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COMMENTARY

SUBCLASSIFICATION OF ALPHA-ADRENERGIC RECEPTORS BY DIRECT BINDING STUDIES

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The characterization and classification of receptors for catecholamines (adrenergic receptors) have depended historically upon biological effect and upon relative potencies of various adrenergic compounds. Alpha-adrenergic receptors show the characteristic order of potency: epinephrine ⩾ norepinephrine ⩾ isoproterenol [1]. Beta adrenergic receptors have the potency order: isoproterenol > epinephrine ⩾ norepinephrine [1]. Antagonists which are relatively selective for either alpha- or beta-adrenergic receptors are well known. In addition to this distinction, beta-adrenergic receptors have been subclassified as $beta_1$ and $beta_2$ [2]. At $beta_1$ receptors (heart, adipose tissue), norepinephrine is approximately equipotent with epinephrine and 3- to 4-fold weaker than isoproterenol. At $beta_2$ receptors (smooth muscle), norepinephrine is somewhat weaker than epinephrine and about 100-fold weaker than isoproterenol. Certain beta-receptor antagonists (such as practolol [3], butoxamine [4] and atenolol [5]) are relatively selective for either $beta_1$ or $beta_2$ receptors, while others (such as propranolol [6] and timolol [6]) have similar affinities for both types.

Physiologic evidence for subclasses of alpha-adrenergic receptors

Subclasses of alpha-adrenergic receptors may also exist. The alpha receptors on pre-synaptic noradrenergic nerve terminals, which mediate a feedback inhibition of nerve-stimulus evoked norepinephrine release, have affinities for certain adrenergic agonists and antagonists which are quite different from those of conventional post-synaptic alpha receptors. These studies are well summarized in recent reviews [7–10]. The pre-synaptic receptor is classified as an alpha receptor because its effect is blocked by the classical alpha antagonists phentolamine, phenoxybenzamine and dihydroergotamine, and because the potency of several beta-phenylethylamine agonists resembles their known potency order at post-synaptic alpha receptors. However, many differences between the pre- and post-synaptic alpha receptors have also been reported. For example, in the spleen, phenoxybenzamine is about 30 times more potent in inhibiting post-synaptic smooth muscle contraction than in blocking the pre-synaptic site [11]. In rabbit heart [12], the alpha agonists oxymetazoline and naphazoline maximally inhibit nerve-stimulus evoked norepinephrine release at concentrations which activate no post-synaptic alpha response, whereas phenylephrine causes a post-synaptic alpha-mediated inotropic response without activating the pre-synaptic alpha response. Comparing pre-synaptic alpha receptors in rat vas deferens with post-synaptic alpha receptors in rat anococcygeus muscle, Doxey *et al.* [13] concluded that the antagonists yohimbine and phentolamine were more potent in blocking pre-synaptic alpha receptors, whereas prazosin and phenoxybenzamine were preferential for post-synaptic receptors.

Starke *et al.* [14–16] made detailed studies of rabbit pulmonary artery, where the pre-synaptic effect (inhibition of nerve-stimulus induced transmitter release) and post-synaptic effect (direct stimulation of smooth muscle contraction) could be observed under identical conditions. In this system, the alpha-agonists, clonidine and (−)alpha-methylnorepinephrine, are seven times more potent for the pre-synaptic than the post-synaptic effect. Similarly, the antagonist, yohimbine, is selective for pre-synaptic receptors, showing a 20-fold higher potency there than post-synaptically. In contrast, the agonists, phenylephrine and methoxamine, and the antagonists, clozapine and azapetine, were at least 10-fold more potent post-synaptically. In contrast to the rat studies mentioned above, in this system phentolamine showed slight post-synaptic preference. Compounds which were approximately equipotent pre- and post-synaptically included the agonists, epinephrine and norepinephrine, and the antagonists, dihydroergotamine and piperoxane.

In 1974, in the light of mounting evidence in favor of distinctions between the two types of alpha receptors, Langer [17] proposed that classical post-synaptic alpha receptors be referred to as $alpha_1$ and the receptors on pre-synaptic nerve terminals as $alpha_2$. Berthelson and Pettinger [18] have summarized recently the physiologic evidence for an $alpha_2$ type of receptor in several additional tissues, such as frog melanocytes, kidney renin-releasing cells, and brain neurons affecting blood pressure. A major feature of these $alpha_2$ receptors is their relatively high affinity for clonidine and alpha-methylnorepinephrine, as compared to the classical smooth muscle alpha receptors.

Use of radioligand binding studies for subclassification of alpha-receptors

So far, the characterization of these distinct types of alpha-adrenergic receptors has utilized physiological response measurements of relative potencies of agonists and antagonists. An alternative method of estimating

Table 1. Dissociation constants of agonists and antagonists for alpha-adrenergic receptors from seven peripheral tissues *

	Agonists						Antagonists			
	Clonidine	(−) Epinephrine	( ) Norepinephrine	(−) Phenylephrine	Methoxamine	(+) Alpha-methylnorepinephrine	DHE	PBZ	Yohimbine	Phentolamine
	K_D (μM)						K_D (μM)			
Rabbit uterus [21]	0.26	0.23	0.65	3.5	7.5	2.3	0.010	0.018	0.22	0.015
Canine aorta [22]	0.33	1	2.6	5.3		130	0.013	0.05	0.2	0.053
Rat liver [27]	0.16	0.12	1.2	2.5	15	74	0.002	0.004	0.08	0.002
Human platelet [25]	0.017	0.26	0.85	0.86	24	4.5	0.011	0.13	0.002	0.014
Rabbit platelet [26]	0.08	0.1	0.4	2.5	28	0.9	0.006	0.1	0.007	0.03
Rat submandibular [23]	0.15	2.9	12		75	20	0.014	1.3	0.19	0.073
Rat parotid [24, 29]	0.13	1.6	10				0.0045	0.50	0.080	0.05

* Suspensions of tissue membranes were incubated at 20° or 25° at pH 7.4 to 7.5 to steady state (18–30 min) with [^{3}H]dihydroergocryptine (DHE) in the absence and presence of different concentrations of the competing adrenergic agents. The suspensions were filtered through glass fiber filters and rinsed, and bound radioactivity was determined by liquid scintillation spectrometry. "Specific" binding of DHE (receptor binding) was defined as the difference in radioactivity bound to membranes in the absence and presence of 10^{-5} M phentolamine in the incubation suspension: specific binding amounted to 50–85 per cent of the total radioactivity bound, depending upon the type of tissue. In each tissue studied, [^{3}H]dihydroergocryptine binding fulfilled the criteria expected for binding to the alpha-adrenergic receptor: binding was rapid, reversible, saturable, of high affinity, and stereospecific for *d*- and *l*-isomers of catecholamines. Dissociation constants (K_D) of competing drugs were calculated according to the formula

$$K_D = \frac{EC_{50}}{1 + \frac{(S)}{K_m}} \quad [28]$$

where EC_{50} = the concentration of competing drug causing a 50 per cent decrease in specific [^{3}H]dihydroergocryptine binding, (S) = concentration of [^{3}H]dihydroergocryptine in the incubation suspension, and K_m = the dissociation constant of [^{3}H]dihydroergocryptine, as determined by a radioligand saturation curve. The K_D for phenoxybenzamine (PBZ) is an "apparent K_D", since PBZ is an irreversible agent and, as such, does not have a true dissociation constant. In aorta and in rabbit platelet, a 10-min preincubation of membranes with PBZ, before [^{3}H]DHE addition, was used. In liver, similar K_D values were obtained either with or without PBZ preincubation.

receptor affinities for adrenergic agents is to measure directly their competition for receptor binding of radiolabeled adrenergic ligands [19]. Dissociation constants derived from such direct binding studies are usually closely comparable with those obtained by the study of physiologic response in the same tissue [20–22].

The use of direct radioligand binding studies to distinguish possible subtypes of alpha-adrenergic receptors in different tissues has several advantages over a similar attempt using drug affinities determined from biological response data. To measure the affinity of a drug for the receptor in an isolated organ system or in the intact animal, one must recognize and be able to correct for the problems of transport of the drug to its site of action, neuronal or extraneuronal uptake systems, metabolism, indirectly mediated responses, and desensitization of the response during the course of the experiment. By contrast, radioligand binding studies can be performed on relatively purified cell membrane fragments, minimizing difficulties in drug transport and metabolism. A less obvious problem with whole tissue data is that of spare receptors, i.e. a maximum biological response may be elicited by an agonist acting on only a small percentage of the available receptors, thus producing an apparent affinity of the agonist much higher than its actual binding affinity for the receptor. Partial agonists, which yield only a fraction of the maximal biological response, even when occupying all of the receptors, further complicate the picture. Radioligand binding studies provide direct measurements of drug–receptor dissociation constants independent of the agonist, partial agonist, or antagonist character of the drug, and, therefore, may provide the most accurate measurement of actual binding affinities. A potential disadvantage of the direct radioligand binding technique is that all alpha receptors in a crude tissue preparation might be labeled, whether of smooth muscle, vascular, neural or other origin. The data, therefore, might represent the composite picture obtained by labeling different types of alpha receptors in proportion to their relative affinities for the ligand and their relative density.

We have compiled radioligand binding data from seven tissues studied in our laboratory in an attempt to see if the directly determined alpha receptor affinities of various drugs conform to the notion of distinct $alpha_1$ and $alpha_2$ receptor subtypes. [^{3}H]dihydroergocryptine, a potent alpha-adrenergic antagonist, was used to label alpha receptors in crude homogenates of rabbit uterus [21], canine aorta [22], rat submandibular gland [23], and rat parotid gland [24]. Human platelets [25] and rabbit platelets [26], separated from serum and other blood elements by various centrifugations, were examined as crude lysates. Rat liver plasma membranes were relatively enriched by an adaptation of the Neville procedure [27].

Table 1 lists dissociation constants for ten adrener-

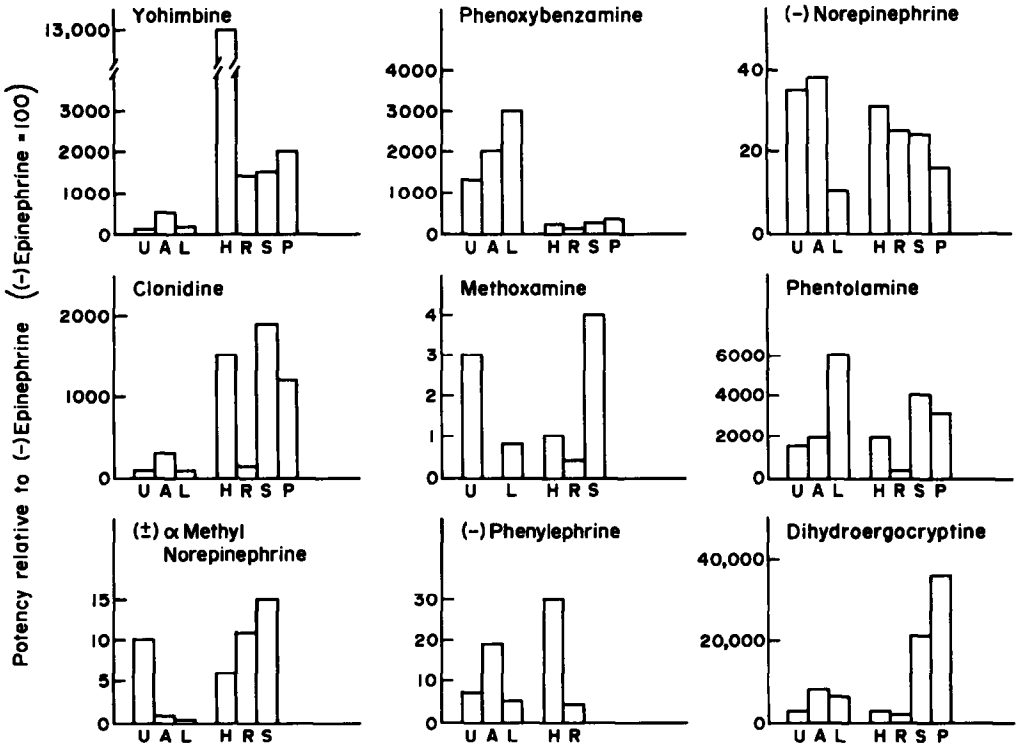

Fig. 1. Drug potencies relative to (−)epinephrine at alpha receptors of seven peripheral tissues. Data from Table 1 were converted to relative potencies [(−)epinephrine = 100] by the formula:

$$\text{Relative potency} = \frac{K_{D(\text{epi})}}{K_{D(\text{drug})}} \times 100$$

Abbreviations used are: U = rabbit uterus; A = canine aorta; L = rat liver; H = human platelet; R = rabbit platelet; S = rat submandibular gland; and P = rat parotid gland.

gic agonists and antagonists in the seven tissues. In order to compare the relative affinities of drugs for the alpha receptors from different tissues, and to minimize difficulties arising from slight methodologic differences (such as the type of buffer and the protein and ligand concentrations in the binding assay), the data were converted to potencies relative to epinephrine by calculating the ratios

$$\frac{K_{D(epi)}}{K_{D(drug)}} \times 100.$$

These ratios are shown graphically in Fig. 1. Normalization of drug potencies to norepinephrine rather than epinephrine gave virtually identical results.

Interpretation of radioligand binding data

It is apparent that radioligand binding studies provide further confirmation of the heterogeneity of alpha-adrenergic receptors first detected in physiologic studies. Yohimbine, clonidine and alpha-methylnorepinephrine are all considered from physiologic studies to be selective for $alpha_2$ (pre-synaptic-like) receptors. On the basis of the potencies of these three drugs in competing for [^{3}H]dihydroergocryptine binding to alpha receptors of the seven tissues studied, we can divide the tissues into two categories. Uterus, aorta and liver tend to have low affinities for these drugs and, therefore, might be classified as $alpha_1$. Human platelet, rabbit platelet, rat submandibular gland and rat parotid gland generally have high affinities for these three compounds, and thus might be considered of the $alpha_2$ type. (The high affinity of uterus receptors for alpha-methylnorepinephrine and the low affinity of rabbit platelet receptors for clonidine are notable exceptions to these generalizations.) Phenoxybenzamine, considered $alpha_1$-selective on the basis of physiologic data, also segregates these seven tissues into the same two groups; it has higher affinity for uterus, aorta and liver (all $alpha_1$), and lower affinity for human and rabbit platelet, and rat sumandibular and parotid glands ($alpha_2$).

Methoxamine and phenylephrine, which in physiologic studies were considerably more potent in contracting post-synaptic smooth muscle than in inhibiting pre-synaptic norepinephrine release [15], do not seem to follow the same trends in the radioligand binding studies. Norepinephrine is highly non-selective between $alpha_1$ and $alpha_2$ in the seven tissues compiled here, as it is in physiologic studies. Phentolamine in physiologic studies behaved as if it were $alpha_1$ selective in one tissue system and $alpha_2$ selective in another (see above); in binding studies it appears similarly non-diagnostic. Dihydroergocryptine potencies in binding studies indicate that it also is not a selective agent: this result is in accord with the physiologic nonselectivity of the closely related ergot alkaloid, dihydroergotamine, in the rabbit pulmonary artery [16].

The subclassification of alpha receptors explains, perhaps, some previously observed aberrancies in the physiologic response of the platelet alpha receptor to certain alpha antagonists. Epinephrine induces platelet aggregation, which is blocked by alpha receptor antagonists [30–32]. However, the potencies of phenoxybenzamine and yohimbine in blocking platelet aggregation are quite different from their potencies in blocking smooth muscle contraction. Phenoxybenzamine potently blocks smooth muscle contraction, but is extremely weak or ineffective in blocking epinephrine-induced platelet aggregation [30, 31]. Yohimbine is 40-fold weaker than phentolamine in blocking aortic contraction [33], while it is more potent than phentolamine in blocking epinephrine-induced platelet aggregation [32]. These observations are in accord with our binding data for platelets and are another reflection of the high affinity of the $alpha_2$ receptor of platelets for yohimbine and its low affinity for phenoxybenzamine. The apparent $alpha_2$ nature of platelet alpha-adrenergic receptors appears not to have been appreciated previously.

Criteria for alpha receptor subclassification by binding data

According to the results compiled here, the most useful criteria for subclassification of alpha receptors by direct binding studies appear to relate to the relative affinities of four of the compounds studied. As a preliminary generalization, $alpha_1$ receptors would be defined as those having high relative affinity for the antagonist phenoxybenzamine and low relative affinities for the agonists clonidine and alpha-methylnorepinephrine and the antagonist yohimbine. By contrast, $alpha_2$ receptors would be those having high relative affinities for clonidine, alpha-methylnorepinephrine and yohimbine and low relative affinity for phenoxybenzamine. Further binding studies are necessary to assess whether the dissociation constants of other adrenergic agents will offer additional criteria for alpha receptor classification. For example, the physiologic selectivities of prazosin, azapetine, oxymetazoline and naphazoline suggest that these drugs may be useful.

Thus, the examination of binding data from seven tissues supports the usefulness of the $alpha_1$–$alpha_2$ subclassification scheme. In agreement with Berthelson and Pettinger [18], we have found $alpha_2$-type receptors in non-neural tissues. As mentioned above, contamination of tissue preparations with neural elements might affect binding data. However, such contamination is obviously absent from the platelet lysates. Moreover, further binding studies in the rat submandibular gland after denervation have revealed no significant changes in drug affinity constants (Arnett and Davis, manuscript in preparation). Therefore, it would appear that "post-synaptic" alpha receptors in the salivary gland are in fact of the $alpha_2$ type. Since many $alpha_2$ type receptors are clearly not on neural pre-synaptic elements, the term "$alpha_2$" seems preferable to "pre-synaptic" for the subclassification of alpha-adrenergic receptors.

It should be recognized, however, that heterogeneity appears to exist within each alpha-receptor subclass, and that the $alpha_1$–$alpha_2$ designation serves only as a first approximation. For example, the binding studies would classify uterus, aorta and liver as $alpha_1$ even though uterus has a high affinity for alpha-methylnorepinephrine. Also, the relatively low affinity of clonidine for rabbit platelet alpha receptors is an exception to the general pattern. Physiologic studies have also demonstrated potency differences between various post-synaptic ($alpha_1$) alpha receptors [33, 34]. In pre-synaptic ($alpha_2$) alpha receptors from different tissues, differences in imidazoline drug potencies have also been described [35].

The classification scheme outlined here depends entirely upon alpha receptor affinities for various selective drugs, not upon the intrinsic activities of the drugs in stimulating biologic effect. While the tissues classified here as $alpha_2$ (human and rabbit platelets and rat submandibular and parotid glands) bear remarkable similarities to pre-synaptic alpha receptors on the basis of receptor affinities for certain drugs, there are also remarkable differences between these receptors, based on intrinsic activities. For example, clonidine and oxymetazoline are full agonists at pulmonary artery presynaptic receptors, and naphazoline is almost a full agonist [14, 15]. In contrast, all three drugs are antagonists of epinephrine-stimulated K^+ release from dispersed rat parotid cells [29], and clonidine is only a partial agonist [25, 26] or an antagonist [36] in platelets. Phenylephrine and methoxamine, which are at least partial agonists pre-synaptically, are partial agonists [25] or antagonists [36] in human platelets. More data may be needed before a classification scheme embracing both affinities and intrinsic activities can be constructed. Also, varying intrinsic activities in different systems may well be a reflection of post-receptor events.

Possible future uses of direct radioligand binding studies to characterize and classify alpha-adrenergic receptors

Further understanding of alpha adrenergic receptors may result from additional application of direct radioligand binding techniques.

First, mentioned above, study of $alpha_1$ and $alpha_2$ receptor affinities of other adrenergic drugs may locate an additional number of selective (and, therefore, diagnostic) compounds, which would be useful in subclassification. In addition, by these techniques drugs can be screened easily for their selectivity; as we learn more about central and peripheral alpha receptors, knowledge of the selectivity of a drug may be useful in the prediction of its therapeutic effects.

Second, as seen in Figure 1, the ligand [^{3}H]dihydroergocryptine is not especially selective for $alpha_1$ or $alpha_2$ receptors, having a very high affinity for both types. Thus, as mentioned above, in a crude membrane preparation (such as uterus or aorta) the ligand would be expected to bind to all alpha receptors, whether of neural, vascular, other smooth muscle, or other cell type. Each type of alpha receptor would contribute to the data in proportion to its relative numbers. The possible presence of more than one type of alpha receptor in a preparation might be detected by at least three different methods.

(1) If feasible, denervation and subsequent degeneration of nerve terminals should eliminate alpha receptors of pre-synaptic origin. Comparison of drug affinities of control and denervated tissues might then reveal several possibilities. For example, if both control and denervated tissue preparations have $alpha_2$-type affinities, the main tissue is probably $alpha_2$ (as in the case of rat submandibular gland). If both preparations appear to be $alpha_1$, either the numbers of pre-synaptic alpha receptors must be small, or both the pre-synaptic and the post-synaptic receptors are $alpha_1$. If control tissue has affinities midway between $alpha_1$ and $alpha_2$, and denervated tissue appears to be $alpha_1$, then the numeric contribution of $alpha_2$ receptors from pre-synaptic terminals must be significant.

(2) In a preparation containing significant numbers of both $alpha_1$ and $alpha_2$ receptors, the competition curve for [^{3}H]dihydroergocryptine binding by a drug selective for either $alpha_1$ or $alpha_2$ would be more shallow than expected. Barnett *et al.* [6] recently reported this type of result for lung beta-adrenergic receptors using competition binding between the non-selective beta-adrenergic ligand, [^{3}H]dihydroalprenolol, and the $beta_1$-selective antagonist, practolol. They derived a method for estimating the amounts and drug affinities of each of the two receptor sub-types present. Application of this technique to alpha-adrenergic receptor studies may yield further interesting information.

(3) An alternative approach to labeling all alpha receptors in a given tissue preparation with a nonselective ligand and detecting different subtypes by competition with unlabeled selective agents would be to develop radioligands which were either $alpha_1$ or $alpha_2$ selective themselves. For example, [^{3}H]clonidine would be a logical possibility for an $alpha_2$-selective ligand, and [^{3}H]WB4101 may be $alpha_1$ selective. In this context it is worth noting that the recently reported differences in binding specificities of brain adrenergic receptors [37, 38] obtained with [^{3}H]clonidine and [^{3}H]WB4101, previously interpreted as representing "agonist" and "antagonist" states of the receptors, might be reinterpreted instead as the selective labeling of $alpha_2$ and $alpha_1$ subpopulations of alpha receptors. The observation that competition for [^{3}H]dihydroergocryptine binding gives drug affinities intermediate to those obtained with the other two ligands [39] would then indicate that this ligand binds both $alpha_1$ and $alpha_2$ receptors in the central nervous system as well as peripherally. Radioligands selective for $alpha_1$ or $alpha_2$ receptors will become very useful if they meet the necessary requirements of high specific radioactivity, high affinity for the intended receptor, and low amounts of nonspecific (non-receptor) binding.

In summary, examination by direct binding techniques of the relative potencies of certain drugs which were shown in physiologic studies to be relatively selective for either pre-synaptic or post-synaptic alpha receptors indicates that peripheral alpha receptors can be divided into at least two subclasses. These differences in affinity between $alpha_1$ and $alpha_2$ receptors may represent structural differences in the receptor molecules themselves, i.e. "iso-receptors".

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Note added in proof—After this Commentary was submitted for publication, the possibility of mixtures of alpha receptor types in a given membrane preparation, discussed above, was investigated in detail using Method 2 (above) [40]. Extensive competition curves for [^{3}H]dihydroergocryptine binding by an $alpha_1$-selective drug (prazosin) and an $alpha_2$ selective drug (yohimbine) were analyzed by a computer modelling technique which quantitated the proportions of each receptor type present. Rat liver plasma membranes were completely $alpha_1$, and human platelet all $alpha_2$, as might be expected

from considerations of membrane purity described above. Crude rabbit uterus membranes were shown to contain both alpha$_1$ and alpha$_2$ receptors. This mixture may account for the previously discussed high affinity of alpha-methylnorepinephrine in the uterus preparation.

Two other laboratories recently demonstrated mixtures of two types of alpha adrenergic receptors in membranes from brain [41,42] and peripheral tissues. [41].

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