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Heterogeneity of α_1-Adrenergic Receptors Revealed by Chlorethylclonidine

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SUMMARY

Chlorethylclonidine (CEC) has previously been shown to inactivate only a subpopulation of the α_1-adrenergic receptor binding sites in rat brain. We compared α_1-adrenergic receptors in different tissues to determine whether such selective inactivation might reveal the presence of distinct receptor subtypes. Pretreatment of broken cell preparations with 10 μM CEC for 10 min caused a 70–80% decrease in the density of specific ^{125}IBE 2254 binding sites in rat liver and spleen, a 25% decrease in neocortex, but no significant loss in kidney, hippocampus, heart, vas deferens, or caudal artery. The effect of CEC in liver was not reversed by extensive washing, suggesting irreversible inactivation. The selectivity between different tissues was due to differences in the efficacy of CEC inactivating the binding sites and not due to differences in binding affinity. To determine whether the effects on ^{125}IBE 2254 binding reflected selective inactivation of functional receptors, contractile responses of rat spleen and vas deferens were examined. Pretreatment of intact tissues with 100 μM CEC for 30 min caused a large decrease in the potency and maximal contraction to norepinephrine in spleen but had no effect in vas deferens. Inhibition of specific ^{125}IBE 2254 binding by various agonists and antagonists was determined in CEC-sensitive (liver, spleen) and insensitive (hippocampus, vas deferens) tissues. Although many drugs had similar affinities in all tissues, others were substantially less potent in the CEC-sensitive tissues. These experiments suggest that there are at least two subtypes of α_1-adrenergic receptors with different pharmacological properties in mammalian tissues, only one of which is inactivated by CEC.

It has become increasingly clear that α_1-adrenergic receptors do not have the same pharmacological properties in all tissues. Agonists and antagonists have been shown to have different potencies in activating or inhibiting the α_1-adrenergic receptors mediating contraction of different smooth muscles (1–8). The α_1-adrenergic receptors increasing inositol phosphate accumulation can be distinguished pharmacologically from those increasing cyclic AMP accumulation in liver (9) and brain (10), and α_1-adrenergic receptor binding sites in brain can be subdivided based on the potencies of certain competitive antagonists (11, 12). These results raise the possibility that there may be distinct subtypes of α_1-adrenergic receptors, possibly using different biochemical mechanisms for signal initiation.

Recently, Johnson and Minneman (13) reported that CEC (14), a reactive derivative of clonidine, selectively inactivated only a subpopulation (35–40%) of the α_1-adrenergic receptor binding sites labeled with ^{125}IBE in rat cerebral cortex. Higher concentrations of CEC caused no further inactivation of α_1-adrenergic receptors, suggesting the presence of two types of binding sites that differed in their sensitivity to CEC. In support of this, pretreatment of other brain regions with CEC caused variable inactivation of binding sites, with no inactivation being observed in hippocampus (13).

In this manuscript we examine the effects of CEC on α_1-adrenergic receptors in various tissues to determine whether the differential effects of CEC reflect the existence of discrete subtypes of α_1-adrenergic receptors.

Methods

Materials. The following drugs were used: (−) norepinephrine bitartrate, (−) epinephrine bitartrate, (±) normetanephrine HCl, desmethylimipramine HCl, (±)-propranolol HCl, yohimbine HCl, and Percoll (Sigma, St. Louis, MO); chlorethylclonidine, WB4101, and benoxathian HCl (Research Biochemicals, Natick, MA); phentolamine and naphazoline HCl (CIBA-Geigy, Summit, NJ); prazosin HCl (Pfizer, Groton, CT); oxymetazoline (Schering, Bloomfield, NJ); clonidine HCl (Boehringer Ingelheim, Ridgefield, CT); Sgd 101/75 (Siegfried Zofingen, Switzerland); α-methylnorepinephrine (Sterling Winthrop, Rensselaer, NY); methoxamine (Burroughs Wellcome, Research Triangle Park, NC); BE-2254 2[β(4-hydroxyphenyl)-ethylaminomethyl]-tetralone, (Beiersdorf, Hamburg, FRG); ARC 239 2-[2,4-(O-methoxyphenyl)-piperazin-l-yl] ethyl-4,4 dimethyl-1,3-(2H,4H)isoquinolindione dihydrochloride (Boehringer-Mannheim, Biberach, FRG).

Measurement of ^{125}IBE binding. Crude particulate fractions were made from brain cortex, hippocampus, heart, spleen, kidney, vas deferens, and caudal artery. Male Sprague-Dawley rats (200–300 g) were

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ABBREVIATIONS: CEC, chlorethylclonidine; ^{125}IBE, ^{125}IBE 2254; KRB, Krebs-Ringer bicarbonate buffer.

killed by cervical dislocation. Organs were removed and homogenized in 20 ml of 20 mM $NaPO_4$ buffer (pH 7.6) containing 154 mM NaCl (PO_4-salt buffer) with a Polytron at speed 6 or 7 for 10 sec. The homogenate was centrifuged at 20,000 × *g* for 10 min, the supernatant discarded, and the pellet resuspended in PO_4-salt buffer to a final tissue protein concentration of (in mg/ml) 0.25 (cortex, hippocampus), 0.9 (heart), 0.5 (spleen), 0.2 (kidney), 0.3 (vas deferens), and 0.05 (caudal artery). The homogenates (except cortex and hippocampus) were filtered through a double layer of surgical gauze to remove connective tissue fragments before use.

Liver membranes were purified by Percoll gradient centrifugation as described in Prpic *et al.* (15) to improve the quality of the binding data. Briefly, 1.5 g liver were homogenized with a Dounce homogenizer in 25 ml 250 mM sucrose, 5 mM Na HEPES, 1 mM EGTA medium. The homogenate was centrifuged at 1500 × *g* for 10 min, the supernatant discarded, and the pellet resuspended with a Dounce homogenizer in 25 ml of the same medium. Two 10.4-ml aliquots were taken and 1.4 ml Percoll added to each aliquot. After mixing, samples were centrifuged at 35,000 × *g* for 30 min and plasma membranes collected. Membranes were then washed and resuspended in the PO_4-salt buffer to a final protein concentration of 0.1 mg/ml. In some experiments kidney membranes were purified using an identical protocol to make certain that the purification procedure did not affect the receptor properties or sensitivity to CEC.

BE-2254 was radioiodinated to theoretical specific activity as described by Engel and Hoyer (16) and stored at −20° in methanol. Measurement of specific ^{125}IBE binding was usually performed (17) by incubating 100 μl of tissue preparation with ^{125}IBE in PO_4-salt buffer in a final volume of 250 μl for 20 min at 37° in the presence or absence of competing drugs. The incubation was terminated by adding 10 ml of 10 mM Tris-HCl (pH 7.4) at room temperature and filtration over a glass-fiber filter (Schleicher and Schuell No. 30) under vacuum. Each filter was washed with 10 ml of 10 mM Tris-HCl (pH 7.4), dried, and radioactivity measured. Nonreceptor binding was determined to be binding in the presence of 10 μM phentolamine.

Saturation curves were determined by incubating tissue with increasing concentrations of ^{125}IBE (10–100 pM) and analyzing the data by the method of Scatchard (18). The potencies of drugs in competing for the specific ^{125}IBE binding sites was determined by incubation of a single concentration of ^{125}IBE (40–50 pM) in the presence or absence of 16 concentrations of competing drug. IC_{50} values were determined as the *x* intercept on a Hill plot, and K_I values calculated by the method of Cheng and Prusoff (19).

Measurement of contractile responses. Male Sprague-Dawley rats (200–300 g) were killed by cervical dislocation and the vasa deferentia and spleen removed and placed in KRB containing (in mM) 120 NaCl, 5.5 KCl, 2.5 $CaCl_2$, 1.2 NaH_2PO_4, 1.2 $MgCl_2$, 20 $NaHCO_3$, 11 glucose, and 0.029 $CaNa_2$ EDTA equilibrated with 95% O_2:5% CO_2. The KRB also contained 0.1 μM desmethylimipramine and 1 μM normetanephrine to block neuronal and extraneuronal uptake of norepinephrine and 1 μM (±)-propranolol to block β-adrenergic receptors. Each vasa deferentia was carefully dissected free of the adherent sheath of connective tissue, and each spleen was cut longitudinally into two pieces. Tissues were suspended in 10-ml organ baths containing KRB maintained at 37°C and continuously equilibrated with 95% O_2/5% CO_2 and attached to Grass FT .03 force displacement transducers. The vas deferens preparations were stretched to a resting tension of 500 mg, allowed to equilibrate for 60 min, and primed twice with 10 μM norepinephrine followed by thorough washing (20). Agonist-induced increases in isometric tension were determined using a noncumulative dosage schedule where each dose was washed out before the next dose was added, using a 6-min interval between doses. Responses were measured as the peak tension developed while the preparation was exposed to the agonist. The spleen preparations were stretched to a resting tension of 1 g, equilibrated for 90 min, and a priming dose-response curve for norepinephrine was generated (21). After thorough washing, the preparations were equilibrated for another 30 min and cumulative dose-response curves for norepinephrine generated.

EC_{50} values for dose-response curves were calculated by linear regression of all points between 20 and 80% of the maximal response to the agonist. Data were compared statistically using a Student's *t* test.

Results

Differential inactivation of ^{125}IBE binding sites by CEC. Pretreatment of broken cell preparations with 10 μM CEC for 10 min caused markedly different effects on specific ^{125}IBE binding sites in different tissues. This treatment decreased the apparent B_{max} for ^{125}IBE by 70–80% in liver and spleen and by 25% in neocortex but had no significant effect in kidney, hippocampus, heart, vas deferens, or caudal artery (Fig. 1, Table 1). There was little effect on the K_D for ^{125}IBE in these tissues, although small increases were observed in liver and neocortex (Table 1) possibly due to residual drug remaining after washout. Tissue concentrations during CEC pretreatment were kept similar in all cases to control for nonspecific tissue inactivation of the drug. The effect of washing on the inactivation caused by CEC in liver is shown in Fig. 2. There was no recovery of specific ^{125}IBE binding sites after repeated washing, consistent with an irreversible inactivation of binding sites. There was no difference in the recovery of CEC-sensitive or insensitive binding sites after each wash.

Dose-response curves for CEC inactivation were examined in purified membranes from liver and kidney to determine whether the apparent selectivity was due to differences in potency or maximum effect. CEC pretreatment caused dose-dependent decreases in the density of specific ^{125}IBE binding sites in both tissues. Although the potency of CEC was similar in both tissues, the maximum inactivation was much greater in liver (84%) than kidney (38%) (Fig. 3). Note that CEC caused a greater inactivation in purified kidney membranes (38%) than in crude particulate fractions from this tissue (15%, Table 1). Similar results were obtained in rat heart, where pretreat-

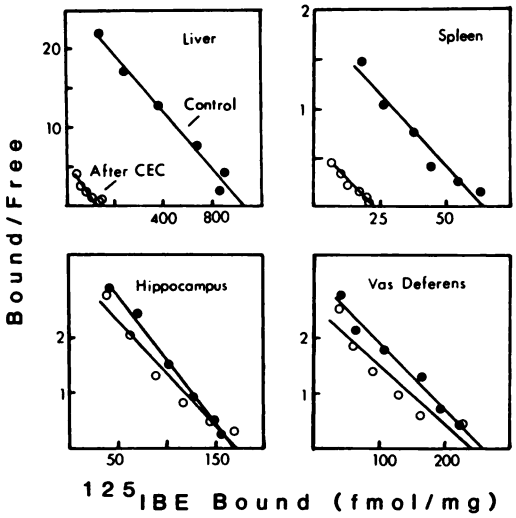

Fig. 1. CEC inactivation of α_1-adrenergic receptors in different tissues. Particulate preparations of spleen, hippocampus, and vas deferens and purified membranes from liver were incubated with or without 10 μM CEC in PO_4-salt buffer at 37°C for 10 min. Reactions were stopped by dilution with cold buffer and centrifugation. After resuspension, saturation isotherms of specific ^{125}IBE binding were determined in control (●) and CEC-pretreated (○) tissues. Each point is the mean of five to nine determinations each performed in duplicate.

TABLE 1

Effect of CEC pretreatment on ^{125}IBE binding sites in different tissues

Purified plasma membranes (liver) or crude particulate fraction (other tissues) were incubated in PO_4-salt buffer in the absence (control) or presence of 10 μM CEC for 10 min at 37°. After addition of 20 ml cold PO_4-salt buffer, preparations were centrifuged at 20,000 × *g* for 10 min. The resulting pellets were resuspended in PO_4-salt buffer. B_{max} and K_D values were obtained from Scatchard analysis of saturation isotherms of specific ^{125}IBE binding. Values are means ± SE of number of observations (*n*).

Tissue	*n*	B_{max}		B_{max} reduction	K_D	
		Control	10 μM CEC		Control	10 μM CEC
		fmol/mg protein		%	*pM*	
Liver	9	690 ± 114	168 ± 34[a]	75.7	28 ± 4	46 ± 10[c]
Spleen	5	67 ± 6	20 ± 2[a]	70.1	37 ± 8	45 ± 8
Neocortex	6	376 ± 33	281 ± 24[b]	25.3	36 ± 4	44 ± 4
Kidney	5	124 ± 13	105 ± 23	15.3	58 ± 10	67 ± 8
Hippocampus	7	233 ± 26	210 ± 21	10.0	48 ± 8	55 ± 6
Heart	5	89 ± 5	82 ± 21	7.9	57 ± 11	113 ± 26
Vas deferens	8	313 ± 58	290 ± 57	7.3	110 ± 15	120 ± 33
Caudal artery	6	507 ± 50	510 ± 80	0	109 ± 27	125 ± 22

[a] $p < 0.001$ compared with control.
[b] $p < 0.01$.
[c] $p < 0.05$.

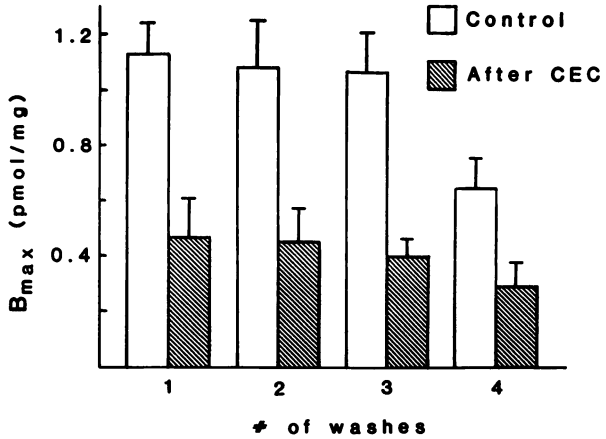

Fig. 2. Effect of washing on inactivation of ^{125}IBE binding caused by CEC. Percoll purified membranes from liver were split into two aliquots and incubated in the absence (*open bars*) or presence (*hatched bars*) of 10 μM CEC at 37° for 10 min in PO_4-salt buffer. Reactions were stopped by dilution with cold buffer and centrifugation. After resuspension, an aliquot of each tissue was removed (one wash) and the remainder subjected to three sequential washes, with an aliquot being removed after each further wash. Saturation analysis of specific ^{125}IBE binding was determined on each fraction and B_{max} values determined by Scatchard analysis. Values are means ± SE from four experiments performed in duplicate.

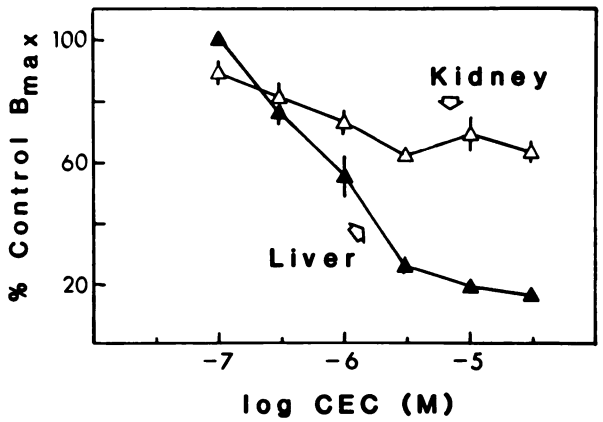

Fig. 3. Dose-response curve for CEC inactivation of ^{125}IBE binding sites in purified membranes from kidney and liver. Percoll purified membranes from kidney and liver were incubated in the absence or presence of the indicated concentrations of CEC at 37° for 10 min in PO_4-salt buffer. Reactions were stopped by dilution with cold buffer and centrifugation. After resuspension, saturation isotherms of specific ^{125}IBE binding were determined in each preparation. Results are expressed as a percentage of the B_{max} in tissues incubated in the absence of CEC (control). Points are means ± SE of four or five determinations performed in duplicate.

ment with 10 μM CEC caused an 8 ± 24% loss of binding sites in a crude particulate preparation (Table 1) but a 27 ± 12% loss in Percoll-purified membranes (data not shown). This may have been due to selective enrichment of the CEC-sensitive population of binding sites in the purified membranes.

The potency of CEC as a competitive antagonist was examined in hippocampus and liver to determine whether the drug had different affinities for binding to the receptors in these tissues. Saturation isotherms of specific ^{125}IBE binding were determined in the presence or absence of 10 μM CEC added directly to the binding assay (no pretreatment). The K_I for CEC was calculated by the change in apparent K_D for ^{125}IBE. Table 2 shows that 10 μM CEC caused a similar 8–10-fold decrease in the apparent K_D for ^{125}IBE in liver (CEC sensitive) and hippocampus (CEC insensitive). The K_I values for CEC were not significantly different in these two tissues. Note that a smaller decrease in the density of binding sites in the liver was observed when CEC was added during the radioligand incubation (Table 2) compared with when tissues were pretreated with CEC (Table 1). This may be due to partial protection of the receptor sites caused by radioligand occupation in the binding assays.

Differential inactivation of contractile responses by CEC. To determine whether the differential inactivation of ^{125}IBE binding sites reflected effects on functional receptors, we examined the effect of CEC pretreatment on contractile responses to norepinephrine in spleen (CEC sensitive) and vas deferens (CEC insensitive). The effects of norepinephrine in both tissues are mediated by α_1-adrenergic receptor activation (unpublished data). A higher concentration of CEC was used to ensure adequate penetration into the intact tissues. Pretreatment with 100 μM CEC for 30 min caused a 27-fold decrease in the potency of norepinephrine and a 25% decrease in the maximal contractile response in spleen but had no effect on the potency of norepinephrine or maximal contractile response in vas deferens (Fig. 4).

TABLE 2

Potency of CEC as a competitive antagonist in liver and hippocampus

Saturation isotherms of specific ^{125}IBE binding to purified liver membranes or a crude particulate fraction from hippocampus were determined in the absence (control) or presence of 10 μM CEC. Tissues were not pretreated, and CEC was present only during the final incubation with radioligand. The K_D and B_{max} for ^{125}IBE were determined by Scatchard analysis. The K_i for CEC was calculated according to the equation $K_i = K_D[I]/(K_{app} - K_D)$, where K_D is K_D for ^{125}IBE in the absence of CEC, K_{app} is the K_D for ^{125}IBE in the presence of CEC, and $[I]$ is the concentration of CEC. There was no significant difference between the K_i for CEC in liver and hippocampus. Values are means ± SE of data from four experiments.

	^{125}IBE K_D		CEC K_i	B_{max}	
	Control	10 μM CEC		Control	10 μM CEC
	pM		μM	fmol/mg	
Liver	27 ± 6	203 ± 43	1.6 ± 0.22	1021 ± 44	531 ± 112
Hippocampus	59 ± 2	621 ± 62	1.1 ± 0.15	222 ± 5	184 ± 13

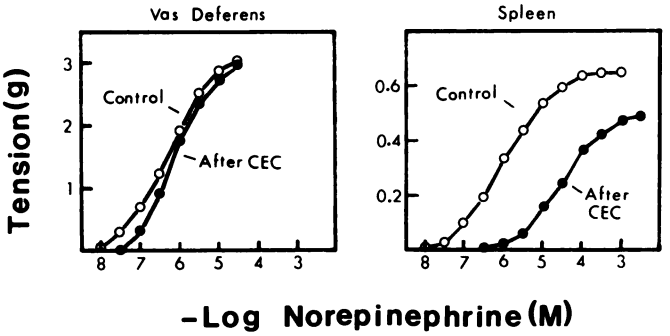

Fig. 4. Effect of CEC on norepinephrine-induced contractions of vas deferens and spleen. Tissues were mounted in the organ bath and equilibrated, and dose-response curves to norepinephrine were determined as described in the text. Tissues were then incubated with 100 μM CEC for 30 min and extensively washed. Dose-response curves to norepinephrine were then repeated. Control tissues were treated identically but without the intervening CEC treatment. The second curve in the control tissues is plotted as control. Values are means ± SE of data from three experiments.

Pharmacological specificity of ^{125}IBE binding sites in different tissues. To determine whether the CEC-sensitive and -insensitive binding sites represented receptors with different binding properties, we compared the potencies of drugs in inhibiting specific ^{125}IBE binding in CEC-sensitive (liver, spleen) and -insensitive (hippocampus, vas deferens) tissues. Hippocampus was compared directly with liver in the same experiments, and spleen was compared directly with vas deferens in other experiments (Tables 3 and 4). Many drugs, including the antagonists BE 2254, prazosin, and yohimbine, showed similar potencies in all tissues. Other drugs, particularly the antagonists WB 4101 and benoxathian, the partial agonist oxymetazoline, and the full agonist methoxamine showed substantially higher affinities in hippocampus and vas deferens when compared with liver and spleen, respectively. It was interesting that Hill coefficients were similar to 1.0 in liver and spleen for all antagonists and for most nonselective drugs in hippocampus and vas deferens. However, for drugs showing selectivity between the two tissues, the Hill coefficients were significantly less than 1.0 in hippocampus and vas deferens, possibly indicating binding site heterogeneity.

Discussion

The experiments reported here suggest that α_1-adrenergic receptors in different rat tissues show a large variation in their sensitivity to inactivation by CEC. Low concentrations of CEC inactivated most of the specific α_1-adrenergic receptor binding sites labeled by ^{125}IBE in broken cell preparations of liver and spleen. This inactivation appeared to be essentially irreversible since there was no recovery after extensive washing. The same concentration of CEC had virtually no effect on the α_1-adrenergic receptor binding sites in hippocampus, heart, vas deferens, and caudal artery. Other tissues, such as cerebral cortex and kidney, showed partial inactivation of receptors after CEC pretreatment.

The observed variations in sensitivity appeared to be caused by differences in the effectiveness of CEC in inactivating the receptors in different tissues and not to differences in binding affinity. We determined dose-response curves for inactivation of ^{125}IBE binding sites by CEC pretreatment in purified membranes from liver and kidney and found that the potency of CEC was similar in both tissues but that there was a much greater maximal inactivation in liver. Competition experiments showed that CEC had approximately the same affinity in reversibly inhibiting specific ^{125}IBE binding in both liver and hippocampus, although it caused a significant inactivation of binding sites only in liver. We also found that CEC had a similar potency in inhibiting ^{125}IBE binding (at a single concentration) in all tissues studied (data not shown). These results suggest that CEC binds equally well to α_1-adrenergic receptors in all tissues but can inactivate the receptors only in some tissues.

To ensure that the inactivation of ^{125}IBE binding sites reflected a loss of functional receptors, we studied the effects of CEC on receptor responsiveness. Stimulation of α_1-adrenergic receptors causes contraction of the smooth muscle in rat vas deferens (20) and spleen (21), two tissues with widely different sensitivities to CEC inactivation. Pretreatment of these tissues with CEC *in vitro* did not cause contraction, showing that CEC has little or no intrinsic activity at the receptors in either tissue. Similarly, CEC pretreatment did not increase inositol phosphate accumulation in any tissue studied (neocortex, vas deferens, or liver; data not shown). However, CEC pretreatment greatly reduced the potency and maximal contractile response to norepinephrine in spleen but had no effect on vas deferens. These results parallel the results from our binding experiments and suggest that both the CEC-sensitive and CEC-insensitive binding sites serve as functional receptors in different tissues.

These results strongly support the hypothesis that α_1-adrenergic receptors in different tissues show a differential sensitivity to inactivation by CEC. There must, therefore, be physical differences between the receptors that cause such differential sensitivity. Such differences could include anything from the existence of completely different receptor subtypes with little sequence homology to the existence of only a single receptor protein with minor posttranslational modifications, such as glycosylation or methylation. The structural basis of these differences remains to be worked out.

To determine whether such physical variations had physiological consequences, it was of interest to determine whether there were differences in the binding properties of these different receptors. Various drugs, including CEC, prazosin, BE 2254, and yohimbine, appeared to be equally potent in competing for the receptor sites in all tissues. Some differences in K_D for the radioligand ^{125}IBE were observed between tissues. In general the CEC-sensitive tissues had slightly higher affinities for

TABLE 3

Inhibition of specific ^{125}IBE binding in hippocampus and liver

Inhibition of specific ^{125}IBE binding by each drug was compared in purified membranes from liver or in crude particulate fractions from hippocampus. IC_{50} and n_H values were determined from the Hill plots, and K_D values calculated by the method of Cheng and Prusoff (19). Values are means ± SE of number of experiments (*n*).

	Hill coefficient (n_H) rectr				-log K_i (molar) rectr		
	Hippocampus	*n*	Liver	*n*	Hippocampus	Liver	Antilog ratio
Antagonists							
BE2254	1.05 ± 0.078	3	1.08 ± 0.027	3	9.20 ± 0.003	9.08 ± 0.028	0.9
Prazosin	0.88 ± 0.026	7	1.02 ± 0.043	7	8.94 ± 0.051	9.09 ± 0.132	0.7
WB4101	0.76 ± 0.050	4	0.95 ± 0.35[a]	3	8.93 ± 0.096	8.13 ± 0.032[c]	6.3
Benoxathian	0.82 ± 0.028	7	1.04 ± 0.069[b]	4	8.56 ± 0.050	7.73 ± 0.046[c]	6.8
ARC239	0.79 ± 0.060	4	1.11 ± 0.052[b]	4	8.19 ± 0.090	7.90 ± 0.035[a]	2.0
Phentolamine	0.74 ± 0.027	6	0.95 ± 0.022[c]	5	7.48 ± 0.118	6.97 ± 0.038[b]	3.2
Yohimbine	1.03 ± 0.085	6	1.01 ± 0.083	5	6.21 ± 0.019	6.20 ± 0.023	1.0
Low efficacy agonists							
Oxymetazoline	0.58 ± 0.028	5	0.92 ± 0.086[a]	5	7.68 ± 0.080	6.75 ± 0.113[c]	8.5
Naphazoline	0.96 ± 0.084	3	0.98 ± 0.097	3	6.87 ± 0.104	6.93 ± 0.044	0.9
Clonidine	0.97 ± 0.045	4	1.04 ± 0.074	4	6.27 ± 0.028	6.21 ± 0.045	1.1
Sgd 101/75	0.83 ± 0.062	7	0.89 ± 0.122	7	5.28 ± 0.063	4.91 ± 0.054[c]	2.3
High efficacy agonists							
Norepinephrine	0.70 ± 0.055	5	0.79 ± 0.042	5	4.90 ± 0.078	5.20 ± 0.46[a]	0.5
Epinephrine	0.70 ± 0.030	5	0.85 ± 0.046[a]	5	5.30 ± 0.058	5.56 ± 0.038[b]	0.5
α-Methyl-norepinephrine	0.83 ± 0.032	3	0.92 ± 0.053	3	4.27 ± 0.033	4.38 ± 0.043	0.8
Methoxamine	0.94 ± 0.018	4	1.05 ± 0.028[a]	4	4.13 ± 0.044	3.39 ± 0.040[c]	5.5

[a] $p < 0.05$.
[b] $p < 0.01$.
[c] $p < 0.001$ compared with hippocampus.

TABLE 4

Inhibition of specific ^{125}IBE binding in vas deferens and spleen

Inhibition of specific ^{125}IBE binding was compared in crude particulate fractions from vas deferens and spleen. IC_{50} and n_H values were determined from Hill plots and K_D values calculated by the method of Cheng and Prusoff (19). Values are means ± SE of number of observations (*n*).

	Hill coefficient (n_H) rectr				-log K_i (molar) rectr		
	Vas deferens	*n*	Spleen	*n*	Vas deferens	Spleen	Antilog ratio
Antagonists							
BE 2254	1.03 ± 0.036	8	1.14 ± 0.045	5	8.99 ± 0.45	9.21 ± 0.114	0.6
Prazosin	1.02 ± 0.080	6	0.84 ± 0.076	5	8.99 ± 0.044	8.89 ± 0.048	1.3
WB 4101	0.63 ± 0.042	9	1.07 ± 0.118[a]	9	8.63 ± 0.069	8.12 ± 0.067[c]	3.2
Benoxathian	0.74 ± 0.038	6	1.05 ± 0.119[b]	6	8.61 ± 0.049	8.18 ± 0.046[c]	2.7
Yohimbine	1.01 ± 0.064	3	0.94 ± 0.057	3	6.19 ± 0.119	6.34 ± 0.074	0.7
Low efficacy agonists							
Oxymetazoline	0.59 ± 0.095	5	1.04 ± 0.078[c]	6	7.18 ± 0.060	6.61 ± 0.042[a]	3.7
High efficacy agonists							
Norepinephrine	0.68 ± 0.015	3	0.82 ± 0.190	3	4.72 ± 0.159	4.73 ± 0.031	1.0
Methoxamine	0.87 ± 0.013	3	1.11 ± 0.083[b]	3	4.16 ± 0.052	3.63 ± 0.041[c]	3.4

[a] $p < 0.01$.
[b] $p < 0.05$.
[c] $p < 0.001$ compared with vas deferens.

^{125}IBE than the CEC-insensitive tissues, although this was not an absolute correlation and the differences were relatively small (Table 1). Scatchard plots appeared to be linear in all tissues. Many other drugs showed substantial differences in potency between the binding sites in the CEC-sensitive and -insensitive tissues. These included the antagonists WB 4101 and benoxathian, the partial agonist oxymetazoline, and the full agonist methoxamine. Other drugs, such as phentolamine and ARC 239, showed significant but slightly lower selectivities. Each of these drugs was more potent in the CEC-insensitive tissues.

The Hill coefficients for all antagonists in inhibiting specific ^{125}IBE binding were close to 1.0 in the CEC-sensitive tissues liver and spleen, suggesting a homogeneous population of binding sites in these tissues. Similarly, the Hill coefficients for apparently nonselective antagonists in inhibiting specific ^{125}IBE binding in the CEC-insensitive tissues hippocampus and vas deferens were also close to 1.0. However, the antagonists showing different affinities in the CEC-sensitive and -insensitive tissues showed low Hill coefficients for inhibition of specific ^{125}IBE binding in hippocampus and vas deferens, consistent with binding site heterogeneity. Previously (13) we had not obtained low Hill coefficients for inhibition of specific ^{125}IBE binding by WB 4101 in rat hippocampus, although we had noticed low Hill coefficients for oxymetazoline in cerebral cortex (17) and vas deferens (20). The low Hill coefficients for WB 4101 observed here may relate to the use of tissues from smaller animals in the current studies. Further analysis of this apparent binding site heterogeneity and comparison with binding constants at receptors mediating functional responses suggest that there are indeed two subtypes of α_1-adrenergic receptor with different affinities for competitive antagonists (22). Similar results have been presented for subdividing ^{3}H-prazosin

binding sites in rat hippocampus on the basis of the affinities of WB 4101 and phentolamine by Morrow and Creese (12). These authors suggested that the binding sites with high and low affinity for WB 4101 be called α_{1a} and α_{1b}, respectively. According to our analysis hippocampus and vas deferens have approximately equal proportions of both subtypes, whereas liver and spleen have only the low affinity (α_{1b}) subtype.

It is tempting to speculate that the CEC-sensitive and -insensitive binding sites represent the two subtypes of α_1-adrenergic receptors that can be distinguished with competitive antagonists. There appears to be a correlation of the existence of CEC-sensitive binding sites with the low affinity (α_{1b}) subtype and of CEC-insensitive sites with the high affinity (α_{1a}) subtype. However, since CEC pretreatment inactivates only 70–80% of the binding sites in liver and spleen, it is surprising that only one subtype of receptor was detected based on affinities for competitive antagonists. More surprisingly, although hippocampus and vas deferens contained similar proportions of both subtypes, CEC caused little or no inactivation of the receptors in these tissues. If CEC inactivated the low affinity (α_{1b}) subtype, we would expect about a 50% inactivation of the binding sites in hippocampus and vas deferens. Therefore we cannot yet quantitatively correlate the sensitivity of α_1-adrenergic receptors to CEC inactivation with the subtypes defined with competitive antagonists, although the general correlation of high affinity subtypes and CEC-insensitive binding sites remains. Both subtypes may exist in both CEC-sensitive and -insensitive forms. Conversely, CEC might selectively inactivate only the low affinity (α_{1b}) subtype, and the apparent discrepancies may come from animal or tissue variations or factors that we cannot yet control. Final resolution of this relationship will await further experiments.

In summary, we have presented evidence for a physical variation in α_1-adrenergic receptor binding sites in different tissues based on their sensitivity to inactivation by CEC. This variation is also apparent in the state of the receptors as they mediate responses in intact tissues. The differences are partially reflected by differences in the affinities of competitive antagonists for binding to the receptors, although the exact relationship between CEC inactivation and different α_1-adrenergic receptor subtypes remains to be clarified.

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