperoxan and phentolamine, were, in addition, potent pre-junctionally, and (3) a few, including prazosin and phenoxybenzamine were relatively more potent post- than pre-junctionally. (For reviews see [37, 85]; for chemical structures of antagonists see Fig. 1.)

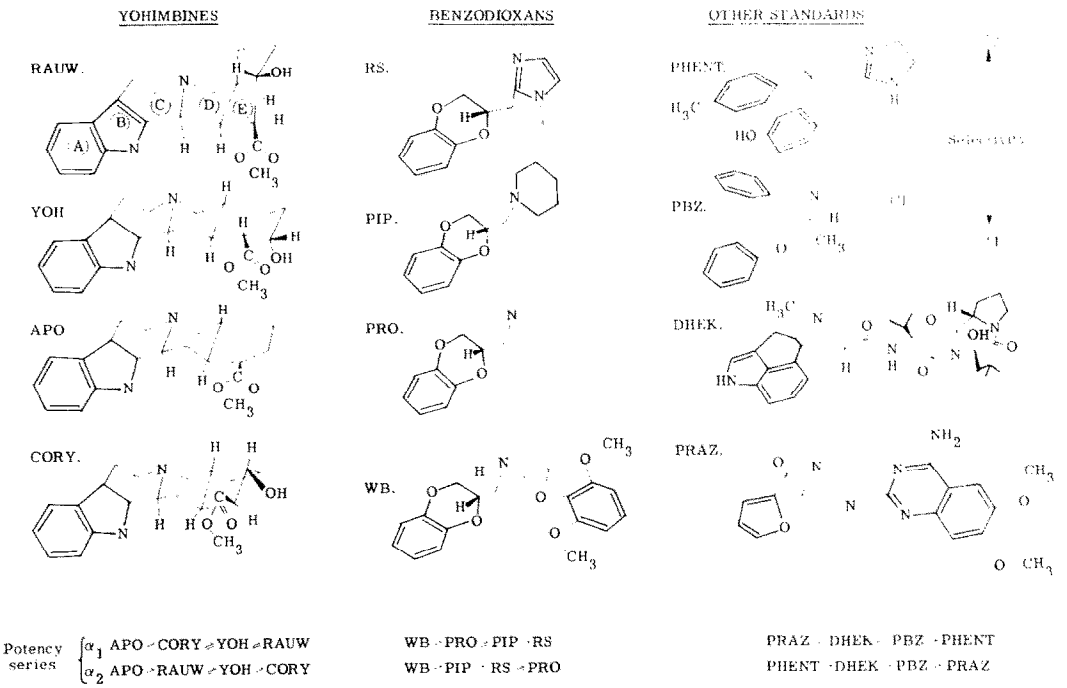
It has long been known that of all the antagonists which could block the post-junctional effects of adrenaline on the cat nictitating membrane or blood pressure, some could, but others, (e.g. yohimbine and piperoxan) could not, block the corresponding response to sympathetic nerve stimulation [37, 93]. This could now be explained in terms of antagonism at pre- and post-junctional α-adrenoceptors. Compounds which block only post-junctional receptors reduce the response to exogenous agonists, e.g. injected adrenaline, or to nerve stimulation. Those which block pre- as well as post-junctional receptors, however, reduce the response to exogenous agonists but reduce less effectively the nerve-induced response. The current explanation is that they interrupt a "negative feedback loop" in which transmitter noradrenaline regulates its own release by acting via the pre-junctional α-adrenoceptors. Thus the post-junctional antagonism may be offset by a greater release of transmitter. This interpretation is not universally accepted since it does not fully explain the different effects of certain agonists and antagonists in some tissues (e.g. [37, 55, 56]) but it serves, at present, to explain a large number of otherwise paradoxical observations. An additional explanation could, for example, be based on differences between the post-junctional receptors activated by nerves compared with those activated by agonists.

Once it was established that receptors which mediate contraction of smooth muscle could be activated by phenylephrine and blocked by prazosin, these two became "markers" for the post-junctional or α_1-adrenoceptors on smooth muscle since neither was potent either as agonist or antagonist at the pre-junctional or α_2-adrenoceptor [37, 85].

In the sub-classification of α-adrenoceptors this seemed, until recently, to be the one fixed point around which further classification could be based. It is the breakdown of this rule and its consequences which form the subject for this commentary.

Evidence that α_1-adrenoceptors, alone, do not explain the effects of noradrenaline on vascular smooth muscle

Noradrenaline produces contraction of isolated strips of human palmar arteries and pressor effects in the pithed rat or anaesthetised cat, which are less susceptible to prazosin than to phentolamine or yohimbine [26, 54, 75]. In the pithed rat, the pressor response to phenylephrine is susceptible to low doses of prazosin [26]. This suggests that, in these preparations, (1) noradrenaline is acting through an α-adrenoceptor, as indicated by the effect of phentolamine or yohimbine, (2) prazosin can block α_1-adrenoceptors, as shown by its effect on phenyl-



Potency series	α_1 APO > CORY ≥ YOH ≥ RAUW	WB > PRO > PIP > RS	PRAZ > DHEK > PBZ > PHENT
	α_2 APO > RAUW ≥ YOH > CORY	WB > PIP > RS ≥ PRO	PHENT > DHEK > PBZ > PRAZ

Fig. 1. The structures of the antagonists which are used to distinguish between α_1- and α_2-adrenoceptors. The three vertical columns represent the yohimbine series, benzodioxan series and other commonly used standard compounds. In each column the compounds are arranged in order of "selectivity" assessed by the method shown in Fig. 2, with those most "selective" for α_2-adrenoceptors at the top. Note that this order does not correspond to "potency" for either receptor: this is shown, for each series, under each column. Abbreviations—RAUW, rauwolscine; YOH, yohimbine; APO, apoyohimbine; CORY, corynanthine; RS, RS 21361; PIP, piperoxan; PRO, prosympal; WB, WB 4101; PHENT, phentolamine; PBZ, phenoxybenzamine; DHEK, dihydroergokryptine; PRAZ, prazosin.

ephrine, but (3) the receptors through which noradrenaline acts cannot be of the α_1-type since they are not blocked by prazosin.

This raised the obvious question that, if these "post-junctional" α-adrenoceptors, presumably situated on vascular smooth muscle, were not α_1, were they α_2 or were they some further, so far uncategorised, species? It was, at first, not possible to clarify this because yohimbine, which was the most selective antagonist of α_2- compared with α_1-adrenoceptors which was available, was not sufficiently "selective" for the purpose, antagonising the effects of both noradrenaline and phenylephrine [26].

However, by using relatively "selective" agonists, we have demonstrated two sets of receptors responsible for pressor effects in the pithed rat, one of which could be categorised as α_1 and the other as similar to the α_2-adrenoceptors which had been found previously at pre-junctional sites. The agonists were chosen on the basis of their "selectivity" for pre-junctional receptors, i.e. guanabenz and xylazine, or for post-junctional receptors, i.e. phenylephrine; the potency of each drug at pre- and post-junctional α-adrenoceptors was established on rat vas deferens and rat heart [16, 19, 64, 67]. The pressor responses to xylazine or guanabenz were more susceptible to blockade by yohimbine than prazosin, but, in contrast, those to phenylephrine were more susceptible to prazosin than to yohimbine [26, 19] (see Fig. 2). This has been independently confirmed by at least three other groups using the alternative α_2-agonists M-7 [25], guanfacine, B-HT 933 [90] and B-HT 920 [80]. The use of a sufficiently "selective" α_2-agonist was critical since several other agents, particularly imidazoline derivatives such as clonidine and oxymetazoline, were potent agonists at α_2-adrenoceptors (pre-junctional, nerve terminal) but were also sufficiently potent at the α_1-adrenoceptors (post-junctional, smooth muscle) that their pressor effects were more susceptible to prazosin than to yohimbine; this was confirmed by their effects in other test systems [16, 19].

In addition to the pithed rat preparation, evidence for α_2-adrenoceptors on vascular smooth muscle has been obtained in conscious or pithed rabbits [43, 71] and in the perfused hind limb of the rabbit [72] or dog [62]. Preliminary evidence for an "adrenoceptor" which is resistant to α_1-adrenoceptor antagonists has been found in rat anococcygeus *in situ* in pithed rats but the evidence from antagonists does not confirm this as α_2 and the same phenomenon cannot be described *in vitro* under standard conditions (unpublished observations). The only report, so far, from *in vitro* experiments, for a vascular smooth muscle α_2-adrenoceptor is from canine vascular smooth muscle [15]: venous smooth muscle was found to respond to α_1- or α_2-adrenoceptor agonists but arterial smooth muscle only to α_1; the effects of antagonists tended to support this but were complicated by the presence of non-competitive antagon-

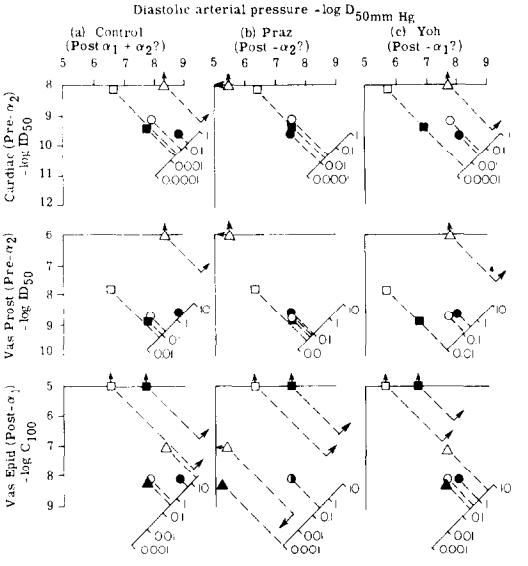


Fig. 2. Comparison of the diastolic pressor effects of α-adrenoceptor agonists in the pithed rat with their effects in other "α-adrenoceptor" test systems. Potency on blood pressure is assessed as the negative logarithm of the dose (moles/kg) to produce an increase in diastolic arterial blood pressure by 50 mm Hg. This is plotted for (a) controls, (b) after prazosin, 1 mg/kg, (c) after yohimbine, 1 mg/kg. Cardiac pre-junctional α_2-agonism is assessed as the negative logarithm of the dose (moles/kg) to produce 50% reduction in the cardioaccelerator response to sympathetic nerve stimulation in the pithed rat. Vas prost—indicates pre-junctional α_2-agonism *in vitro* assessed as the negative logarithm of the concentration (molar) producing a 50% reduction in the response of the prostatic portion of rat vas deferens to a single field stimulus. Vas epid—indicates post-junctional α_1-agonism *in vitro* assessed as the negative logarithm of the concentration producing a 100% increase in the contractile response of the epididymal portion of the rat vas deferens to a single field stimulus. The agonists are clonidine (○), oxymetazoline (●), xylazine (□), guanabenz (■), phenylephrine (△) and amidephrine (▲ bottom row only). An arrow attached to a symbol indicates that no potency could be detected at the dose indicated so that the real position of the point lies in the direction indicated. Since the plots are log/log the ratio of potencies can be found by projecting each point at right angles to the diagonal scales indicated; this is calibrated as the pressor potency as a fraction of the other indices.

ism. The classification of "post-junctional α_2-adrenoceptors" is thus still tentative since they have not undergone rigorous pharmacodynamic analysis *in vitro*.

Properties of the "post-junctional" α_2-adrenoceptors

(a) Agonist specificity. The effects of thousands of compounds which are chemically related to the natural agonists, noradrenaline and adrenaline, have been tested for their ability to stimulate or block α- or β-adrenoceptors in smooth muscle and other target tissues (e.g. [10]).

Since it has been realised that α-adrenoceptors are present also at autonomic nerve terminals, this exercise has been repeated comparing the pre- and post-junctional effects of, in particular, those compounds which were known already to be agonists for post-junctional α-adrenoceptors (for reviews see [85, 37]). The rank order of potency for agonism at each receptor varied according to the experimental preparation and conditions employed but, in general, phenylephrine and methoxamine emerged as the compounds which were potent at the post-junctional but not the pre-junctional receptors [24, 86, 95] and xylazine consistently demonstrated greater potency for pre- rather than post-junctional α-adrenoceptors [24, 95]. Clonidine was not sufficiently selective for the purpose of discriminating between post-junctional α-adrenoceptors [19] but the availability or tritiated clonidine with a high specific activity has led to the use of this compound in identifying ligand binding sites, which are analogous to α_2-adrenoceptors, on membrane preparations [91]. Guanabenz was found to have a high potency at displacing clonidine from such sites [53]. When guanabenz was assessed as an agonist at pre- and post-junctional α-adrenoceptors in the rat vas deferens and heart, its relative potency (selectivity) at the different receptors was similar to that of xylazine but it was ten times more potent. For these reasons phenylephrine (α_1) and xylazine and guanabenz (α_2) were chosen as the compounds most likely to allow discrimination between different receptors of these types.

Figure 2 illustrates that the potencies of these agonists at pre- and post-junctional "α_2-adrenoceptors" in different tissues can be correlated. When the pressor effects in the pithed rat were compared with pre- or post-junctional effects in rat vas deferens or the pre-junctional effect on the cardiac sympathetic nerves, the relative potencies of the different compounds showed no clear relationship (Fig. 2a). The effects were then examined after large doses (1 mg/kg) of the antagonists prazosin and yohimbine which were chosen for their ability to virtually abolish responses to α_1- or α_2-agonists, respectively. After administration of prazosin the pressor response correlated well with the pre-junctional response in either the heart or vas deferens (α_2), while any semblance of correlation with the post-junctional response in vas deferens (α_1), disappeared (Fig. 2b). After yohimbine, the pressor response correlated best with the post-junctional (α_1) response in vas deferens and no longer showed a close relationship to the pre-junctional α_2 responses (Fig. 2c).

The weakest part of this latter correlation was the relative potency of phenylephrine. We had been concerned from our earlier experiments in rat vas deferens that phenylephrine might not be the most suitable choice as a "selective" α_1-adrenoceptor agonist since it had failed to produce as great a maximum response as the other agonists [64]. When this was investigated further, in the vas deferens and the cardiovascular system, phenylephrine exhibited pre-junctional α_2-adrenoceptor agonism and pre- and post-junctional β-adrenoceptor agonism, each of which appeared within the range of phenylephrine's α_1-adrenoceptor concentration/response curve and, thus, distorted this relationship. This was confirmed by comparing phenylephrine with another "selective" α_1-adrenoceptor agonist, amidephrine [13].

Amidephrine could be shown to act almost entirely

via α_1-adrenoceptors, lacking the α_2-, β_2- and β_1-adrenoceptor-mediated effects of phenylephrine [30]. Indeed, one factor which makes phenylephrine's pressor effect more susceptible to α_1-adrenoceptor antagonists than that of noradrenaline is the greater β_2-mediated vasodilation induced by the former, which tends to exaggerate the apparent degree of antagonism by reducing the response which remains after partial blockade. When amidephrine was plotted in Fig. 2c the correlation of the pressor response after an α_2-adrenoceptor antagonist with the post-junctional α_1-mediated response in vas deferens was confirmed. As illustrated by the case of phenylephrine, when preparations contain several adrenoceptors, deviations from the "potency series" can arise. It is however such aberrations from the "expected" pattern which assist the refinement of receptor classification.

An analogous exercise with the anococcygeus highlights further pitfalls in this type of analysis. In contrast to the pressor responses, the contractile responses of the *in situ* anococcygeus did not show such a clear-cut relationship when agonist potencies were compared with the other preparations. Following prazosin, the remaining response had a potency series which was more closely correlated with the α_2-adrenoceptor-mediated responses at other sites but yohimbine had no effect apart from a slight antagonism of the effects of phenylephrine, clonidine and oxymetazoline and which could be ascribed to its α_1-antagonism. Thus, although a prazosin-resistant response can be shown, no part of the response could be ascribed to "α_2-adrenoceptors". To investigate this further, the rat anococcygeus was studied *in vitro*. pA_2 values were obtained for antagonism of responses to the agonists amidephrine and xylazine by the antagonists corynanthine and rauwolscine. The latter are stereoisomers of yohimbine which, in other preparations, have been shown to be relatively more potent against α_1- and α_2-agonists, respectively [23, 29, 70, 74]. The pA_2 values for corynanthine against each agonist were similar at 7.0–7.5 and for rauwolscine were again similar for each agonist at 6.0–6.5 (unpublished observations). These values are consistent with the relative potency of these antagonists against α_1-agonists in other tissues and reveal no evidence for a post-junctional α_2-adrenoceptor. Furthermore the "α_1" agonism by xylazine stands in contrast to its effect *in vivo* which was resistant to prazosin [19]. These observations may be critical for the further study of putative post-junctional α-adrenoceptors of a type other than α_1, indicating that they may be more easily demonstrated in blood-perfused tissues *in situ* than in isolated tissues bathed in saline. Finding the conditions under which such receptors can be demonstrated *in vitro* may provide the key to the cellular mechanisms which they mediate and, hence, to their physiological role. The possibility of interconversion of α-adrenoceptor sub-types, according to experimental conditions, cannot be excluded.

As the evidence stands, in anococcygeus there is no evidence to justify classification of the *in situ*, prazosin-resistant response to the "α_2-agonists" as an "adrenoceptor", let alone an α_1- or α_2-adrenoceptor. This problem arises whenever a response is found which is resistant to the known antagonists. For example, adrenaline can produce a residual pressor response [28] or a contraction of anococcygeus, which is resistant to all but excessively large, non-specific doses of α_1- and α_2-adrenoceptor antagonists. This need not, however, be via the same mechanism as for guanabenz and xylazine. For the time being, perhaps these receptors should remain unclassified. The temptation to classify prematurely all responses of "α-agonists" as α_1 or α_2 should be resisted. In a similar situation in isolated arteriolar preparation, Hirst and Nield [44] have found an excitatory effect of noradrenaline which is resistant to phentolamine (α_1- and α_2-adrenoceptor antagonist) and have suggested the term γ-adrenoceptor [45]. No doubt α_3 will be suggested soon.

An important factor when using complex *in vivo* preparations is that the pressor responses have complex time courses which rarely reflect an equilibrium at the effector cells. When comparing different compounds and different tissues, "peak" responses may be misleading [21]. The relationships shown in Fig. 2 would be distorted if pre- and post-junctional effects had not been assessed at the same time after administration of the agonist. This makes it even more difficult to quantify the effects of antagonists since, after an antagonist, responses may "peak" at different times. Quantitative assessment of the effect of an antagonist depends on the elimination of the effector mechanism, as a factor, by measurement of the concentrations of agonist producing equal "responses". This becomes virtually impossible in an *in vivo* system, even one as "simple" as the pithed rat. Add to this the difficulty in determining the concentration of either the agonist or the antagonist at the receptor and quantification of antagonism in the pithed rat becomes, at best, a rough estimate. This is not an argument against the use of effector systems as a means of assessing adrenoceptor mechanisms but is a plea for caution.

There may be a difference in the time course of α_1- and α_2-mediated effects (see [21]). The pressor responses to several α_2-adrenoceptor agonists tend to be slower in onset and longer lasting than those to phenylephrine or amidephrine [19]. This also applies to the slow onset of the *in vitro* actions of xylazine and clonidine on the rat anococcygeus, which is apparently α_1-mediated. It is, therefore, not yet clear whether such differences in time course are due to the rate of establishment of an equilibrium at the receptor (influenced by diffusion properties and metabolism of agonist) or to the post-receptor events. If the latter is the case then possible differences in the roles of different calcium stores and channels might be of great interest (see [15]).

(b) Antagonist specificity. Given the above reservations, it is essential to use antagonists to establish whether the α_1-adrenoceptor-resistant responses of agonists are due to α_2-adrenoceptors or to something else. In this respect yohimbine and piperoxan had been used commonly as antagonists which were less potent at α_1 than at α_2 until it was found that rauwolscine (α-yohimbine) was slightly less potent than yohimbine at α_1-adrenoceptors, while retaining potency at α_2-adrenoceptors [74]. Much of the evidence for the "α_2" nature of the prazosin-resistant

pressor effect of catecholamines and other agonists arises from the use of these three compounds, i.e. piperoxan, yohimbine, rauwolscine [16, 19, 28, 58, 90, 97]. None of these compounds are, however, as "selective" for α_2-adrenoceptors as prazosin or corynanthine are for α_1-adrenoceptors. Rauwolscine has the greatest ratio of potency at the two receptors [94] but there is only a narrow concentration range (3×10^{-8} M – 3×10^{-7} M) over which it can be regarded as a "selective" α_2-antagonist *in vitro* since it has a pre-junctional α_2-adrenoceptor pA_2 of approx. 7.5 but a post-junctional α_1-adrenoceptor pA_2 value of approx. 6; in a variety of tissues including rabbit pulmonary artery and rat anococcygeus and vas deferens [74, 94, McGrath unpublished]. Rauwolscine has its slight advantage over yohimbine since it is approx. five times less potent than yohimbine at post-junctional α_1-adrenoceptors but is almost equipotent at pre-junctional α_2-adrenoceptors in these same preparations. A new series of imidazoyl-substituted benzodioxans have recently been synthesised and shown to be even more selective than rauwolscine for α_2, cf. α_1-adrenoceptors, e.g. RS 21361 [74]. The potencies of some antagonists at post-junctional α_1- and pre-junctional α_2-adrenoceptors in rat vas deferens are compared in Fig. 3 and the structures of the main series are shown in Fig. 1.

In examining the effects of catecholamines on post-junctional α-adrenoceptors the choice of antagonists is critical, since the results of experiments can

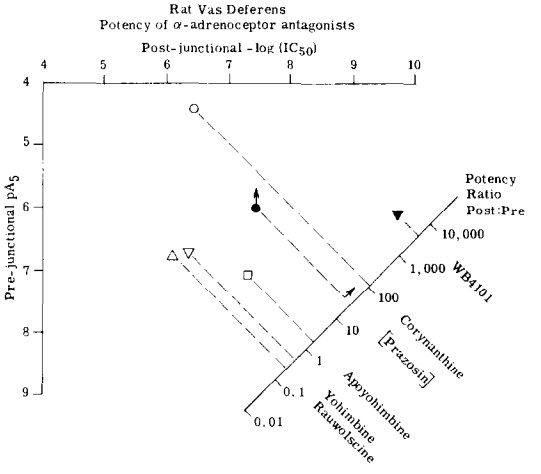


Fig. 3. Comparison of the pre- and post-junctional antagonism of "α"-adrenoceptor antagonists in the rat vas deferens, *in vitro*. The post-junctional effect is assessed as the negative logarithm of the concentration (molar) producing a 50% reduction of the adrenergic component of the response of the epididymal portion to a single field stimulus. The pre-junctional effect is the pA_5 for antagonism of the inhibitory effect of xylazine against the response of the prostatic portion to a single stimulus. Note that WB 4101 has a greater potency ratio, post: pre, than corynanthine despite being considerably more potent pre-junctionally and that the yohimbine stereoisomers which are apparently "selective" for pre-junctional receptors owe this mainly to a lack of post-junctional potency. Prazosin could not be tested adequately in this system since it has excitatory effects on the smooth muscle in concentrations $\geq 10^{-6}$ M.

become open to different interpretations due to the relative lack of "specificity" of the compounds available. It is interesting that one of the most common tests for "adrenolytic" activity (which we would now interpret as α-adrenoceptor blockade) in the 1930's and 1940's, when a vast number of compounds were prepared, was the ability to produce adrenaline reversal in the anaesthetised cat [10]. It is possible that some compounds with α_2-antagonism might have been missed since they blocked only part of the response. The uniqueness of yohimbine and piperoxan (and more recently, phentolamine) as being "adrenolytique" but not "sympatholytique" can be interpreted as due to their possession of antagonism for both α_1-adrenoceptors (post-junctional) and α_2-adrenoceptors (pre- and post-junctional), [Bacq, personal communication; 3, 93]. Since the neurotransmitter, noradrenaline, released from pressor nerves seems to act mainly at α_1-adrenoceptors [19, 20], if an exclusively α_2-adrenoceptor antagonist had been tested it might have been discarded as weak in both its adrenolytic and sympatholytic qualities. In the quest for α_2-antagonists, which would be useful as pharmacological tools if for nothing else, we may, therefore, have to look no further than to retest some of the compounds which were expected to block responses to adrenaline on structure/activity grounds, but which proved ineffective, or to test further derivatives or analogues of existing blockers and agonists.

The pithed rat provides a simple and effective screen for this purpose, compounds being tested against the pressor responses to one of the α_2-adrenoceptor agonists such as guanabenz, xylazine, B-HT 920, B-HT 933 or M-7 [20, 25, 58, 89, 90] or, if "physiological" receptors are of importance, against adrenaline in the presence of an α_1-adrenoceptor antagonist, e.g. prazosin [28]. Adrenaline has the additional advantage that it can be compared with any existing, historical data obtained on cats and dogs in the absence of α_1-blockade. When employing adrenaline (as with phenylephrine, see above) the β-mediated vasodilation must be considered (although this is a physiological factor). This can be prevented by a β-blocker or avoided by replacing adrenaline with noradrenaline, which lacks β_2-agonism [5, 28,]. Similarly, α_1-antagonism can be assessed against amidephrine or phenylephrine but the possibility of different sub-groups of α_1-adrenoceptors indicates caution (see below).

Although many antagonists have some ability to discriminate between different α-adrenoceptors, blockade by them of the pressor effect of a compound should not be taken to indicate that that compound acts via a particular α-adrenoceptor, e.g. the yohimbine stereoisomers are all reasonably potent antagonists of 5-hydroxytryptamine receptors in both non-vascular [60] and vascular tissues (unpublished observations). These antagonists are not, therefore, "selective" in a broad sense and cannot, on their own, be used to identify the receptor activated by an agonist. Resistance to prazosin or corynanthine, sensitivity to rauwolscine and resistance to a 5-hydroxytryptamine antagonist such as methysergide or mianserin would be the best indicators currently available for the characterisation of a vascular,

post-junctional α_2-adrenoceptor-mediated effect [5, 29].

(c) Effects of catecholamines. The effects of synthetic agonists at α_2-adrenoceptors on smooth muscle are given physiological significance by the observation that part of the pressor response to circulating adrenaline or noradrenaline is mediated by this receptor. Exogenous catecholamines, administered parenterally, or endogenous catecholamines, released from the adrenal medulla by stimulation of the appropriate sympathetic nerves, act partly through α_1- and partly through α_2-adrenoceptors as shown by the blockade by different antagonists, alone and in combination [19, 20, 28, 29]. In contrast, under identical experimental conditions the α_1-adrenoceptor antagonists, prazosin and corynanthine, have a proportionately greater effect against the response to vasopressor nerve stimulation than would be expected from their more limited effects against intravenously administered noradrenaline [19, 20, Docherty and McGrath, unpublished]. This is shown schematically in Fig. 4a. These observations have important consequences for the physiological role of adrenoceptors and the effects of drugs thereon.

Do nerves activate only α_1? A key question concerns why the nerve-mediated response appears to be mainly α_1. Several possibilities will need to be tested in a wide range of preparations. If noradrenaline released from vascular sympathetic terminals produces a pressor response by acting predominantly at α_1-adrenoceptors, then this implies either (i) that α_1 are located nearer to the nerve varicosities than

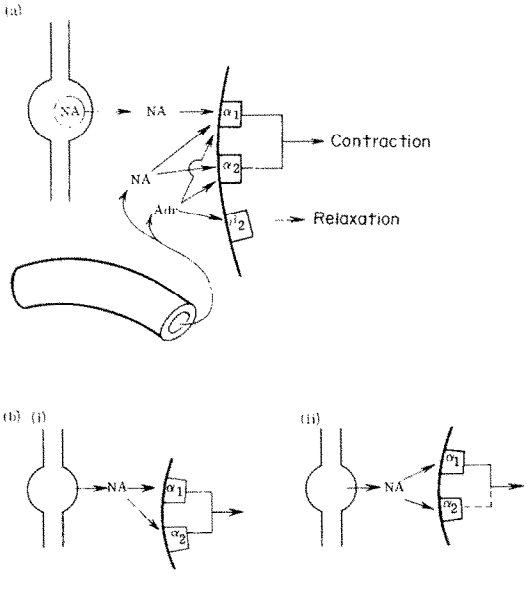


Fig. 4. Schematic representations of roles of α_1- and α_2-adrenoceptors in modulating tone of smooth muscle. (a) The simplest concensus from current results. (b) (i) Continuing from (a), noradrenaline from nerves reaches only α_1; (ii) an alternative to (i), noradrenaline from nerves can reach α_2 but α_2 requires longer activation for its effect to develop: this effectively castrates the hypotheses in Fig. 5 and cannot yet be discounted.

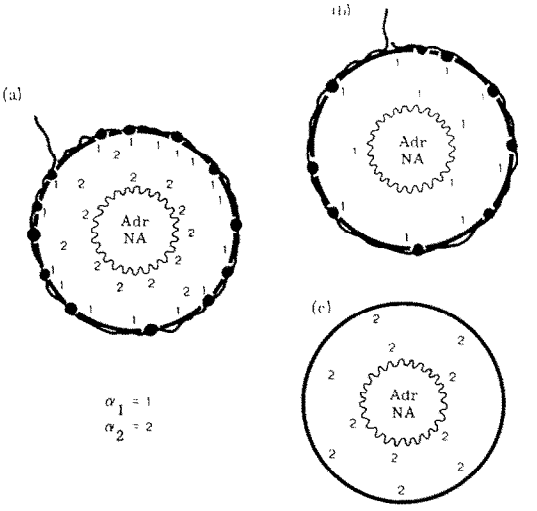


Fig. 5. Possible anatomical reasons for the different effects of α_1- and α_2-antagonists against sympathetic nerves and circulating catecholamines. (a) In innervated vessels α_1-adrenoceptors may be relatively closer to the nerve varicosities or confined to the outer layers, while α_2-adrenoceptors are spread more diffusely or confined to inner layers. (b) Innervated vessels contain only α_1. (c) Non-innervated vessels contain only α_2.

are α_2, or (ii) that the distribution of receptors is similar but the receptor–contraction coupling is such that noradrenaline from the nerves is more effective on α_1 than on α_2 (Fig. 4b). Possibility (i) in turn, could be due to location of each receptor in different vessels or to different distribution of receptors within each vessel (Fig. 5).

To distinguish between these alternatives will require the study of the effects of uptake blockade and denervation on the responses to nerve stimulation and to a range of α_1- and α_2-agonists some of which are, and others not, substrates for the neuronal uptake process for noradrenaline. If α_1-adrenoceptors are relatively nearer to the nerve varicosities (Fig. 5a or b), then such procedures should have more influence on the responses to α_1-agonists. This would still not distinguish whether α_2-adrenoceptors were located in innervated vessels but distantly from the nerve varicosities (Fig. 5a) or located in non-innervated vessels (Fig. 5c). It might still be necessary to devise preparations which could discriminate between innervated and non-innervated vessels. It seems that this has not yet been answered because the pressor, post-junctional α_2-adrenoceptors have been satisfactorily demonstrated only in blood-perfused, *in situ* or *in vivo* preparations [19, 43, 72]. In these circumstances removing the influence of the neuronal uptake process for noradrenaline has the additional effect of prolonging the presence, in the bloodstream, of agonists which are normally eliminated by this route. For example, the pressor effects of both phenylephrine (α_1) and xylazine (α_2) in the pithed rat are increased by blocking neuronal uptake but interpretation is made difficult for the above reasons [20]. Such experiments would be easier in isolated vascular preparations; it is not yet clear

whether the absence of many such reports of α_2-mediated effects is due to a critical property of α_2-adrenoceptors (or their excitation–contraction coupling mechanism), which is disrupted in *in vitro* conditions, or is because such receptors are restricted to small resistance vessels, which are not the type usually studied.

The second possibility, that the α_2-adrenoceptors might be accessible to, but less susceptible to activation by, neurally released noradrenaline, cannot be dismissed easily (Fig. 4b—ii). If the neurotransmitter is present at the receptors for a period of, at most, a few milliseconds, as is suggested by the electrophysiological evidence, then the nature of the post-receptor events might critically determine whether contraction of muscle could be initiated via each receptor type. In contrast the relative contribution from each receptor would be radically different in the face of prolonged exposure to the circulating agonist. If the α_2-system was slower to activate but the effect was cumulative, this would explain the difference between the effects of neural and circulating noradrenaline. This factor is not always considered, when comparisons are made between responses to nerve stimulation and to agonists. Even where two populations of receptors have not yet been proposed, alternative modes of receptor–contraction coupling can result in radically different responses. For example, in rabbit ear artery two phases of the contractile response to noradrenaline or to nerve stimulation are well documented [8]; with exogenous noradrenaline both an immediate, short-lived response and a more prolonged second phase are produced whereas with nerve stimulation the second phase will occur only after unphysiologically long trains of pulses. *In vitro* this problem can be reduced by applying the exogenous noradrenaline iontophoretically [46], an approach which can yield more detailed information on the receptors and post-receptor mechanisms involved in vascular neurotransmission. It will be interesting to see the effects of "selective" α_1- and α_2-adrenoceptor antagonists on responses to iontophoretic noradrenaline or on "biphasic" responses to noradrenaline. In the context of more than one type of α-adrenoceptor, of course, the prospect of more than one post-receptor mechanism for each type is daunting.

Evidence which has been published so far on the relative contribution of α_1- and α_2-adrenoceptors to the vascular response to sympathetic nerve stimulation is tenuous and open to several interpretations, for the above reasons and also because the effects of antagonists have been tested against the responses to trains of stimuli. This is open to the objection that the effects of α_2-antagonists cannot be assessed adequately against effector responses since blockade at pre-junctional receptors might interfere with the release of transmitter. In attempting to answer the question "what proportions of the nerve-mediated response are mediated by post-junctional α_1- and α_2-adrenoceptors on vascular smooth muscle?", there is currently an impasse. Madjar, Docherty and Starke [72] have examined the effects of "selective" antagonists and agonists in the rabbit, perfused hind limb. They concluded that blockade of nerve-mediated responses by rauwolscine could be attributed to participation of post-junctional α_2-adrenoceptors in the response to transmitter noradrenaline. This is based on the impotence of rauwolscine against phenylephrine and hence on its "selectivity" for α_2-adrenoceptors. However, if rauwolscine has any effect at post-junctional α_1-adrenoceptors, this argument does not hold. Post-junctional α_2-adrenoceptor involvement cannot, therefore, be absolutely confirmed. In contrast, Langer *et al.* [62] have carried out similar experiments in the dog, perfused hindquarters. They demonstrated, as we had in the pithed rat [19, 20], that prazosin was relatively more effective against nerve-induced than noradrenaline-induced pressor responses and concluded that on vascular smooth muscle α_1-adrenoceptors are mainly "intrasynaptic" while α_2-adrenoceptors are mainly "extrasynaptic". Unfortunately in this brief communication they did not mention the effect of an α_2-antagonist against the nerve-induced response so that the possibility of "intrasynaptic" post-junctional α_2-adrenoceptors could not be excluded.

Neither of the above two studies could, therefore, confirm or exclude the possibility of "innervated" post-junctional α_2-adrenoceptors on vascular smooth muscle. The standard method of avoiding the problem of feedback, when analysing the effector response to nerve stimulation, is to employ single pulses or low frequencies [18, 21, 69]. With vascular preparations, however, this is technically difficult. We have produced pressor responses to single pulses of stimulation via the lower thoracic sympathetic outflow in the pithed rat or to trains of pulses at low (< 0.5 Hz) frequencies in the pithed rabbit. In each case the response was reduced by between 80 and 100% by doses of prazosin which, according to their effects against injected agonists, were "selective" for α_1-adrenoceptors but they were reduced by between 10 and 60% by doses of rauwolscine, which, against agonists, were "selective" for α_2-adrenoceptors [N. A. Flavahan, J. C. McGrath and C. E. McKean, unpublished]. Clearly there is inadequate data from which to arrive at a definitive conclusion. However, an important consequence of these results may be to put in question, yet again, the validity of extrapolating from the effects against agonists to those against nerves. For example, recent evidence from the isolated anococcygeus of the rat indicates that there may be two populations of post-junctional "α_1"-adrenoceptors (α_{1a}, α_{1b}): (see later section—"anococcygeus"). If one sub-group, e.g. α_{1b}, is present at the vascular neuroeffector "junction", but is in a minority over the rest of vascular smooth muscle, then the effects of circulating agonists might never reliably predict the events at the neuroeffector junction. This is supported by the effects of antagonists against the pressor effects of phenylephrine or noradrenaline in the pithed rabbit or rat. Corynanthine was less potent against phenylephrine than against noradrenaline while prazosin showed no such differential effect; blocking each. This suggests that phenylephrine, particularly in low doses, activated receptors which were blocked by prazosin but not by corynanthine while noradrenaline activated receptors which were susceptible to each antagonist. Each of these receptors will be α_1 according to the

effects of prazosin but the effects of low doses of phenylephrine may not represent activation of the receptors activated by nerves. This may be a further factor in the difficulty of predicting the effect of an "α-antagonist" against the response to nerve stimulation. Antagonists might have little or no effect against phenylephrine yet be antagonists at the sub-group of α_1-adrenoceptors which are activated by noradrenaline from nerves.

Even in the rat heart, where a positive chronotropic α_1-adrenoceptor-mediated component, but no equivalent α_2-adrenoceptor-mediated component, can be demonstrated, rauwolscine can inhibit, to a small extent, the cardioaccelerator response to nerve stimulation [31; Flavahan and McGrath, unpublished].

$\alpha_1 + \alpha_2 = ?$ The responses to α_1- and α_2-adrenoceptor activation do not appear to summate in a simple manner. This complicates pharmacological interpretation of their relative significance. For example, in the pithed rat, α_1-antagonists, e.g. prazosin, corynanthine, and α_2-antagonists, e.g. rauwolscine, yohimbine, were tested against the pressor response to adrenaline. The effect of the combination of antagonists was the same irrespective of order of addition while the effect of α_2-antagonism, on its own, seemed smaller than when it followed α_1-antagonism [28, 29]. Since prazosin produced no clear "potentiation" of the effects of α_2-antagonists against "selective" agonists, e.g. xylazine, it seemed unlikely that this represented any interaction of the antagonists at the α_2-adrenoceptor. An explanation can, however, be based on the observation that the effects are non-additive. α_1- and α_2-adrenoceptors which are present together in a proportion of resistance vessels might produce, on simultaneous activation, a response which is less than the sum of their individual responses. This could arise from one or more of several common steps between receptor activation and constriction of the vessel. The greater effect of α_1-antagonism, i.e. a proportionately larger effect via α_1-adrenoceptors, can be due either to further beds, in series or in parallel, containing only α_1-adrenoceptors, or to a proportionately greater effect by α_1 than α_2 when the two occur together. At its most extreme, a situation could exist in which the sum of the responses was equal to the response of the dominant receptor. Thus, if α_1 was dominant, an α_2-antagonist would have no effect on the response to an agonist which activates both receptors but an α_1-antagonist would produce a shift in the dose–response curve to coincide with the effect of α_2-agonism. In this way an agonist can produce an effect through two receptors but total blockade of only one receptor may modify the net response by very little. Subsequent blockade of the other receptor, however, should now eliminate the response. The effect of a "selective" antagonist, taken in isolation, may, therefore, give no information on the potency of an agonist on that receptor unless potential effects at other receptors are eliminated. In the original demonstration of "prazosin-resistant" pressor responses to noradrenaline in the rat and cat [26], responses were found to be resistant to yohimbine and, therefore, difficult to classify as "α_2". This obstacle is, however, overcome by studying the effects of the combination of α_1- and α_2-antagonism.

It should be noted that, once this principle of "non-summation" of response is accepted, it is but a small step to observe that some responses may result from effects which "more than summate", viz. since resistance is inversely proportional to the fourth power of the radius (and hence circumference) of the individual vessels, small changes in length of circularly-arranged smooth muscle cells will be more than additive in their effect on resistance and hence pressure changes. Until the locations of the individual receptors and their effects within each blood vessel are established, therefore, the interpretation of pharmacological effects, particularly of drug combinations, would appear to be largely at the discretion of the investigator.

(d) Physiological role. Clearly the pattern of post-junctional α-adrenoceptors is not yet resolved. Its current practical importance, however, is that no ubiquitous explanation for the effects of "selective" α-adrenoceptor antagonists should be expected. It is likely that the various sub-groups will be distributed in a different fashion between different organs, between different vessels in a particular organ and with species. Already it is clear that in man prazosin is "selective" for α_1-adrenoceptors but that certain cardiovascular reflexes, exerted in part via sympathetic nerves, can be maintained during therapy [63, 73]. Furthermore, the clinical use of α_1-adrenoceptor antagonists as a means of avoiding interference with pre-junctional α_2-adrenoceptor-mediated feedback may run into the problem of post-junctional α_2-adrenoceptor activation. For example, in the removal of phaeochromocytoma, the effect of a non-selective α-antagonist on α_2-mediated feedback could become a problem, particularly if a muscle relaxant was used which blocked the neuronal reuptake of noradrenaline [22]. An α_1-adrenoceptor antagonist would not, however, suppress all the effects of circulating noradrenaline and it might be dangerous to attempt its use. A partial solution would be appropriate selection of the muscle relaxant and other adjuvants to anaesthesia. The clinical ideal, however, would be to obtain an antagonist which was selective for post-junctional α_2-adrenoceptors to use in combination, as required, with an α_1-antagonist.

The multiplicity of post-junctional α-adrenoceptors, together with the β-adrenoceptors, gives considerable scope for subtlety in the control of the vascular system by the sympathetic nervous system. As shown in Fig. 4a, circulating adrenaline, circulating noradrenaline and adrenergic nerve stimulation each represent a different pattern of adrenoceptor activation. The study of the differences in the proportionate activation of post-junctional α_1- and α_2-adrenoceptors by adrenaline and noradrenaline are complicated by the adrenaline's β_2-adrenoceptor agonism and the greater susceptibility of noradrenaline to neuronal uptake. Blocking β-adrenoceptors or the uptake process will not provide a straightfoward answer since these manoeuvres will, respectively, alter blood flow to different areas and vary the anatomical disposition of the catecholamines with respect to the nerve terminals. In most mammalian species adrenaline is the dominant circulating

catecholamine released from the adrenal medulla while noradrenaline is derived mainly from the post-ganglionic sympathetic nerve terminals. In many species, however, a significant proportion of adrenal catecholamines is comprised of noradrenaline, which is stored in different groups of cells from adrenaline and can be independently released according to the nature of the activating physiological stimulus. It will be interesting to see whether there is a different distribution of α_1-, α_2- and β_2-adrenoceptors in the blood vessels involved in the responses to these stimuli, e.g. exercise, cold, fear.

Two further interactions may provide clues to the distribution and physiological roles of different adrenoceptors.

β_2 vs α_2. First, when β-adrenoceptors are not blocked, rauwolscine almost abolishes the pressor response to adrenaline [28]. This contrasts with its failure to block the response to adrenaline after β-blockade. Since the effect of rauwolscine against noradrenaline is not changed by β-blockade, it appears that this "non-summation" of α_1- and α_2-adrenoceptor mediated responses is influenced by adrenaline's β-agonism. This implies that the presence of β-agonism increases the contribution from α_2-adrenoceptors. The explanation of this could be biochemical or anatomical, e.g. (1) α_2- and β_2-adrenoceptors have opposing effects on the same biochemical process in the cell membrane (e.g. adenylcylase) whereas the α_1-adrenoceptors do not act through this same mechanism (e.g. increased phosphatidylinositol turnover) [52] (2) it is also possible to pursue the hypothesis developed above, of the distribution of receptors among different beds. All that would be required would be a vascular bed which, under resting conditions, had a high resistance, but which was capable of considerable vasodilation. If this bed had α_2- and β_2-adrenoceptors, acting in opposition, then its α_2-component, on its own, would contribute little to the overall peripheral resistance and thus, in the absence of β-agonism, would still allow the dominance of α_1. In the face of β_2-adrenoceptor mediated vasodilation, however, this bed would be a major determinant of peripheral resistance and thus α_2-adrenoceptors could have a greater modulatory role. The obvious candidate for such a vascular bed would be skeletal muscle. There would be no objection to there also being a small α_1-component, which might be involved in neurogenic vasoconstriction but whose effects would be easily overriden by the β_2-effect from circulating adrenaline and by local factors.

Acid/base changes α_2/α_1. The second observation, which may have considerable significance for the study of post-junctional α_1/α_2-adrenoceptors, is the influence of blood gases. In our standard procedure, pithed rats are ventilated with pure O_2 at a minute volume which maintains an arterial blood pH of 7.4 ± 0.05, a P_aCO_2 of 35–40 mm Hg and a P_aO_2 in excess of 300 mm Hg. The balance of α_1- to α_2-adrenoceptor mediation of responses has, however, been found to vary according to the degree of ventilation and, hence, with blood gases [71, 32]. Rats were hyperventilated or hypoventilated in order to vary the P_aCO_2 and the arterial pH. As the pH went from 7.6 to 7.2 the responses to catecholamines decreased, the effect on this of prazosin remained approximately constant (in percentage terms) but the effect of rauwolscine steadily increased. This could have been due either to an increase in the α_2-mediated response or to a decline in the α_1-influence. Since the overall response to catecholamines declined and since the responses to α_1-agonists declined but to α_2-agonists increased, our preliminary interpretation is that as the rats became acidotic the α_2 component from adrenaline has increased while the α_1 component has decreased [33, 71].

This observation has several consequences. It may explain quantitative differences between the observations of different groups of workers who are using similar preparations, but with slight variations in technique, and must be an important factor in any studies involving organ perfusion at a fixed rate unrelated to metabolic demand. From our own earlier experience employing air-ventilated pithed rats [38, 39] it is often necessary to hyperventilate in order to maintain an adequate P_aO_2. This results in a low P_aCO_2 and an arterial pH in the region of 7.5–7.6. Under these conditions the effect of α_2-antagonists against catecholamine response is at a minimum.

The physiological significance may be that in local acidotic conditions the influence of sympathetic tone, exerted via α_1-adrenoceptors, will decline. Consequently, the effects of a generalised increase in sympathetic neural tone could be overriden in discrete areas of high metabolic activity. The reason for the relative resistance to acidosis of the α_2-mediated effect is not immediately obvious but clearly it leaves open the possibility of a continued influence of circulating catecholamines and, perhaps, of nerve-activated α_2-adrenoceptors in certain specialised areas. If the pre-junctional α_2-adrenoceptors are similarly resistant to acidosis, as they might be if they are chemically identical with the post-junctional ones, then this would be advantageous in preventing the loss of any negative feedback which might occur at high frequencies of sympathetic discharge. Under conditions of stress this might be a factor in restraining both vascular tone and the heart rate [17].

In view of the "dominance" of α_2 under acidotic conditions, it is interesting that the first *in vitro* demonstration of post-junctional α_2-adrenoceptors is in veins [15]. Teleologically, it seems reasonable that α_2-adrenoceptors should be employed in veins where conditions are likely to be acidotic. The same argument could apply to tissues which are metabolically active such as skeletal muscle. Among the commonly used vascular preparations this also raises several interesting questions. For example we have been unable to demonstrate post-junctional α_2-adrenoceptors in portal vein but of course this "vein" does not carry acidotic blood. The pulmonary "artery" is commonly used to assess the effects of drugs at post-junctional α_1-adrenoceptors *in vitro*; in the acidotic conditions *in vivo*, however, these might remain ineffective, thus protecting pulmonary blood flow. These examples also raise the question of the suitability of the usual 95% O_2:5% CO_2 gas mixture employed *in vitro*. Perhaps it will be necessary to reduce the O_2 and to vary the CO_2 according to the tissue employed.

The effect of acidosis is one of the few clues currently available in the pursuit of differences between the α_1- and α_2-adrenoceptors. The site of the effect could be a change in the properties of (a) the agonist molecule, modifying its affinity for, or "activity" at the receptor; (b) the antagonist molecule (this cannot be the only change since the responses to the agonists are reduced); (c) the receptor; (d) the events following receptor activation (here a link between pH and Ca^{2+} activation might be pertinent); (e) the intrinsic tone of the blood vessels.

Sub-classification of "post-junctional" α-adrenoceptors in vitro

Adrenoceptors have been sub-classified as α_1 and α_2 according to the order of potency of agonists and antagonists established by Starke and co-workers for pre- and post-junctional α-adrenoceptors, particularly in the rabbit pulmonary artery [37, 85]. Clearly tissues as diverse as frog skin [6] and blood platelets [42] have responses mediated by receptors akin to those at the terminals of the adrenergic nerves in the rabbit pulmonary artery (α_2). There is, however, a danger, that by applying only some of the criteria, receptors may be wrongly classified. This particularly applies to the tendency to equate "prazosin-resistance" with "α_2". If an attempt is made to classify a receptor by the use of agonists and antagonists and if it fits neither the α_1 nor the α_2 category then it will be more helpful for future categorisation if it remains unclassified, e.g. α_x, than if it is fitted into the "nearest" category. This could also present problems of interpretation in ligand-binding experiments if these were based on the assumption of only two possible sub-types.

So far, there is no substantial evidence to require the re-categorisation of α-adrenoceptors mediating contraction of non-vascular smooth muscle as "α_2". On the other hand, such experiments are usually carried out *in vitro* and the evidence for post-junctional α_2-adrenoceptors, even in vascular tissue, *in vitro* is inconclusive. There is, however, preliminary evidence from the anococcygeus and vas deferens *in vitro* that there are two distinct post-junctional α-adrenoceptors each of which fits the gross category of "α_1" but between which there are differences in the potency series of both agonists and antagonists [64, 65; MacDonald and McGrath, unpublished]. This latter observation has a bearing on previous hypotheses for sub-classification of α-adrenoceptors on smooth muscle.

Existing hypotheses

Three different means of distinguishing between sub-types of α-adrenoceptors have been (a) whether or not dopamine is an agonist [80]; (b) whether or not optical isomers are equipotent [4]; (c) differences between the agonist effects of imidazolines and phenylethylamines [82, 83]. It will be interesting to see whether these three categories can be amalgamated with the α_1/α_2 system to give a comprehensive set of criteria for classification.

At present it seems that none of these three methods corresponds to an α_1/α_2 split. Post-junctional effects of dopamine which are affected by "α-blockers" are antagonised by "α_1-selective" antagonists such as prazosin [36; McGrath, unpublished]. (−)-Amidephrine is a potent α_1-agonist on the blood pressure of the pithed rat but (+)-amidephrine is virtually totally inactive despite the system's sensitivity to α_2-agonists [30; Flavahan and McGrath, unpublished]. The effects of both phenylethylamines and imidazolines on smooth muscle *in vitro* are readily antagonised by prazosin [McGrath, unpublished]. It seems rather that these are methods for the sub-classification of α_1-adrenoceptors.

The amalgamation of methods (a) and (c) is already possible since Ruffolo *et al.* [82] have demonstrated that dopamine acts more like imidazolines than like phenethylamines. Since dopamine does not contain an asymmetrical centre it would be possible to refine (c) to distinguish between dopamine and imidazolines (which generally have no asymmetry) on the one hand and the (*l*) isomers of phenethylamines on the other. There is insufficient evidence for a general incorporation of isomerism into the categorisation but it is interesting that, whereas imidazolines are generally considered to be partial agonists at post-junctional α-adrenoceptors, tetrahydrazoline, which has a centre of asymmetry, and oxymetazoline, naphazoline and (3,4-dihydroxyphenylamino)-2-imidazoline, which have no chiral centre but have asymmetrical ring systems, are all imidazolines which can be shown to behave as full agonists in some test systems [35, 77, 81].

Where should we look for a general hypothesis?

Can a general hypothesis incorporate these diverse mean of categorising α_1-adrenoceptors? It seems, at present, that there is no complete split, as there is between α_1 and α_2. Some compounds, particularly the (*l*)-isomers of the phenylethanolamines, noradrenaline and phenylephrine (and the less common amidephrine) appear universally as α_1-agonists irrespective of the test system and it is possible that they act at all "α_1"-adrenoceptors. In fact together with their antagonism by prazosin, they probably contribute to the safest definition of an α_1-adrenoceptor. In contrast, the effects of the imidazolines and of the other compounds which are α_2-agonists, e.g. where the imidazoline has been replaced by another heterocyclic group (xylazine, Bay$_a$ 6781) or by a guanidinium group (guanabenz, guanfacine), are not consistent. For example, oxymetazoline is at least as potent as noradrenaline or phenylephrine in rat or cat anococcygeus [35, 68], rat vas deferens [64], rat blood pressure after blocking α_2-adrenoceptors [19] and rabbit pulmonary artery [86], but is less potent on guinea pig aorta [95] and rat aorta [81] and has virtually no effect on rabbit basilar artery [7]. This variation between noradrenaline and oxymetazoline could be due to the existence of different sub groups of α_1-adrenoceptors whose distribution varies between tissues. There is also evidence for more than one component in the effector responses to "α_1"-agonists in some cases of these tissues.

Anococcygeus. In both the rabbit basilar artery [7] and the rat anococcygeus [McGrath, unpublished] phenylethanolamines produce dose–response curves with a "shoulder" indicating two components. In contrast, the "non-phenylethanolamine" agonists

produce virtually no response in the rabbit basilar artery but a monophasic curve in rat anococcygeus. It seems possible that in anococcygeus the "low concentration" component of the response to phenylethanolamines and the response to "non-phenylethanolamines" is mediated by one type of α_1-adrenoceptor (α_{1a}) while the response to high concentrations of phenylethanolamines is mediated by a second type (α_{1b}) at which "non-phenylethanolamines" are relatively poor agonists. This is supported by the phasic nature of the response to low concentrations of phenylethanolamines and to all but "maximal" concentrations of non-phenylethanolamine agonists. The responses to low concentrations of indirect sympathomimetics also are "phasic" and after chemical sympathectomy or in the presence of an uptake I blocker, the increased sensitivity to noradrenaline is largely due to a greater phasic component at low concentrations, suggesting that these "α_{1a}-adrenoceptors" are readily activated by prolonged exposure to low concentrations of agonist within the tissue and that they mediate a phasic response. On the other hand, the adrenergic nerve-mediated response is not phasic and might therefore involve α_{1b}-adrenoceptors. This is reinforced by the resistance of both nerves and high concentrations of noradrenaline to certain antagonists, including rauwolscine. If this tentative hypothesis proves accurate, it will provide a further explanation, in addition to α_2-mediated feedback, for the actions of antagonists which are "adrenolytic" but not "sympatholytic".

However, this provides no evidence for post-junctional receptors which, *in vitro*, are resistant to α_1-antagonists. This raises the interesting possibility that the post-junctional α_2-adrenoceptors, as demonstrated *in vivo*, are transmuted, under *in vitro* conditions, to resemble α_1-adrenoceptors, at least as far as affinity of antagonists is concerned; they might even become one of the sub-groups. This would mark a difference from pre-junctional α_2-adrenoceptors, whose distinction from α_1-adrenoceptors, is, if anything, easier to demonstrate *in vitro* than *in vivo*.

Rabbit basilar artery. This has a "low dose component" of the response to noradrenaline which corresponds more closely to the "α_{1b}" than "α_{1a}" adrenoceptors in anococcygeus, i.e. "non-phenylethanolamines" have little agonist activity [7]; the extreme lack of activity may, however, indicate yet another receptor.

If this hypothetical subdivision of α_1-adrenoceptors is correct, presumably those tissues, such as rat and guinea pig aorta, in which imidazolines are relatively impotent, owe the greater part of their response to α_{1b}-adrenoceptors. This subdivision of post-junctional α_1-adrenoceptors does not imply that imidazolines lack affinity for α_{1b}-adrenoceptors. Clearly imidazolines can competitively antagonise the responses to phenylephrine, even in a tissue such as rat aorta in which the agonist potency of imidazolines is low [81]. An alternative to partial agonism could be agonism at some but antagonism at other sub-groups of α_1-adrenoceptors. This could also explain why some agonists are "full" or "partial" according to the preparation.

If this is true then it has importance for ligand-binding studies. First, if imidazolines have affinity for two types of α_1-adrenoceptor plus α_2-adrenoceptors, it is going to be difficult to use these compounds to differentiate among receptor subtypes since the system relies entirely on affinity. Secondly, if receptors on intact cells can change their properties so radically from *in vivo* to *in vitro*, then considerable further changes can be expected in their transfer to purified membrane fragments. If this change is a further subtle change in affinity for antagonists (and it is known that the precise composition of the environment is crucial for binding of "antagonist" ligands) [88] then it is possible that some sub-types of α_1-adrenoceptors might appear to be classifiable as α_2. For example, the different binding characteristics of dihydroergotamine and prazosin for membrane fragments originating from rabbit uterus have been used as a basis for differentiation between α_1- and α_2-adrenoceptors in this organ [49]. However, WB 4101, which has been shown to be "selective" as a ligand for α_1-adrenoceptors in membranes from calf brain [92] is not selective in the membranes from rabbit uterus [50]. While it can be argued that this shows non-selectivity of WB 4101, it might also indicate that, under the experimental conditions to which these membranes are subjected, prazosin is distinguishing between α_1-adrenoceptor subtypes. This illustrates the difficulty in utilising, for ligand-binding studies, organs in which the physiological role of adrenoceptor subtypes is not known; there is no evidence which implicates α_2-adrenoceptors in any effector response in rabbit uterus.

The vas deferens. This provides further evidence for subdivision of α_1-adrenoceptors. The post-junctional "agonist" effects of phenylethanolamines and imidazolines on rat vas deferens are complex. The excitatory effects of agonists are not directly related to contraction. This is of relevance to the interpretation of any study carried out on this organ since "dose–response" curves are often carried out after an exposure which is short enough to allow only an initial transient contraction whose disappearance is often described as "desensitisation", even though phasic activity and potentiation of nerve-induced responses may continue for several hours. Current evidence [64–66; MacDonald and McGrath, unpublished] suggests that the initial and long-term responses involve different mechanisms of receptor–contraction coupling, which, by implication, suggests different receptors, in their "function" if not necessarily in their affinity for drugs. There is also evidence for an anatomical separation of these functionally different receptors since, after transverse bisection, the two ends of the vas have different properties. For example the epididymal portion is more susceptible than the prostatic portion to the excitatory effects of oxymetazoline whereas the two portions are equally sensitive to noradrenaline [64; MacDonald and McGrath, unpublished].

Ruffolo *et al.* [82] have shown a "lack of cross-desensitization" between imidazolines and phenylethanolamines in the rat whole vas. The authors interpreted this to mean that the two classes of agonist interact at different sites on a single receptor [82]. While there may be such a receptor, the

anatomical separation of receptors indicated above suggests that at least part of the explanation for the apparent lack of cross-desensitization is that phenylethanolamines can produce a contraction through a receptor which starts insensitive to oxymetazoline (similar to the α_{1b}-adrenoceptor of anococcygeus?). We have attempted to reproduce their findings using bisected vasa but have met with no success.

Further evidence for more than one type of α_1-adrenoceptor in the rat vas deferens comes from a comparison of the effects of different antagonists. The adrenergic nerve-mediated response [69] and the contractile responses to various "α" agonists can be shown to be broadly α_1 in that they are blocked by antagonists such as prazosin and WB 4101 [69, 64]. The order of potency of antagonists against the two forms of stimulus are not, however, identical. For example, comparing the threshold concentrations of antagonists producing inhibition of nerve-induced response with the published pA_2 values against phenylethanolamines: (i) for prazosin the concentration is similar, (ii) for yohimbine, WB 4101 or rauwolscine the effect is greater against the nerves, (iii) for ajmalicine the effect is greater against the agonists [57, 70, 74; McGrath, unpublished]. This is another case in which the α_1-adrenoceptors activated by the neurotransmitter, noradrenaline, cannot be shown to be identical with those activated by exogenous agonists. Considerable work remains to be done, for example, comparing the pA_2 values for antagonists against the contractile effects of the imidazolines, before the receptors activated by the adrenergic nerves can be identified: however, the greater adrenergic nerve induced contractile component in the epididymal portion points to the type of receptor which can be activated by imidazolines as well as by phenylethanolamines, i.e. "α_{1a}". While the receptors activated by nerves in the anococcygeus were postulated as α_{1b}, there is a clear difference in the functional capabilities of the adrenergic nerves in the two cases; in anococcygeus repetitive stimulation of the adrenergic nerves can maintain contractile tone for several minutes without signs of fatigue whereas in the vas the "adrenergic" component of the nerve-induced contraction starts to decline after 2 sec [D. H. Brown and J. C. McGrath, unpublished]. This may be related to the respective physiological functions of the nerves in each organ. It will be interesting to see whether such parallels stand up to examination in other organs and what is the relationship to mobilisation of different sources of Ca^{2+}.

Human palmar arteries. In vitro these provided one of the original observations for "prazosin-resistant" responses to noradrenaline [54, 76]. Although this has been cited as a precognition of post-junctional α_2-adrenoceptors, it may have been, rather, a premonition of the diversity of post-junctional α-adrenoceptors. Subsequent studies by Moulds and co-workers [87; Moulds, personal communication] do not confirm that the "prazosin-resistant" response is "α_2" but rather that, in the human palmar vessels, the order of potency of agonists and antagonists do not coincide with either the "α_1" or "α_2", which are defined in the animal studies. The receptors appear to be similar to "α_1-adrenoceptors" in respect of agonist potency but, among the antagonists, yohimbine is more potent, and prazosin less potent, than expected. It is not yet clear whether this points to species differences among α-adrenoceptors or to some feature of this particular experimental preparation. However, as in the animal studies, the lack of pre-junctional α_2-adrenoceptors *in vitro* does not preclude their existence *in vivo*.

Classification of drugs

It has become common practice to assess the "selectivity" of agonists or antagonists for α_1- and α_2-adrenoceptors as the ratio of some index such as ED_{50} or pA_2, often obtained in different preparations, e.g. as shown in Fig. 2. If the post-junctional α_1-adrenoceptors are not a homogeneous population and if the proportions of these sub-groups vary between organs, then these ratios will carry no general significance. Conflicting "data" from different groups of workers should be expected.

Approaches to further research

The environment of the "receptor". A start has been made towards studying adrenoceptors at the molecular level using ligand-binding techniques at a time when studies involving effector responses are resulting in a multiplication of the number of post-junctional α-adrenoceptors. It must be the aim of future research to link the molecular events which occur between the "drug–receptor" interaction and the effector response. If ligand-binding is taken as the first approximation to the "drug–receptor" interaction, it is important that the ligands are thoroughly tested for their pharmacological profile against "effector" responses, preferably in the same tissue. Otherwise there is no guarantee that the "specific" binding site, presumably representing a particular molecular conformation, is connected in any way with the next stages in the "activation" process, i.e. is a receptor for any known process. Such conformations may occur in molecules involved with physiological processes which are, as yet, unknown, or even as part of structural elements. If attention is, however, paid to the "pharmacological" effects of all the substances employed in binding studies, the results may become more refined and capable of picking out subtleties such as sub-groups of α_1-adrenoceptors. It may eventually prove unfortunate that the α_1/α_2 categories have been applied in binding studies. Since "post-junctional" receptors seem likely to be the most abundant type in homogenised tissues, it could be predicted from isolated organ studies *in vitro* that different types of α_1-adrenoceptors might be found and that some of these might be the same molecular elements which would appear, by the same definition, as α_2-adrenoceptors *in vivo*. However, it seems that "binding sites" are being categorised by the α_1/α_2 system, particularly according to the affinity for antagonists [96]. It might be helpful, before this goes too far, to distinguish between "binding sites" and "adrenoceptor". Perhaps α_2-binding sites will be the same things as α_2-adrenoceptors which are found *in vivo* but, if they are not, then it is going to be difficult, in a few years time, to comprehend the present literature.

The resolution of this problem of relating information derived (i) *in vivo*, (ii) *in vitro*, but with cells

intact in "physiological saline" and (iii) in semi-purified sub-cellular fragments, may lie in defining the environment and the adjacent elements to which it is believed that the "receptors" are connected. A considerable advance towards this has already been made in binding studies with the observation that affinity of "agonists" for binding sites is modified by the presence of GTP and inorganic cations [41, 88].

As well as providing evidence on the molecular level for the elements involved with drug–receptor interaction this also makes a start towards the study of the "receptors" under physiological conditions. Initially, ligand binding is facilitated by studying washed membrane fragments in a simple buffered medium, but to arrive at the reactions which occur in functioning cells it is necessary to replace environmental factors until "near-physiological" conditions pertain. In this context the reduction in binding of "antagonists" to "α_2-binding sites" produced by the addition of monovalent cations and GTP is of great interest, given the possibility of interconversion suggested by the "effector" studies. It would be interesting to know whether, in some circumstances, these "α_2-binding sites" can acquire affinity for "α_1-agonists" at the same time as losing it for "α_2-agonists".

In intact cells the problem is further complicated by the influence on the receptors of adjacent cellular elements. If the interaction of the drug and receptor can modify performance of such cellular elements, e.g. opening a particular "ion channel", then it must be possible that, conversely, such elements can modify the conformation of the receptor. An understanding of these "post-receptor" processes may be necessary for complete identification of "binding sites" with "adrenoceptors" and for a thorough understanding of the "drug–receptor interaction". It should thus be noted that receptor–contraction coupling involving α-adrenoceptors (to take one example) is not fully understood. Differences in agonist-mediated effector responses and possible different roles of Ca^{2+} influx in α_1- and α_2-mediated responses were mentioned above. In particular, however, the events which follow from activation of α-adrenoceptors by noradrenaline released from sympathetic nerves are under debate. By analogy with the cholinergic system, electrophysiological methods have been used to study the effect of adrenergic nerve stimulation on the intracellularly-recorded membrane potential in smooth muscle. Few organs are, however, suitable for this type of study. In two preparations which can be used, the vas deferens and arteriolar smooth muscle, excitatory junction potentials (ejp's) can be produced by stimulating the sympathetic nerves. These ejp's summate and, when the membrane potential reaches a threshold level, an action potential is fired and is accompanied by contraction of the muscle. In the cholinergic system these processes are sequential: the entire process can be stopped by an antagonist which interferes with the initial agonist–receptor activation. In both vas deferens and arteriolar smooth muscle, however, α_1-adrenoceptor antagonists can reduce the contractile response to nerve stimulation without reducing the ejp's [9, 12, 44, 47, 48, 51]. This suggests two things which are crucial to the role of α-adrenoceptors in neurotransmission: (i) the ejp's induced by stimulation of sympathetic nerves are not initiated by activation of α-adrenoceptors; (ii) the involvement of α-adrenoceptors in the contraction induced by sympathetic nerve stimulation is either independent of, or subsequent to, the ejp.

In the case of the arteriolar smooth muscle, the α_1-antagonist prazosin can increase the threshold for firing of an action potential [48]. If this is confirmed as an "α_1-antagonist" action then a link of α_1-adrenoceptors with the electrical events will be maintained but this will be associated with the action potential rather than the ejp. The mechanism of activation of the ejp remains obscure but Hirst and Neild [48] have suggested another adrenoceptor (γ). A co-transmitter or even another set of (non-adrenergic) nerves cannot yet, however, be ruled out.

In the vas deferens, dissociation of the post-junctional α-adrenoceptors and the electrical events is even clearer. Here the contractile response to nerve stimulation consists of two components, adrenergic and "non-adrenergic" [2, 69].

Recently it has been found that nifedipine, a Ca^{2+} entry blocker, can selectively block this "non-adrenergic" contraction leaving the adrenergic component [34]. Nifedipine does not, however, act by interfering with the ejp's but it does prevent the occurrence of the muscle action potential [9]. Since the adrenergic (α_1-mediated) contraction is not modified, it can be inferred that the α_1-induced contraction does not require an action potential but utilises, perhaps, a more direct excitation–contraction coupling process. If this is the case it could suggest a different type of α-adrenoceptor compared with those involved in depolarising the cell membrane. This might also explain why the vas deferens has such a dense adrenergic innervation, since the "adrenergic" response would not be transmitted between cells.

The transition from *in vitro* to *in vivo* can be expected to produce further changes in the environment which might modify the properties of receptors. pH has already been mentioned as modifying the α_1/α_2 balance but temperature, O_2, the composition of the extracellular fluid compared with "physiological" salines and the presence of blood-borne factors including hormones are all likely to play a part. Certain inorganic cations, which are already known to influence "ligand binding", e.g. Mg^{2+}, are rarely present in the plasma or extracellular fluid in the same concentrations as in the commonly used salines.

Structure/activity relationships. Since Easson and Steadman [27] first postulated a three-point attachment theory for the "adrenoceptor", many hypotheses have been advanced for the structural requirements of drug molecules which interact at this site.

Have any further clues been given now that more than one α-adrenoceptor has been found?

At every stage of the search for structure/activity relationships among the analogues of adrenaline a constant feature has been the ability of small structural changes or substitutions to convert agonists to antagonists and vice versa [10]. Many analogues of

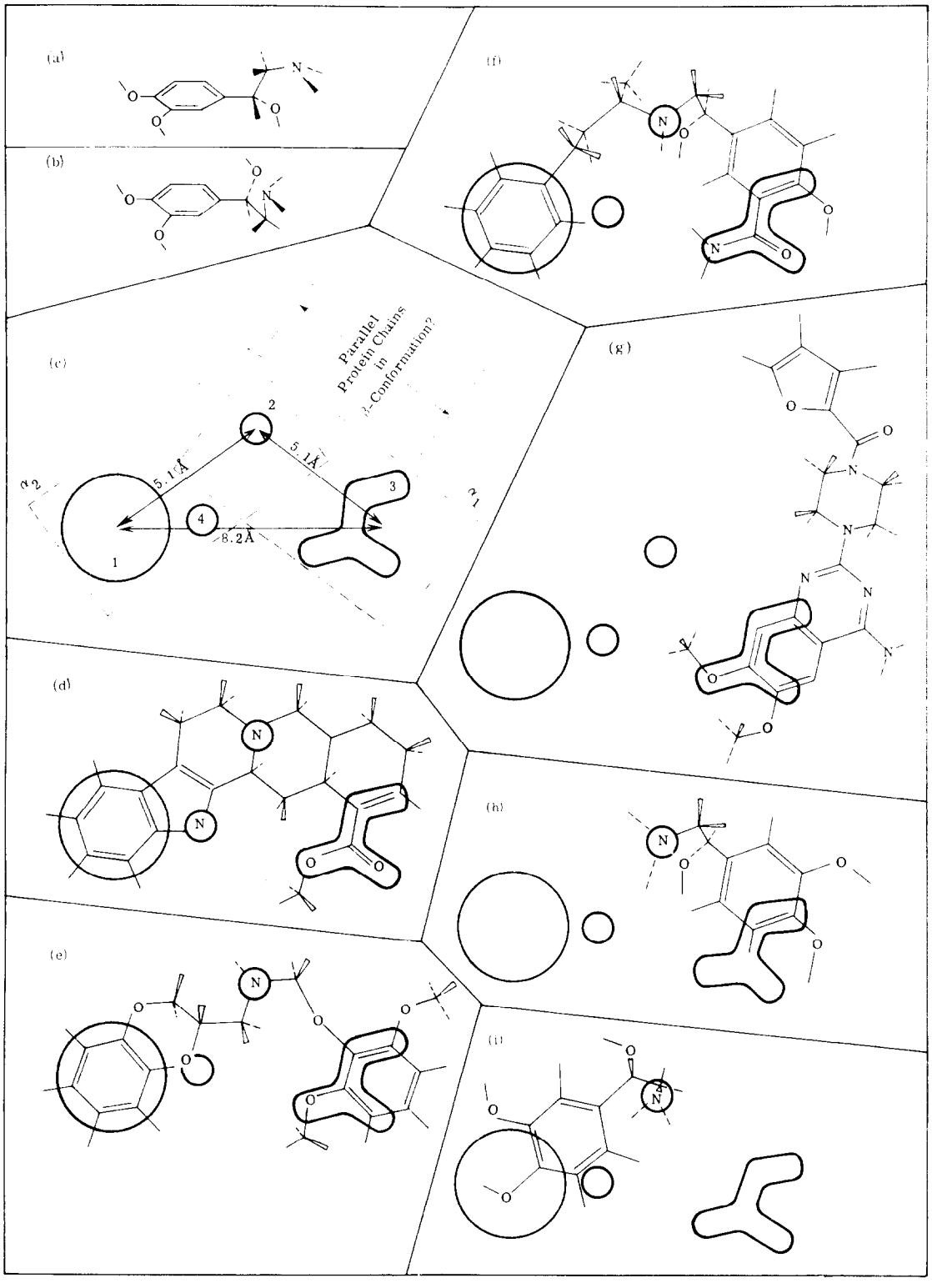


Fig. 6. (a) Noradrenaline, extended; (b) noradrenaline, folded; (c) hypothetical "α"-adrenoceptor binding site, viewed from within. Critical areas are based on features in apoyohimbine—(1) aromatic group, (2) amine, (3) carboxymethyl, (4) ring B nitrogen; (d) apoyohimbine; (e) WB 4101; (f) labetalol (SR form); (g) prazosin; (h) noradrenaline, extended at "α_1" site; (i) noradrenaline, folded at "α_2" site.

adrenaline have been devised on the principle of an amine group separated by two or three atoms (usually C but sometimes including O or N) from a benzyl or other aromatic group. For example, substitution of *meta* and *para* hydroxyl groups on the phenyl group commonly converts antagonists into agonists. When this is interpreted according to current receptor theory it indicates that such substitutions increase "intrinsic activity" although there might actually be a loss of "affinity" for the receptor [81]. Among such compounds, therefore, it has seemed likely that attachment of agonists or antagonists to the receptor is in an orientation similar to that of the physiological agonists.

This is generally viewed in terms of the extended conformation of noradrenaline, i.e. with the amine at its maximum possible distance from the ring. However, a survey of some of the rigid or semi-rigid analogues suggests that this conformation is impossible for certain α-agonists and that, coincidentally, this group includes several α_2-agonists, e.g. B HT 933 or tetrahydro-naphthalene derivatives. Instead, such compounds have a structure which is analogous to the folded form of noradrenaline or adrenaline. On quantum mechanical grounds the folded form of noradrenaline, i.e. with the terminal amino rotated towards the ring rather than pointing away from it, is at only a slight disadvantage compared with the extended form [79]. This produces several critical differences for structure/activity relationships. First, the distance from the phenol ring to the nitrogen is less in the folded form (Figs. 6a and b). Secondly the stereometric position of substituents at the amine or at the adjacent carbon are radically different in the two forms. Furthermore, substitution at the carbon adjacent to the amino group, as occurs in α-methyl noradrenaline, favours the folded conformation and it is known that α-methyl noradrenaline is relatively potent at α_2- compared with α_1-adrenoceptors. Thirdly, the influence of the chiral centre at the carbon adjacent to the aromatic ring may differ in the two conformations. Since the negatively charged β-OH and positively charged amino group are likely to remain *cis* to each other in either form, the presence of this OH group eliminates or greatly decreases the relative stability of one of the two, otherwise likely, folded forms [79]. Thus, in the folded form, the β-OH group might not necessarily be a "point of attachment" in each case but could play a different role: influencing the absolute conformation. It is, therefore, possible that noradrenaline and analogous compounds may interact with the α_1-adrenoceptor in the extended form and the α_2-adrenoceptor in the folded form. Imidazoline-derived agonists are in the interesting position that they can adopt folded or extended forms but the favourability of each will depend on the nature of their link to the benzyl group and the substituents on the latter.

The structures of α-adrenoceptor antagonists which are obvious analogues of catecholamines have, so far, given few clues to the structure of the "receptor" or to the requirements for "selectivity" between α_1 and α_2. It might, therefore, be worthwhile to speculate from the structures of some of the potent antagonist which have been synthesised and tested in the last few years and which are, generally, larger molecules. A hypothetical representation of some critical areas for binding of antagonists to the "α"-adrenoceptor can be based on similarities in the structure of apoyohimbine and WB 4101 (Figs. 6c–e), both of which are potent antagonists at α_1- and α_2-adrenoceptors (Figs. 1 and 3). Apoyohimbine is interesting because it has a very rigid structure but is more potent at α_1 and approximately equipotent at α_2 than the less rigid stereoisomers of yohimbine (Figs. 1 and 3). WB 4101, on the other hand is extremely flexible and has only one centre of asymmetry. Despite this, the three sites which are likely to be important for binding in the yohimbine analogues, i.e. the aromatic ring A, the amine and the carboxymethyl substituent on ring E are precisely superimposable with corresponding groups in WB 4101. This may yet prove to be a red herring but, since it was proposed [80], it has been found that only one of the four possible isomers of labetalol (SR) has potent α-adrenoceptor antagonism [11] and that this is, coincidentally, the only one which can satisfy these same stereometric criteria (Fig. 6f).

What can this predict about "α_1- and α_2-selectivity"? No one hypothesis is likely to fit all the diverse compounds which block α-adrenoceptors but there are some clues available from the most "selective" compounds which are, currently, available. In the yohimbine series, the most striking feature is the lack of α_2-antagonism by corynanthine (Figs. 1 and 3 [74, 70]). Comparison of the stereoisomers indicates that the unique feature of corynanthine is its inability to place its ring E carboxymethyl substituent below the plane of the rest of the rather flat molecule (Fig. 1). The binding site here may thus differ between α_1 and α_2, being below the plane in α_1 but above or on the plane in α_2. Corynanthine's impotence at α_2 would thus result from the loss of affinity at this site. Since prazosin cannot fit the model indicated by apoyohimbine and WB 4101, either the model is wrong or prazosin has a different type of attachment. The latter can be demonstrated by fitting prazosin only to one part of the site and by allowing it to have an additional site, in the same plane, but outside the immediate vicinity (Fig. 6g). Since this allows prazosin to occupy part of the "receptor area" without occupying the important "amino" binding area, it can explain why prazosin, whose structure is not based on phenylamines and was not synthesised with the intention of blocking adrenoceptors, is so selective. The critical area for antagonism of α_2 may be the aromatic ring and amino groups: this is supported by the newly reported "selective" α_2-antagonist, RS 21361, whose benzodioxan and imidazoyl groups could be expected to bind here [74] and also by substituted analogues of clonidine which show some α_2-antagonism [14].

Although it appears that α_1- and α_2-"selectivity" have been achieved by precise specification at different ends of the "receptor area", this need not imply that these are the critical areas for agonism. Nevertheless, it is possible to continue the speculation and combine it with the agonist data to suggest that α_2-agonists may act in the area of the aromatic and amino groups while the α_1-agonists still attach in the amino area but with a different second site.

i.e. in the region where prazosin can bind. This could also explain why high concentrations of prazosin demonstrate agonism. Interestingly, labetalol (SR form) (Fig. 6f), when fitted to this "receptor", captures the extended conformation of noradrenaline within its structure, strengthening the possibility that this represents the agonist conformation (Fig. 6h).

Labetalol is notable among α-adrenoceptor antagonists for producing excitatory effects at concentrations which are within its antagonist concentration/response relationship. No such obvious clue is available for the "fit" of the α_2-agonist. It could occupy the receptor either with its amine and phenyl ring in the same orientation as for α_1 or it might take up an alternative position in the same orientation as the α_2-antagonists (Fig. 6i). In either case, if the amine in (*l*)-noradrenaline is kept projecting towards the viewer out of the surface in Fig. 6, then the β-OH group in the folded form (α_2) is projecting up and, in the extended form (α_1) is projecting down. In (*d*)-noradrenaline this β-OH switches to the other side of the plane in the folded form but remains on the same side in the extended form. This might underlie differences in the stereoselectivity of agonists between α_1- and α_2- or between different types of α_1-adrenoceptors. For the same reason, an α-Me substitution will intrude above the plane in the extended but not the folded form. Another correlation is that neither of these possibilities envisages α_2-agonists and α_2-antagonists as binding to the receptor in the same way: in ligand binding studies it has been suggested that the affinity of "α_2"-ligand-binding sites for agonists but not for antagonists can be modified by several components of the incubation medium and that this is not the case with α_1 [41, 88].

As defined so far, the conformation of the antagonists and hence of the matching "receptor" area is fairly flat with only a gentle depression in the area of the "amine" binding site. Since the upper surface as viewed in Fig. 6 has fewer projections, when all antagonists are considered, and since the amine is situated on this side in the yohimbines, it seems likely that this will contact the receptor. A flat surface rather than a narrow cleft seems to be indicated if bulky three-dimensional structures such as the ergot alkaloids are to be accommodated in the same way. The distances between the three main binding areas would seen to be ideal to correspond to the adjacent side groups in two parallel protein chains in the β-conformation as in the "Kusnetsov–Ghokov Grids" proposed as the basis for various other "receptors" [59, 84]. There is also a remarkable symmetry in the distribution of the binding sites postulated in Fig. 6c. Clearly a relatively small antagonist molecule could interact in either position 1–2 or 3–2 and its "selectivity" would be determined by its stereochemistry. It is already known that, for potency against methoxamine in rabbit aortic strips, stereostructure was more critical for the isomers of piperoxan (relatively more α_2-antagonism) than for those of prosympal (relatively more α_1-antagonism) [78]. A different orientation of these two closely related compounds at one receptor or at two sub-groups could explain this. Stereoselectivity of antagonists for α_1 and α_2 or among sub-groups of α_1 may be of great interest.

Does this mean that there is one α-adrenoceptor and that the different sub-groups arise from different interactions with the same site? This could not explain why α_1-agonism is still possible in the presence of an α_2-antagonist, unless, of course, there is another, adjacent, amino-binding site. A more attractive proposition would be one generic type whose properties can be modified according to local environmental conditions or in which conformational changes can be induced by environmental cofactors or adjacent molecules. These, in turn, could be modulated by cellular or circulating factors. This has a bearing on the fundamental question of whether different types of adrenoceptors are interconvertible *per se* or whether they are part of separate protein entities which have superficial similarities in their conformations, as would be required of recognition sites for similar agonists. This applies not only to α_1/α_2 but to many other permutations of adrenoceptors including α_1/β_1 in the heart, α_2/β_2 in blood vessels and any other cases where related effector systems are under control of more than one type of "adrenoceptor". The isolation of "receptors", which is an objective of ligand-binding studies, should answer this question. Initial results suggest that α_1- and α_2-ligand-binding sites are different protein entities [40] but, in the systems used for such studies, it is not known whether the two groups come from the same cells: thus attachment to different effector systems rather than fundamental differences between the generic "receptor" site could explain this.

Whatever the biochemical background turns out to be, it is certain that different types of α-adrenoceptors, the systems which they modulate, their developmental background and their interconversion or changing balance by environmental, nervous and hormonal factors (and no doubt, by drugs) will preoccupy many pharmacologists for the next few years.

Summary

The concept of two types of α-adrenoceptor, α_1 located on smooth muscle and mediating contraction and α_2 located on nerve terminals and mediating inhibition of transmitter release, has broken down. *In vivo* it has been shown that post-junctional receptors, with characteristics closely related to those of the α_2-adrenoceptors at nerve terminals, can mediate pressor responses and are, "post-junctional α_2-adrenoceptors". Several differences among agonists *in vitro* have superficial similarities to the *in vivo* α_1/α_2 system but do not correspond precisely and seem to point to a subdivision of post-junctional α_1-adrenoceptors. A preliminary hypothesis is: *in vivo* α_1 is rapid in onset, short-lived, utilises internal Ca^{2+}, prefers alkalosis and responds to short-term stimuli such as short bursts of nerve impulses or bolus injections of catecholamines; α_2 is slower in onset, longer-lived, utilises external Ca^{2+}, prefers acidosis and responds to more prolonged stimuli such as circulating catecholamines; *in vitro* these categories of response occur but antagonists fail to define an α_1/α_2 split, suggesting that some critical factor is missing *in vitro*. The implications of these trends in α-adrenoceptor classification are discussed in relation to current pharmacological and biochemical

methods for receptor typing, to the possible physiological actions and roles of such receptors and to structure/activity relationships among agonists and antagonists.

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