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Comparison of α_1-Adrenergic Receptor Subtypes Distinguished by Chlorethylclonidine and WB 4101

KENNETH P. MINNEMAN, CHIDE HAN,[1] and PETER W. ABEL[2]

Department of Pharmacology, Emory University School of Medicine, Atlanta, Georgia 30322

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SUMMARY

We showed previously that subtypes of α_1-adrenergic receptors can be differentiated by selective inactivation with chlorethylclonidine (CEC) [*Mol. Pharmacol.* **32**:505–510 (1987)] or by their affinities for the competitive antagonist WB 4101 [*Nature (Lond.)* **329**:333–335 (1987)]. Examining eight rat tissues, the proportions of ^{125}IBE 2254-binding sites sensitive to inactivation by CEC correlated significantly ($p < 0.05$) with the proportion having a low affinity for WB 4101. However, the proportion of CEC-sensitive sites was always smaller than the proportion of low affinity WB 4101 sites. Further experiments showed that repetitive pretreatment with CEC or pretreatment under hypotonic conditions caused a larger inactivation of binding sites, suggesting that CEC did not access all sites under the isotonic conditions used previously. The proportions of binding sites inactivated by 10 μM CEC under hypotonic conditions were quantitatively similar to and correlated significantly ($p < 0.01$) with the proportion having a low affinity for WB 4101. Pretreatment of hippocampus and vas deferens with CEC caused a loss of all low affinity WB 4101-binding sites, leaving only high affinity sites. In vas deferens, CEC pretreatment decreased the potency of norepinephrine in stimulating ^{3}H-inositol phosphate accumulation but not contractile responses. In rat liver slices, CEC inactivated norepinephrine-stimulated ^{3}H-inositol phosphate accumulation in parallel with ^{125}IBE-binding sites. These results suggest that: 1) the CEC-sensitive and -insensitive ^{125}IBE 2254-binding sites are equivalent to those with a low and high affinity for WB 4101, respectively, and 2) the CEC-sensitive binding sites with a low affinity for WB 4101 are the α_1-adrenergic receptors linked to inositol phospholipid hydrolysis.

Recently, attention has been focused on the possibility that α_1-adrenergic receptors might not have the same pharmacological properties in all tissues. Differences in the potencies of drugs in affecting contraction of smooth muscle (1, 2), second messenger formation in liver and brain (3, 4), and inhibiting radioligand binding (5, 6) have been reported.

We recently found that α_1-adrenergic receptor-binding sites and functional responses in different tissues could be distinguished by two different criteria. The site-directed alkylating agent CEC (7) was found to inactivate α_1-adrenergic receptor-binding sites and functional responses in some tissues but not in others (8, 9). Previously, Morrow and Creese (6) had reported that α_1-adrenergic receptor-binding sites in rat brain could be subdivided into two classes based on their affinities for the competitive antagonists WB 4101 and phentolamine. We tested the potencies of WB 4101 and one of its congeners, benoxathian, on the α_1-adrenergic receptor-binding sites and functional responses in tissues with different sensitivities to CEC (10). We found that WB 4101 and benoxathian were much more potent in tissues where the receptors were not inactivated by CEC than in those where the receptors were inactivated by CEC. Only those receptors with a low affinity for WB 4101 appeared to stimulate inositol phospholipid metabolism (10).

These results suggested that there might be two subtypes of α_1-adrenergic receptors with different sensitivities to inactivation by CEC, different affinities for the competitive antagonists WB 4101 and benoxathian, and linked to different mechanisms for signal transduction. However, despite the general correlation between CEC sensitivity and the proportion of low affinity WB 4101-binding sites in each tissue, there was not quantitative agreement between these two approaches. In all tissues, a substantially greater proportion of the receptors had a low affinity for WB 4101 than could be inactivated by CEC.

In this paper we show that this discrepancy was probably caused by incomplete access of the highly water-soluble CEC under the inactivation conditions used in our previous experiments, and we present further evidence that only the CEC-

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[1] Permanent address: Department of Pathophysiology, Beijing Medical University, Beijing, China.
[2] Present address: Department of Pharmacology, Creighton University Medical School, Omaha, NB 68178.

ABBREVIATIONS: CEC, chlorethylclonidine; WB 4101, 2-(2,6-dimethoxyphenoxyethyl)-aminomethyl-1,4 benzodioxane; ^{125}IBE, ^{125}IBE 2254: ^{125}I-(2-β-(4-hydroxyphenyl)-ethylaminomethyl)-tetralone; Hepes, 4-(2-hydroxyethyl)-1-piperazineethane sulfonic acid; %WB$_{Low}$, percentage of ^{125}IBE-binding sites with a low affinity for WB 4101; %CEC$_{SENS}$, percentage of ^{125}IBE-binding sites inactivated by 10 μM CEC.

sensitive receptor with a low affinity for WB 4101 is linked to inositol phospholipid hydrolysis in different tissues.

Experimental Procedures

Materials. The following drugs were used: (−)-norepinephrine bitartrate, desmethylimipramine hydrochloride, and Percoll (Sigma Chemical Co., St. Louis, MO); CEC and WB 4101 (Research Biochemicals Inc., Natick, MA); phentolamine mesylate (CIBA-Geigy, Summit, NJ); BE 2254 (2-β(4-hydroxyphenyl)-ethylaminomethyl)-tetralone (Beiersdorf, Hamburg, FRG); and ^{3}H-inositol and carrier-free Na ^{125}I (Amersham Corp., Arlington Heights, IL).

Tissue preparation for radioligand binding. Male Sprague-Dawley rats (200–300 g) were killed by decapitation and crude particulate fractions were made from brain cortex, hippocampus, heart, spleen, kidney, vas deferens, and caudal artery as described previously (9). Briefly, tissues were homogenized with a Polytron in 20 ml of 20 mM $NaPO_4$ buffer (pH 7.6) containing 154 mM NaCl (PO_4-salt buffer), centrifuged at 20,000 × *g* for 10 min, and resuspended either in the same buffer or in 10 mM Na Hepes (pH 7.6), where specified, to the appropriate tissue concentration (9). Tissue preparations (except brain regions) were then filtered through a double layer of surgical gauze. Liver membranes were purified by Percoll gradient centrifugation (11) as described previously (9), and washed and resuspended in PO_4-salt or Na Hepes buffer in the same manner.

CEC treatment. Aliquots (usually 4 ml) of the resuspended preparations were incubated for 10 min at 37° with or without 10 μM CEC for 10 min in either PO_4-salt or Na Hepes buffer. Reactions were stopped by diluting with 20 ml of cold PO_4-salt buffer, centrifuging at 20,000 × *g* for 10 min, and resuspending in 4 ml of PO_4-salt buffer. In some experiments this inactivation protocol was repeated several times on the same tissue preparation.

^{125}IBE binding. BE 2254 was radioiodinated to theoretical specific activity (12) and stored at −20° in methanol. Measurement of specific ^{125}IBE binding was performed by incubating 0.1 ml of tissue preparation with ^{125}IBE in PO_4-salt buffer in a final volume of 0.25 ml for 20 min at 37° in the presence or absence of competing drugs (12, 13). The incubation was terminated by adding 10 ml of 10 mM Tris-HCl (pH 7.4) and filtering 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) and dried; then the radioactivity was measured. Nonreceptor binding was determined to be binding in the presence of 10 μM phentolamine.

Analysis of binding data. Saturation curves were determined by incubating tissue with increasing concentrations of ^{125}IBE (25–800 pM) and analyzing the data by the method of Scatchard (14). The potencies of drugs in competing for the specific ^{125}IBE-binding sites were 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 were calculated by the method of Cheng and Prusoff (15). The best two-site fit for a binding curve was calculated by minimizing the sum of squares of the errors using nonlinear regression analysis. Two-site models were compared to one-site models to determine whether the increase of goodness of fit was significantly more than would be expected on the basis of chance alone (16) using a partial *F* test. The *p* values less than 0.05 were considered significant.

^{3}H-Inositol phosphate accumulation. Norepinephrine-stimulated accumulation of ^{3}H-inositol phosphates (17) was determined in rings of vas deferens and slices of rat liver. Briefly, 3-mm rings of vas deferens (18) or 350 × 350 μm slices of rat liver were preincubated at 37° for 30 or 10 min, respectively, in the absence or presence of varying concentrations of CEC. Aliquots of tissue were then incubated in Krebs-Ringer bicarbonate buffer containing 10 mM LiCl under 95% O_2/5% CO_2 for 2 hr at 37°. Vas deferens incubations also contained 0.1 μM desmethylimipramine, 0.1 mM pargyline, and 0.1 mM pyrogallol to block uptake and inactivation of norepinephrine. Reactions were stopped by organic extraction, and ^{3}H-inositol phosphates were isolated from the aqueous phase by Dowex chromatography as previously described (18). An aliquot of the organic phase was counted to determine total incorporation of ^{3}H-inositol into each tissue preparation. In experiments with liver, after incubation with or without CEC, aliquots of slices were also taken for homogenization, centrifugation, resuspension, and determination of specific ^{125}IBE binding as described above.

Results

Two-site analysis of WB 4101 inhibition curves in different tissues. The distribution of α_1-adrenergic receptor-binding sites with different affinities for WB 4101 was determined by examining the inhibition of ^{125}IBE binding by this drug in eight rat tissues. As previously reported (10), WB 4101 inhibition curves were best fit by a one-site model in preparations of liver and spleen. In the other six tissues, however, the inhibition curves were significantly better fit by a two-site model (Table 1). Although the proportions of the two sites varied among tissues, the K_D values for each site did not vary substantially. The K_D values for the high affinity site varied less than 3.6-fold and averaged (−log K_D) 9.58 ± .094 M while those for the low affinity sites varied less than 2.2-fold and averaged 8.26 ± .047 M.

Comparison of $\%CEC_{SENS}$ and $\%WB_{LOW}$ in different tissues. We correlated the $\%CEC_{SENS}$ in PO_4-salt buffer (9) and the $\%WB_{LOW}$ (Table 1) in all 8 tissues. From Fig. 1 it is clear that, although there is a significant ($p < 0.05$) correlation between these two parameters, they are not quantitatively identical. Some tissues with a high $\%WB_{LOW}$ showed no significant inactivation by CEC. In fact, the $\%WB_{LOW}$ was greater than the $\%CEC_{SENS}$ in all tissues studied.

Retention of biological activity of CEC. If CEC was lost or destroyed during the inactivation period it might not fully inactivate all of the "sensitive" binding sites. Therefore, we tested the biological activity of CEC following exposure to tissues. When the supernatant from CEC-treated tissues (hippocampus or liver) was added to fresh liver membranes, there was no loss in the ability of CEC to inactivate the ^{125}IBE-binding sites (data not shown), suggesting that the biological activity was retained.

Repetitive inactivation with CEC in hippocampus. It was possible that CEC was not able to inactivate all "sensitive" binding sites during a single incubation. Therefore, we deter-

TABLE 1

Two-site analysis of inhibition of specific ^{125}IBE binding by WB 4101 in rat tissues

Inhibition of specific ^{125}IBE binding by 16 concentrations of WB 4101 (3×10^{-11} to 1.8×10^{-7} M) was determined as described under Experimental Procedures. All inhibition curves for a single tissue were averaged and analyzed by nonlinear regression analysis to determine K_H, K_L, and % R_H for the best two-site fit. The best two-site fit was compared to the best one-site fit (determined from a Hill plot) using a partial *F* test.

Tissue	*n*	−log K_H	−log K_L	% R_H	% R_L	*p* Value for 2-site fit over 1-site fit
			M			
Spleen	8		8.16	0	100	
Liver	4		8.08	0	100	
Heart	4	9.88	8.19	16	84	<0.001
Neocortex	4	9.66	8.40	29	71	<0.001
Kidney	4	9.78	8.28	30	70	<0.005
Caudal artery	4	9.35	8.15	30	70	<0.05
Vas deferens	12	9.46	8.40	38	62	<0.025
Hippocampus	10	9.33	8.42	64	36	<0.01

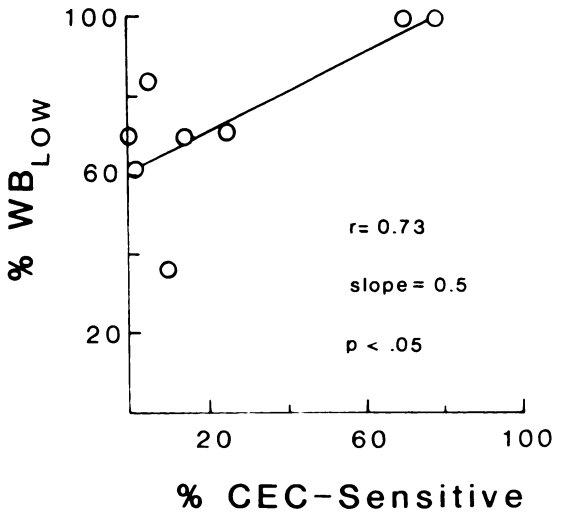

Fig. 1. Comparison of the proportion of ^{125}IBE-binding sites with a low affinity for WB 4101 (from Table 1) with the proportion sensitive to inactivation by CEC in PO_4-salt buffer (from Ref. 9) in eight rat tissues.

TABLE 2

Repetitive inactivation with CEC in hippocampus

A crude particulate fraction from rat hippocampus was prepared as described under Experimental Procedures and incubated with or without 10^{-5} M CEC for 10 min at 37°. The incubation was terminated by dilution with 20 ml of cold PO_4-salt buffer and centrifugation. After resuspension, an aliquot of tissue was removed from each preparation, and the remaining tissue was treated again with or without fresh 10 μM CEC for 10 min. This procedure was repeated two more times so that the tissue was subjected to up to four periods of incubation with or without CEC. Saturation analysis of specific ^{125}IBE binding was determined on each preparation. Each value is the mean ± standard error of data from four experiments.

No. of treatment periods	B_{max}		% Control
	Control	10 μM CEC	
	fmol/mg protein		
1	411 ± 53.8	419 ± 14.0	106 ± 12.4
2	479 ± 44.1	406 ± 43.3	86 ± 8.9
3	500 ± 22.7	428 ± 29.1	86 ± 5.9
4	449 ± 38.6	340 ± 14.1[a]	76 ± 5.8

[a] $p < 0.05$ compared to control.

mined whether multiple treatments with CEC would inactivate more ^{125}IBE-binding sites than a single treatment. Particulate preparations of hippocampus were treated from one to four times with 10 μM CEC (Table 2). Although there was no significant loss of ^{125}IBE-binding sites after a single period of inactivation, four sequential treatments with CEC caused a significant 24% loss of binding sites.

CEC inactivation in hypotonic medium. In previous experiments, inactivation with CEC was always done in the same PO_4-salt buffer used for ^{125}IBE binding. Since such isotonic conditions promote resealing of vesicles following tissue homogenization, the water solubility of CEC might have prevented it from gaining access to binding sites enclosed in such vesicles. We tested the ability of CEC to inactivate ^{125}IBE-binding sites in a hypotonic buffer which would promote vesicle lysis (10 mM Na Hepes, pH 7.6). CEC caused a much greater inactivation of ^{125}IBE-binding sites under hypotonic conditions in all tissues examined (Table 3) than under the isotonic conditions used previously (9). In the presence of isotonic NaCl, 10 μM CEC had no effect on the density of sites in hippocampus (see Ref. 9 and Table 2), while under hypotonic conditions this treatment caused a 41% loss of binding sites (Fig. 2). Similar

TABLE 3

CEC inactivation in hypotonic medium

A crude particulate fraction from most tissues and purified membranes from liver were prepared as described in the text, centrifuged, and resuspended in 10 mM Na Hepes buffer (pH 7.6). Aliquots of tissue were incubated in this buffer with or without 10 μM CEC for 10 min at 37°, diluted with 20 ml of cold 10 mM Na Hepes (pH 7.6), centrifuged, and resuspended in the appropriate amount of PO_4-salt buffer. Saturation analysis of specific ^{125}IBE binding was determined in PO_4-salt buffer as described under Experimental Procedures. Each value is the mean ± standard error of four to six observations.

	B_{max}			K_D	
	Control	CEC	% Control	Control	CEC
	fmol/mg of protein			pM	
Spleen	107 ± 9	6 ± 6[a]	5 ± 5	41 ± 4	
Liver	866 ± 48	124 ± 27[a]	14 ± 2.8	36 ± 4	140 ± 13[a]
Heart	192 ± 10	66 ± 11[a]	35 ± 6.3	39 ± 2	87 ± 31
Neocortex	976 ± 104	419 ± 46[b]	44 ± 5	24 ± 2	37 ± 5
Kidney	214 ± 14	120 ± 20[b]	55 ± 7	68 ± 13	101 ± 22
Caudal artery	742 ± 122	335 ± 89[c]	46 ± 9	74 ± 19	63 ± 20
Vas deferens	584 ± 107	276 ± 46[c]	49 ± 5	71 ± 13	57 ± 6
Hippocampus	470 ± 57	273 ± 31[c]	59 ± 5	58 ± 24	70 ± 20

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

increases in inactivation were observed in all other tissues. However, the $\%CEC_{SENS}$ was still quite variable in different tissues (Table 3).

Comparison of the $\%WB_{LOW}$ and the $\%CEC_{SENS}$ in hypotonic medium. There was a highly significant correlation ($p < 0.01$) between the $\%WB_{LOW}$ and the $\%CEC_{SENS}$ in hypotonic medium in different tissues (Fig. 3). The best fit line is close to the line of identity, with a slope of 0.98, suggesting that these two methods are distinguishing the same subpopulations of binding sites.

Effect of CEC inactivation in hypotonic medium on WB 4101 inhibition curves in vas deferens and spleen. If CEC inactivates only the α_1-adrenergic receptors with a low affinity for WB 4101, then tissues containing both types of sites should selectively lose their low affinity sites after CEC inactivation. Hippocampus and vas deferens, which contain 38–64% high affinity sites, respectively, were pretreated with 10 μM CEC for 10 min in hypotonic medium and the inhibition of ^{125}IBE binding by WB 4101 was determined. Composite inhibition curves for these and other tissues are shown in Fig. 4. CEC pretreatment caused a significant increase in the potency of WB 4101 in competing for the remaining sites. Computer modeling of the resulting competition curves showed some suggestion of a small component of low affinity WB 4101 sites remaining; however, a two-site fit was not significantly better than a one-site fit in either tissue (Table 4). The −log K_D for WB 4101 in both CEC-pretreated tissues resembled the high affinity −log K_D observed in tissues with both receptor types (Table 1).

Effect of CEC on norepinephrine-stimulated ^{3}H-inositol phosphate accumulation. The potencies of WB 4101 in blocking norepinephrine-stimulated ^{3}H-inositol phosphate accumulation in a variety of tissues suggested that the receptor involved was of the low affinity, CEC-sensitive type (10). However, CEC pretreatment did not reduce norepinephrine-stimulated ^{3}H-inositol phosphate accumulation in brain slices (8). Because of problems in tissue access of the water-soluble CEC, it was possible that this was related to a problem of tissue penetration. Therefore, we tested to see whether CEC could block norepinephrine-stimulated ^{3}H-inositol phosphate accu-

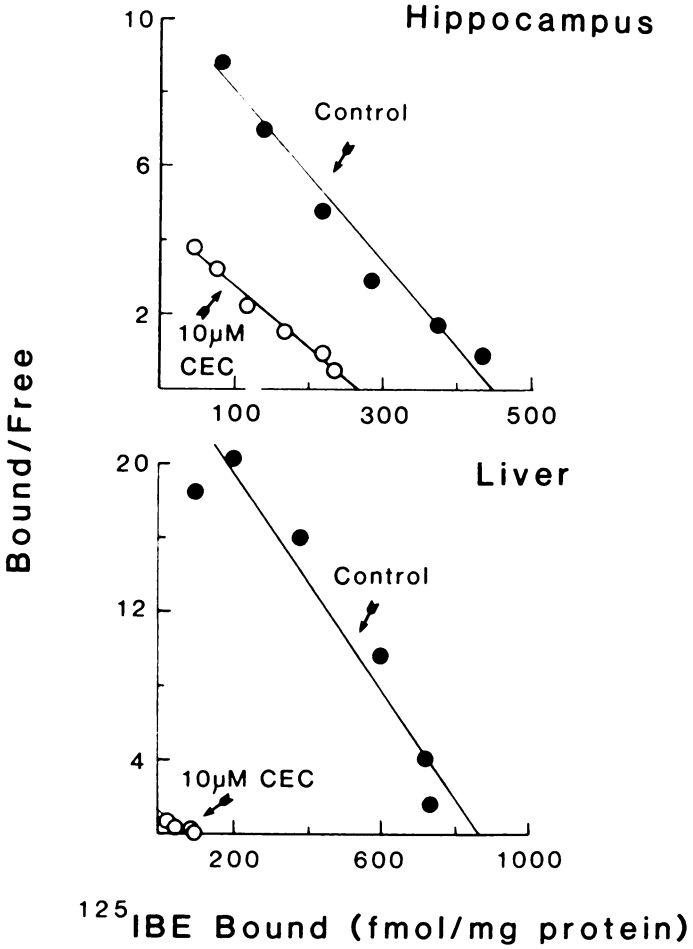

Fig. 2. CEC inactivation of ^{125}IBE-binding sites in hypotonic buffer. A crude particulate fraction from hippocampus and purified membranes from rat liver were centrifuged and resuspended in 10 mM Na Hepes (pH 7.6). Aliquots of each tissue were incubated in this buffer for 10 min with (○) or without (●) 10 μM CEC, diluted with 20 ml of cold Na Hepes buffer, and centrifuged. Tissues were resuspended in the appropriate volume of PO_4-salt buffer and saturation analysis of specific ^{125}IBE binding was determined as described under Experimental Procedures. Each value is the mean of duplicate determinations from four experiments.

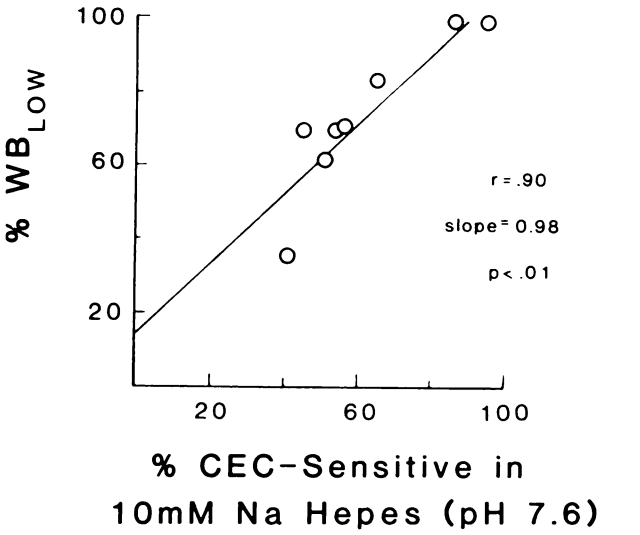

Fig. 3. Comparison of the proportion of ^{125}IBE-binding sites with a low affinity for WB 4101 (from Table 1) with the proportion sensitive to inactivation by CEC in hypotonic buffer (from Table 4) in eight rat tissues.

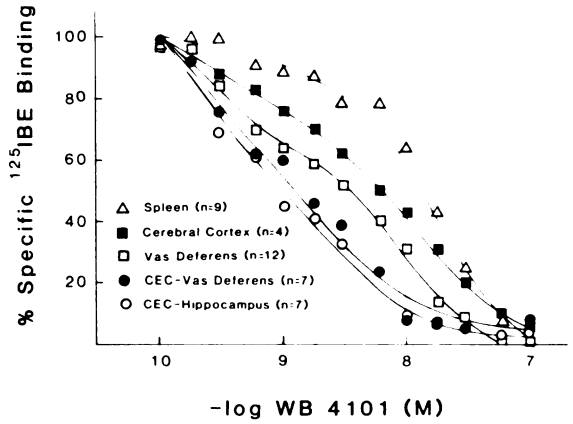

Fig. 4. Inhibition of specific ^{125}IBE binding by WB 4101 in control and CEC-treated tissues. Particulate preparations of hippocampus (○) and vas deferens (●) were pretreated with 10 μM CEC for 10 min, diluted, centrifuged, and resuspended as described under Experimental Procedures. Control preparations of particulate fractions of vas deferens (□), cerebral cortex (■), and spleen (△) were prepared without preincubation. Each value is the mean of duplicate determinations from 4 (cerebral cortex), 7 (CEC-treated hippocampus, CEC-treated vas deferens), 9 (spleen), or 12 (control vas deferens) experiments.

mulation in rat liver, which contains only the CEC-sensitive receptor subtype with a low affinity for WB 4101. Fig. 5 shows that pretreatment of liver slices with 10 or 30 μM CEC causes a dose-dependent loss of norepinephrine-stimulated ^{3}H-inositol phosphate accumulation, and that the density of ^{125}IBE-binding sites decreases in parallel with the decrease in responsiveness. Complete inactivation is not observed, probably because of problems of access of CEC into the slices. Rat vas deferens contains approximately equal proportions of both subtypes, although only the subtype with a high affinity for WB 4101 mediates the contractile response (10). Fig. 6 shows that pretreatment of vas deferens with 100 μM CEC, a concentration that has no significant effect on norepinephrine-induced contractions (9), causes a substantial inactivation of the receptor reserve (76%) for stimulation of ^{3}H-inositol phosphate accumulation in this tissue.

Discussion

The results presented here suggest that the two methods we have used to differentiate α_1-adrenergic receptors are distinguishing the same receptor subtypes. In support of the results of Morrow and co-workers (5, 6), we found that WB 4101 clearly distinguishes two different α_1-adrenergic receptor-binding sites (α_{1a} and α_{1b}) in a variety of mammalian tissues. Although the relative proportions of sites varies in different tissues, the K_D values for WB 4101 at the "high" (α_{1a}) and "low" (α_{1b}) affinity binding sites do not vary substantially. Most importantly, these K_D values correlate strikingly with the pA_2 values for blockade of norepinephrine-induced contractions of vas deferens and spleen, respectively (10). These observations strongly suggest that there are two subtypes of α_1-adrenergic receptors with different affinities for this competitive antagonist.

However, we initially chose the tissues in which different affinities for WB 4101 were demonstrated on the basis of their differential sensitivity to inactivation by CEC (7–9). Although there is a general correlation between the $\%WB_{Low}$ and the

TABLE 4

Inhibition of specific ^{125}IBE binding by WB 4101 in CEC-pretreated hippocampus and vas deferens

Particulate preparations from rat hippocampus and vas deferens were pretreated with 10 μM CEC for 10 min in hypotonic Na Hepes as described in the text. Inhibition of specific ^{125}IBE binding by WB 4101 was determined in each tissue (Table 1). Seven curves for each tissue were averaged and analyzed by nonlinear regression analysis to determine K_H, K_L, and % R_H for the best two-site fit. This was not significantly better ($p > 0.2$) than the best one-site fit (determined from a Hill plot) using a partial F test to compare the residual sum of squares (SOS) for either curve.

	CEC-treated hippocampus	CEC-treated vas deferens
One-site analysis		
$-\log K_D$	9.15	9.08
Residual SOS	476	636
Two-site analysis		
$-\log K_H$	9.36	9.19
$-\log K_L$	8.36	8.23
% R_H	81%	87%
Residual SOS	375	575
Partial F test	1.34 at 2 + 10 df NS	0.50 at 2 + 10 df NS

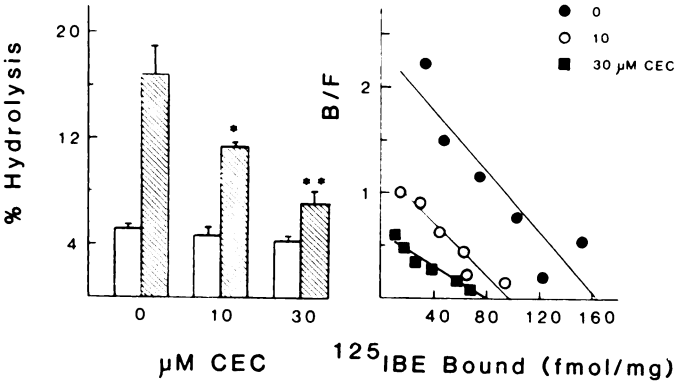

Fig. 5. Parallel inactivation of norepinephrine-stimulated ^{3}H-inositol phosphate accumulation (*left*) and specific ^{125}IBE-binding sites (*right*) by CEC in slices of rat liver. Liver slices were preincubated with 0, 10, or 30 μM CEC for 10 min, and ^{3}H-inositol phosphate accumulation was determined in the presence (▨) and absence (□) of 10 μM norepinephrine. Other slices were homogenized in PO_4-salt buffer, centrifuged, and resuspended in PO_4-salt buffer, and saturation analysis of specific ^{125}IBE binding was determined. Each value is the mean ± standard error (*left*) or mean (*right*) of data from four experiments determined in triplicate (*left*) or duplicate (*right*).

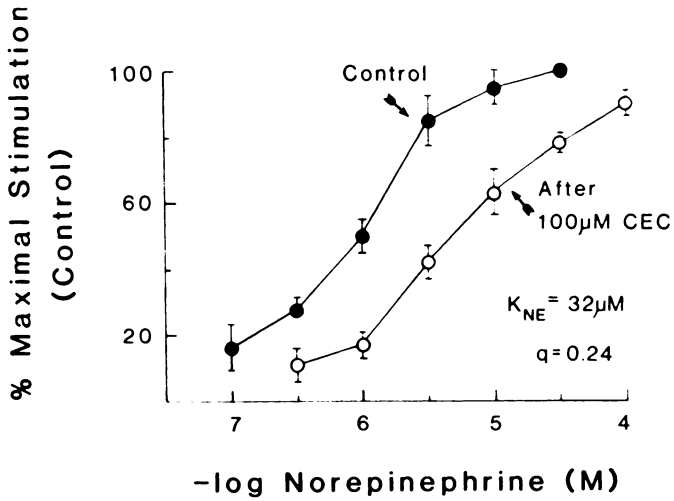

Fig. 6. Effect of CEC on norepinephrine-stimulated ^{3}H-inositol phosphate accumulation in rat vas deferens. Rings of vas deferens were pretreated for 30 min with (○) or without (●) 100 μM CEC at 37° and washed, and ^{3}H-inositol phosphate accumulation was determined in the presence of varying concentrations of norepinephrine. Each value is the mean ± standard error of data from four experiments. Analysis of the curves by the method of Furchgott (23) gave a K_A value of 32 μM for norepinephrine, and a q value of 0.24.

%CEC_{SENS}, there is not quantitative agreement between the proportions of each subtype calculated using each of these two approaches. Many tissues had a substantial %WB_{LOW} but were totally insensitive to CEC inactivation, and all tissues had a greater %WB_{LOW} than %CEC_{SENS}.

Our current results suggest that this discrepancy was probably caused by an incomplete access of the highly water-soluble CEC to α_1-adrenergic receptor-binding sites under the isotonic inactivation conditions used previously. This is supported by the fact that substantially greater inactivation is observed when tissue preparations are subjected to multiple treatments with CEC in isotonic medium, or when exposure to CEC is performed under hypotonic conditions. Although more binding sites become sensitive to CEC under hypotonic conditions, there are clearly still "sensitive" and "insensitive" binding sites. The fact that the %CEC_{SENS} under hypotonic conditions correlates highly with, and is quantitatively similar to, the %WB_{LOW} suggests that these two different drugs are distinguishing the same subpopulations of α_1-adrenergic receptor-binding sites.

The observation that pretreatment of hippocampus and vas deferens with CEC under hypotonic conditions essentially eliminates all low affinity WB 4101-binding sites strongly supports this conclusion.

We conclude from these results that there are two pharmacologically distinct subtypes of α_1-adrenergic receptors. One subtype (α_{1a}) has a relatively high affinity for WB 4101 and is not inactivated by CEC, whereas the other (α_{1b}) has a lower affinity for WB 4101 and is inactivated by CEC. Previously (10), we had shown that the potency of WB 4101 in blocking norepinephrine-stimulated inositol phospholipid hydrolysis in different tissues suggested that only the α_{1b} subtype was involved in this response. We would predict, therefore, that CEC would inactivate α_1-adrenergic receptor-stimulated inositol phosphate production. However, our initial studies on CEC showed that pretreatment of slices of cerebral cortex with 100 μM CEC did not reduce the magnitude of norepinephrine-stimulated ^{3}H-inositol phosphate accumulation (8). Since it was possible that the physicochemical characteristics of CEC might have restricted its penetration in brain slices, we repeated these experiments with liver (only α_{1b}) and vas deferens (a mixture of both subtypes). As predicted, we found that CEC inactivates the receptors stimulating inositol phosphate accumulation at concentrations similar to those necessary for inactivation of α_{1b}-adrenergic receptor binding sites in intact tissues. The decrease in the inositol phosphate response in liver slices was similar in dose dependence to the decrease in ^{125}IBE-binding sites in the same slices. The dual observations that the receptors increasing inositol phospholipid hydrolysis can be inactivated by CEC and have a low affinity for WB 4101 (10) support the conclusion that this response is mediated solely by the α_{1b} subtype. Since the contraction of vas deferens (α_{1a}) is blocked by nifedipine, we think it likely that this receptor subtype utilizes a completely different molecular mechanism for increasing intracellular calcium (10). The fact that CEC inactivation of the small population of α_{1b}-adrenergic receptors in vas deferens decreases norepinephrine-stimulated inositol

phospholipid hydrolysis (α_{1b}) but not norepinephrine-stimulated contractile responses (α_{1a}) (9) strongly supports this possibility.

In conclusion, these results strongly support the existence of two subtypes of α_1-adrenergic receptors with different sensitivities to CEC, different affinities for WB 4101, and linked to different mechanisms for signal transduction. The relationship of these two receptor subtypes to the well established variability in the importance of extracellular calcium in receptor-stimulated smooth muscle contraction (19–21) and the relatively small amount of inositol (1,4,5)-trisphosphate formed in response to receptor stimulation in some cells (22) are topics of current interest in our ongoing studies. With appropriate cognizance of possible access problems and careful validation of selectivity and completeness of receptor inactivation, CEC should prove to be a valuable tool for inactivating the α_{1b}-receptor subtype linked to inositol phosphate formation and exposing responses to the other (α_{1a}) receptor subtype.

Acknowledgments

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Send reprint requests to: Dr. Kenneth P. Minneman, Department of Pharmacology, Emory University School of Medicine, Atlanta, GA 30322.