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[^{3}H]DIHYDROERGOCRYPTINE BINDING TO ALPHA-ADRENERGIC RECEPTORS OF HUMAN PLATELETS

A REASSESSMENT USING THE SELECTIVE RADIOLIGANDS [^{3}H]PRAZOSIN, [^{3}H]YOHIMBINE, AND [^{3}H]RAUWOLSCINE

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Abstract—Which subtype(s) of the alpha-adrenergic receptor occurs on human platelets? Studies of platelet responsiveness to adrenergic compounds and indirect radioligand binding studies addressing this question have yielded contradictory conclusions. These binding studies employed the ligand [^{3}H]dihydroergocryptine ([^{3}H]DHE), an alpha-adrenergic antagonist that does not select between alpha$_1$- and alpha$_2$-adrenergic receptors and that also binds to other receptor types in some tissues. To determine the subtype of the platelet alpha-adrenergic receptor, we have examined the binding to intact human platelets of [^{3}H]prazosin (alpha$_1$-selective), [^{3}H]yohimbine (alpha$_2$-selective), and [^{3}H]rauwolscine (alpha$_2$-selective), and we have compared the binding of these selective radioligands with that of [^{3}H]DHE. [^{3}H]Yohimbine and [^{3}H]rauwolscine both bound with high affinity (K_D = 2.7 and 4.6 nM, respectively) to an equal number and a single class (Hill coefficient ~1.0) of sites (~300 per platelet), but [^{3}H]yohimbine yielded lower nonspecific binding than did [^{3}H]rauwolscine. In paired experiments, [^{3}H]DHE bound to 1.5 times as many (phentolamine-displaceable) sites as did [^{3}H]yohimbine or [^{3}H]rauwolscine. Unlabeled yohimbine and epinephrine competed for fewer [^{3}H]DHE binding sites than did phentolamine. Thus, in addition to binding to the alpha$_2$-adrenergic receptors identified by [^{3}H]yohimbine and [^{3}H]rauwolscine, [^{3}H]DHE seems to bind to other sites on human platelets. The nature of these sites is not clear. We found that [^{3}H]prazosin did not identify alpha$_1$-adrenergic receptors on platelets, and that phenoxybenzamine only inhibited [^{3}H]yohimbine and [^{3}H]DHE binding at higher concentrations than usually observed for alpha$_1$-adrenergic receptors. We conclude that (1) all alpha-adrenergic sites on human platelets are of the alpha$_2$ subtype, (2) [^{3}H]DHE may bind to additional, as yet ill-defined, sites in addition to those sites identified by [^{3}H]yohimbine and [^{3}H]rauwolscine, and (3) [^{3}H]yohimbine is the preferred antagonist radioligand for studying the alpha$_2$-adrenergic receptors on human platelets.

Human platelets contain alpha-adrenergic receptors. When exposed to agonists such as epinephrine, these receptors promote platelet aggregation and secretion, enhance aggregation caused by unrelated agents (ADP, thrombin), increase calcium influx, and inhibit adenylate cyclase [1–5]. Alpha-adrenergic receptors in other tissues have been classified into alpha$_1$ and alpha$_2$ subtypes [6, 7] but the nature of the alpha-adrenergic receptors on platelets has been unclear. Some investigators have concluded that all platelet alpha-adrenergic receptors are of the alpha$_2$ type [8, 9], others have concluded that both alpha$_1$ and alpha$_2$ receptors are present [10, 11], and still others have concluded that the platelet alpha-adrenergic receptor is a unique ("alpha$_3$") type of receptor [12]. Several investigators have used radioligand binding to study the alpha-adrenergic receptors on human platelets. In most of these studies, the investigators used the radioligand [^{3}H]dihydroergocryptine (DHE) [13–15], a ligand that does not select between alpha$_1$ and alpha$_2$ receptors and that binds to other types as well in some tissues [16, 17]. Several selective alpha-adrenergic radioligands have become available recently. To directly determine the subtype of the platelet receptors, we compared the binding to platelets of [^{3}H]DHE with three selective alpha-adrenergic antagonists: [^{3}H]prazosin (alpha$_1$-selective), [^{3}H]yohimbine (alpha$_2$-selective), and [^{3}H]-rauwolscine (alpha$_2$-selective). We found that alpha-adrenergic receptors on platelets were exclusively of the alpha$_2$ subtype, and that [^{3}H]yohimbine was the preferred radioligand for studying these sites in intact platelets.

MATERIALS AND METHODS

Materials. [^{3}H]DHE (33 Ci/mmole), [^{3}H]-yohimbine (82 Ci/mmole), and [^{3}H]rauwolscine (84 Ci/mmole) were obtained from the New England Nuclear Corp., Boston, MA. Unlabeled and labeled prazosin were gifts from Pfizer, New York, NY. Purity of the radioligands was determined with thin-layer chromatography. Phentolamine mesylate was a gift from Ciba-Geigy, Ardsley, NY. (+) and (−)epinephrine were gifts from Sterling-Winthrop, Rensselaer, NY. All other reagents were from standard sources.

Radioligand binding. Radioligand binding was performed as previously reported [18]. Blood (60 ml)

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from normal men or women (aged 22–44) who had taken no medications in at least 2 weeks was drawn into 0.38% (final) sodium citrate. The platelets were isolated [14], washed twice in an isotonic buffer, and resuspended in a buffer containing 50 mM Tris–HCl, 100 mM NaCl, and 5 mM EDTA, at pH 7.5. Intact platelets ($\sim 5 \times 10^7$) were incubated in a volume of 0.25 ml with the radioligands at 25° for 30 min in polypropylene test tubes. Buffer (10 ml; at 22° for [^{3}H]prazosin, [^{3}H]yohimbine, and [^{3}H]rauwolscine; at 37° for [^{3}H]DHE) was then added to each tube, and the contents were immediately filtered through fiberglass filters (Whatman GF/C), which were then rapidly washed with a further 10 ml of buffer. Under these conditions, virtually no specific binding of the ligands was lost during filtration and washing. We performed parallel incubations in the presence of 10 μM phentolamine to determine nonspecific binding, and subtracted this from the total binding to determine the specific binding. Because DHE is photosensitive we took care to keep our test tubes covered. Maximum binding capacities and radioligand affinities (dissociation constants, K_Ds) were determined by Scatchard analyses of the specific binding data. Competition binding experiments were analyzed with a computer program [19] that uses non-linear regression to fit the radioligand binding data to the mass-action binding equation.

Partition coefficients. Radioligand (2 μCi) was incubated with 0.5 ml of water and 0.5 ml of *n*-octanol at 25° for 30 min with frequent and vigorous mixing. The radioactivities in 25 μl of the octanol and 25 μl of the aqueous phases were determined; the ratio is the octanol:water partition coefficient.

Statistics. Results are expresed as means ± standard deviation, and statistical significance was calculated by a two-tailed Student's *t*-test.

RESULTS

[^{3}H]Rauwolscine binding to platelets. In preliminary experiments (not shown), we found that [^{3}H]rauwolscine behaved very similarly to [^{3}H]yohimbine [18] in binding to intact platelets. [^{3}H]Rauwolscine bound rapidly to platelets, reaching equilibrium in 20–30 min, and the binding was completely reversible in 1 hr. (−)Epinephrine (the physiological enantiomer) competed for [^{3}H]rauwolscine binding sites on platelets over 10-fold more potently than did (+)epinephrine. In saturation binding experiments, [^{3}H]rauwolscine bound with an affinity of 4.6 ± 1.2 nM (N = 7) and a Hill coefficient of 1.00 ± 0.03 to 344 ± 70 sites per platelet. Unlabeled yohimbine competed for all of the specific [^{3}H]rauwolscine binding with a K_D of 2.1 nM. Because this value is virtually identical with the K_D with which [^{3}H]yohimbine binds to platelets (2.7 ± 0.7 nM, [18]) we have concluded that [^{3}H]rauwolscine binds to alpha$_2$-adrenergic receptors.

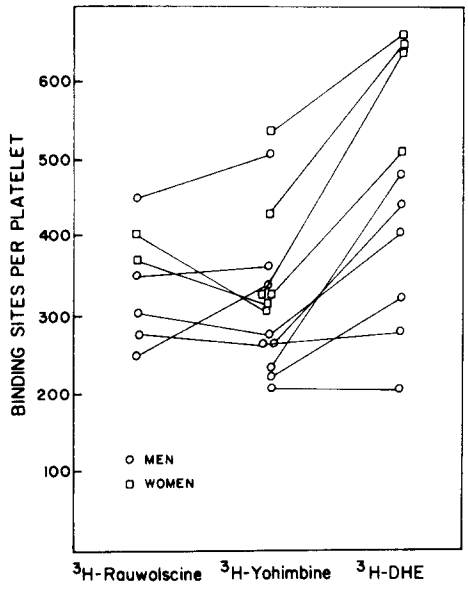

Fig. 2. Comparison of maximum binding of [^{3}H]DHE, [^{3}H]yohimbine, and [^{3}H]rauwolscine to platelets from male (○) and female (□) donors. The maximum binding of the radioligand was determined by parallel saturation binding isotherms analyzed by the method of Scatchard as in Fig. 1.

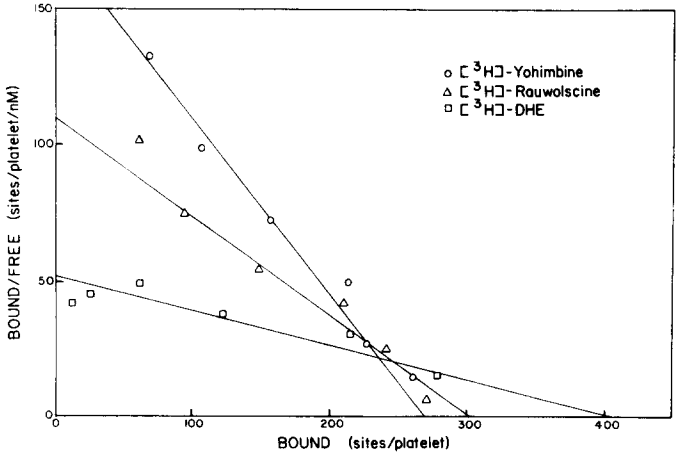

Fig. 1. Scatchard analysis of binding of [^{3}H]DHE, [^{3}H]yohimbine, and [^{3}H]rauwolscine to platelets from one donor.

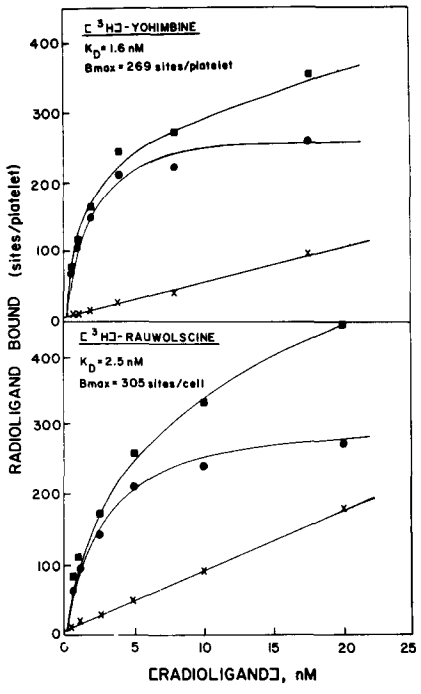

Fig. 3. Total, specific, and nonspecific binding of [³H]rauwolscine and [³H]yohimbine to platelets. The two radioligands were used in parallel, and the points shown are the mean of duplicate determinations.

Comparison of [³H]DHE, [³H]yohimbine, and [³H]rauwolscine binding. The results of paired comparisons between [³H]yohimbine and the other radioligands are shown in Figs. 1 and 2. On average [³H]DHE bound to 1.5 ± 0.4 (N = 10, P = 0.002) times as many sites as did [³H]yohimbine. Similar results were obtained with several batches of each radioligand in experiments performed over a 1-year period. [³H]Rauwolscine, on the other hand, bound to the same number of sites as did [³H]yohimbine (ratio = 1.0 ± 0.2, N = 7). The nonspecific binding observed with [³H]yohimbine was much lower than that observed with [³H]rauwolscine (Fig. 3). Platelets from female donors had somewhat more receptors than those from male donors, but the difference was not statistically significant.

Further evidence that [³H]yohimbine binds to fewer sites than does [³H]DHE was obtained by studying the competition of unlabeled drugs for [³H]DHE binding sites (Fig. 4). Although specific binding at alpha-adrenergic receptors is conventionally defined as binding for which the alpha-adrenergic antagonist phentolamine competes, we found that both unlabeled yohimbine and epinephrine (up to concentrations of 100 μM) competed for fewer [³H]DHE sites than did phentolamine. This difference was more striking in experiments where we used a high [³H]DHE concentration (Fig. 4). Thus, both labeled and unlabeled yohimbine bound to fewer (phentolamine-competable) sites than did [³H]DHE. The difference in number of sites detected by the two radioligands, therefore, cannot be attributed solely to an incorrect determination of their specific activities.

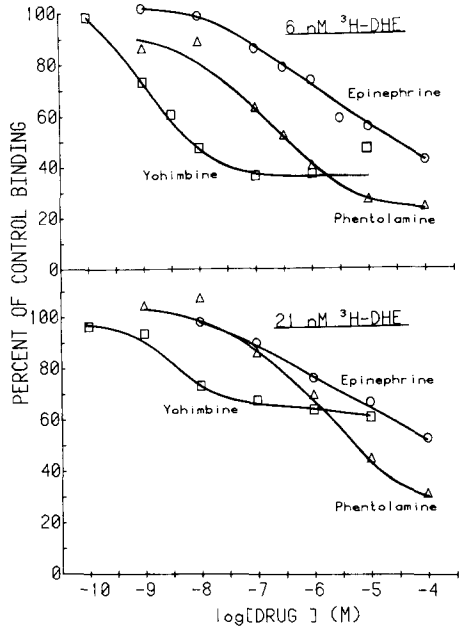

Fig. 4. Competition of unlabeled adrenergic drugs for [³H]DHE binding to intact platelets. Platelets were incubated with 6 nM (top) or 21 nM (bottom) [³H]DHE and various concentrations of yohimbine (□), phentolamine (△), or epinephrine (○), and binding was determined as in Fig. 1. The results shown are expressed as a percentage of total binding determined without competing drugs. The data points are the mean of triplicate determinations and are typical of three such experiments. The dissociation constant of [³H]DHE for platelet sites was 5 nM. Ascorbic acid (0.8 mM) was included in the incubations with epinephrine.

Possible existence of alpha$_1$-adrenergic receptors in platelets. To determine whether platelets possess alpha$_1$-adrenergic receptors, we examined the binding of the alpha$_1$-selective antagonists [³H]prazosin and unlabeled phenoxybenzamine. The binding of [³H]prazosin to platelets was quite unlike that expected at alpha$_1$-adrenergic receptors in several

Table 1. Competition of adrenergic compounds for [³H]prazosin binding to intact platelets*

Drug	Concn (μM)	% of control binding
Phentolamine	1	91 ± 15
	10	73 ± 5
Dihydroergocryptine	1	90 ± 16
	10	45 ± 9
(+)Epinephrine	10	95 ± 6
	100	65 ± 2
(−)Epinephrine	10	97 ± 7
	100	71 ± 11

* Binding was performed exactly as described for [³H]yohimbine binding in Fig. 1 except that 10 nM [³H]prazosin was used and 0.8 mM ascorbate and the competing drugs were added to the incubation buffer. The results shown are percentages of the binding that was found in the control experiments without the competing drugs, and they are the means and standard deviations of three experiments run in triplicate.

respects: (1) the competition of [^{3}H]prazosin binding by epinephrine was not stereoselective (Table 1); (2) 1 μM phentolamine or 1 μM DHE (concentrations that compete for almost all specific [^{3}H]yohimbine and [^{3}H]DHE binding) did not compete for [^{3}H]prazosin binding at all (Table 1); and (3) 'specific' [^{3}H]prazosin binding (defined either as binding competed for by 10 μM phentolamine or by 10 μM prazosin) was not saturable with increasing radioligand concentrations.

We tested the ability of phenoxybenzamine, which is generally considered to be an alpha$_1$-selective antagonist [6], to block [^{3}H]DHE and [^{3}H]yohimbine sites in intact platelets (Fig. 5). Phenoxybenzamine blocked these sites in an irreversible manner (blockade was identical whether the phenoxybenzamine was washed away or not) but the concentrations required for blockade ($\sim$1 μM) were far higher than those observed ($\sim$10 nM) at alpha$_1$-adrenergic receptors [20] in other tissues. Thus, results with both [^{3}H]prazosin and phenoxybenzamine indicated that human platelets do not contain alpha$_1$-adrenergic receptors.

[^{3}H]DHE binding and other amine sites on platelets. To explain the difference between [^{3}H]DHE and [^{3}H]yohimbine binding, we wondered whether [^{3}H]DHE binds to serotonin receptors or uptake sites in platelets as it does in other tissues [17]. We therefore compared the competition of many concentrations of serotonin and imipramine for [^{3}H]DHE and [^{3}H]yohimbine sites on platelets (Fig. 6). Both compounds competed identically for sites recognized by the two radioligands. In four experiments, the dissociation constant of serotonin averaged 82 μM for competition with [^{3}H]DHE and 69 μM with [^{3}H]yohimbine; the dissociation constants for competition by imipramine were 9.2 and 5.0 μM respectively. Inspection of the data (Fig. 6) reveals no evidence of a biphasic binding curve; computer analyses of the data confirm that the drugs competed for a single class of radioligand binding sites. Thus, the difference in the number of [^{3}H]DHE and [^{3}H]yohimbine sites does not appear to have resulted from the binding of [^{3}H]DHE to these other sites involved in amine action in the platelet [21–25].

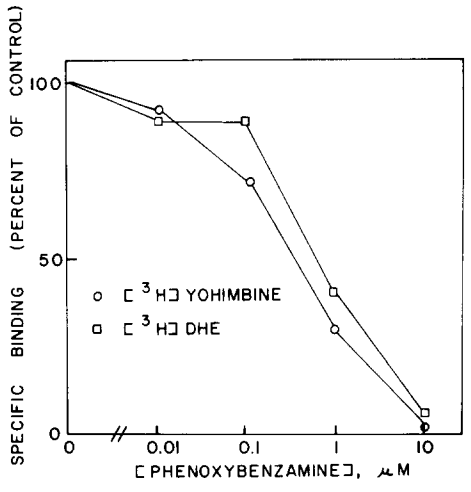

Fig. 5. Effect of preincubating platelets with phenoxybenzamine on [^{3}H]DHE and [^{3}H]yohimbine binding. We incubated washed platelets (7×10^8/ml) with various concentrations of phenoxybenzamine for 40 min at 25°, washed the platelets twice over the next 90 min, and then resuspended them to the same volume. Radioligand binding was performed with 10 nM [^{3}H]yohimbine (○) or 16 nM [^{3}H]DHE (□). Specific binding was that for which 10 μM phentolamine competed; the data points shown are the mean of three (for [^{3}H]yohimbine) or five (for [^{3}H]DHE) replicates. In parallel experiments (without phenoxybenzamine), [^{3}H]yohimbine bound to 276 sites/platelet with a K_D of 2.3 nM and [^{3}H]DHE bound to 454 sites/platelet with a K_D of 6.1 nM.

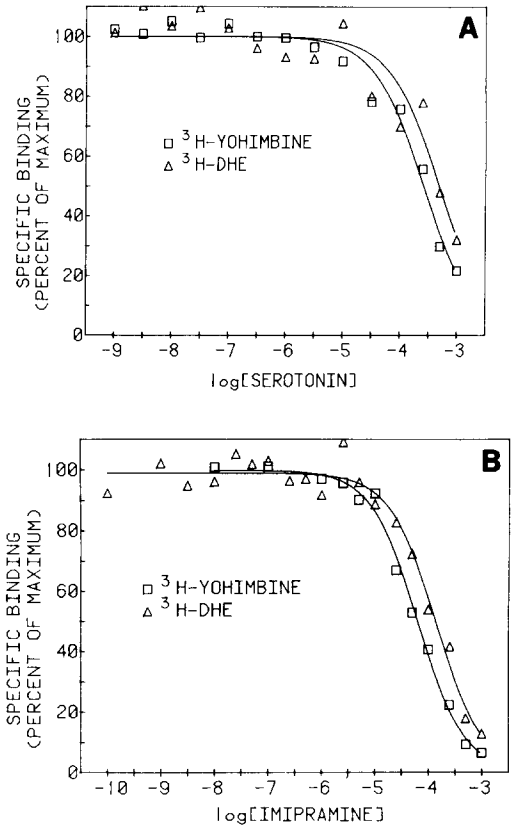

Fig. 6. Competition of [^{3}H]yohimbine and of [^{3}H]DHE binding with serotonin (A) and imipramine (B). Intact platelets were incubated with 25 nM [^{3}H]DHE or 15 nM [^{3}H]yohimbine and various concentrations of serotonin (A) or imipramine (B). The data points are the means of two (for [^{3}H]yohimbine) or five (for [^{3}H]DHE) replicates, and the curves were drawn by computer [19]. Nearly saturating concentrations of the radioligands were used so that the binding of the drugs to all the receptors, and not just a subset, was examined. In the experiments shown, the dissociation constant of serotonin was 51 μM for competition with [^{3}H]yohimbine and 88 mM for competition with [^{3}H]DHE, and the dissociation constants of imipramine were 5.4 μM and 26 μM. In all cases, computer analysis indicated that the drugs were competing for a single class of radioligand binding sites; a two-site model did not yield a statistically better fit. Experiments with serotonin were done in the presence of 1 μM imipramine to prevent uptake. This concentration of imipramine had no effect on radioligand binding.

We also found that dopamine competed identically for [^{3}H]DHE and [^{3}H]yohimbine binding with dissociation constants of 22 and 32 μM respectively.

Hydrophobicity of alpha-adrenergic radioligands. We determined that the octanol:water partition coefficient was 46:1 for [^{3}H]DHE, 12:1 for [^{3}H]yohimbine, and 8:1 for [^{3}H]rauwolscine.

DISCUSSION

Until recently, radioligand binding studies of alpha-adrenergic receptors on platelets were performed with non-selective ligands such as [^{3}H]DHE. Results of such studies together with physiological measurements have led to conflicting conclusions regarding the subtype of platelet alpha-adrenergic receptors [8–12]. We reasoned that one approach to resolving this controversy was to use subtype-selective radioligands in studies with intact platelets. We have shown previously that [^{3}H]yohimbine, an alpha$_2$-selective ligand, binds rapidly, and reversibly, to a single class of binding sites on intact platelets and platelet membranes [18]. The binding saturated at 207 ± 41 sites/platelet; the affinity of [^{3}H]yohimbine for these sites was 2.7 ± 0.7 nM, the Hill coefficient was 1.0, and adrenergic drugs competed for these sites stereoselectively and with a rank order of potency expected for alpha$_2$-adrenergic receptors. In the current study, we have investigated two other selective radioligands, [^{3}H]prazosin (alpha$_1$-selective) and [^{3}H]rauwolscine (alpha$_2$-selective). The two alpha$_2$-selective radioligands, [^{3}H]yohimbine and [^{3}H]rauwolscine, bound to a similar number of receptors, whereas [^{3}H]prazosin failed to detect alpha$_1$-adrenergic receptors on platelets. Moreover, phenoxybenzamine blocked platelet alpha-adrenergic receptors only at concentrations far higher than those generally required to block alpha$_1$-adrenergic receptors. These findings thus support previous observations indicating that all platelet alpha receptors are of the alpha$_2$ subtype [8, 9] and refute the claim that platelets have other types of alpha-adrenergic receptors [10–12]. Additional studies, perhaps including purification of the receptors, will be necessary to determine whether the alpha$_2$-adrenergic receptors on platelets are identical to those on other tissues [6].

Rauwolscine, the α-isomer of yohimbine, is a selective alpha$_2$-adrenergic antagonist that, in some systems, is somewhat more potent than yohimbine [26, 27]. [^{3}H]Rauwolscine has recently been made available, and no report of its characteristics in binding assays has yet been published. Compared with [^{3}H]yohimbine binding to alpha$_2$-adrenergic receptors on intact platelets, [^{3}H]rauwolscine has a somewhat lower affinity and binds to more nonspecific sites (in spite of an identical specific activity). Thus, [^{3}H]rauwolscine appears to offer no advantages over [^{3}H]yohimbine for studies of the alpha$_2$-adrenergic receptors on intact platelets.

We found that [^{3}H]DHE bound to more sites on platelets than did [^{3}H]yohimbine or [^{3}H]rauwolscine. This difference is consistent with published data. Pooling all our results, we found that [^{3}H]yohimbine bound to 272 ± 95 (N = 34) sites per platelet [18] whereas in four comparable studies [^{3}H]DHE bound to a mean of 341–464 sites per platelet [14, 28–30]. The nature of the "extra" [^{3}H]DHE sites is not clear, but our studies of [^{3}H]DHE binding were frustrated by the lower reproducibility and consistency of the data compared to the crisp results obtained with [^{3}H]yohimbine and [^{3}H]rauwolscine. The agonist radioligands [^{3}H]clonidine [31], [^{3}H]epinephrine [32], and [^{3}H]norepinephrine [33] bind to fewer sites than do antagonist ligands, because detectable binding of labeled agonists occurs preferentially to the "high-affinity" state of the receptor [18, 34]. Yohimbine, however, is not an agonist; in platelet aggregation experiments it is a pure antagonist [2, 18]. Moreover, the results of competition experiments with dopamine, serotonin, and imipramine seem to rule out the possibility that [^{3}H]DHE binds to dopamine or serotonin receptors or uptake sites.

A discrepancy between the number of sites identified by [^{3}H]DHE and by selective radioligands has been noted in some, but not all, previous studies of other tissues. Tharp *et al.* characterized the binding of [^{3}H]yohimbine to human abdomen adipocyte membranes and found that it bound to 85% as many sites as did [^{3}H]DHE, although the difference was not statistically significant [35]. The data for the alpha$_2$-adrenergic receptors on membranes prepared from rat heart are contradictory: in one study, [^{3}H]DHE bound to more sites than did [^{3}H]prazosin [36]; in another the number of sites identified by the two ligands was nearly equal [37]. Similarly, the data for receptors in rat liver membranes are contradictory, with a major discrepancy between [^{3}H]DHE and selective ligands found in one study [38] but not in another [39].

We were surprised to find that phentolamine competed for more [^{3}H]DHE binding than did epinephrine (Fig. 3). Similarly, Newman *et al.* [15] previously reported that phentolamine competes for more [^{3}H]DHE binding to platelets than does 1 mM epinephrine. We found, however, that phentolamine, epinephrine, norepinephrine, and yohimbine all competed for precisely the same number of [^{3}H]yohimbine binding sites. Since high concentrations of epinephrine—by definition—must bind to all the functional adrenergic receptors, these results suggest that some phentolamine-competible [^{3}H]DHE binding may be to nonadrenergic sites or to adrenergic receptors that are not functional. Our use of phentolamine to define specific binding with [^{3}H]DHE cannot, however, be the only explanation of why [^{3}H]DHE bound to more sites on platelets than did [^{3}H]yohimbine. While this manuscript was being prepared, Daiguji *et al.* [40] published a comparison of [^{3}H]DHE and [^{3}H]yohimbine binding to platelet membranes using 100 μM norepinephrine to define specific binding. They found, as we did, that [^{3}H]DHE bound to 1.5 times as many sites as did [^{3}H]yohimbine.

It is possible that [^{3}H]DHE, [^{3}H]yohimbine, and [^{3}H]rauwolscine all bind only to alpha$_2$-adrenergic receptors but that some of these receptors, perhaps those recently synthesized or internalized, are in locations that can only be reached by [^{3}H]DHE. If so, then the selective ligands might underestimate the total alpha$_2$-adrenergic receptor number. If

[^{3}H]DHE were much more lipid soluble than the alpha$_2$-selective ligands, this explanation would be quite appealing, but all the ligands are hydrophobic, making it difficult to attribute the substantial differences in their binding to the small differences in their lipid solubility.

The experiments reported here were performed with intact platelets. We also performed all these experiments (except those with [^{3}H]rauwolscine) using a particulate or 'membrane' preparation (using ~100 μg membrane protein per tube; prepared as in Ref. 18). Although we found fewer binding sites in the membranes than in the intact platelets, the qualitative conclusions in all these experiments were identical to those obtained in intact platelets.

The study of platelet alpha-adrenergic receptors is of considerable interest both because of the role platelets may play in disease and because human platelets are a convenient model for the study of less accessible alpha$_2$-adrenergic receptors (such as those in brain, liver, kidney, adipose tissue, and thyroid). Several laboratories have used [^{3}H]DHE binding to platelets or platelet membranes to study clinical disorders, receptor desensitization, and the molecular events involved in receptor function [41–45] (reviewed in Ref. 46). Our current results with intact platelets, as well as results by others using platelet membranes [40], show that [^{3}H]DHE binds to more sites than do the selective alpha$_2$ ligands. This leads to an obvious question: does [^{3}H]DHE bind to too many sites or does [^{3}H]yohimbine bind to too few? Our efforts designed to identify the nature of the "extra" [^{3}H]DHE sites have been unsuccessful, and therefore we cannot definitely answer that question. Perhaps future studies with solubilized or purified receptors or with as yet unavailable affinity probes or antibodies will ultimately allow the question to be clearly answered. For now, we believe that [^{3}H]DHE recognizes too many sites and that [^{3}H]yohimbine, therefore, is the preferred ligand. Our opinion is based on three "soft" arguments. First, platelet aggregation experiments have shown that yohimbine can completely block epinephrine-induced aggregation and the potentiation of ADP-induced aggregation caused by lower concentrations of epinephrine [10, 18]. Thus, one need not invoke the participation of the "extra" [^{3}H]DHE sites (to which yohimbine does not bind) to account for epinephrine-induced aggregation or potentiation of aggregation. Second, results with [^{3}H]DHE depend heavily on which drug is used to define nonspecific binding (Fig. 4, and Ref. 15). Phentolamine—the drug commonly used to define specific binding to alpha-adrenergic receptors—competed for more [^{3}H]DHE sites than did epinephrine, the physiologic agonist which activates the receptors. Thus, some phentolamine-competable [^{3}H]DHE sites may not be functional adrenergic receptors. With [^{3}H]yohimbine, in contrast, epinephrine and phentolamine competed for an identical number of sites. Third, results with [^{3}H]DHE were often of poor quality—especially with intact platelets—making precise experiments difficult. The results with [^{3}H]yohimbine were of consistently high quality.

In summary, we set out to use selective radioligands to resolve a controversy regarding the subtype(s) of the alpha-adrenergic receptors on human platelets. We found that alpha$_2$-selective ligands bound specifically to platelets, but that the alpha$_1$-selective ligand [^{3}H]prazosin did not, and so we conclude that all platelet alpha-adrenergic receptors are of the alpha$_2$ subtype. While doing these experiments, we noted a puzzling discrepancy between the numbers of sites identified by two alpha$_2$-selective radioligands and by the non-selective ligand [^{3}H]DHE; the basis for this discrepancy is obscure. [^{3}H]Yohimbine appears to be the preferred radioligand for quantitating platelet alpha$_2$-adrenergic receptors.

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