use of *in vitro* methods to obtain nucleic acids alkylated with metabolites or labeled xenobiotics has a définite advantage over *in vivo* and organ per fusion methods, in that much lower amounts of radioactivity are necessary for an individual experiment.

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An assay for alpha-adrenergic receptor subtypes using [^{3}H]dihydroergocryptine

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In 1948, Ahlquist [1] made the initial demarcation between alpha- and beta-adrenergic receptors. Subsequently, two subtypes of beta [2] and alpha [3, 4] receptors have been identified. A number of drugs have been proposed to discriminate in physiological experiments between these two alpha receptor subtypes by virtue of a relatively greater affinity for one or the other alpha receptor subtype [5–7]. We have recently described a method for quantitatively determining the alpha-adrenergic receptor subtypes using computer modelling of competition curves of prazosin with the non-selective antagonist [^{3}H]dihydroergocryptine ([^{3}H]DHE) [8]. Prazosin was found to be ~ 10,000-fold more potent at alpha$_1$ receptors, whereas yohimbine was ~ 500-fold more potent at alpha$_2$ receptors in rabbit uterus [8].

We now propose and validate a new and simpler method for quantifying the alpha-adrenergic receptor subtypes. This method has the advantage of not requiring complex computational techniques.

Rabbit uterine membranes were prepared and binding assays were performed as described previously [9]. Prazosin was a gift from Pfizer Inc., New York, NY. In each experiment a competition curve of [^{3}H]DHE (present at a concentration of ~ 5 nM) by prazosin was constructed; in the same membrane preparation, [^{3}H]DHE saturation curves (1–15 nM) were performed in the presence and the absence of a fixed concentration of prazosin (10^{-7}M). The data from the saturation curves were subjected to Scatchard analysis [10] to obtain estimates of the number of alpha receptor sites in the presence of prazosin (R_{alpha_2}) and the absence of prazosin (R_{total}). The difference between R_{total} and R_{alpha_2}, ($R_{total} - R_{alpha_2}$), was taken as an estimate of the number of alpha$_1$ receptors (R_{alpha_1}). Independent estimates of the proportion of alpha$_1$ and alpha$_2$ receptors were also generated by the computer modelling of prazosin competition curves. The computer modelling has been described previously in detail [8].

Briefly, using a PDP 11/45 computer, data were analyzed by a nonlinear least squares curve fitting technique [11] using a generalized model for complex ligand–receptor interactions [12] to determine the proportion of alpha receptor subtypes present in the prazosin competition curve, and to determine the number of alpha receptors in each of the saturation curves. The deviation of the observed data points from the predicted values was weighted according to the reciprocal of the predicted variance [13]. The computer simulations were based on the above computer techniques. All experiments were performed in duplicate.

The basic premises underlying the method to be described here include the following: (1) the affinity of prazosin for alpha$_1$ receptors ($K_{D_{alpha_1}} \sim 5 \times 10^{-10}$M) is so much higher than its affinity for alpha$_2$ receptors ($K_{D_{alpha_2}} \sim 5 \times 10^{-6}$M) [8] that, when it is present at a concentration of 10^{-7}M, essentially all the alpha$_1$ and virtually none of the alpha$_2$ receptors will be occupied by prazosin; (2) the binding of prazosin to the alpha$_1$ receptors is so tight that over the usual range of [^{3}H]DHE concentrations used in constructing saturation curves (1–15 nM) prazosin will not be displaced from the alpha$_1$ receptors; and hence (3) in the presence of 10^{-7}M prazosin, [^{3}H]DHE saturation curves will measure only the alpha$_2$ receptors in uterine membranes.

Computer simulations were done to test the validity of these premises. For the purposes of computer simulation, typical experimentally determined affinities of prazosin and [^{3}H]DHE were assumed: for prazosin these were $K_{D_{alpha_1}} = 5 \times {}^{-10}$M and $K_{D_{alpha_2}} = 5 \times 10^{-6}$M; and for the non-selective radioligand [^{3}H]DHE [9] the affinities were $K_{D_{alpha_1}} = K_{D_{alpha_2}} = 5 \times 10^{-9}$M. Also, the proportions of alpha receptor subtypes were set at alpha$_1$ = 20 per cent and alpha$_2$ = 80 per cent. The simulations revealed that even at the highest [^{3}H]DHE concentrations used in actual experiments (15 nM), prazosin filled more than 97 per cent of the alpha$_1$ sites and less than 5 per cent of the alpha$_2$ sites. Thus, the computer simulations substantiate the basic assumptions of the 'double' saturation curve or Scatchard plot technique proposed here.

Fig. 1 illustrates the results of experiments with the same membrane preparation, wherein both a detailed prazosin competition curve (Fig. 1A) and [^{3}H]DHE Scatchard plots

in the presence and the absence of 10^{-7}M prazosin (Fig. 1B) were made. The proportions of alpha$_1$ and alpha$_2$ receptors in the membranes were determined to be: (1) by computer modelling of the prazosin competition curve—alpha$_1$ = 24 per cent, alpha$_2$ = 76 per cent and (2) by linear regression analysis of the Scatchard plots of saturation curves—alpha$_1$ = 16 per cent, alpha$_2$ = 84 per cent. In a total of four such experiments using four different membrane preparations with varying proportions of alpha$_1$ and alpha$_2$ receptors (Table 1), there was very close agreement between estimates provided by the computer modelling of prazosin competition curves and those obtained by routine linear regression analysis of the [^{3}H]DHE saturation curves. The marked variability in alpha-adrenergic subtype proportions from one membrane preparation to another has been a consistent observation and remains unexplained; it is not a result of estrogen or progesterone treatment (B. B. Hoffman and R. J. Lefkowitz, unpublished observations).

It is also noteworthy that the K_D of [^{3}H]DHE was unchanged by the presence or the absence of 10^{-7}M prazosin, being 4.7 ± 1.0 and 4.8 ± 0.4 nM, respectively ($P < 0.48$). This finding is consistent with several of the basic premises underlying our method. First, it confirms that [^{3}H]DHE binds with equal affinity to alpha$_1$ and alpha$_2$ receptors. Second, it agrees with the notion that prazosin

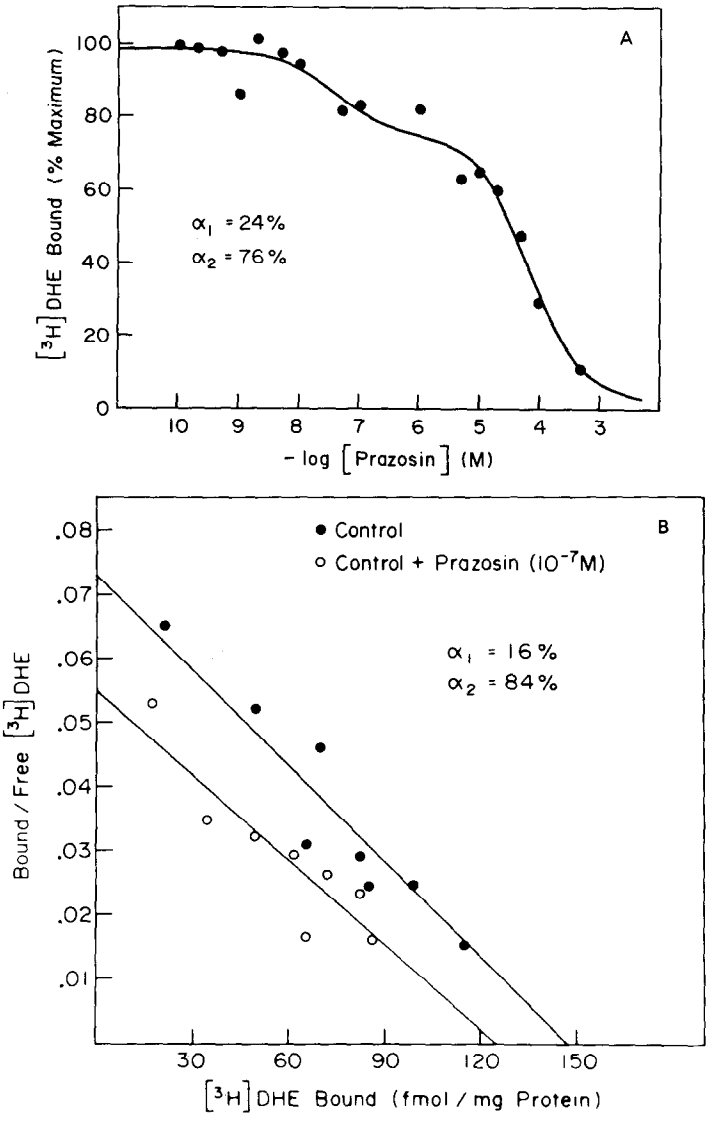

Fig. 1. Estimates of the proportions of alpha-adrenergic receptor subtypes determined by (A) computer modelling of a prazosin competition curve and (B) Scatchard analysis of [^{3}H]DHE saturation curves. In panel (A), the concentration of [^{3}H]DHE in the assay was 5 nM. The complex biphasic competition curve modelled to two classes of receptors with the alpha$_1$ component = 24 per cent and the alpha$_2$ component = 76 per cent. In the same membrane preparation, [^{3}H]DHE saturation curves in the presence and the absence of prazosin (10^{-7}M) were constructed and the derived Scatchard plots were fitted by linear regression analysis. In panel (B), the Scatchard plot of the control membranes indicated the presence of 147 fmoles/mg of protein ($r = 0.93$) of total alpha receptors, and the plot in the presence of 10^{-7}M prazosin yielded alpha$_2$ = 124 fmoles/mg of protein ($r = 0.89$). Thus, the alpha receptor proportions were: alpha$_1$ = 16 per cent, and alpha$_2$ = 84 per cent, in good agreement with the results of the computer modelling.

Table 1. Comparison of quantification of alpha-adrenergic receptor subtypes by computer modelling of prazosin competition curves and Scatchard analysis of [^{3}H]DHE binding*

Expt. No.	Proportion of alpha-adrenergic receptor subtypes			
	Scatchard analysis of [^{3}H]DHE saturation curves		Prazosin competition curve analysis	
1	$alpha_1$ = 16%	$alpha_2$ = 84%	$alpha_1$ = 24%	$alpha_2$ = 76%
2	$alpha_1$ = 12%	$alpha_2$ = 88%	$alpha_1$ = 0%	$alpha_2$ = 100%
3	$alpha_1$ = 0%	$alpha_2$ = 100%	$alpha_1$ = 0%	$alpha_2$ = 100%
4	$alpha_1$ = 41%	$alpha_2$ = 59%	$alpha_1$ = 43%	$alpha_2$ = 57%
Mean	$alpha_1$ = 17 ± 9%	$alpha_2$ = 83 ± 9%	$alpha_1$ = 17 + 10%	$alpha_2$ = 83 ± 10%

* The proportions of $alpha_1$ and $alpha_2$ receptors in each experiment were determined as indicated in the text. The good agreement in the estimates of the proportions of alpha receptor subtypes in each experiment (and overall for the meaned data) for the two techniques is evident. A different membrane preparation was used for each experiment.

at a concentration of 10^{-7}M is not competitively interacting with the $alpha_2$ receptors, since such an interaction would have decreased the apparent affinity of [^{3}H]DHE binding.

In separate computer simulations, we assessed the feasibility of using an analogous technique with the $alpha_2$ selective antagonist yohimbine to measure directly $alpha_1$ receptors in uterine membranes with the [^{3}H]DHE saturation curve analysis as described above. These simulations revealed that over the range of [^{3}H]DHE concentrations routinely employed in our assays (1–15 nM), there was no concentration of yohimbine which exclusively occupied most of the $alpha_2$ receptors but spared the $alpha_1$ receptors. This reflects the fact that, while yohimbine has about a 500-fold higher potency at $alpha_2$ than $alpha_1$ receptors [8], its discriminatory power is still inadequate for use in this fashion.

It is important to have available reliable methods for determining the alpha-adrenergic receptor subtypes in tissues with a view to understanding their detailed regulation. We have demonstrated previously that computer modelling of detailed [^{3}H]DHE competition curves of such selective antagonists as prazosin and yohimbine [8] could be used to define the proportions of $alpha_1$ and $alpha_2$ receptors in membrane preparations. We have now demonstrated that traditional Scatchard plots may also be used to estimate reliably the proportions of $alpha_1$ and $alpha_2$ receptors in membranes from rabbit uterus. This method is simpler and also provides estimates of receptor subtype proportions which are in good agreement with the values obtained from computer modelling of competition curves. The use of a similar 'double' Scatchard plot technique was proposed previously to determine $alpha_1$ and $alpha_2$ receptor subtypes in brain membranes [6]. However, in that study no theoretical justification was provided for the technique nor were the estimates compared with those obtained from competition curves. Indeed, it is necessary to be cautious in using this approach. Prazosin with about a 10,000-fold higher affinity at $alpha_1$ than $alpha_2$ sites was found to be adequate for this purpose, whereas yohimbine (~ 500-fold $alpha_2$ selective in the uterus) was found by computer simulation to be inadequate. Thus, for other ligands in other tissues, the estimates obtained by the two techniques need to be compared to assure their equivalence.

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