0026-895X/87/030239-08$02.00/0

MOLECULAR PHARMACOLOGY, 31:239–246

Differentiation of α_1-Adrenergic Receptors Linked to Phosphatidylinositol Turnover and Cyclic AMP Accumulation in Rat Brain

RONALD D. JOHNSON and KENNETH P. MINNEMAN
Department of Pharmacology, Emory University School of Medicine, Atlanta, Georgia 30322
Received June 10, 1986; Accepted December 23, 1986

SUMMARY

Activation of α_1-adrenergic receptors in slices of rat brain increases inositol phosphate accumulation, increases basal cyclic AMP accumulation, and potentiates the increase in cyclic AMP caused by adenosine. We compared these three responses to determine whether they are mediated by the same receptors. The increase in inositol phosphates and the potentiation of cyclic AMP accumulation in cerebral cortex were largely blocked by chelation of extracellular calcium, whereas the increase in basal cyclic AMP was not affected. The magnitude of the increase in inositol phosphates in different brain regions correlated with the magnitude of the potentiation of cyclic AMP accumulation (r = 0.80), but neither of these correlated with the magnitude of the increase in basal cyclic AMP. Although other alkylating agents inactivated all of the α_1-adrenergic receptor-binding sites labeled with ^{125}IBE 2254 in membrane preparations of cerebral cortex, chlorethylclonidine (CEC) potently and selectively inactivated only half of these sites. Pretreatment with CEC partially blocked the increase in basal cyclic AMP, but not the increase in inositol phosphates or potentiation of cyclic AMP accumulation in slices of cerebral cortex. Comparing different brain regions, there was a better correlation between the density of ^{125}IBE 2254-binding sites not inactivated by CEC with the magnitude of the increase in inositol phosphates or potentiation of cyclic AMP accumulation than with the increase in basal cyclic AMP. Although the largest increase in inositol phosphates was observed in slices of hippocampus, there was only a small increase in basal cyclic AMP in this region, and CEC did not inactivate any ^{125}IBE-binding sites in hippocampus. Phentolamine and WB 4101 were significantly more potent in inhibiting specific ^{125}IBE 2254 binding in hippocampus than in cerebral cortex. After treatment of cerebral cortical membranes with CEC, however, these drugs had potencies similar to those observed in hippocampus. The results suggest that the α_1-adrenergic receptors mediating increases in basal cyclic AMP accumulation can be differentiated from those mediating increases in inositol phosphate accumulation and potentiating adenosine stimulated cyclic AMP accumulation by their binding properties, calcium dependency, regional distribution, and sensitivity to the alkylating agent CEC.

Several lines of evidence suggest that α_1-adrenergic receptors may comprise a heterogeneous group (1). Differences in the potencies and intrinsic activities of drugs have been reported for α_1-adrenergic receptors in smooth muscle (2–6), liver (7), and brain (8, 9). In addition, several studies have reported differences in the importance of external calcium concentration on α_1-adrenergic receptor-mediated responses in both smooth muscle (6, 10) and liver (7, 11–13).

α_1-Adrenergic receptors are thought to belong to the class of receptors which initiate their physiological effects by hydrolyzing phosphatidylinositol 4,5-bisphosphate in the cell membrane (14). This results in production of inositol 1,4,5-trisphosphate which mobilizes intracellular calcium (15) and diacylglycerol which activates protein kinase C (16). In mammalian brain, however, activation of α_1-adrenergic receptors also increases cyclic AMP accumulation and potentiates increases in cyclic AMP accumulation caused by other agonists such as adenosine (17–21). A similar α_1-adrenergic receptor-mediated increase in cyclic AMP levels can be observed in rat liver under certain conditions (7, 22, 23).

α_1-Adrenergic receptor-mediated increases in cyclic AMP accumulation may be secondary to phosphoinositide hydrolysis and increases in intracellular calcium (21). Conversely, α_1-adrenergic receptors might be linked to more than one signal transduction mechanism, or there might be distinct subtypes of α_1-adrenergic receptors linked to different signal transduction mechanisms (1, 7, 13).

This work was supported by National Institutes of Health Grant NS 21325. R. D. J. is a recipient of a predoctoral fellowship from the Pharmaceutical Manufacturers Association Foundation.

ABBREVIATIONS: BAAM, bromoacetylalprenololmenthane; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid; KRB, Krebs-Ringer bicarbonate buffer; ^{125}IBE, ^{125}IBE 2254, {2-[β-(4-hydroxyphenyl)-ethylaminoethyl]tetralone}; CEC, chlorethylclonidine; IBMX, isobutylmethylxanthine; PMA, 4β-phorbol-12-myristate-13-acetate; DIB, dibenamine; EEDQ, *n*-ethoxy-carbonyl-2-ethoxy-1,2-dihydroquinone; BXT, benextramine.

We recently compared the pharmacological properties of the α_1-adrenergic receptors in rat cerebral cortex linked to phosphatidylinositol hydrolysis to those increasing basal cyclic AMP accumulation and potentiating the increase in cyclic AMP accumulation caused by adenosine (24). In order to examine the α_1-adrenergic receptors linked to cyclic AMP accumulation we first inactivated the β-adrenergic receptors by treatment with the alkylating agent, BAAM (25). We found small but significant differences in the potencies of agonists and certain antagonists in activating or inhibiting these different responses (24). Also, in studying the adaptive regulation of these responses following changes in synaptic input, the different responses did not always covary (26). Therefore, we have examined the relationship between these responses to determine whether they are mediated by the same receptor population.

Methods

Tissue preparation. Male Sprague-Dawley rats (150–300 g) were killed by cervical dislocation and decapitated; then, the brain and cervical spine cord were removed. The cerebral cortex or other indicated regions were dissected and chopped into 350 × 350 μm slices on a McIlwain tissue chopper. Slices were dispersed in KRB (120 mM NaCl, 5.5 mM KCl, 2.5 mM $CaCl_2$, 20 mM $NaHCO_3$, 11 mM glucose, 0.029 mM $CaNa_2$ EGTA, 1.2 mM $MgCl_2$, and 1.2 mM NaH_2PO_4) previously equilibrated at 37° with 95% O_2/5% CO_2. The KRB was decanted, and the slices were resuspended in fresh KRB and incubated under O_2/CO_2 at 37° for 20 min.

Cyclic AMP accumulation. Increases in cyclic AMP accumulation were determined by the prelabeling method of Shimizu *et al.* (27) as described by Johnson and Minneman (24). Aliquots of slices were prelabeled with ^{3}H-adenine and then pretreated with 30 μM BAAM to inactivate the β-adrenergic receptors (24, 25). The slices were again washed and resuspended in KRB and cyclic AMP accumulation was determined (24, 28). Cyclic AMP accumulation was expressed as percentage of total tritium incorporated into the slices which was converted to ^{3}H-cyclic AMP (percentage conversion).

^{3}H-Inositol phosphate accumulation. Increases in ^{3}H-inositol phosphates were determined in parallel preparations by the method of Berridge *et al.* (29) as previously described (30). An aliquot of the organic phase was evaporated to dryness and counted for determination of total ^{3}H-inositol incorporated into lipid. Results were expressed as counts per minute or as percentage of the total radioactivity incorporated which was converted to inositol phosphates (percentage hydrolysis).

A prelabeling protocol was also used for the measurement of ^{3}H-inositol phosphates in some experiments on calcium dependence. Slices (1.2 g wet weight) were incubated for 60 min under O_2/CO_2 at 37° in 30 ml of KRB containing 300 μCi of ^{3}H-inositol. The KRB was decanted, and the slices were washed and resuspended in KRB and divided into three equal aliquots. One aliquot was suspended in regular Li-KRB, one in Li-KRB without added calcium, and the other in Li-KRB without added calcium and containing 1 mM EGTA. Aliquots of 75 μl of packed slices were then added to tubes containing 285 μl Li-KRB with or without added calcium and/or EGTA, 300 μM ascorbate, 0.1 μM desmethylimipramine, and drugs as indicated. Tubes were incubated at 37° under O_2/CO_2 for 15 min, and the reaction was stopped as described above. ^{3}H-Inositol phosphates were isolated as described above.

^{125}IBE binding. Tissues were homogenized in 10–30 volumes of 20 mM sodium phosphate buffer (pH 7.6) containing 154 mM NaCl ("phosphate-salt buffer"), centrifuged at 20,000 × *g* for 10 min, and resuspended in 200–400 volumes of phosphate-salt buffer. Aliquots of membrane preparations (100 μl; 20–40 μg of protein) were incubated in a final volume of 0.25 ml of phosphate-salt buffer containing 25–800 pM ^{125}IBE (31) and drugs as indicated. After 20 min at 37°, reactions were stopped by addition of 10 ml of 10 mM Tris-Cl (pH 7.4) and filtration over glass fiber filters (Schleicher and Schuell no. 30). The filters were washed with 10 ml buffer and dried, and radioactivity was determined. Specific ^{125}IBE binding was defined as binding displaced by 10 μM phentolamine (32) and usually represented 80–90% of total binding. Protein content was determined by the method of Bradford (33) using bovine serum albumin as the standard.

Materials. CEC was obtained from Research Biochemicals Inc., and BAAM was generously provided by Dr. Josef Pitha (National Institute on Aging). Carrier-free Na^{125}I was obtained from Amersham, and ^{3}H-inositol and [2,8-^{3}H]adenine were obtained from New England Nuclear. Phentolamine mesylate was provided by Ciba-Geigy. All other chemicals were from standard commercial sources.

Results

Effect of removing extracellular calcium. Fig. 1 shows the effect of omitting calcium, or omitting calcium and adding 1 mM EGTA to the incubation medium on α_1-adrenergic receptor-mediated responses in slices of rat cerebral cortex. Omitting calcium caused small decreases in the ^{3}H-inositol phosphate response and in the potentiation of adenosine-stimulated cyclic AMP accumulation in BAAM-pretreated slices. However, omitting calcium did not reduce the increase in basal cyclic AMP accumulation caused by α_1-adrenergic receptor stimulation in BAAM-pretreated slices. Addition of EGTA to chelate extracellular calcium abolished the inositol phosphate response and markedly reduced the potentiation of cyclic AMP accumulation. Again, there was no effect on the stimulation of basal cyclic AMP accumulation, despite a small increase in baseline values (Fig. 1).

Since there is a substantial difference in final incubation time for measurement of cyclic AMP and inositol phosphates (15 min versus 2 hr), it was necessary to control for the time during which the tissues were exposed to low calcium. The experiments were therefore repeated using a prelabeling protocol to measure inositol phosphate accumulation. Tissues were prelabelled with ^{3}H-inositol for 60 min in normal calcium KRB, and exposed to norepinephrine in the presence or absence of calcium and EGTA for only 15 min. Although the response to norepinephrine was smaller due to the shorter stimulation period, the results were qualitatively identical to those obtained with the standard protocol (Table 1). Removal of extracellular calcium caused a small decrease in the inositol phosphate response, whereas addition of 1 mM EGTA for 15 min completely abolished the response.

Effects of cyclooxygenase inhibitors. Previous work (34) suggested that α_1-adrenergic receptor-stimulated cyclic AMP accumulation in brain might be blocked by inhibitors of prostaglandin synthesis. We pretreated slices for 30 min with 0.1 mM indomethacin or 0.3 mM acetylsalicylic acid and also added these drugs during the stimulation period. Although these treatments reduced basal cyclic AMP accumulation by 24–30%, they did not significantly reduce the increases in basal cyclic AMP or the potentiation of the response to adenosine in response to α_1-adrenergic receptor stimulation in BAAM-pretreated slices (data not shown).

Effect of IBMX. α_1-Adrenergic receptor stimulation has been shown to alter cyclic AMP degradation in cardiomyocytes (35). To determine whether the effects of norepinephrine on cyclic AMP levels in brain slices were due to a decrease in cyclic AMP degradation, we studied the effect of the cyclic

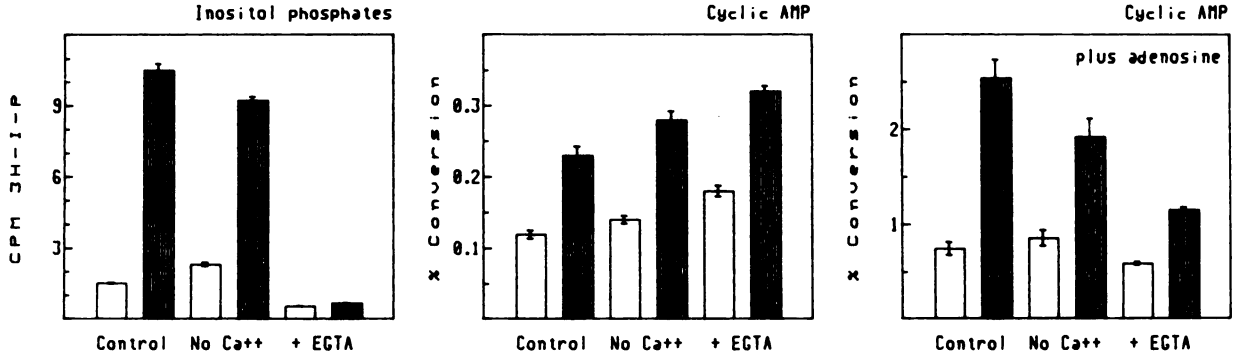

Fig. 1. Effect of reducing extracellular calcium on α_1-adrenergic receptor responses in rat cerebral cortex. The effects of omitting calcium (*No Ca^{++}*), or omitting calcium and chelating extracellular calcium with 1 mM EGTA (+ *EGTA*) on norepinephrine-stimulated inositol phosphate accumulation (*left*), norepinephrine-stimulated cyclic AMP accumulation (*center*), and norepinephrine potentiation of cyclic AMP accumulation in response to 0.3 mM adenosine (*right*) in BAAM-pretreated slices of rat cerebral cortex are shown. Experimental details are described in the legend to Table 1. □, values in the absence of norepinephrine; ■, values in the presence of 0.3 mM norepinephrine. Each *bar* represents the mean ± standard error of quadruplicate determinations from each of four experiments.

TABLE 1

Effect of removal of calcium on α_1-adrenergic receptor-mediated responses in rat cerebral cortex

Norepinephrine (0.3 mM)-stimulated increases in basal cyclic AMP accumulation and potentiation of 0.3 mM adenosine-stimulated cyclic AMP accumulation in BAAM-pretreated slices were determined as described in the text. Slices were resuspended in calcium-free KRB with or without EGTA only for the final 15-min incubation with norepinephrine. In control KRB, percentage conversion was 0.12 ± 0.01 in the absence of drug, 0.23 ± 0.02 with 0.3 mM norepinephrine, 0.64 ± 0.09 with 0.3 mM adenosine, and 2.30 ± 0.22 with both norepinephrine and adenosine. Norepinephrine (0.3 mM)-stimulated inositol phosphate accumulation was determined as described in the text where the final 2-hr incubation with ^{3}H-inositol and norepinephrine was performed in calcium-free KRB with or without EGTA (regular protocol). To control for the time of exposure to low Ca^{2+}, slices were preloaded with ^{3}H-inositol in normal KRB for 1 hr, and exposed to norepinephrine (0.3 mM) in calcium-free KRB with or without EGTA for only 15 min (prelabeling protocol). Each value is the mean ± standard error of the per cent increase over basal observed in three to four experiments performed in triplicate.

	Percentage stimulation			
	Increase in basal cyclic AMP	Potentiation of adenosine-stimulated cyclic AMP	Increase in inositol phosphates (Regular protocol)	Increase in inositol phosphates (Prelabeling protocol)
Control KRB	86 ± 10.9	240 ± 15.2	589 ± 5.6	56 ± 5.1
No Ca^{2+}	91 ± 8.6	122 ± 20.5[a]	296 ± 3.4[a]	43 ± 5.1
No Ca^{2+} + 1 mM EGTA	80 ± 4.7	97 ± 5.0[a]	25 ± 4.5[a]	2 ± 4.7[a]

[a] $p < 0.01$ compared to control KRB.

nucleotide phosphodiesterase inhibitor IBMX. Increasing doses of IBMX caused a dose-dependent increase in both basal and norepinephrine-stimulated cyclic AMP accumulation in BAAM-pretreated slices (Fig. 2). The increase in basal accumulation was almost exactly matched by the increase in norepinephrine-stimulated cyclic AMP accumulation. It was not possible to measure the effect of IBMX on cyclic AMP accumulation in the presence of adenosine, since IBMX blocked the response to adenosine (data not shown).

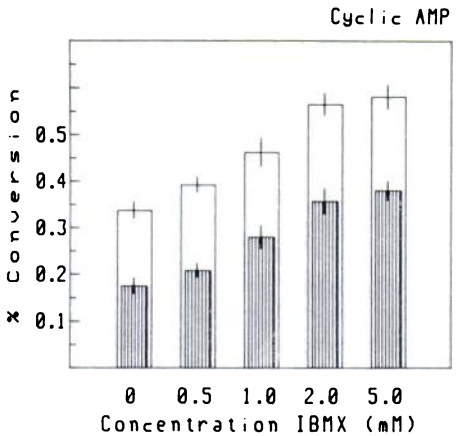

Fig. 2. Effect of IBMX on cyclic AMP accumulation in rat cerebral cortex. IBMX was present at the indicated concentrations for the final 15-min incubation. ■, basal cyclic AMP accumulation; □, cyclic AMP accumulation in the presence of 0.3 mM norepinephrine in BAAM-pretreated slices. Each *bar* represents the mean ± standard error of quadruplicate determinations from each of three experiments.

Effect of PMA. Treatment with phorbol esters has been shown to mimic (36–38) or block (39–41) α_1-adrenergic receptor-mediated responses. We found no effect of 15 min pretreatment with, and continued presence of, 0.01 or 1 μM PMA on α_1-adrenergic receptor-mediated increases in inositol phosphates, in basal cyclic AMP, or in potentiation of adenosine effects on cyclic AMP in BAAM-pretreated slices of cerebral cortex (data not shown).

Regional distribution in brain. There was considerable variation in the magnitude of basal cyclic AMP accumulation and in the response to norepinephrine and adenosine separately or in combination in BAAM-pretreated slices from different brain regions (Table 2). Basal cyclic AMP accumulation was highest in cerebellum and lowest in hypothalamus. The largest α_1-adrenergic receptor-mediated basal increases were found in spinal cord (151% increase), striatum (117%), and cerebral cortex (107%), whereas the smallest increases were observed in hippocampus (30%) and cerebellum (39%). The largest α_1-adrenergic receptor-mediated potentiation of adenosine-stimulated cyclic AMP accumulation was observed in hypothalamus, striatum, and spinal cord (250% increase), with the smallest effect observed in cerebellum (14%).

TABLE 2

Regional distribution of receptor-mediated increases in cyclic AMP accumulation in slices of rat brain after BAAM pretreatment

The indicated brain regions were dissected and tissues from two to eight animals were pooled and chopped into 350 × 350 μm slices. After prelabeling with ^{3}H-adenine and pretreatment with 30 μM BAAM, slices were incubated for 15 min with the indicated drugs, and cyclic AMP accumulation was determined as described. Each value is the mean ± standard error of triplicate determinations from four experiments.

Region	Percentage Conversion			
	Control	0.3 mM Norepinephrine	0.3 mM Adenosine	Norepinephrine + Adenosine
Cortex	0.20 ± 0.016	0.41 ± 0.025 (207)[a]	0.66 ± 0.052 (334)	1.86 ± 0.178 (281)
Cerebellum	0.84 ± 0.195	1.17 ± 0.301 (139)	1.51 ± 0.253 (179)	1.72 ± 0.248 (114)
Pons-medulla	0.37 ± 0.042	0.64 ± 0.035 (174)	0.94 ± 0.071 (255)	1.92 ± 0.241 (205)
Spinal cord	0.28 ± 0.017	0.70 ± 0.114 (251)	0.48 ± 0.047 (173)	1.68 ± 0.359 (348)
Striatum	0.17 ± 0.019	0.37 ± 0.171 (217)	0.62 ± 0.044 (365)	2.16 ± 0.021 (348)
Hypothalamus	0.14 ± 0.017	0.24 ± 0.086 (167)	0.71 ± 0.061 (506)	2.50 ± 0.198 (350)
Hippocampus	0.16 ± 0.027	0.21 ± 0.079 (130)	0.36 ± 0.033 (223)	1.70 ± 0.126 (472)
Thalamus	0.17 ± 0.015	0.32 ± 0.019 (188)	0.55 ± 0.055 (329)	1.51 ± 0.126 (274)

[a] Numbers in parentheses, per cent of control.

We have previously determined the magnitude of norepinephrine-stimulated inositol phosphate accumulation in these brain regions (42). Therefore, we compared the magnitude of each of the three responses in the different brain regions. There was clearly no correlation between the magnitude of the increase in basal cyclic AMP accumulation and either the potentiation of adenosine-stimulated cyclic AMP accumulation ($r = -0.24$) or the increase in inositol phosphates ($r = -0.19$). However, there was a significant correlation between the magnitude of the inositol phosphate response and the potentiation of adenosine-stimulated cyclic AMP accumulation ($r = 0.80$; $p < 0.05$).

Effect of irreversible alkylating agents on α_1-adrenergic receptor density. We examined the effects of several irreversible alkylating agents on the density of specific ^{125}IBE-binding sites in membrane preparations from rat cerebral cortex, to determine whether all of the binding sites were equally sensitive to inactivation. Pretreatment of membranes with DIB, EEDQ, and BXT caused a dose-dependent loss of specific ^{125}IBE-binding sites (Fig. 3). Each of these compounds was able to inactivate essentially all of the specific ^{125}IBE-binding sites in this preparation, and there was no evidence for heterogeneity in the susceptibility of the binding sites to alkylation. However, pretreatment of membranes with CEC (43) inactivated only a portion of the specific ^{125}IBE-binding sites in a dose-dependent manner. At doses as small as 3 μM, approximately half of the specific ^{125}IBE-binding sites were lost, and little further inactivation was observed even when the dose of CEC was increased 100-fold (Fig. 3).

Effect of CEC pretreatment on α_1-adrenergic receptor responses. To determine whether the subpopulation of binding sites selectively inactivated by CEC was linked to a particular response, we studied the effect of CEC pretreatment on α_1-adrenergic receptor-mediated responses in brain slices. CEC was less potent in inactivating specific ^{125}IBE-binding sites when added to slices of cerebral cortex, probably due to restricted diffusion into the slices. However, pretreatment of slices with 10 or 100 μM CEC significantly reduced the density

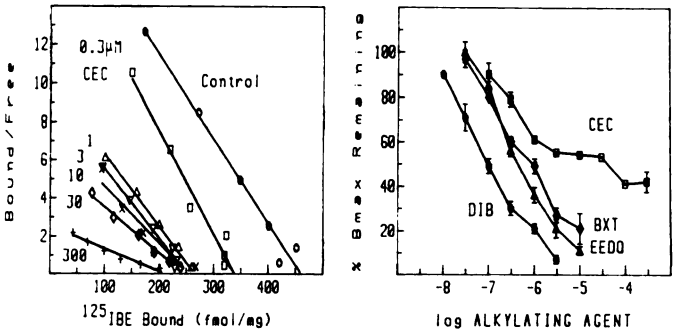

Fig. 3. Inactivation of α_1-adrenergic receptor-binding sites by CEC, DIB, BXT, and EEDQ. Cerebral cortices from four rats were homogenized in 20 ml of 20 mM phosphate buffer (pH 7.6) and centrifuged for 10 min at 20,000 × *g*; then, the pellet was resuspended in 10 ml of the same buffer. Aliquots of 1 ml were diluted to 10 ml and warmed to 37°. The indicated doses of alkylating agent were added and the tissue was incubated 10 min at 37°. Reactions were stopped by addition of 10 ml of cold buffer and centrifugation at 20,000 × *g* for 10 min. Each pellet was resuspended in 20 ml of phosphate-salt buffer, and Scatchard analysis of saturation isotherms of specific ^{125}IBE binding was determined as described. *Left:* CEC inactivation. Each point is the mean of duplicate determinations from three to seven experiments. *Right:* Dose dependence of the inactivation of ^{125}IBE-binding sites. Scatchard analysis of saturation isotherms of specific ^{125}IBE binding was performed on each tissue preparation after treatment as described above. The percentage of the control B_{max} remaining after treatment with each dose of drug is indicated on the *ordinate*. Each value is the mean ± standard error of three to seven experiments performed in duplicate.

of specific ^{125}IBE-binding sites in membrane preparations from the slices. Both concentrations also reduced norepinephrine-stimulated increases in basal cyclic AMP accumulation in BAAM-pretreated slices, but did not significantly affect the inositol phosphate response or the potentiation of adenosine-stimulated cyclic AMP accumulation (Fig. 4).

Inactivation of ^{125}IBE-binding sites by CEC in different brain regions. Since some of the specific ^{125}IBE-binding sites in cerebral cortex were not inactivated by pretreatment with CEC, it was of interest to determine the proportion of sites sensitive to CEC inactivation in each brain region. Follow-

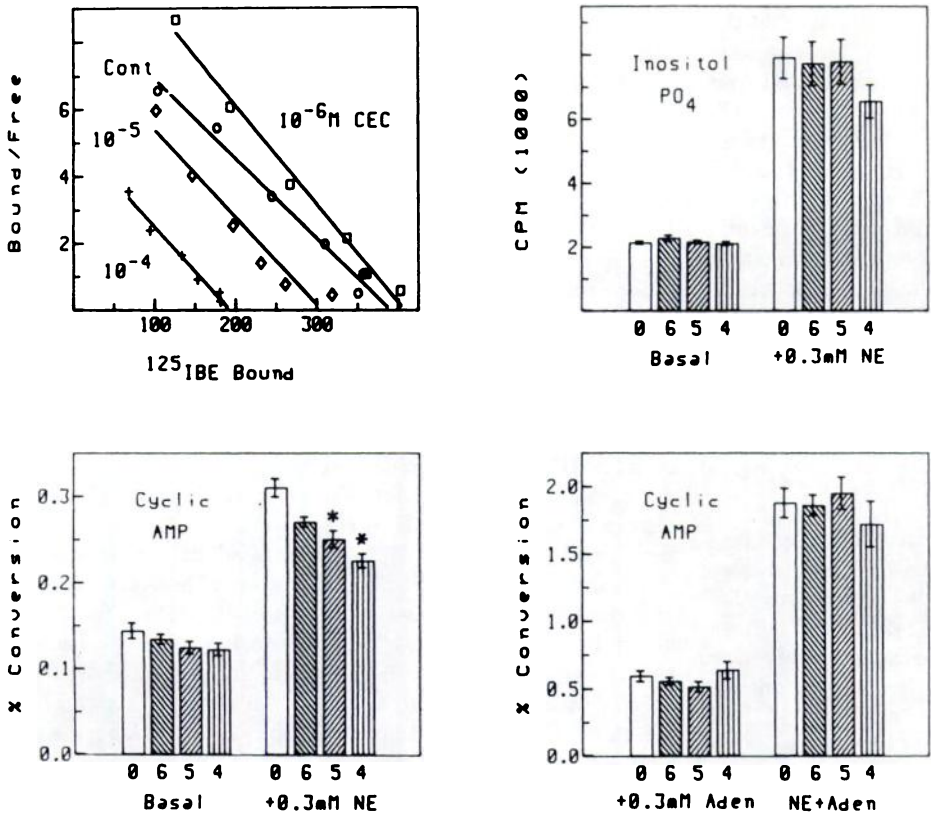

Fig. 4. Effect of CEC on α_1-adrenergic receptor-mediated responses in slices of rat cerebral cortex. Slices of cerebral cortex were incubated with 0, 10^{-6} (6), 10^{-5} (5), or 10^{-4} (4) M CEC for 10 min and washed extensively. A 100-μl aliquot of slices from each tissue preparation was homogenized in 10 ml of phosphate-salt buffer, centrifuged at 20,000 × *g* for 10 min, and resuspended in 3 ml of buffer. Scatchard analysis of specific ^{125}IBE binding was determined as described (*upper left*). Other slices were taken for measurement of norepinephrine (*NE*, 0.3 mM)-stimulated accumulation of ^{3}H-inositol phosphates (*upper right*) or ^{3}H-cyclic AMP in the absence (*lower left*) or presence (*lower right*) of 0.3 mM adenosine (*Aden*) in BAAM-pretreated slices. Each point is the mean or mean ± standard error of five to six experiments performed in duplicate (^{125}IBE binding) or triplicate (cyclic AMP and inositol phosphate accumulation). *, $p < 0.05$ compared to control (0).

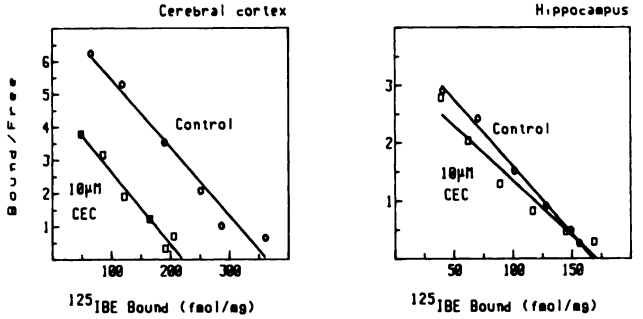

Fig. 5. Comparison of the effect of CEC pretreatment on specific ^{125}IBE-binding sites in rat cerebral cortex and hippocampus. One-half cerebral cortex or three pair hippocampi were homogenized in 20 ml of phosphate-salt buffer, centrifuged at 20,000 × *g* for 10 min, and resuspended in 22 ml. Five-ml aliquots were incubated with or without 10^{-5} M CEC for 10 min at 37°. Reactions were stopped by addition of 20 ml of cold buffer and centrifugation. Pellets were resuspended in 3 ml of phosphate-salt buffer and Scatchard analysis of saturation isotherms of specific ^{125}IBE binding was determined as described. Each point is the mean of duplicate determinations from four experiments.

ing pretreatment of membrane preparations with 10 μM CEC, there was no significant reduction in the density of specific ^{125}IBE-binding sites in rat hippocampus (Fig. 5), but a 20–40% reduction in the other regions examined (Table 3). We correlated the density of ^{125}IBE-binding sites which are sensitive or insensitive to inactivation by 10 μM CEC to the magnitude of the different responses in each brain area. There were no significant relationships ($p > 0.05$) between the density of CEC-sensitive or -insensitive ^{125}IBE-binding sites and the magnitude of any functional response studied. It was interesting, however, that the highest correlation coefficients were observed between the density of CEC-insensitive sites and the magnitude of the inositol phosphate and potentiated cyclic AMP responses ($p < 0.25$).

TABLE 3

Effect of pretreatment with CEC on the density of ^{125}IBE-binding sites in rat brain

The indicated brain regions were dissected and homogenized in 10 ml of phosphate-salt buffer, centrifuged at 20,000 × *g* for 10 min, and resuspended in 60 (cortex), 100 (cerebellum, pons-medulla, hypothalamus), 150 (hippocampus, thalamus, spinal cord) or 200 (striatum) volumes of buffer. Half of each tissue preparation was incubated with or without 10^{-5} M CEC for 10 min at 37°. Twenty ml of cold buffer were added and tissues were centrifuged again. Tissues were resuspended in 30 (pons-medulla, cerebellum), 40 (hypothalamus), 60 (cortex, hippocampus, thalamus, spinal cord), or 80 (striatum) volumes of buffer. Scatchard analyses of saturation isotherms of specific ^{125}IBE binding were determined as described. Each value is the mean ± standard error of data from four to seven experiments performed in duplicate. No significant changes in K_D values were observed.

Region	^{125}IBE 2254 B_{max}		Δ	Per cent insensitive to CEC
	Total	After 10^{-5} M CEC		
	fmol/mg of protein			
Cortex	452 ± 49	279 ± 35[a]	173	62
Cerebellum	184 ± 16	125 ± 12[b]	59	68
Pons-medulla	210 ± 13	168 ± 11[b]	42	80
Spinal cord	196 ± 27	156 ± 8	40	80
Striatum	303 ± 24	217 ± 12[b]	86	72
Hypothalamus	466 ± 35	306 ± 36[b]	160	66
Hippocampus	233 ± 26	210 ± 21	23	90
Thalamus	465 ± 34	282 ± 9[a]	183	60

[a] $p < 0.01$ compared to total.
[b] $p < 0.05$ compared to total.

Differences in pharmacological properties of ^{125}IBE-binding sites. Phentolamine and WB 4101 have been reported to have heterogeneous affinities for α_1-adrenergic receptor-binding sites in rat brain (8, 9). We compared the potencies of these drugs in competing for specific ^{125}IBE-binding sites in rat cerebral cortex and hippocampus. Rat hippocampus was chosen

because it had the largest inositol phosphate response, but the smallest increase in basal cyclic AMP and no binding sites sensitive to CEC inactivation. Both phentolamine and WB 4101 were slightly but significantly more potent (2.1- and 3.8-fold, respectively) in competing for specific ^{125}IBE-binding sites in hippocampus than in cortex (Table 4). However, the Hill coefficients for both drugs in both tissues were not significantly different than 1.0, and a two-site model did not significantly improve the goodness of fit for the inhibition curves in either tissue using a partial F test (44). Treatment of membranes of cerebral cortex for 10 min with 10 μM CEC significantly increased the potency of both phentolamine (Table 4) and WB 4101 (Fig. 6) by 1.9- and 2.6-fold, respectively, such that the values were no longer significantly different from those in untreated hippocampus.

Discussion

Activation of α_1-adrenergic receptors in slices of rat cerebral cortex increases inositol phosphate accumulation, increases cyclic AMP accumulation, and potentiates the increase in cyclic AMP accumulation caused by adenosine (17–21, 30, 45, 46). In this paper we have described several experiments suggesting that these effects are not necessarily mediated by the same α_1-adrenergic receptor population.

Increased cyclic AMP accumulation might be a consequence of phosphoinositide hydrolysis through increases in intracellular calcium or activation of protein kinase C. Chelation of calcium by EGTA blocks α_1-adrenergic receptor-mediated potentiation of isoproterenol or histamine stimulation of cyclic AMP accumulation (47) and increased inositol phosphate accumulation in cortical slices (48). However, the effects on α_1-adrenergic receptor-mediated increases in basal cyclic AMP accumulation are less clear (47). We confirmed that EGTA abolished the increase in inositol phosphates and greatly reduced the potentiation of adenosine-stimulated cyclic AMP accumulation. However α_1-adrenergic receptor-mediated increases in basal cyclic AMP accumulation were not affected by EGTA, suggesting that they are not secondary to the phosphoinositide response. α_1-Adrenergic receptor-mediated potentiation of enzyme induction in rat pineal gland and stimulation of calcium efflux in smooth muscle cells are mimicked by phorbol esters which activate protein kinase C (36–38). Conversely, phorbol esters uncouple α_1-adrenergic receptors from phosphoinositide metabolism in many tissues (38, 39, 41), possibly by receptor phosphorylation (40). However, we observed no effects of the phorbol ester PMA, suggesting that short-term activation of protein kinase C neither uncouples α_1-adrenergic receptors nor is responsible for any of the responses examined in this system.

Increases in phosphoinositide metabolism also increase arachidonic acid levels in some tissues (16). Partington *et al.* (34) reported that α_1-adrenergic receptor-mediated increases in cyclic AMP accumulation in brain slices were reduced by cyclooxygenase inhibitors. We found, however, that indomethacin and acetylsalicylic acid reduced basal cyclic AMP accumulation but did not alter the response to α_1-adrenergic receptor stimulation, suggesting that the increases in cyclic AMP are not secondary to increased prostaglandin synthesis.

Changes in cyclic AMP levels may be due to changes in the rate of degradation. Activation of both muscarinic cholinergic (49) and α_1-adrenergic (35) receptors reduces cyclic AMP levels in various cell types by increasing degradation. The increase in cyclic AMP accumulation observed in our experiments was not due to decreased degradation, since the effects of the phosphodiesterase inhibitor IBMX, at concentrations which maximally increased basal cyclic AMP levels, were additive with those of α_1-adrenergic receptor-mediated increases. These experiments also show that the increase in basal cyclic AMP accumulation is not dependent on activation of adenosine receptors. Daly *et al.* (21, 45) found that α_1-adrenergic receptor-mediated increases in cyclic AMP accumulation in slices of rat cerebral cortex were blocked by 8-phenyltheophylline or adenosine deaminase. In other brain regions, however, the response is not blocked by these manipulations (21, 50, 51). The high concentrations of IBMX used here completely blocked the increase in cyclic AMP accumulation caused by 0.3 mM adenosine (data not shown) but did not affect the increase in basal cyclic AMP accumulation. Obviously the potentiation of the effect of adenosine could not be examined in the presence of IBMX. We also tested the effects of 10 μM 8-phenyltheophylline, and found that this compound reduced basal levels and reduced but did not abolish the effect of α_1-adrenergic receptor stimulation (data not shown).

The α_1-adrenergic receptor-mediated increases in basal cyclic AMP accumulation in our experiments are not affected by chelation of extracellular calcium, inhibition of eicosanoid synthesis, activation of protein kinase C, inhibition of cyclic AMP degradation, or blockade of adenosine receptors. This response does not, therefore, appear to be secondary to increases in phosphoinositide metabolism. However, α_1-adrenergic receptor-mediated potentiation of adenosine-stimulated cyclic AMP accumulation is markedly reduced by chelation of extracellular calcium and may therefore be due to effects on mediator release or on cellular calcium.

TABLE 4

Potency of WB 4101 and phentolamine in competing for specific ^{125}IBE-binding sites in cerebral cortex before or after treatment with 10^{-5} M CEC for 10 min and in untreated hippocampus

Inhibition of specific ^{125}IBE binding (60–70 pM) by 16 concentrations of each drug was determined as described in the text. IC_{50} values and Hill coefficients (n_H) were determined from Hill plots and K_i values were calculated. Each value is the mean of four (WB 4101) or five (phentolamine) separate determinations.

	WB 4101		Phentolamine	
	$-\log K_i$	n_H	$-\log K_i$	n_H
	M		M	
Control cortex	8.15 ± 0.058	0.98 ± 0.065	6.94 ± 0.029	0.96 ± 0.019
Cortex after CEC	8.56 ± 0.102[a]	0.89 ± 0.066	7.22 ± 0.099[a]	0.80 ± 0.024[b]
Control hippocampus	8.73 ± 0.076[c]	0.93 ± 0.078	7.26 ± 0.108[b]	0.83 ± 0.060

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

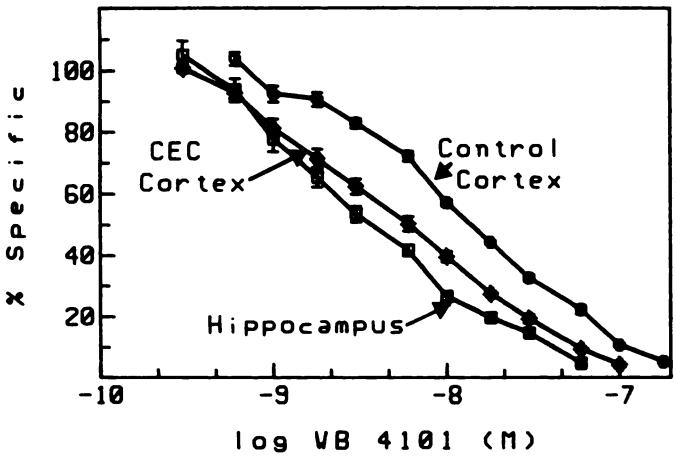

Fig. 6. Inhibition of specific ^{125}IBE binding by WB 4101 in hippocampus and in cerebral cortex before and after CEC pretreatment. One-half cerebral cortex or three pair hippocampi were homogenized in 20 ml of phosphate-salt buffer and centrifuged at 20,000 × *g* for 10 min. Pellets were resuspended in 22 (cortex) or 8.5 (hippocampus) ml of phosphate-salt buffer. Cortex was split into two 10-ml aliquots and incubated with or without 10^{-5} M CEC for 10 min at 37°. Twenty ml of cold buffer were added, samples were centrifuged, and each was resuspended in 8.5 ml. Inhibition of specific ^{125}IBE binding by WB 4101 was determined as described. Each value is the mean ± standard error of duplicate determinations from four experiments.

We reported previously that there was not a good correlation between the density of specific ^{125}IBE-binding sites and the magnitude of the inositol phosphate response to α_1-adrenergic receptor stimulation in different rat brain regions (42). In this study we found that the increase in inositol phosphates was significantly correlated with the potentiation of adenosine-stimulated cyclic AMP, but that the increase in basal cyclic AMP did not correlate with either of the other responses. This discrepancy was most apparent in rat hippocampus, which had the largest increase in inositol phosphates, but the smallest increase in basal cyclic AMP.

The most direct differentiation of α_1-adrenergic receptors would be provided by drugs which selectively activate or inhibit specific responses. Sgd 101/75 (*N*-2-methyl-indazol-4-imino)-imidazolidine hydrochloride) has been suggested to discriminate between putative α_1-adrenergic receptor subtypes mediating smooth muscle contraction (1–3, 10). However, we found this compound to have little activity in stimulating or blocking any of the responses in rat cerebral cortex (data not shown). We also screened a variety of irreversible alkylating agents for their ability to inactivate α_1-adrenergic receptor-binding sites in cortical membranes. Alkylating agents such as DIB, BXT, and EEDQ caused a complete inactivation of specific ^{125}IBE-binding sites, as previously reported for phenoxybenzamine (52). However, when membranes of cerebral cortex were pretreated with low concentrations of CEC, only about half of the total binding sites could be inactivated. Increasing the concentration of CEC by more than 100-fold caused little further loss of binding sites. Although, as might be expected, CEC was less potent in slice preparations, pretreatment with this compound decreased (but did not abolish) α_1-adrenergic receptor-mediated increases in basal cyclic AMP.

If CEC is selective for α_1-adrenergic receptors mediating increases in basal cyclic AMP accumulation, it would be expected to have little effect on the receptors in hippocampus where there is little or no basal cyclic AMP response. This prediction was clearly fulfilled (Fig. 5). However, the density of ^{125}IBE-binding sites in each brain region, which were sensitive to alkylation by CEC, did not correlate well with the magnitude of the basal cyclic AMP response. There were better correlations between the density of ^{125}IBE-binding sites insensitive to CEC inactivation and the magnitude of the other two responses, although these did not reach statistical significance. Regardless of the correlations, these data suggest that the α_1-adrenergic receptors in rat hippocampus are not identical to those in cerebral cortex.

Morrow and Creese (9) recently reported that phentolamine and WB 4101 appeared to distinguish two different α_1-adrenergic receptor-binding sites labeled with ^{3}H-prazosin in rat brain. Hippocampus had a different proportion of these sites than did cortex, although both sites were found in both regions. We found that both phentolamine and WB 4101 were significantly more potent in competing for ^{125}IBE-binding sites in hippocampus than in cerebral cortex. A two-site model did not, however, provide a significantly better fit of the inhibition curves for either drug in either tissue. Interestingly, pretreatment of cortical membranes with CEC significantly increased the potencies of both drugs and made them similar to those observed in hippocampus. This evidence also suggests a difference in the receptors in these two brain regions. It is not clear why, if there are two distinct receptor types, there are not two distinct binding affinities for these competing antagonists in cerebral cortex. One possibility is that ^{125}IBE is slightly selective between such binding sites and cancels out the small selectivity of the competing drug.

Our results show many similarities to those obtained in liver. α_1-Adrenergic receptor stimulation in liver causes a calcium-dependent increase in phosphoinositide metabolism (52) and a calcium-independent increase in cyclic AMP levels (11). In addition, there appear to be both calcium-dependent and calcium-independent metabolic effects of α_1-adrenergic receptor stimulation in rat liver (12, 13). There also appear to be small differences in the potencies of agonists and antagonists at the different responses in liver (7).

In summary, we have found significant differences between the α_1-adrenergic receptors mediating increases in cyclic AMP accumulation and those mediating increases in inositol phosphate accumulation and potentiating adenosine-stimulated cyclic AMP accumulation in cerebral cortex. These include differences in their sensitivity to removal of external calcium, their regional distribution, their sensitivity to the alkylating agent CEC, and their binding properties. It is therefore likely that there are significant differences between the receptors mediating these different responses.

Acknowledgments

We thank Dr. Josef Pitha (National Institute on Aging) for bromoacetylalprenololmenthane, and Christopher Mowry and Morris James for excellent technical assistance.

References

1. Hieble, J. P., R. M. DeMarinis, and W. D. Matthews. Evidence for and against heterogeneity of α_1-adrenoceptors. *Life Sci.* **38**:1339–1350 (1986).
2. Coates, J., U. Jahn, and D. F. Weetman. The existence of a new subtype of α-adrenoceptor on the rat anococcygeus is revealed by Sgd 101/75 and phenoxybenzamine. *Br. J. Pharmacol.* **75**:549–552 (1982).
3. Weetman, D. F., U. Jahn, S. Ismail, M. A. Chadwich, J. Coates, K. Lawson, N. Turner, and K. Thiele. Sgd 101/75: a sympathomimetic that can be used to identify a new subtype of α-adrenoceptor, the α_{1B}-adrenoceptor. *Methods Find. Exp. Pharmacol.* **5**:425–434 (1983).
4. Gibson, A., and O. Yu. Pharmacology of postsynaptic α-adrenoceptors in the mouse anococcygeus muscle. *Br. J. Pharmacol.* **3**:1–5 (1983).

5. McGrath, J. C. Evidence for more than one type of post-junctional α-adrenoceptor. *Biochem. Pharmacol.* **31**:467–484 (1982).
6. McGrath, J. C. Ca-free and Ca-requiring α_1-responses in rat anococcygeus have differing agonist potency series. *Br. J. Pharmacol.* **79**:227P (1983).
7. Morgan, N. G., L. E. Waynick, and J. H. Exton. Characterization of the α_1-adrenergic control of hepatic cAMP in male rats. *Eur. J. Pharmacol.* **96**:1–10 (1983).
8. Morrow, A. L., G. Battaglia, A. B. Norman, and I. Creese. Identification of subtypes of ^{3}H-prazosin-labeled α_1-receptor binding sites in rat brain. *Eur. J. Pharmacol.* **109**:285–287 (1985).
9. Morrow, A. L., and I. Creese. Characterization of α_1-adrenergic receptor subtypes in rat brain. A reevaluation of ^{3}H-WB 4101 and ^{3}H-prazosin binding. *Mol. Pharmacol.* **29**:321–330 (1986).
10. Timmermans, P. B. M. W. M., M. J. M. C. Thoolen, M.-J. Mathy, B. Wilffert, A. de Jonge, and P. A. van Zwieten. Sgd 101/75 is distinguished from other selective α_1-adrenoceptor agonists by the inhibition of its pressor responses by calcium entry blockade and vasodilatation in pithed rats and cats. *Eur. J. Pharmacol.* **96**:187–192 (1983).
11. Morgan, N. L., P. F. Blackmore, and J. H. Exton. Age-related changes in the control of hepatic cyclic AMP levels by α_1- and β_2-adrenergic receptors in male rats. *J. Biol. Chem.* **258**:5103–5109 (1983).
12. Reinhart, P. H., W. M. Taylor, and F. L. Bygrave. The role of calcium ions in the mechanism of action of α-adrenergic agonists in rat liver. *Biochem. J.* **223**:1–13 (1984).
13. Garcia-Sainz, J. A., and S. M. T. Hernandez-Sotomayor. Adrenergic regulation of gluconeogenesis: possible involvement of two mechanisms of signal transduction in α_1-adrenergic action. *Proc. Natl. Acad. Sci. USA* **82**:6727–6730 (1985).
14. Berridge, M. J. Inositol triphosphate and diacylglycerol as second messengers. *Biochem. J.* **220**:345–360 (1984).
15. Streb, H., R. F. Irvine, M. J. Berridge, and I. Schulz. Release of calcium from a non-mitochondrial intracellular store in pancreatic acinar cells by inositol 1,4,5 triphosphate. *Nature (Lond.)* **306**:67–69 (1983).
16. Nishizuka, Y. The role of protein kinase C in cell-surface signal transduction and tumor promotion. *Nature (Lond.)* **308**:693–698 (1984).
17. Perkins, J. P., and M. M. Moore. Characterization of the adrenergic receptors mediating a rise in cyclic 3′5′ adenosine monophosphate in rat cerebral cortex. *J. Pharmacol. Exp. Ther.* **185**:371–378 (1973).
18. Schultz, J., and J. W. Daly. Adenosine 3′5′ monophosphate in guinea pig cerebral cortical slices: effects of α- and β-adrenergic agents, histamine, serotonin and adenosine. *J. Neurochem.* **21**:573–579 (1973).
19. Sattin, A., T. W. Rall, and J. Zanella. Regulation of cyclic adenosine 3′5′ monophosphate levels in guinea pig cerebral cortex by interaction of alpha adrenergic and adenosine receptor activity. *J. Pharmacol. Exp. Ther.* **192**:22–32 (1975).
20. Davis, J. N., C. D. Arnett, E. Hoyler, L. P. Stalvey, J. W. Daly, and P. Skolnick. Brain α-adrenergic receptors: comparison of ^{3}H-WB4101 binding with norepinephrine-stimulated cyclic AMP accumulation in rat cerebral cortex. *Brain Res.* **159**:125–135 (1978).
21. Daly, J. W., W. Padgett, C. R. Creveling, D. Cantacuzene, and K. L. Kirk. Cyclic AMP-generating systems: regional differences in activation by adrenergic receptors in rat brain. *J. Neurosci.* **1**:49–59 (1981).
22. Chan, T. M., and J. H. Exton. α-Adrenergic-mediated accumulation of adenosine 3′:5′-monophosphate in calcium depleted hepatocytes. *J. Biol. Chem.* **252**:8645–8651 (1977).
23. Blair, J. B., M. E. James, and J. L. Foster. Adrenergic control of glucose output and adenosine 3′5′ monophosphate levels in hepatocytes from juvenile and adult rats. *J. Biol. Chem.* **254**:7579–7584 (1979).
24. Johnson, R. D., and K. P. Minneman. Characterization of α_1-adrenergic receptors which increase cyclic AMP accumulation in rat cerebral cortex. *Eur. J. Pharmacol.* **129**:293–305 (1986).
25. Pitha, J., B. A. Hughes, J. W. Kusiak, E. M. Dax, and S. P. Baker. Regeneration of β-adrenergic receptors in senescent rats: a study using an irreversible binding antagonist. *Proc. Natl. Acad. Sci. USA* **79**:4424 (1982).
26. Johnson, R. D., P. M. Iuvone, and K. P. Minneman. Regulation of α_1-adrenergic receptor density and functional responsiveness in rat brain. *J. Pharmacol. Exp. Ther.*, in press.
27. Shimizu, H., C. R. Crevelling, and J. W. Daly. A radioisotopic method for measuring the formation of adenosine 3′5′ cyclic monophosphate in incubated slices of brain. *J. Neurochem.* **16**:1609–1616 (1969).
28. Salomon, Y. C., C. Londos, and M. Rodbell. A highly sensitive adenylate cyclase assay. *Anal. Biochem.* **58**:541–548 (1974).
29. Berridge, M. J., C. P. Downes, and M. R. Hanley. Lithium amplifies agonist-dependent phosphatidylinositol responses in brain and salivary glands. *Biochem. J.* **206**:587–595 (1982).
30. Minneman, K. P., and R. D. Johnson. Characterization of α_1-adrenergic receptors linked to ^{3}H-inositol metabolism in rat cerebral cortex. *J. Pharmacol. Exp. Ther.* **230**:317–323 (1984).
31. Engel, G., and D. Hoyer. ^{125}I BE 2254, a new high affinity radioligand for α_1-adrenoceptors. *Eur. J. Pharmacol.* **73**:221–224 (1981).
32. Minneman, K. P. Binding properties of α_1-adrenergic receptors in rat cerebral cortex: similarity to smooth muscle. *J. Pharmacol. Exp. Ther.* **227**:605–612 (1983).
33. Bradford, M. M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **72**:248–254 (1976).
34. Partington, C. R., M. W. Edwards, and J. W. Daly. Regulation of cyclic AMP formation in brain tissue by α-adrenergic receptors: requisite intermediacy of prostaglandins of the E series. *Proc. Natl. Acad. Sci. USA* **77**:3024–3028 (1980).
35. Buxton, I. L. O., and L. L. Brunton. Action of the cardiac α_1-adrenergic receptor: activation of cyclic AMP degradation. *J. Biol. Chem.* **26**:6733–6737 (1985).
36. Sugden, D., J. Vanecek, D. C. Klein, T. P. Thomas, and W. B. Anderson. Activation of protein kinase C potentiates isoprenaline-induced cyclic AMP accumulation in rat pinealocytes. *Nature (Lond.)* **314**:359–361 (1985).
37. Zatz, M. Phorbol esters mimic α-adrenergic potentiation of serotonin-*N*-acetyltransferase induction in the rat pineal. *J. Neurochem.* **45**:637–639 (1985).
38. Colucci, W. S., M. A. Gimbrone, Jr., and R. W. Alexander. Phorbol diester modulates α-adrenergic receptor coupled calcium efflux and α-adrenergic receptor number in cultured vascular smooth muscle cells. *Circ. Res.* **58**:393–398 (1986).
39. Lynch, C. J., R. Charest, S. B. Bocckino, J. H. Exton, and P. F. Blackmore. Inhibition of hepatic α_1-adrenergic effects and binding by phorbol myristate acetate. *J. Biol. Chem.* **260**:2844–2851 (1985).
40. Leeb-Lundberg, L. M., S.Cotecchia, J. W. Lomasney, J. F. de Bernardis, R. J. Lefkowitz, and M. G. Caron. Phorbol esters promote α_1-adrenergic receptor phosphorylation and receptor uncoupling from inositol phospholipid metabolism. *Proc. Natl. Acad. Sci. USA* **82**:5651–5655 (1985).
41. Corvera, S., K. R. Schwarz, R. M. Graham, and J. A. Garcia-Sainz. Phorbol esters inhibit α_1-adrenergic effects and decrease the affinity of liver cell α_1-adrenergic receptors for (−)-epinephrine. *J. Biol. Chem.* **261**:520–526 (1986).
42. Johnson, R. D., and K. P. Minneman. α_1-Adrenergic receptors and stimulation of ^{3}H-inositol metabolism in rat brain: regional distribution and parallel inactivation. *Brain Res.* **341**:7–15 (1985).
43. LeClerc, G., B. Rouot, J. Schwartz, J. Velly, and C. G. Wemuth. Studies on some *para*-substituted clonidine derivatives that exhibit an α-adrenoceptor stimulant activity. *Br. J. Pharmacol.* **71**:5–9 (1980).
44. Munson, P. J., and D. Rodbard. LIGAND: a versatile computerized approach for characterization of ligand-binding systems. *Anal. Biochem.* **107**:220–239 (1980).
45. Daly, J. W., W. Padgett, Y. Nimitkitpaisan, C. R. Creveling, D. Cantacuzene, and K. L. Kirk. Fluoronorepinephrines: specific agonists for the activation of α and β adrenergic-sensitive cyclic AMP-generating systems in brain slices. *J. Pharmacol. Exp. Ther.* **212**:382–389 (1980).
46. Brown, E., D. A. Kendall, S. R. Nahorski. Inositol phospholipid hydrolysis in rat cerebral cortical slices. I. Receptor characterisation. *J. Neurochem.* **42**:1379–1387 (1984).
47. Schwabe, U., and J. W. Daly. The role of calcium ions in accumulation of cyclic adenosine monophosphate elicited by alpha and beta adrenergic agonists in rat brain slices. *J. Pharmacol. Exp. Ther.* **202**:134–143 (1977).
48. Kendall, D. A., and S. R. Nahorski. Inositol phospholipid hydrolysis in rat cerebral cortical slices. II. Calcium requirement. *J. Neurochem.* **42**:1388–1394 (1984).
49. Meeker, R. B., and T. K. Harden. Muscarinic cholinergic receptor-mediated activation of phosphodiesterase. *Mol. Pharmacol.* **22**:310–319 (1982).
50. Jones, D. G., and L. F. McKenna. Alpha adrenergic receptor mediated formation of cyclic AMP in rat spinal cord. *J. Cyclic Nucleotide Res.* **6**:133–141 (1980).
51. LeBlanc, G. G., and R. D. Ciaranello. α-Noradrenergic potentiation of neurotransmitter stimulated cAMP production in rat striatal slices. *Brain Res.* **293**:57–65 (1984).
52. Minneman, K. P. Phenoxybenzamine is more potent in inactivating α_1- than α_2-adrenergic receptor binding sites. *Eur. J. Pharmacol.* **94**:171–174 (1983).
53. Prpic, V., P. F. Blackmore, and J. H. Exton. Phosphatidylinositol breakdown induced by vasopressin and epinephrine in hepatocytes is calcium-dependent. *J. Biol. Chem.* **257**:11323–11331 (1982).

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