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Estradiol Reduction of the Agonist High Affinity Form of the α_2-Adrenoceptor in the Hypothalamus of Female Rats: Identification as the α_{2D} Subtype

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SUMMARY

These studies examined which α_2-adrenoceptor subtype is expressed in the hypothalamus and preoptic area and the influence of estradiol administration on α_2-adrenoceptors in the hypothalamus of female rats. The α_2-adrenoceptor antagonist [^{3}H]RX821002 bound to a single site in hypothalamus, preoptic area, and cortex membranes, with high affinity and low nonspecific binding, as determined by Scatchard and kinetic binding analyses. Competition for [^{3}H]RX821002 binding in the hypothalamus and preoptic area by various noradrenergic agonists and antagonists revealed a unique pharmacological specificity with a high degree of similarity to that of the α_{2D}-adrenoceptor. Norepinephrine displacement of [^{3}H]RX821002 binding in hypothalamic membranes from ovariectomized animals was monophasic and characterized by high affinity. In contrast, norepinephrine competition for [^{3}H]RX821002 binding sites in the hypothalamus from rats exposed to estradiol for 48 hr was biphasic, and norepinephrine bound to both a high (18%) and a low (82%) affinity site in these membranes. Thus, the formation of agonist high affinity α_{2D}-adrenoceptor complexes was inhibited by prior exposure to estrogen. In both control and estradiol-exposed hypothalamic membranes, 100 μM 5′-guanylylimidodiphosphate [Gpp(NH)p] converted the norepinephrine competition curves to ones characterized by monophasic, low affinity binding. In addition, binding of the full α_2-adrenoceptor agonist [^{3}H]UK-14,304 in the hypothalamus and preoptic area of female rats was concentration-dependently diminished by Gpp(NH)p treatment. Complete loss of [^{3}H]UK-14,304 binding was effected by 100 μM Gpp(NH)p. This suggests that [^{3}H]UK-14,304 may be useful in labeling the agonist high affinity state of α_2-adrenoceptors. Decreasing the incubation temperature in saturation studies from 25° to 0° increased [^{3}H]UK-14,304 binding in hypothalamic membranes of control rats but not in membranes from estradiol-treated rats. Estradiol treatment for 48 hr decreased [^{3}H]UK-14,304 binding in hypothalamic membranes by 34% (0°) to 60% (25°), without changing the K_d. These results suggest that the α_{2D}-adrenoceptor is the predominant subtype in the hypothalamus and preoptic area of female rats and that estradiol treatment markedly reduces the number of α_{2D}-adrenoceptors in the agonist high affinity state.

A major regulator of NE release at both central and peripheral sites is the α_2-adrenoceptor (1). Activation of α_2-adrenoceptors can potently inhibit NE release from a variety of tissues (2–4). Furthermore, these receptors have been localized on the nerve terminals of noradrenergic neurons (5–7). This provides the possibility that released NE can act presynaptically to inhibit its own release. This mechanism has been termed autoinhibition of neurotransmitter release. We previously demonstrated that NE release in HYP slices of OVX rats is under potent α_2-adrenoceptor-mediated inhibition (8). In those studies, the imidazoline α_2 antagonists idazoxan and RX were far more effective in increasing NE release than were the alkaloid α_2 antagonists yohimbine and rauwolscine. The pharmacology of this response suggested mediation by the α_{2D}-adrenoceptor subtype. In addition, we demonstrated that *in vivo* administration of E_2, a steroid hormone known to increase hypothalamic NE release, may do so in part by attenuating α_2-adrenoceptor inhibition of NE release (8). Surprisingly, however, the same E_2 doses failed to measurably down-regulate α_2-adrenergic binding sites when [^{3}H]idazoxan was used in ligand binding assays (9).

There are presently four known subtypes of α_2-adrenoceptor (α_{2A}, α_{2B}, α_{2C}, and α_{2D}), as recently reviewed by Bylund (10). The α_{2A}-adrenoceptor was cloned from human platelet cells by Kobilka *et al.* (11). The rat homologue of the human α_{2A}-adrenoceptor has been termed the α_{2D}-adrenoceptor. It has 89%

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ABBREVIATIONS: NE, norepinephrine; HYP, hypothalamus; OVX, ovariectomized; RX, RX821002; E_2, estradiol; POA, preoptic area; CTX, cortex; UK-14,304, 5-bromo-6-(2-imidazoline-2-yl-amino)quinoxaline; Gpp(NH)p, 5′-guanylylimidodiphosphate.

amino acid sequence identity with the α_{2A} receptor and demonstrates a similar pharmacological specificity, with one notable exception; the α_{2D}-adrenoceptor binds the alkaloid α_2 antagonists with markedly lower affinity than does the human α_{2A}-adrenoceptor.

There is also increasing evidence that α_{2D}-adrenoceptors mediate inhibition of NE release in several tissues. For example, in both the rat vas deferens (12) and isolated perfused rat kidney (13) the pharmacology of the α_2-adrenoceptor mediating inhibition of NE release was similar to that of the α_{2D}-adrenoceptor. The present study was undertaken to identify and characterize the α_2-adrenoceptor subtype expressed in the HYP and POA of female rats and to test the hypothesis that E_2 attenuation of α_2-adrenoceptor-mediated inhibition of NE release is achieved by regulating the agonist high affinity state of the α_2-adrenergic receptor.

Materials and Methods

Animals and hormone treatments. Female Sprague-Dawley rats (150–175 g) were obtained from Taconic Farms (Germantown, NY) and were bilaterally OVX, under Metofane anesthesia, 4–7 days before use. OVX rats were given two subcutaneous injections of either 2 μg of E_2 benzoate or peanut oil (controls), 24 and 48 hr before sacrifice.

Membrane preparation. Animals were killed by decapitation, and the brains were rapidly removed and placed in ice-cold buffer containing 50 mM Tris and 10 mM $MgCl_2$, pH 7.4. The entire HYP and POA were dissected over ice and removed as a block. Based on anatomical landmarks, the block was divided (with a McIlwain tissue chopper) into separate portions containing the middle HYP and POA, as described previously by Etgen and Petitti (14). The HYP slices included the arcuate nucleus, the ventromedial nucleus, the dorsomedial nucleus, and much of the lateral HYP. A sample of frontal CTX was also removed from some animals. Tissue from the HYP, POA, and CTX of OVX control and E_2-treated female rats was homogenized separately and centrifuged for 10 min at 20,000 × *g*, the supernatant was discarded, and the pellet containing the crude membrane fraction was frozen at −70°. Before use the pellets were resuspended in buffer containing 50 mM Tris and 10 mM $MgCl_2$, pH 7.4.

Binding studies and data analysis. For Scatchard analysis aliquots of suspended membranes were incubated with either 0.2–10 nM [^{3}H]RX or 0.1–15 nM [^{3}H]UK-14,304 for 4 hr at 25° or overnight at 0°. Nonspecific binding was determined in a parallel set of incubations that included a 1000-fold excess of nonradioactive RX or UK-14,304. Specific binding was determined by subtracting nonspecific binding from total binding. Receptor affinities (K_d) and numbers (B_{max}) were determined by computer-assisted curve fitting using the relationship (15):

$$\frac{[B]}{[L]} = \frac{B_{max}}{K_d} - \frac{[B]}{K_d}$$

where [B] is the concentration of bound radioligand at a given ligand concentration, [L]. For competition studies, 5.0 nM [^{3}H]RX was incubated with 12 concentrations (10^{-11} to 10^{-3} M) of nonradioactive noradrenergic agonists or antagonists in triplicate for 2 hr at 25° or overnight at 0°. Curves were fit using a four-parameter logistic equation (16):

$$[B] = \sum_{i=1}^{2} \left(\frac{[B_0] - NS}{1 + ([I]/IC_{50})^H} + NS \right)$$

where [B] is equal to the concentration of receptor-ligand complex at various concentrations of unlabeled inhibitor ([I]), $[B_0]$ is equal to bound radioligand when [I] = 0, NS represents nonspecific binding, the IC_{50} is [I] where [B] = ½$[B_0]$, H is the pseudo-Hill coefficient (H = 1 for single-affinity models and $H < 1$ when multiple affinity states are present), and i is 1 for a one-site model or 2 for a two-site model. Binding reactions were terminated by rapid filtration through glass fiber filters that had been presoaked in 1% polyethylenimine for 1 hr. Aliquots of each membrane suspension were analyzed for protein content by the method of Lowry *et al.* (17). B_{max} was expressed as fmol/mg of protein.

Statistics. Analysis of variance was used to evaluate significant differences between means. When the analysis of variance was significant ($p < 0.05$), between-group differences were determined by planned *post hoc* comparisons using the Newman-Keuls multiple-range test. Testing for statistical differences between various binding models was with an F test (18).

Materials. E_2 benzoate was purchased from Steraloids, Inc. (Wilton, NH). Metofane was obtained from Pitman-Moore, Inc. (Atlanta, GA). Radiolabeled [^{3}H]RX (specific activity, 55 Ci/mmol) was obtained from Amersham (Arlington, IL), and [^{3}H]UK-14,304 (specific activity, 70.9 Ci/mmol) was obtained from New England Nuclear (Boston, MA). Unlabeled idazoxan, RX, rauwolscine, WB-4101, phentolamine, and UK-14,304 were purchased from Research Biochemicals, Inc. (Natick, MA). Yohimbine, propranolol, and oxymetazoline were purchased from Sigma Chemical Co. (St. Louis, MO). Prazosin was obtained from Pfizer (Groton, CT).

Results

Kinetic and equilibrium studies indicate that [^{3}H]RX binding to α_2-adrenoceptors in HYP, POA, and CTX from female rats is saturable and of high affinity. The association and dissociation of [^{3}H]RX binding to HYP membrane preparations were monoexponential, as determined by nonlinear least squares analysis; likewise, a semilogarithmic transformation of the data was linear, indicating that binding was probably to a single site (Fig. 1). Dissociation was slow at 0°, with a dissociation rate constant (k_{-1}) of 1.36 ± 0.07 × 10^3 min^{-1}. Association experiments indicated that, at 0°, 40–60 min was necessary to reach steady state. Binding was stable for at least 24 hr. Similar results were obtained from CTX and POA membrane preparations (Table 1). Hormone treatment had no effect on the rate constants for [^{3}H]RX binding. The equilibrium dissociation constant (K_d) was in close agreement with the kinetically determined K_d (Table 1). Equilibrium binding experiments with [^{3}H]RX showed that binding to HYP membranes was saturable, with low levels of nonspecific binding. Scatchard and Hill plot analyses indicated a single affinity for binding (Fig. 1). Exposure to E_2 for 48 hr did not decrease α_2-adrenoceptor number in the HYP of female rats (Table 1).

The α_{2D}-adrenoceptor is the major subtype present in the HYP and POA. The competition for [^{3}H]RX binding by various nonradioactive adrenergic ligands showed a distinct pharmacological profile (Fig. 2). All competing nonradioactive antagonists had slope factors near unity, indicating a single affinity for interaction with the RX-labeled site. Table 2 presents a summary of pK_i values for several agonists and antagonists, in order of decreasing affinity. The rank order of potency of these agents in both the HYP and POA markedly resembles the affinities displayed by the α_{2D} receptor subtype (19). This is demonstrated by a direct plot of the affinities of these drugs in competing for [^{3}H]RX binding in HYP membranes versus the affinities of these drugs in cell types known to express specific α_2 receptor subtypes. A strong correlation of affinities was obtained when the HYP was compared with bovine pineal gland and rat submaxillary gland, tissues known to contain the α_{2D} receptor subtype (Fig. 3). All other subtype comparisons yielded poor correlations. These data strongly suggest that [^{3}H]

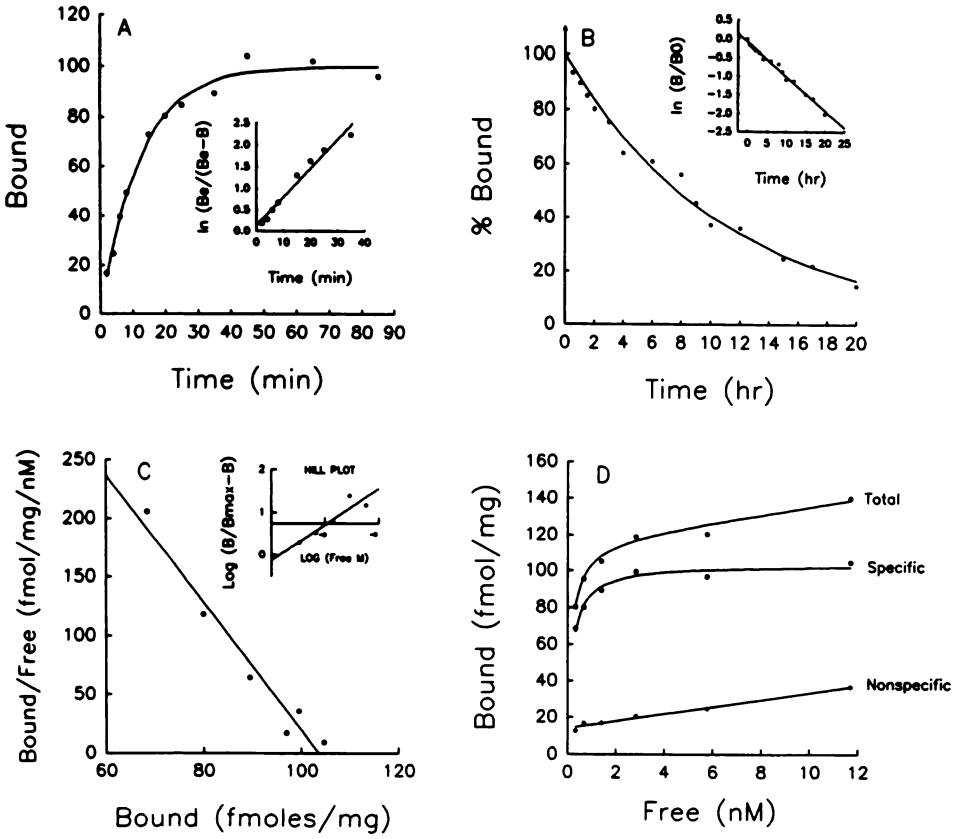

Fig. 1. Kinetic and Scatchard analyses of [^{3}H]RX binding in the HYP of female rats. A, Association experiments were conducted by mixing 5.0 nM [^{3}H]RX with membrane aliquots for various lengths of time and terminating the reaction by filtration with ice-cold Tris-$MgCl_2$ buffer. Nonspecific binding was determined with membrane aliquots that had been preincubated with 5 μM nonradioactive RX. Specific binding was calculated both by subtracting a linear least squares determination of nonspecific binding from total binding and via computer-aided curve fitting of total bound radioligand. Bound radioligand is plotted as a percentage of radioligand bound at steady state. Inset: semilogarithmic transformation. B, Dissociation experiments were conducted by mixing 5.0 nM [^{3}H]RX with membrane aliquots on ice for 2 hr; at various times, dissociation was initiated by the addition of a large excess of unlabeled RX to prevent reassociation of [^{3}H]RX. Nonspecific binding was determined in tubes incubated with 5 μM unlabeled RX. Specific binding was determined both by subtracting nonspecific binding from total binding and by using computer-aided curve fitting of total bound radioligand. Inset: semilogarithmic transformation. *B0*, amount of [^{3}H]RX bound before any dissociation; *Be*, amount of [^{3}H]RX bound at steady state. C and D, saturation plots (D) and Scatchard analysis (C) of [^{3}H]RX binding in aliquots of suspended membranes incubated with 0.2–10 nM [^{3}H]RX overnight at 0°; 1000-fold excess unlabeled RX was used to define nonspecific binding. Inset C: Hill plot.

TABLE 1

Rate constants and kinetically derived K_d values of [^{3}H]RX binding to HYP, POA, and CTX membranes from control and E_2-treated rats

The kinetically derived K_d values were in good agreement with the K_d values derived from Scatchard analysis of saturation isotherms conducted at 0° (HYP control, 0.22 ± 0.03 nM; HYP with E_2, 0.28 ± 0.01 nM). Data shown are the means ± standard errors of two to four independent replications.

Tissue	Treatment	Dissociation constant, K_{-1}	Association constant, K_{+1}	Kinetically derived K_d	B_{max}
		$min^{-1} \times 10^3$	$nM^{-1}\ min^{-1}$	nM	fmol/mg
HYP	Control	1.36 ± 0.07	0.009 ± 0.002	0.17 ± 0.06	112.3 ± 2.8
	E_2	1.89 ± 0.06	0.009 ± 0.001	0.21 ± 0.01	147.3 ± 34
POA	Control	1.61 ± 0.02	0.005 ± 0.001	0.38 ± 0.12	
	E_2	1.89 ± 0.01	0.011 ± 0.006	0.24 ± 0.15	
CTX	Control	1.48 ± 0.04	0.005 ± 0.002	0.33 ± 0.11	
	E_2	1.80 ± 0.07	0.005 ± 0.001	0.39 ± 0.07	

RX labels the α_{2D} receptor subtype and that this is the major subtype expressed in the HYP and POA of female rats.

E_2 diminishes high affinity binding of NE to α_2-adrenergic receptors. NE displaced [^{3}H]RX in HYP membranes prepared from OVX control females with high affinity (pK_i = 7.69 ± 0.06) and in a monophasic fashion (Fig. 4). In contrast, NE competition for [^{3}H]RX binding sites in the HYP from E_2-treated rats was biphasic, indicating that NE binds to both high and low affinity sites (Fig. 4). Resolution of the biphasic competition curves revealed a high affinity (pK_i = 8.11 ± 0.43) site (R_H) comparable to that seen in OVX rats, as well as a low affinity (pK_i = 5.60 ± 0.05) site (R_L). R_H accounted for only 18% of the binding sites in the HYP from E_2-treated rats (Table 3). In the absence of Gpp(NH)p, NE competition curves for HYP membranes from control rats had slopes less than unity, and the pK_i was lower than in E_2-exposed membranes. This suggests the presence of some R_L in OVX control HYP, although resolution of competition curves into two sites was not a statistically improved fit. Treatment of membranes from both control and E_2-treated rats with 100 μM concentrations of the nonhydrolyzable GTP analogue Gpp(NH)p eliminated the high affinity component, leaving a single site of low affinity (Fig. 4).

Hormone treatment reduces UK-14,304 binding in HYP membranes. UK-14,304 is a full α_2-adrenoceptor agonist that is useful in specifically labeling the agonist high affinity state of α_2-adrenoceptors (20, 21). We found that [^{3}H]UK-14,304 binding was only to high affinity sites in the HYP and POA membranes of female rats. In fact, conversion of high affinity sites to low affinity sites by treatment with Gpp(NH)p resulted in a concentration-dependent loss of [^{3}H]UK-14,304 binding (Fig. 5). The pK_i values for Gpp(NH)p-induced loss of binding were as follows: in the HYP, control, 7.79 ± 0.16; E_2, 7.86 ± 0.05; in the POA, control, 7.43 ± 0.08; E_2, 7.53 ± 0.26 (means ± standard errors of three independent determinations).

E_2 treatment significantly lowered the B_{max} of [^{3}H]UK-14,304 binding in the HYP, compared with OVX control HYP ($p < 0.0001$), but did not alter the K_d (Fig. 6; Table 4). Binding of [^{3}H]UK-14,304 to both control and hormone-treated membranes was saturable and demonstrated low nonspecific binding; Hill plots indicated that binding was probably to a single noncooperative site (Fig. 6). Interestingly, the difference be-

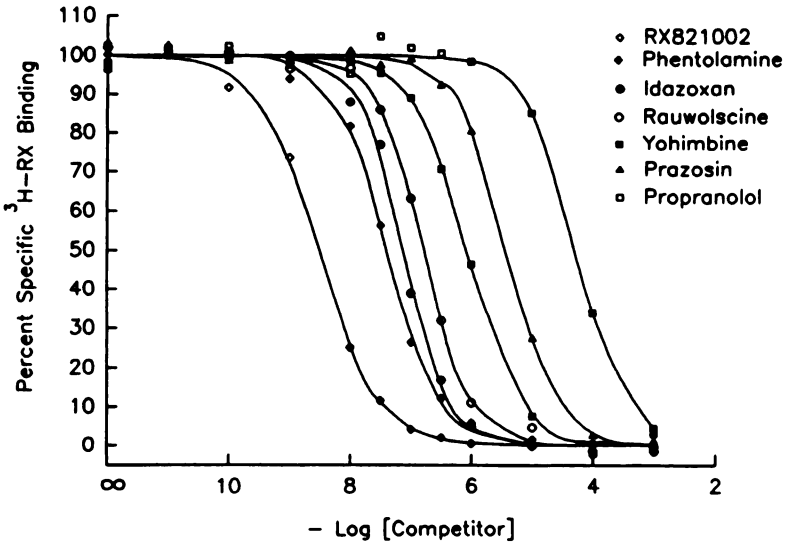

Fig. 2. Competition with [^{3}H]RX binding by adrenergic ligands, showing a distinct pharmacological profile. HYP membranes were incubated for 4 hr at 25° with 5.0 nM [^{3}H]RX and competing nonradioactive ligands (over a concentration range from 1 mM to 10 pM). Specific binding was defined as the amount of [^{3}H]RX bound in the absence of competing ligand minus the amount bound in the presence of 1000-fold excess RX.

TABLE 2

Rank order of potencies of antagonists and agonists in competing for [^{3}H]RX binding sites in membranes from HYP and POA

Binding of 5.0 nM [^{3}H]RX was determined at 25° in the presence of a range of concentrations (1 mM to 10 pM) of nonradioactive antagonists and agonists, as described in the legend to Fig. 2. Data represent means ± standard errors of three or four independent replications.

	pK$_i$ for competition with [^{3}H]RX binding			
	HYP		POA	
	Control	E_2	Control	E_2
Antagonists				
RX	9.15 ± 0.02	9.27 ± 0.07	9.39 ± 0.24	9.46 ± 0.17
Phentolamine	7.95 ± 0.24	8.26 ± 0.13	8.11 ± 0.17	8.21 ± 0.17
Idazoxan	7.97 ± 0.06	7.77 ± 0.04	8.02 ± 0.02	8.17 ± 0.03
Rauwolscine	7.39 ± 0.07	7.60 ± 0.01	7.32 ± 0.01	7.62 ± 0.01
Yohimbine	6.81 ± 0.01	6.83 ± 0.03	6.83 ± 0.05	6.77 ± 0.02
WB4101	6.08 ± 0.05	6.18 ± 0.22	6.59 ± 0.40	6.51 ± 0.32
Prazosin	5.99 ± 0.05	6.16 ± 0.05	6.07 ± 0.11	6.39 ± 0.09
Propranolol	4.98 ± 0.03	5.23 ± 0.28	5.13 ± 0.02	5.05 ± 0.05
Agonists				
Oxymetazoline	8.38 ± 0.11	8.21 ± 0.14	8.38 ± 0.14	8.23 ± 0.11
UK-14,304	7.61 ± 0.06	7.80 ± 0.09	ND[a]	ND

[a] ND, not done.

tween the B_{max} values in control and hormone-treated membranes was accentuated when the saturation studies were carried out at 0°. As the temperature was lowered from 25° to 0°, the control B_{max} in HYP membranes increased significantly, from 147 to 202 fmol/mg ($p < 0.02$). In contrast, the B_{max} for E_2-treated HYP membranes did not change as a function of temperature (Table 4).

Discussion

The present study demonstrates that 1) the α_{2D}-adrenoceptor is the major subtype present in the HYP and POA of female rats and 2) E_2 treatment markedly inhibits the formation of the agonist high affinity state of the α_{2D}-adrenoceptor in the HYP of female rats. The first conclusion is supported by the finding that a plot of the pK_i values of various noradrenergic agonists and antagonists in competing for [^{3}H]RX binding in the HYP and POA of female rats versus the pK_i values seen in tissues that express specific α_2-adrenoceptor subtypes (10, 19, 22, 23) yields a good correlation only for rat submaxillary gland and bovine pineal gland (Fig. 3), two tissues known to express the α_{2D}-adrenoceptor (24, 25). The unique pharmacological specificity displayed in both the HYP and POA and the demonstration that [^{3}H]RX binds to only a single saturable site, with low nonspecific binding (Fig. 1), strongly suggest that the α_{2D}-adrenoceptor is the major subtype present in these brain regions. This conclusion is further supported by our earlier findings that the imidazoline α_2 antagonists idazoxan and RX can dramatically enhance KCl-evoked NE release from HYP slices, whereas the alkaloid α_2 antagonists are far less effective at facilitating NE release (8). The α_{2D}-adrenoceptor is believed to be the rat homologue of the human platelet α_{2A}-adrenoceptor (10, 19, 26–28). Pharmacologically, the rat α_{2D}-adrenoceptor displays 10- and 20-fold lower affinity for the alkaloid α_2 antagonists yohimbine and rauwolscine, respectively, than does the human α_{2A} receptor (27, 28). Thus, the pharmacology of our functional measurement of α_2-adrenoceptor regulation of NE release also supports the conclusion that the α_{2D}-adrenoceptor is the major subtype present in the HYP of female rats.

This study did not determine the α_2-adrenoceptor subtype expressed in the CTX of female rats. However, previous work from our laboratory[1] and others (29–31) suggests that the CTX may express a mixed population of α_2 receptor subtypes. Scatchard analysis of [^{3}H]RX binding in CTX membranes incubated at 37° indicated that this ligand may display a lower affinity than that observed in HYP and POA membranes. In addition, the enthalpy and entropy contributions to [^{3}H]RX binding in CTX membranes differ from those measured in the HYP and POA.[1]

Several lines of evidence also support the conclusion that E_2 treatment potently inhibits the formation of the agonist high affinity state of the α_{2D}-adrenoceptor in the HYP. First, NE binding in HYP membranes from control OVX rats is characterized almost entirely by binding to a single, high affinity site. In contrast, NE competition for [^{3}H]RX binding in HYP membranes from E_2-treated animals is biphasic (Fig. 4). Resolution of these biphasic curves shows that R_H accounts for only 18% of the binding isotherm. The remaining low affinity component, R_L, predominates (82%) in membranes from E_2-treated

[1] G. B. Karkanias and A. M. Etgen. A novel mechanism of estrogen regulation of signal transduction revealed by thermodynamics of α_2-adrenoceptor binding. Submitted for publication.

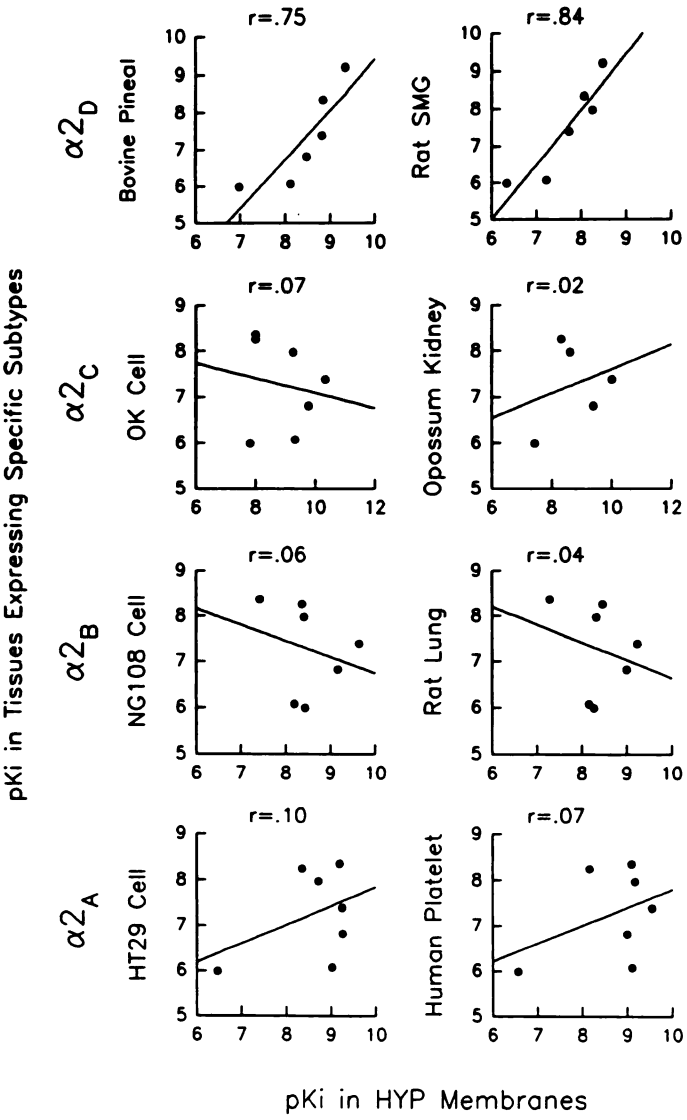

Fig. 3. Correlation plots of the pK_i values in the HYP versus the pK_i values in tissues known to express specific α_2-adrenoceptor subtypes. The pK_i values for a variety of adrenergic ligands closely match the pK_i profile seen in tissues known to contain the α_{2D}-adrenoceptor. There is a poor correlation of the pK_i values with those of other subtypes. The correlation plots were produced by graphing the pK_i values in the HYP, in order of increasing affinity (prazosin, WB4101, yohimbine, rauwolsciine, idazoxan, oxymetazoline, and phentolamine), against the pK_i values for the same drugs in tissues known to express specific α_2 receptor subtypes (19). The following values were not available: WB4101 values for opossum kidney and rat submaxillary gland (*SMG*), phentolamine value for bovine pineal gland, and oxymetazoline value for opossum kidney.

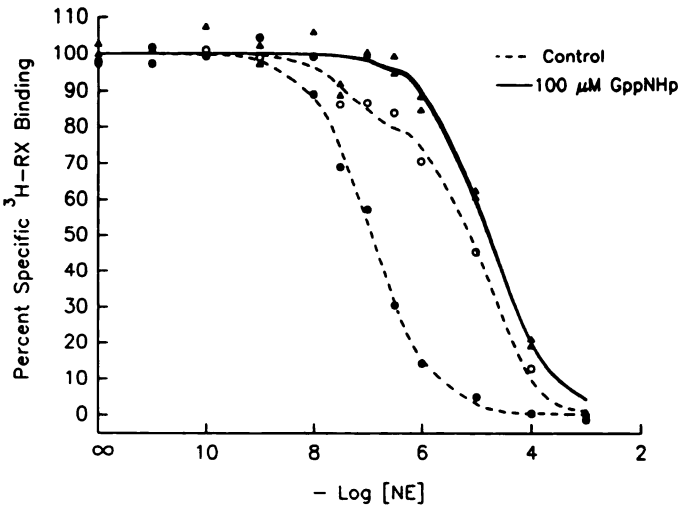

Fig. 4. Reduction of the high affinity component of NE binding by E_2 treatment. Nonlinear least squares regression analysis of NE competition for [^{3}H]RX in the absence of Gpp(NH)p (– – –) in membranes from E_2-treated female rats (*open symbols*) indicated that a two-site model was the best fit. The high affinity site had affinity similar to that seen in curves for HYP membranes from OVX control rats (*closed symbols*). The low affinity site resembled sites seen when membranes were pretreated with 100 μM Gpp(NH)p (——). These plots are representative of three independent replications.

TABLE 3

Reduction of agonist high affinity binding in hypothalamic membranes by E_2 treatment

In the absence of Gpp(NH)p, NE competition for [^{3}H]RX binding in HYP membranes from control rats was characterized entirely by high affinity binding (R_H). In membranes from E_2-exposed females, R_H decreased to 18%. Treatment with 100 μM Gpp(NH)p converted the binding sites to the low affinity form (R_L) in OVX control and hormone-treated membranes. Competition studies were carried out as described in the legend to Fig. 2. Data represent the means ± standard errors of three independent replications.

Treatment	pK_H	pK_L	R_H/R_L	Slope
			%	
−Gpp(NH)p				
Control	7.69 ± 0.06		100/0	0.67 ± 0.10
E_2	8.11 ± 0.43	5.60 ± 0.05	18.4/81.6	0.57 ± 0.06
+Gpp(NH)p				
Control		5.50 ± 0.02	0/100	0.78 ± 0.03
E_2		5.47 ± 0.01	0/100	0.79 ± 0.02

animals and resembles the single site seen when α_{2D}-adrenoceptors are converted to low affinity by Gpp(NH)p (Table 3). These results demonstrate that 48-hr exposure to E_2 markedly inhibits the formation of the R_H state of the α_{2D}-adrenoceptor, converting the receptor population to a predominantly R_L state. The pK_i of the R_L form is similar to that observed when membranes are incubated with Gpp(NH)p to induce uncoupling of receptors from G proteins.

The conclusion that membranes from E_2-treated females have a diminished number of agonist high affinity α_{2D}-adrenoceptor complexes is further supported by our studies with the full α_2 agonist [^{3}H]UK-14,304. E_2 treatment significantly reduced [^{3}H]UK-14,304 binding in the HYP of female rats, compared with OVX controls (Fig. 6). It has been reported that [^{3}H]UK-14,304 labels only the agonist high affinity form of the α_2-adrenoceptor in postmortem human brain (20) and rat cerebral CTX (21). Loftus *et al.* (21) reported that treatment with divalent cations, especially Mn^{2+}, can shift the receptor population into the high affinity form, with a concomitant increase in [^{3}H]UK-14,304 binding. Conversely, treatment with Gpp(NH)p, which dissociates G proteins and hence induces receptor uncoupling, causes a concomitant loss of [^{3}H]UK-14,304 binding (20). Consistent with these findings, we observed that Gpp(NH)p decreases [^{3}H]UK-14,304 binding in both the HYP and POA of female rats, in a concentration-dependent fashion (Fig. 5). Thus, [^{3}H]UK-14,304 binding can serve as a useful index for measuring the agonist high affinity receptor population (i.e., R_H). The loss of [^{3}H]UK-14,304 binding in HYP membranes from estrogen-treated rats provides additional evidence that E_2 inhibits the formation of the agonist high affinity state of the α_{2D}-adrenoceptor.

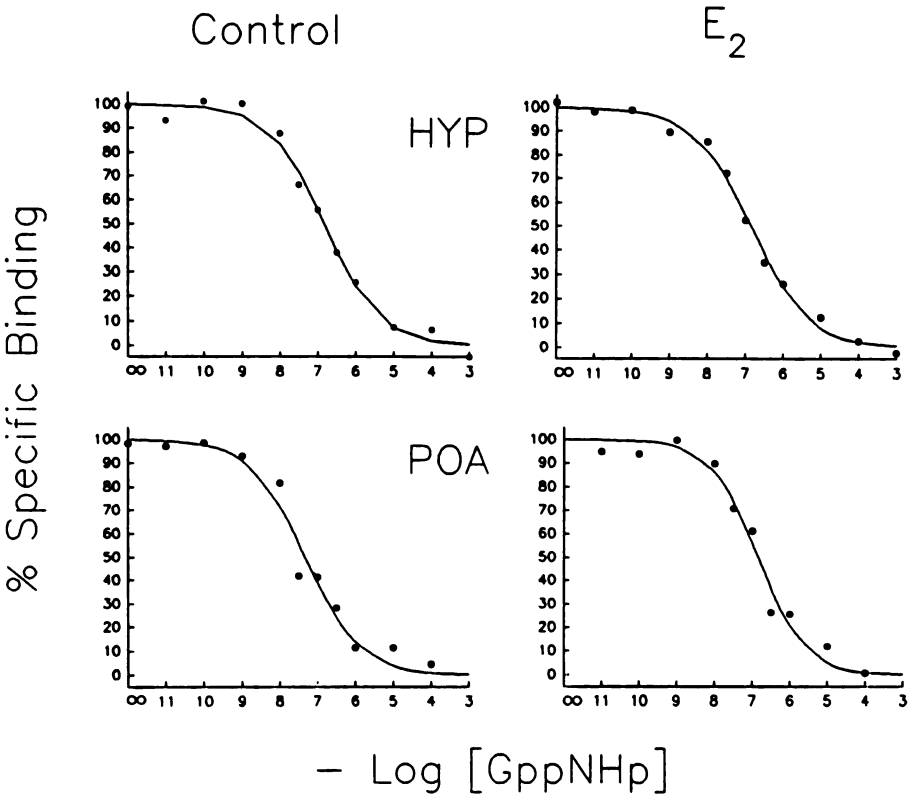

Fig. 5. Decrease of [^{3}H]UK-14,304 binding in both HYP and POA membranes by Gpp(NH)p treatment. These plots are representative of results obtained when HYP and POA membranes were incubated at 25° with 5.0 nM [^{3}H]UK-14,304 and 12 concentrations (10^{-11} to 10^{-3} M) of Gpp(NH)p. [^{3}H]UK-14,304 binding was concentration-dependently inhibited by Gpp(NH)p treatment. The Gpp(NH)p-induced inhibition of [^{3}H]UK-14,304 binding is characterized by slopes less than unity (HYP control, 0.61 ± 0.13, three experiments; HYP with E_2, 0.50 ± 0.01, three experiments; POA control, 0.79 ± 0.15, three experiments; POA with E_2, 0.79 ± 0.12, three experiments.

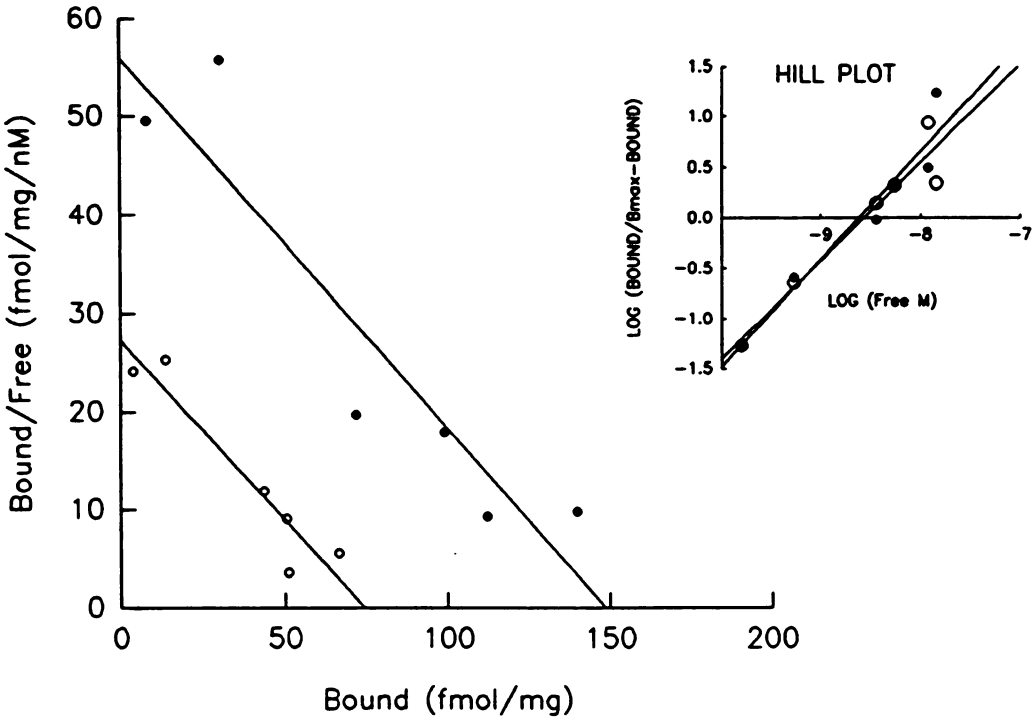

Fig. 6. Reduction of UK-14,304 binding in HYP membranes by hormone treatment. [^{3}H]UK-14,304 binding was diminished in estrogen-treated rats (○), compared with OVX control animals (●). Aliquots of suspended membranes from individual animals were incubated with 0.1–15 nM [^{3}H]UK-14,304 for 4 hr at 25°; 1000-fold excess unlabeled UK-14,304 was used to define nonspecific binding. These results are representative of six independent replications. Inset: Hillplot.

It is noteworthy that E_2 induced an approximately 50% reduction in high affinity sites in HYP membranes, as assessed by [^{3}H]UK-14,304 binding, whereas competition experiments with NE indicate an 80% reduction in high affinity binding. This apparent difference may be reconciled if one considers that there is likely to be some R_L in OVX control HYP. Resolution of the NE competition curves from OVX control HYP into two sites did not produce a statistically improved fit. However, the observations that NE competition curves in HYP membranes from control rats had slopes less than unity and that the pK_i of R_H was higher in E_2-exposed membranes than in control membranes suggest that some R_L is present in OVX control HYP. It is therefore likely that the reduction in high affinity binding assessed by NE competition experiments is a slight overestimate. Because [^{3}H]UK-14,304 binding is not complicated by binding to the low affinity site, it may represent

TABLE 4

E_2 reduction of [^{3}H]UK-14,304 binding measured at both 0° and 25°

Scatchard analysis of [^{3}H]UK-14,304 binding was carried out as described in the legend to Fig. 6. The number of [^{3}H]UK-14,304 binding sites was reduced by estrogen treatment, as measured at both 0° and 25°. Incubation of membranes at 0° increased [^{3}H]UK-14,304 binding in control membranes but not in membranes from E_2-treated animals. Values are the means ± standard errors of three (0°) or six (25°) independent replications.

Treatment	B_{max}	K_d
	fmol/mg	*nM*
0°		
Control	202.4 ± 11.3[a]	3.7 ± 1.9
E_2	82.9 ± 7.0[b]	5.1 ± 2.6
25°		
Control	147.0 ± 28.7	2.8 ± 0.54
E_2	96.4 ± 25.6[b]	3.0 ± 1.2

[a] Significantly different from 25° control ($p < 0.02$).
[b] Significantly different from either control ($p < 0.0001$).

a more accurate estimate of the magnitude of E_2 reduction of agonist high affinity binding in the HYP of female rats.

It is interesting to note that the difference in [^{3}H]UK-14,304 binding between control and hormone-treated females was greater when Scatchard analysis was carried out on binding reactions conducted at 0° rather than 25°. According to the principle of Le Chatelier, a decrease in temperature favors enthalpy-driven reactions (32). It has been demonstrated that the formation of high affinity receptor complexes is an enthalpy-driven reaction (33). The increase in [^{3}H]UK-14,304 binding in HYP membranes from OVX control rats as the temperature is lowered from 25° to 0° is thus consistent with the notion that low temperature favors the formation of high affinity receptor complexes, thereby increasing [^{3}H]UK-14,304 binding. In contrast, no temperature-dependent increase in [^{3}H]UK-14,304 binding was observed in membranes from E_2-treated animals. These data suggest that one facet of E_2 action may be inhibition of temperature-induced formation of high affinity receptor complexes in the HYP of female rats. Interestingly, we have demonstrated that E_2 treatment can also alter the thermodynamics of α_2-adrenergic ligand-receptor binding in the HYP of female rats.[1] In HYP membranes from E_2-exposed females, the entropic contribution to both agonist and antagonist binding is increased, whereas the enthalpy component is reduced. These changes may be indicative of a decreased capacity of α_2-adrenoceptors to adopt the conformation necessary for effective signal transduction. Therefore, E_2 modulation of α_2-adrenoceptors may be at the level of the ligand-receptor interaction, as well as at the receptor-G protein interface.

Overall, the present data are consistent with the interpretation that exposure to E_2 promotes uncoupling of α_2-adrenergic receptors from G proteins in HYP membranes. Other receptors have been reported to be modulated in a similar fashion. For example, the β-adrenoceptor agonist isoproterenol is a potent stimulator of cAMP formation in HYP slices and membranes from OVX control but not E_2-treated female rats (34, 35). In these E_2-treated HYP membrane preparations, binding studies with Gpp(NH)p have shown that hormone treatment uncouples the β-adrenoceptor from its G protein (35). Furthermore, chronic estrogen treatment promotes a similar functional uncoupling of the D_2 dopamine receptor from adenylyl cyclase in the anterior pituitary gland (36). Therefore, interference with receptor-G protein coupling may be a general mechanism of steroid hormone regulation of transmembrane signal transduction by catecholamine receptors.

The present results also indicate that caution is necessary in the interpretation of α_2 receptor autoradiography studies, particularly those utilizing only a single ligand concentration to determine receptor B_{max}. This study shows that the B_{max} measured with certain ligands, such as the α_2 agonist UK-14,304, can be affected by factors other than actual changes in receptor number, e.g., temperature and the extent of receptor-G protein coupling. In fact, we find that the B_{max} measured with [^{3}H]UK-14,304 is significantly decreased (Fig. 6), whereas the B_{max} measured with the antagonist [^{3}H]RX does not change or may even increase in response to E_2 treatment (Table 1). These apparently divergent actions of E_2 would be difficult to detect and reconcile on the basis of autoradiographic determinations of receptor number alone. For example, despite the increase in [^{3}H]RX binding in the HYP, α_2-adrenoceptor function in the HYP is markedly attenuated by E_2 (8). This attenuation may result from reduced α_2-adrenoceptor-G protein coupling, as indicated by decreased [^{3}H]UK-14,304 binding and the alteration in NE competition for [^{3}H]RX sites.

In conclusion, these findings demonstrate that the α_{2D}-adrenoceptor is the major subtype present in the HYP and POA of female rats, and they support the conclusion that estrogen can reduce the agonist high affinity form of the α_{2D}-adrenoceptor. Functionally, this E_2-induced receptor uncoupling may reduce the ability of released NE to bind to presynaptic α_2-adrenoceptors and inhibit additional neurotransmitter release (8). These results may in part provide a mechanism to explain E_2 facilitation of hypothalamic NE release (8, 37).

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