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Differential Localization of α_2-Adrenergic Receptor Subtypes in Brain

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WAMSLEY, J. K., M. E. ALBURGES, M. A. E. HUNT AND D. B. BYLUND. *Differential localization of α_2-adrenergic receptor subtypes in brain.* PHARMACOL BIOCHEM BEHAV 41(2) 267-273, 1992.—The pharmacological identification and characterization of subtypes of α_2-adrenergic receptors have been confirmed by molecular biological investigations. Using receptor autoradiographic techniques, it has been possible to show regions of the brain where α_2 agonist binding ([^{3}H]para-aminoclonidine) is preferentially labeling the presumed guaninenucleotide-sensitive, high-affinity conformations of the α_2 receptor. Careful examination of autoradiograms generated using the tritiated antagonists yohimbine, idazoxan, and rauwolscine also indicates some disparity in the regions occupied by these radiolabeled ligands. Inhibition of [^{3}H]rauwolscine binding with the subtype selective compounds, ARC-239, or oxymetazoline demonstrates that there are discrete regions of the brain where one receptor subtype predominates over the other. These studies indicate that previous investigations utilizing the agonist para-aminoclonidine as the ligand for obtaining labeling of α_2 receptors have overlooked some regions of binding due to the subtype selectivity of this ligand. A more complete localization of α_2-adrenergic receptors can be obtained using the tritiated antagonist rauwolscine, and the differential distribution of at least two subtypes of the α_2 receptor can be obtained by selective inhibition of this binding.

α_2-Adrenergic receptors α_2-Receptor subtypes α_{2A} α_{2B} Para-aminoclonidine Idazoxan Rauwolscine Yohimbine α_2-Receptor localization

ALPHA$_2$-adrenergic receptors, along with α_1 and β_1, are the three major types of adrenergic receptors. Alpha$_2$, as well as the other two adrenergic receptor types, are not homogeneous receptor populations, but can be further divided into at least three subtypes (5). Pharmacological studies have indicated the presence of at least three and possibly four α_2-adrenergic receptor subtypes [for reviews, see (4,6)]. The existence of at least three α_2-receptor subtypes has been confirmed by molecular biologic studies [for review, see (10)].

Autoradiographic localization of α_2 receptors has been accomplished using the tritiated agonist para-aminoclonidine (18,21,22,27,28). This compound has been shown to have a preference for the high-affinity state of the α_2 receptor as verified by the demonstration that the binding is sensitive to guanine nucleotides (20,23). Alpha$_2$-adrenergic receptors are thought to be coupled to the inhibitory G-protein (G_i) representing the second messenger system of these receptors (8,15). Agonists, at low concentrations, should preferentially bind to the high-affinity conformation of the receptors and be sensitive to the presence of guanine nucleotides, whereas antagonists should not. The α_2-receptor antagonists [^{3}H] rauwolscine and [^{3}H]idazoxan have also been used for autoradiographic studies of α_2-receptors. Since these two ligands label similar, but not identical, populations of receptors, some preferential labeling or subtype selectivity appears to be present (1-3).

In the present study, the localization of α_2-adrenergic receptors labeled with [^{3}H]idazoxan, [^{3}H]rauwolscine, and [^{3}H]yohimbine were compared. Experimental conditions were discovered that provide a signal-to-noise ratio superior to that previously reported in the literature. The binding of the antagonists were then compared to the binding of a tritiated agonist, para-aminoclonidine, either in the presence or absence of a guanine nucleotide. In addition, the subtype-selective compounds oxymetazoline and ARC-239 were used to inhibit specific binding of [^{3}H]rauwolscine for differential localization of two receptor subtypes in rat brain. The results of these studies indicate that para-aminoclonidine is subtype selective, as well as preferentially labeling the high-affinity conformational state of the α_2 receptor. In addition, there are several regions of the brain where one α_2-receptor subtype predominates over the others.

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METHOD

Male Sprague-Dawley rats (150–250 g) were anesthetized with sodium pentobarbital (100 mg/kg, IP; Abbott Labs; North Chicago, IL) and perfused intracardially with ice-cold, isotonic saline. The brains of the animals were rapidly dissected from surrounding tissues and the cortex was scraped free from the surface of the corpus callosum with a spatula. The cortical regions from several animals were pooled and gently homogenized by using four strokes with a glass homogenizing tube and a Teflon pestle. The tissues were then frozen in plastic centrifuge tubes after spinning at 500 rpm for 5 min to remove any air bubbles that resulted from the homogenization procedure. Subsequently, the end of the plastic tube was cut off and the frozen cylinder of tissue extruded and sectioned in a cryostat. These sections of "cortical mash" were then thaw mounted on microscope slides and used for the biochemical experiments. This procedure provided uniform sections of mixed cellular populations in the pooled regions of cortex from several animals. Binding to these sections gave consistent results, thus providing reliable and more relevant data. For autoradiographic studies, the same procedure was followed except the entire brain was frozen intact on a cryostat chuck, by submerging in −70°C isopentane, and sectioned (each 20 μm in thickness) for subsequent thaw mounting on microscope slides.

In the biochemical experiments, several different buffer conditions were analyzed for their effects on the signal-to-noise ratio of [^{3}H]yohimbine, [^{3}H]idazoxan, [^{3}H]rauwolscine, and [^{3}H]para-aminoclonidine binding. These different buffer systems included Tris-HCl buffers (50 mM, pH 7.7, or pH 7.1 with 0.5 mM EDTA), a physiological buffer (118.1 mM NaCl, 2.5 mM $CaCl_2$, 1.1 mM $MgSO_4$, 2.1 mM KCl, 0.93 mM KH_2PO_4, 25 mM $NaHCO_3$, 11 mM glucose, 0.1 mM ascorbate, pH 7.4, gassed with 5% CO_2/95% O_2), and glycylglycine buffer (25 mM, pH 7.4), as well as many other combinations of these buffers with minor adjustments in the concentration of monovalent and divalent cations. The effect of pH was determined for [^{3}H]yohimbine and [^{3}H]idazoxan binding in both the glycylglycine (25 nM) and Tris-HCl (50 mM) buffers. The pH of each buffer was varied from 7.2 to 7.8. From these experiments, the optimal conditions for obtaining labeling of slide-mounted tissue sections were obtained. In the subsequent binding steps, a 25-mM glycylglycine buffer at pH 7.6 was utilized. This buffer improved the specific binding of three of the four radioligands analyzed in this study (yohimbine, rauwolscine, and idazoxan).

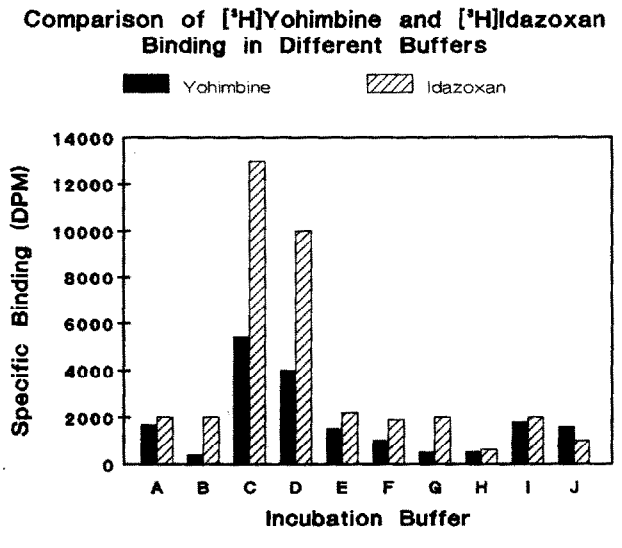

FIG. 1. Preparations of brain mash were used for labeling under the following conditions. The section were wiped from the slides and the radioactivity determined. The experiments were performed in triplicate and repeated twice with results varying less than 10% from the mean values indicated. A, Tris buffer plus 120 mM NaCl; B, Tris buffer plus 120 mM NaCl including a 20-min preincubation in the presence of 200 mM NaCl; C, GlyGly (pH 7.6); D, GlyGly (pH 7.6) with preincubation; E, GlyGly (120 mM NaCl); F, GlyGly (120 mM NaCl) with preincubation; G, physiological buffer (1.1 mM $MgSO_4$); H, physiological buffer (1.1. mM $MgSO_4$) with preincubation; I, physiological buffer; J, physiological buffer with preincubation.

[^{3}H]Rauwolscine (86.2 Ci/mmol; Du Pont NEN; Boston, MA) was included in the incubation buffer at a concentration of 1.0 nM. This binding assay was performed using a 20-min preincubation in buffer solution, followed by a 90-min incubation at room temperature and two subsequent 5-min rinses in fresh-cold buffer without added radioactivity. The labeled tissue sections were individually wiped from the microscope slides and placed in plastic vials along with 10 ml Ecolume (ICN Biomedical, Inc., Irvine, CA) cocktail followed by vigorous shaking. The vials were counted in a Beckman LS 5000TD liquid/scintillation spectrometer for the determination of receptor-bound radioactivity. Binding of [^{3}H]rauwolscine was also accomplished in the presence of various concentrations (10^{-4}–10^{-11} M) of oxymetazoline or ARC-239. These sections were also wiped from the slides for calculation of bound radioactivity as determined by liquid scintillation counting. Nonspecific binding was defined by using 10 μM concentration of phentolamine in the incubation medium of adjacent tissue sections.

For the autoradiographic experiments, a concentration obtained by doubling (approximately) the K_d value (2,21) of each radioligand (rauwolscine 1.2 nM; idazoxan 1.6 nM; yohimbine 3.8 nM; and para-aminoclonidine 0.5 nM), was used, with optimal conditions for obtaining labeling, to bind α_2 receptors in sagittal sections of whole brain. The binding conditions described above were the same for the [^{3}H]yohimbine (79.1 Ci/mmol; DuPont NEN; Boston, MA) and [^{3}H]idazoxan (42.4 Ci/mmol; Amersham Corp.; Arlington Heights, IL) with the exception that in the [^{3}H]idazoxan binding experiment two 1-min rinses rather than two 5-min rinses were performed after the incubation step. Labeling with [^{3}H]para-aminoclonidine (58.2 Ci/mmol; DuPont NEN; Boston, MA) was performed using Tris-HCl buffer (170 mM) with 20 mM $MgCl_2$ at pH 7.6 and the same preincubation, incubation, and rinse conditions used for the binding of [^{3}H]rauwolscine and [^{3}H]yohimbine. Binding studies with these radioligands were also carried out in the presence of 10 μM guanylyl-5′-imidodiphosphate [Gpp(NH)p]. Furthermore, labeling experiments with a 0.6 nM [^{3}H]rauwolscine (K_d concentration) in the presence of 7.8 nM oxymetazoline (IC_{50} concentration) or 9.0 nM ARC-239 (IC_{50} concentration) were performed. Labeled tissue sections were dried by blowing cooled, desiccated air over the tissue surface, then storing overnight in desiccated containers at 4°C. The sections were then exposed to tritium-sensitive film (Amersham Hyperfilm; Amersham, Arlington Heights, IL). After an appropriate exposure period (5–12 weeks), the films were developed and autoradiograms quantitated using a microcomputer imaging densitometry system (MCID; Imaging Systems, Inc.; St. Catherines, Ontario).

RESULTS

The evaluation of 10 different buffer systems disclosed the ionic sensitivity and pH dependency of the binding (Fig. 1).

TABLE 1

α_2-ANTAGONIST AND AGONIST BINDING TO RAT BRAIN AREAS (fmol/mg TISSUE)

	Radioligand [2 × K_d]							
	Yohimbine	Yohimbine + Gpp (NH) p	Idazoxan	Idazoxan + Gpp (NH) p	Rauwolscine	Rauwolscine + Gpp (NH) p	Para-Aminoclonidine	Para-Aminoclonidine + Gpp (NH) p
FrP I-III	15.2 ± 7.8	23.0 ± 5.2	57.6 ± 7.1	46.2 ± 7.9	8.9 ± 1.0	6.2 ± 0.9	3.7 ± 0.8	1.0 ± 0.0
FrP IV	20.7 ± 4.1	24.4 ± 6.0	44.2 ± 7.7	30.9 ± 8.5	13.6 ± 2.7	6.2 ± 0.9	1.6 ± 0.4	0.2 ± 0.0
FrP V-VI	17.4 ± 6.2	23.8 ± 4.0	43.1 ± 5.2	29.4 ± 4.0	8.5 ± 0.9	5.2 ± 0.2	1.2 ± 0.4	0.1 ± 0.0
RSA	19.1 ± 4.9	19.5 ± 2.0	63.8 ± 7.6	45.9 ± 6.8	15.0 ± 2.7	6.8 ± 1.5	4.2 ± 0.9	*
CPU	20.9 ± 6.1	29.0 ± 2.6	44.2 ± 4.7	32.2 ± 3.3	11.9 ± 2.1	10.7 ± 0.9	1.7 ± 0.5	*
GP	22.9 ± 4.0	32.1 ± 3.2	23.0 ± 3.1	17.6 ± 4.3	11.8 ± 1.3	9.1 ± 0.8	0.8 ± 0.2	*
LMol	36.9 ± 3.1	33.1 ± 7.6	87.8 ± 17.2	55.1 ± 8.9	16.4 ± 4.2	11.6 ± 1.5	5.3 ± 0.7	*
CA_1	19.7 ± 4.1	27.3 ± 5.4	42.9 ± 6.1	32.2 ± 2.8	16.3 ± 2.9	15.7 ± 0.9	0.6 ± 0.3	*
DG	15.5 ± 6.3	19.3 ± 3.2	25.4 ± 5.0	21.4 ± 2.4	27.0 ± 10.0	17.2 ± 1.3	0.9 ± 0.2	*
St	28.2 ± 1.0	27.4 ± 6.5	50.5 ± 7.0	38.0 ± 3.2	13.3 ± 1.5	9.1 ± 0.9	1.8 ± 0.5	*
LD	33.8 ± 3.2	33.0 ± 5.0	68.8 ± 7.8	60.0 ± 7.0	17.2 ± 3.4	9.8 ± 1.2	8.5 ± 3.1	0.8 ± 0.2
DLG	28.7 ± 1.4	29.5 ± 2.3	55.5 ± 4.5	52.0 ± 3.2	7.1 ± 1.9	8.5 ± 0.3	3.8 ± 1.6	*
MGD	29.5 ± 3.6	31.9 ± 3.2	58.1 ± 10.7	50.8 ± 10.2	10.4 ± 1.9	7.4 ± 0.8	4.5 ± 1.5	*
SuG	25.2 ± 10.7	22.7 ± 6.0	55.2 ± 6.3	46.5 ± 7.8	12.3 ± 0.9	8.3 ± 1.0	2.7 ± 0.9	0.5 ± 0.1
ECIC	20.1 ± 6.3	27.7 ± 4.2	55.8 ± 5.9	42.8 ± 2.5	11.4 ± 1.9	8.5 ± 1.6	2.0 ± 1.1	*
SNR	30.2 ± 4.0	30.1 ± 6.0	48.9 ± 4.4	32.7 ± 5.6	18.8 ± 2.2	13.2 ± 1.2	1.3 ± 0.5	*
AHipA	45.5 ± 8.1	41.9 ± 11.6	96.0 ± 25.4	83.3 ± 21.8	25.2 ± 6.4	13.0 ± 2.7	14.9 ± 2.6	1.4 ± 0.5
LC	35.2 ± 1.4	37.8 ± 7.8	70.2 ± 26.7	51.2 ± 15.5	17.1 ± 0.8	17.1 ± 2.7	10.8 ± 1.2	*
GLC	14.5 ± 4.8	20.8 ± 2.9	31.2 ± 2.0	16.1 ± 2.0	4.0 ± 1.0	3.4 ± 1.0	1.2 ± 1.0	*
MLC	6.7 ± 2.1	13.2 ± 3.3	17.2 ± 4.4	5.0 ± 2.1	1.9 ± 0.2	1.2 ± 0.2	0.2 ± 0.2	*
Ce,BL	39.3 ± 3.6	41.2 ± 9.8	80.4 ± 21.8	74.3 ± 19.4	25.5 ± 1.8	15.3 ± 2.4	12.8 ± 1.8	1.1 ± 0.1

Values are expressed as the mean ± SEM determined from densitometric measurements made on autoradiograms generated from sagittal sections. The experiments were repeated twice, each representing 12 sections/condition from 4 animals. Abbreviations: see legend for Table 2.

*Not above background.

Addition of a high concentration of sodium chloride (to dissociate any endogenous ligand that may already be occupying the receptors) invariably caused a slight decrease in overall binding. Optimal conditions for both yohimbine and idazoxan involved the use of glycylglycine buffer at a pH of 7.6. The pH curve (not shown) indicated a gradual increase in binding (50% of the highest level of binding was obtained at pH 7.3) that peaked at 7.6 and then sharply declined (returning to the 50% of optimal binding between pH 7.7 and 7.8). The optimal binding conditions for the radioactive antagonists were obtained by preincubating slide-mounted tissue sections for 20 min in a glycylglycine buffer at pH 7.6. This was followed by two 5-min rinses in fresh buffer. The sections were then subjected to a 90-min incubation in the presence of a 1-nM concentration of the radioactive compound, followed by two rinses.

Labeling with [^{3}H]yohimbine for autoradiography proved to be somewhat abstruse. The conditions described above provided a very high specific to nonspecific binding ratio with [^{3}H]yohimbine and yet the autoradiograms left something to be desired. Most of the areas of α_2-adrenergic receptor binding localized with the other two radiolabeled compounds (idazoxan and rauwolscine) were identified by [^{3}H]yohimbine, but there was also a high diffuse background involving most regions of the brain. This diffuse binding appeared to be specific as it was readily inhibited with phentolamine, but did not show any identifiable association with specific brain structures. In the presence of the guanine nucleotide, the binding of [^{3}H]yohimbine was slightly, but not significantly, increased (Table 1).

The binding of [^{3}H]idazoxan was accomplished with high specific to nonspecific ratios. Utilizing the glycylglycine buffer at a pH of 7.6, autoradiographic localization of [^{3}H]idazoxan binding showed discretely labeled nuclei throughout the central nervous system (CNS), and this followed a pattern that would be expected of α_2 receptors in most cases. There were, however, a few areas labeled with [^{3}H]idazoxan that did not fit with the previously identified distribution of α_2-adrenergic sites. With [^{3}H]rauwolscine, a high specific to nonspecific ratio was also obtained in a pattern in keeping with labeling of α_2-adrenergic receptors. Both of the latter two antagonists showed a slight decrease in the receptor binding in the presence of 10 μM Gpp(NH)p (Table 1).

The highest concentrations of α_2-receptor binding seen with [^{3}H]rauwolscine were in the locus ceruleus, bed nucleus of the stria terminalis, substantia nigra-pars reticulata, amygdalohippocampal area, median eminence, nucleus of the solitary tract, subfornical organ, laterodorsal thalamic nucleus, basolateral amygdaloid nucleus, dentate gyrus, the CA_1 region, and lacunosum molecular layer of the hippocampus (Table 2, Fig. 2). The lowest binding densities were found in the granular and molecular layers of the cerebellum and the superficial and deep layers of the cerebral cortex. The localization of this tritiated antagonist followed the binding pattern of the tritiated agonist ([^{3}H]para-aminoclonidine), although some differences did exist. For instance, we were able to demonstrate substantial binding of the tritiated antagonist in the CA_1 region of the hippocampal formation, both in the rostral and caudal extents. The binding was increased slightly in the caudal-most regions of the hippocampal formation and was very dense near the subiculum. This is in contrast to the results obtained with [^{3}H]para-aminoclonidine, where the binding

TABLE 2

PERCENT OCCUPANCY OF α_2 RECEPTORS BY THE NONRADIOACTIVE COMPOUND (INHIBITION OF RECEPTORS LABELED WITH [^{3}H] RAUWOLSCINE)

	Drugs (IC_{50} nM)	
	Oxymetazoline (7.8) (mean ± SEM)*	ARC-239 (9.0) (mean ± SEM)*
FrP I-III	44.5 ± 5.1	19.8 ± 2.5
FrP IV	60.7 ± 5.9	72.1 ± 9.3
FrP V-VI	32.7 ± 4.0	32.7 ± 4.4
RSA	67.6 ± 7.2	66.7 ± 8.1
CPu	48.3 ± 5.8	33.6 ± 4.2
LMol	74.6 ± 8.4	52.0 ± 6.0
CA_1	80.6 ± 8.6	70.2 ± 5.3
DG	67.7 ± 7.3	67.1 ± 8.2
St	22.6 ± 3.5	51.4 ± 6.4
LD	68.5 ± 7.8	42.7 ± 3.9
DLG	67.1 ± 7.6	42.9 ± 5.0
MGD	59.9 ± 6.5	50.1 ± 4.9
SuG	46.4 ± 5.7	24.7 ± 3.6
SNR	60.3 ± 8.0	87.9 ± 7.9
AHipA	82.3 ± 9.3	64.3 ± 7.6
GLC	33.4 ± 6.2	36.5 ± 4.8
MLC	79.7 ± 8.6	99.7 ± 12.6
Ce BL	83.1 ± 9.8	62.0 ± 8.5
LC	70.4 ± 8.3	41.2 ± 6.0

FrP I-III, frontoparietal cortex laminae I-III; FrP IV, frontoparietal cortex laminae IV; FrP V-VI, frontoparietal cortex laminae V-VI; RSA, retrosplenial agranular cortex; CPu, caudate-putamen; GP, globus pallidus; LMol, lacunosum molecular layer of the hippocampus; CA_1, field CA_1 of Ammon's horn; DG, dentate gyrus; St, stria terminalis; LD, laterodorsal thalamic nucleus; DLG, dorsal lateral geniculate nucleus; MGD, medial geniculate nucleus, dorsal; SuG, superior gray layer of the superior colliculus; ECIC, external cortex inferior colliculus; SNR, substantia nigra-pars reticulata; AHipA, amygdalohippocampal area; GLC, granular layer of the cerebellum; MLC, molecular layer of the cerebellum; Ce BL, amygdaloid nucleus (central, basolateral); LC, locus ceruleus.

*Values are expressed as the mean (± SEM). Experiments were repeated twice, each representing 12 sections of cortical mash from 4 animals.

was undetectable in the most rostral regions of the hippocampal formation. Also, the tritiated antagonist ([^{3}H]rauwolscine) densely labeled the subfornical organ and the substantia nigra-pars reticulata, whereas [^{3}H]para-aminoclonidine recognized very few sites in these regions. In addition, [^{3}H]para-aminoclonidine did not have as great a propensity to recognize receptors in lamina IV of the cerebral cortex, dentate gyrus, and the bed nucleus of the stria terminalis. These same regions were densely labeled by both [^{3}H]idazoxan and [^{3}H]rauwolscine. There was also a distinct band of labeling found in the piriform cortex and stria terminalis with the tritiated antagonists that was not evident with the tritiated agonist.

By utilizing [^{3}H]rauwolscine to obtain specific labeling of α_2-adrenergic receptors on slide-mounted tissue sections, it was possible to inhibit binding with the subtype-selective compounds oxymetazoline and ARC-239 (Fig. 3). The IC_{50} value concentrations determined for oxymetazoline and ARC-239 were 7.8 ± 1.0 and 9.0 ± 1.2, nM, respectively (n = 4). Utilizing these concentrations to obtain selective inhibition of [^{3}H]rauwolscine binding from α_2-receptor subtypes demonstrated the existence of subtypes in the CNS (Table 2, Fig. 2). Oxymetazoline-sensitive binding of [^{3}H]rauwolscine predominated in the superficial layers of the cerebral cortex (I-III), the caudate-putamen, stratum lacunosum moleculare and CA_1 of the hippocampus, several thalamic regions, the superior colliculus, amygdalohippocampal area, and the locus ceruleus. The sites recognized by ARC-239 predominated in lamina IV of the cortex, the stria terminalis, the substantia nigra-pars reticulata, and the cerebellum.

DISCUSSION

Since all labeled compounds used in this study were present in tritiated form, the possible contributions of white matter to differential quenching of the signal obtained from these ligands could not account for the differences seen. Consistent with previous work, the tritiated α_2 agonist para-aminoclonidine appears to label preferentially the high-affinity, guanine-nucleotide-sensitive state of the α_2 adrenoceptor. However, the use of this agonist may provide a qualitatively and a quantitatively incomplete profile of the total α_2-adrenergic receptor population. Therefore, in the present study, we elected to use the tritiated antagonist, rauwolscine, for the localization and inhibition experiments.

Molecular biological approaches have verified the presence of at least three α_2-adrenergic receptor subtypes (10,14). Three human α_2 subtypes have been cloned and sequenced. They are designated C10, C4, and C2 based on their chromosomal localization. C10 was isolated from human platelets and has a pharmacology consistent with that of an α_{2A}-adrenergic receptor (11). cDNAs homologous to C10 have been cloned from porcine (9) and rat (7). Weinshank et al. (24) cloned a human α_{2B} receptor identical to the C2 clone from Lefkowitz's group (13), and the rat homologue has also been cloned (29). The pharmacologic characteristics for C4, cloned from the human kidney (19), are not yet sufficiently known to allow it to be assigned to one of the four pharmacological α_2 subtypes, although it is most similar to the α_{2C}. Since in these initial inhibition studies we have used only two subtype-selective ligands, firm identification of the receptor subtypes cannot be expected. The oxymetazoline-sensitive binding is most likely α_{2A} (although it could be α_{2D}) and the ARC-239-sensitive sites could be either α_{2B} or α_{2C}.

Results obtained in previous studies indicate that [^{3}H]yohimbine may be labeling sites other than α_2 receptors. This would seem apparent from our attempts to autoradiographically localize the binding of [^{3}H]yohimbine since we could not account for all the binding on the basis of α_2 receptors alone. Good labeling was obtained with [^{3}H]idazoxan but, again, there is evidence in the literature that this compound may be labeling sites other than the α_2-adrenergic receptor (12,16, 17,25,26). This might possibly account for the differences in binding seen in previous studies attempting to compare [^{3}H]rauwolscine with [^{3}H]idazoxan (1,2). We likewise found such differences between these two ligands, but suggest that [^{3}H]rauwolscine more closely follows the distribution expected of α_2-adrenergic receptors.

In comparing the binding of the antagonists with [^{3}H]para-aminoclonidine, such discrepancies surface again. Previously, these differences have been attributed to the existence of conformational states of the α_2 receptor that are known to be sensitive to guanine nucleotides. By occupying the guanine nucleotide binding protein with Gpp(NH)p, it is possible to shift the high-affinity conformation of the α_2 receptor (which is preferentially labeled by [^{3}H]para-amino-

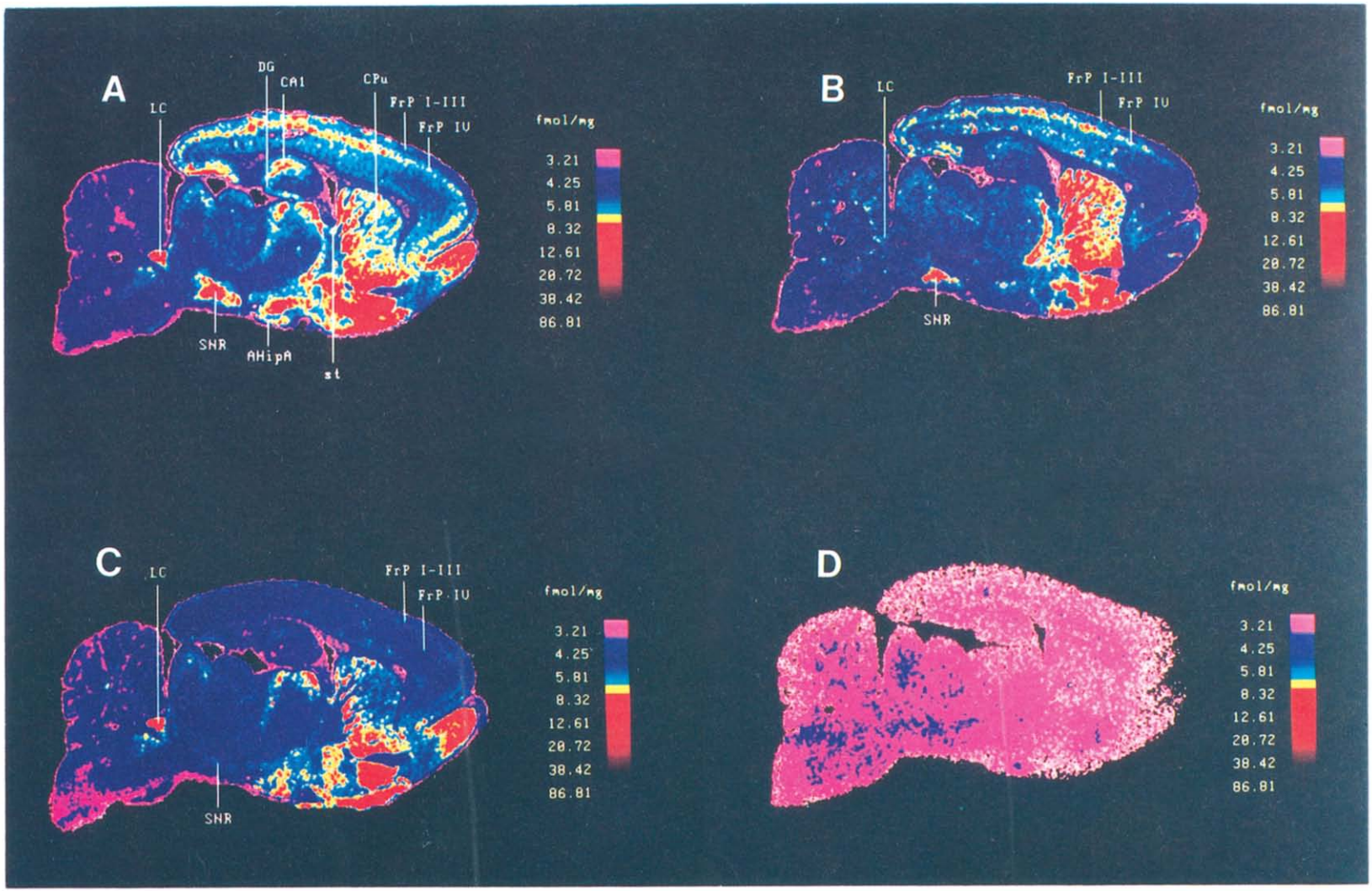

FIG. 2. Color-coded computerized reconstructions of autoradiograms depicting the density and distribution of α_2-receptor subtypes as localized with [^{3}H]rauwolscine. (A) *Total binding showing* α_2 *receptors in several regions including* cortex, substantia nigra, *and locus ceruleus. (B) Binding of* [^{3}*H*]rauwolscine *in the presence of* oxymetazoline. The sites sensitive to oxymetazoline represent the α_{2A} subtype. Note the binding in the locus ceruleus is inhibited with little change in cortex and nigra. (C) Inhibition of binding in the presence of ARC-239 indicates the presence of α_{2B} or α_{2C} sites. Note cortex and nigra binding are now inhibited (α_{2B} or α_{2C}), while the binding in the locus ceruleus is undisturbed (α_{2A}). (D) [^{3}H]Rauwolscine binding in the presence of phentolamine to show specific binding (areas of nonspecific binding remain labeled with the radioligand). Abbreviations: see legend for Table 2.

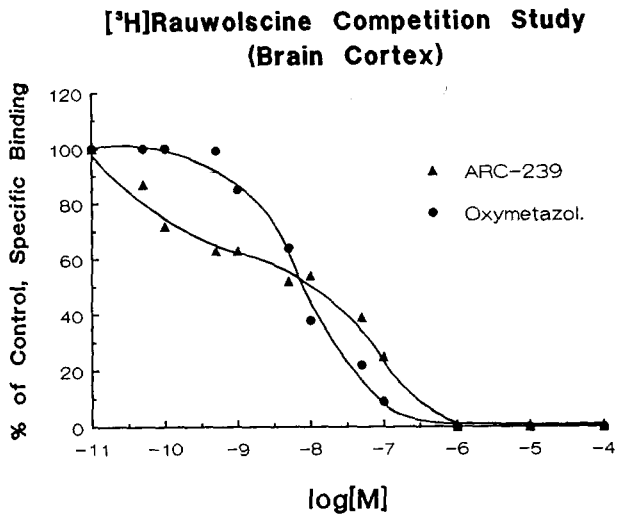

FIG. 3. Competition study performed with cortical mash preparation. Both compounds (ARC-239 and oxymetazoline) show shallow inhibition curves against [^{3}H]rauwolscine binding. Each point represents the sum of three experiments (with triplicate samples in each) performed on pooled cortices from five animals.

clonidine) to the low-affinity state to which little, if any, of the tritiated agonist in the incubation medium binds. Thus, differences between tritiated agonist and antagonist binding could be due to a variable fraction of the receptors in various brain regions being in the high-affinity state. Alternatively, differences in binding could be due to either or both of the radioligands binding with some degree of selectivity to the various subtypes. In comparing the distributions, it was apparent that the tritiated agonist was selectively labeling sites not shared by the binding of the tritiated antagonist. For example, [^{3}H]rauwolscine labeled the substantia nigra-pars reticulata, whereas [^{3}H]para-aminoclonidine recognizes very few sites in this area. This is in keeping with previous reports of the localization of α_2-adrenergic receptors that show little binding in this region (22). Thus, not only do α_2-adrenergic receptors exist in the substantia nigra-pars reticulata, they are quite prevalent there. The same holds true for several regions of the hippocampal formation and lamina IV of the cerebral cortex.

In the competition experiments, we were able to show that the α_2-receptor subtype sensitive to ARC-239 (α_{2B} or α_{2C}) predominates in the substantia nigra. In contrast, the binding in the locus ceruleus is more sensitive to oxymetazoline than to ARC-239, suggesting a predominance of α_{2A} receptors. This is consistent with relatively high [^{3}H]para-aminoclonidine binding. Based on this evidence, we submit that para-aminocloni dine may be selective for the α_{2A}-receptor subtype, an observation that may be supported by evidence already in the literature, but not recognized previously. From our experiments, performed with oxymetazoline and ARC-239, it can be seen that the α_{2B}-receptor subtype predominates in a few regions of the brain where relatively little labeling is obtained with [^{3}H]para-aminoclonidine. The isolation of these areas in future studies should allow binding to be accomplished to one receptor independent of the other. This would provide a method for teasing out the actual contribution of the α_{2B} or other α_2-receptor subtypes that might be contributing to the binding in these areas.

The fact that a subtype-selective agonist ([^{3}H]para-aminoclonidine) has been used in most of the previous receptor autoradiographic studies could explain why α_2-adrenergic receptors in some regions of the brain, such as the substantia nigra, have been overlooked. We have recently obtained results that indicate that another agonist, [^{125}I]para-iodoclonidine, does not share this subtype selectivity (Alburges, Hunt, Bylund, and Wamsley, unpublished observations). The observation that the oxymetazoline-sensitive receptor (α_{2A}) predominates in the locus ceruleus, which contains the vast majority of the noradrenergic cell bodies, may be of therapeutic interest. It may be possible to selectively activate or inhibit the α_2 receptors of the cell body with a drug that might have little or no effect at other postsynaptic sites. Thus, subtype-selective drugs may have very different effects in terms of noradrenergic stimulation or inhibition within the CNS. The present investigation suggests regions of the brain upon which these future studies should focus.

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