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Heterogeneity of Angiotensin II AT_2 Receptors in the Rat Brain

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SUMMARY

Angiotensin II (AT) receptor subtypes (AT_1, selectively displaced by DuP 753, and AT_2, selectively displaced by PD123177 and CGP42112A) were characterized by quantitative autoradiography after incubation with the AT agonist ^{125}I-Sar^1-AT, in specific brain nuclei of young (2-week-old) rats. Binding to AT_1 receptors was sensitive (decreased affinity) to incubation in the presence of guanosine 5′-*O*-(3-thio)triphosphate (GTPγS). Only the AT_1 receptors in the paraventricular nucleus were sensitive to pertussis toxin, indicating the possibility of the existence of AT_1 receptor subtypes. The sensitivity of AT_2 receptors to GTPγS was heterogeneous. In the ventral thalamic and medial geniculate nuclei and in the locus coeruleus, binding to AT_2 receptors was sensitive to GTPγS and to pertussis toxin pretreatment. Conversely, in the inferior olive, binding was insensitive to GTPγS and to pertussis toxin pretreatment. We propose the nomenclature of AT_{2A} receptors for those receptors sensitive to guanine nucleotides and pertussis toxin and that of AT_{2B} receptors for those showing no sensitivity to guanine nucleotides or pertussis toxin treatment.

The recent development of selective AT receptor antagonists (1–3) allowed the characterization of different AT receptor subtypes in peripheral tissues (1–3) and in the brain (4–12). AT_1 receptors are selectively displaced by DuP 753 and AT_2 receptors by CGP42112A and PD123177 (1–12). The brain contains both receptor subtypes (4–12). Their distribution (4–12) and development (6, 8, 9, 11) suggest a different role for each subtype. AT_1 receptors are located in brain areas outside the blood-brain barrier related to fluid regulation, such as the subfornical organ, and in brain areas related to blood pressure control and stress, such as the paraventricular nucleus and the nucleus of the solitary tract (6–9, 11–14). AT_2 receptors are expressed in areas involved in sensory and motor control, such as the ventral thalamic and medial geniculate nuclei, the locus coeruleus, and the inferior olive (6–9, 11, 12). In the rat, the developmental patterns of brain AT_1 and AT_2 receptors are different (6, 8, 9). AT_2 receptors are already present during embryonic life (11) and they are more abundantly expressed in young (2-week-old) animals, compared with adult rats (8, 9), indicating a possible role during maturation of brain structures related to sensory and motor control; AT_1 receptor expression is similar in young and adult rats (8, 9). The enhanced expression of AT receptors (15), and particularly that of AT_2 receptors, in fetal skeletal muscle, skin (11, 16, 17), and aorta (18) further supports the role of AT_2 receptors in developing tissues.

In peripheral tissues, binding to AT_1 receptors is influenced by guanine nucleotides, indicating a possible association with G proteins (11, 19–21), and increased phosphoinositide hydrolysis is one of the signal-transduction mechanisms through AT_1 receptors (11, 21, 22). Conversely, in fetal (11, 18) and adult (20, 23) peripheral tissues, binding to AT_2 receptors is not influenced by guanine nucleotides, and the mechanism of signal transduction is currently unknown. Preliminary observations indicated that guanine nucleotides could also differentially affect AT_1 and AT_2 receptor binding in the brain. We have characterized AT binding in a number of brain nuclei containing AT_1 and AT_2 receptors, comparing their affinity for selective AT_1 and AT_2 antagonists and the binding sensitivity to guanine nucleotides and to pretreatment with pertussis toxin. We chose young 2-week-old rats because of the higher AT receptor expression in brain, in particular that of the AT_2 receptors (8, 9). Our observations indicate that rat brain AT_2 receptors, and probably AT_1 receptors, are heterogeneous and that the modulation of signal transduction by each subtype is probably exerted through different molecular entities.

Materials and Methods

Animals. Groups of 10-day-old Sprague Dawley male rats, along with lactating females, were purchased from Zivic Miller (Alliston Park, PA) and were provided with standard rat food and water *ad libitum*, with a 12/12-hr light/dark cycle with lights on at 6:00 a.m. Rats were killed when 14 days old, by decapitation, between 9:00 and 10:00 a.m., and the brains were removed immediately. One group of five rats were injected intraperitoneally, when 11 days old, with pertussis toxin (List Biological Laboratories, Inc., Campbell, CA), at 0.5 μg/g of body weight, dissolved in 0.01 M sodium phosphate buffer, pH 7.0, with 0.05 M

ABBREVIATIONS: AT, angiotensin II; GTPγS, guanosine 5′-*O*-(3-thio)triphosphate; ATP, adenosine 5′-*O*-(3-thio)triphosphate; G protein, guanine nucleotide-binding protein.

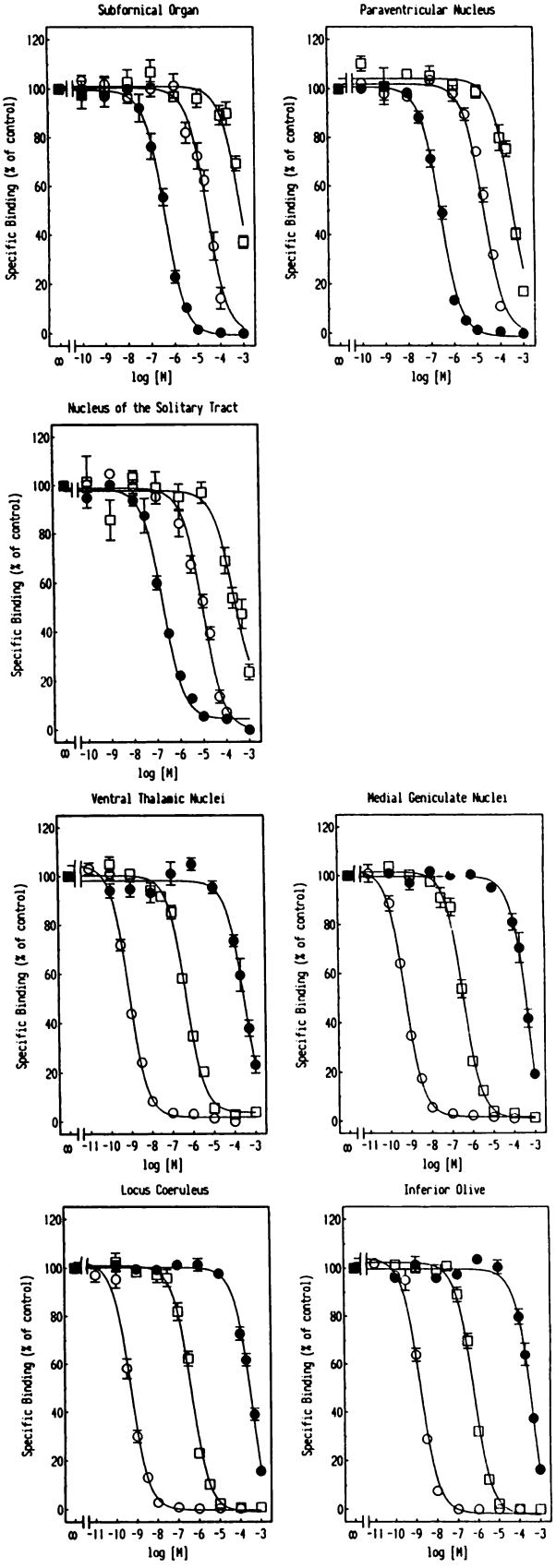

Fig. 1. Characterization of AT receptor subtypes (AT_1 and AT_2) in rat brain by quantitative autoradiography. The figure represents competition curves obtained from consecutive sections from rat brains incubated in the presence of 5×10^{-10} M ^{125}I-Sar^1-AT and increasing concentrations of AT antagonists. Results are expressed as means ± standard errors of groups of eight rats, measured individually. ●, DuP 753; ○, CGP42112A; □, PD 123177.

sodium chloride (150–180 μl/rat). A control group of five rats were injected with a similar volume of vehicle. Rats were killed 3 days later, when 14 days old.

Quantitative autoradiography. For binding studies, the brains were frozen immediately after removal, in isopentane, at −30°. The brains were stored at −70° for <1 week. Coronal sections (16 μm thick) were cut in a cryostat at −15°, thaw-mounted on gelatin-coated glass slides, and dried overnight in a desiccator at 4°.

Sections were labeled *in vitro* with ^{125}I-Sar^1-AT (Peninsula Laboratories, Belmont, CA; iodinated by DuPont-New England Nuclear Laboratories, Wilmington, DE; specific activity, 2200 Ci/mmol), using experimentally determined incubation parameters previously described (24). Briefly, sections were preincubated for 15 min at 22° in 10 mM sodium phosphate buffer, pH 7.4, containing 120 mM NaCl, 5 mM Na_2EDTA, 0.005% bacitracin (Sigma), and 0.2% proteinase-free bovine serum albumin (Sigma), followed by incubation for 120 min in fresh buffer containing the appropriate ligand. After incubation, slides were rinsed four consecutive times, 1 min each, in fresh ice-cold 50 mM Tris·HCl buffer, pH 7.6, followed by a dip in ice-cold distilled water, and the sections were dried under air.

To characterize AT receptor subtypes, consecutive sections were incubated with 5×10^{-10} M ^{125}I-Sar^1-AT, a concentration close to the K_d value (7–9), in the presence of increasing concentrations (10^{-10} to 10^{-3} M) of DuP 753 [2-*n*-butyl-4-chloro-5-hydroxymethyl-1-[2′-((1*H*-tetrazol-5-yl)biphenyl-4-yl)methyl]imidazole] (DuPont, Wilmington, DE), 10^{-11} to 10^{-4} M CGP42112A [nicotinic acid-Tyr-(*N*′-benzyloxycarbonyl-Arg)Lys-His-Pro-Ile-OH] (CIBA-Geigy, Basle, Switzerland), or 10^{-10} to 10^{-3} M PD123177 [1-(4-amino-3-methylphenyl)methyl-5-diphenylacetyl-4,5,6,7-tetrahydro-1*H*-imidazo[4,5-*c*]pyridine-6-carboxylic acid-2HCl] (Parke Davis, Ann Arbor, MI).

To determine maximum binding capacity (B_{max}) and apparent dissociation constants (K_d), consecutive brain sections containing the subfornical organ, the paraventricular nucleus, the nucleus of the solitary tract, the medial geniculate nucleus, or the inferior olive were incubated in the presence of increasing concentrations of ^{125}I-Sar^1-AT ($0.25–8 \times 10^{-9}$ M). Nonspecific binding was determined in the presence of 5×10^{-6} M unlabeled AT (Peninsula). A preliminary experiment revealed a relatively lower affinity for AT binding in the ventral thalamic nucleus and the locus coeruleus. For this reason, for saturation studies in these areas we used ^{125}I-Sar^1-AT in concentrations ranging from 0.25 to 16×10^{-9} M. Incubation conditions for these experiments were similar to those used to determine guanine nucleotide sensitivity (see below).

We studied the possible involvement of G proteins in the regulation of AT binding to its receptor subtypes by performing two different experiments. For determination of the effects of guanine nucleotides on AT binding to its receptors, brain sections were incubated in the absence or presence of increasing concentrations (10^{-8} to 10^{-3} M) of metabolically stable analogues of GTP (GTPγS) and ATP (ATPγS) (Sigma) (16). The incubation conditions were similar to those used for characterization of AT receptor subtypes, with the exception that the preincubation and incubation buffers consisted of 50 mM sodium phosphate buffer, pH 7.4, containing 120 mM NaCl, 10 mM $MgCl_2$, 0.005% bacitracin (Sigma), and 0.2% proteinase-free bovine serum albumin (Sigma). To determine the effects of guanine nucleotides on AT binding, we determined B_{max} and K_d as described above, with the addition of a single concentration of GTPγS. In areas expressing AT_1 receptors, where the GTPγS effect was obtained at relatively lower nucleotide concentrations, we used 3×10^{-7} M GTPγS. In areas expressing AT_2 receptors, where the guanine nucleotide effect was shown at higher concentrations, we used 10^{-5} M GTPγS.

The experiments to test for pertussis toxin sensitivity were performed under the same incubation conditions, with a single concentration (5×10^{-10} M) of ^{125}I-Sar^1-AT.

The dry labeled sections, together with a set of ^{125}I-labeled standards (Amersham Co., Arlington Heights, IL) were apposed against ^{3}H-Hyperfilm (Amersham) in X-ray cassettes (CGR Medical Corp., Baltimore, MD). Films were developed with D19 Kodak developer for 4 min at 4°. Optical densities of the autoradiograms were determined by computerized microdensitometry, and the results were expressed in fmol/mg of protein after comparison with the ^{125}I standard curve (25). IC_{50} values were calculated with the GraphPAD InPlot program (GraphPAD, San Diego, CA). To determine the B_{max} and the K_d from saturation experiments, binding data were analyzed using the LIGAND program (26). Results were expressed as means ± standard errors and were analyzed by unpaired *t* test.

Results

Characterization of brain AT_1 and AT_2 receptor subtypes by competition with selective AT receptor blockers. The selective AT_1 antagonist DuP 753 readily competed for AT binding in the subfornical organ, paraventricular nucleus, and nucleus of the solitary tract (Fig. 1). The selective AT_2 displacer CGP42112A showed much lower affinity for AT binding in areas containing AT_1 receptors (Table 1). The affinity of the selective nonpeptidic AT_2 displacer PD123177 in these areas was 1 order of magnitude lower than that of CGP42112A (Fig. 1; Table 1).

Conversely, the AT_2-selective displacers CGP42112A and PD123177 competed for AT binding to the ventral thalamic nuclei, medial geniculate nucleus, locus coeruleus, and inferior olive with high affinity, whereas the affinity of DuP 753 for AT binding in these areas was several orders of magnitude lower (Fig. 1; Table 1). The different areas containing AT_2 receptors were heterogeneous in their affinity for AT_2-selective blockers. CGP42112A competed for AT binding with slightly (2–3 times) lower affinity in the inferior olive, compared with the ventral thalamic nuclei, medial geniculate nucleui, and locus coeruleus (Table 1). In addition, in all areas rich in AT_2 receptors, the affinity for CGP42112A was several orders of magnitude higher than that for PD123177 (Table 1).

Effect of guanine nucleotides on AT binding to AT_1 and AT_2 receptors. Incubation of brain sections containing AT_1 receptors with increasing concentrations of GTPγS, but not ATPγS, resulted in a progressive loss (up to 90%) of specific AT binding (Figs. 2 and 3). Analysis of AT binding to the subfornical organ, the paraventricular nucleus, and the nucleus of the solitary tract revealed decreased binding affinity after incubation in the presence of GTPγS (Fig. 4; see Table 3), with no apparent differences in maximum binding capacity. The effects of GTPγS on AT binding to AT_2 receptors depended on the area studied. In the ventral thalamic and medial geniculate nuclei and in the locus coeruleus, incubation with GTPγS, but not ATPγS, resulted in a partial loss (up to about 50%) of AT binding (Figs. 2 and 3; Table 2). In these areas, GTPγS decreased binding affinity but did not modify the maximum binding capacity, with the exception of the locus coeruleus, where the maximum binding capacity was increased (Table 3). In addition, the sensitivity of AT binding to guanine nucleotides was higher in the locus coeruleus, compared with the ventral thalamic or medial geniculate nuclei (Table 2). Conversely, there were no effects of guanine nucleotides on AT binding in another brain area containing AT_2 receptors, the inferior olive (Figs. 2, 3, and 4; Tables 2 and 3).

In the presence of GTPγS, we could not reach saturation in some areas because of decreased affinity, even after incubation in the presence of 16×10^{-9} M ^{125}I-Sar^1-AT (Fig. 4), due to limitations of the autoradiographic method.

Effect of pertussis toxin pretreatment on AT binding. Treatment with pertussis toxin produced marked, and selective, alterations in AT binding in specific brain areas. In areas containing AT_1 receptors, binding after pertussis toxin treatment was decreased only in the paraventricular nucleus, and there was no difference in binding in the subfornical organ or the nucleus of the solitary tract (Table 4). In areas containing AT_2 receptors, AT binding after pertussis toxin treatment was reduced in the ventral thalamic and medial geniculate nuclei and in the locus coeruleus, but not in the inferior olive (Table 4).

Discussion

The rat brain has been recently shown to contain two AT receptor subtypes, AT_1 and AT_2 (4–9, 11, 12). These receptor subtypes are similar to the previously described peripheral AT_1 and AT_2 receptors, at least in terms of their relative affinities for the selective AT_1 and AT_2 receptor antagonists (4–9, 11, 12). Most brain areas contain only one AT receptor subtype (7–9, 11, 12). The selective anatomical distribution of the brain AT receptor subtypes indicates that they may play different roles. AT_1 receptors are located in areas involved in well known AT effects, such as cardiovascular and fluid regulation and

TABLE 1

Competition of selective AT_1 and AT_2 antagonists for AT binding in rat brain nuclei

Consecutive coronal sections from 2-week-old rats were incubated with 5×10^{-10} M ^{125}I-Sar^1-AT and increasing concentrations of DuP 753 (from 10^{-10} to 10^{-3} M), CGP42112A (from 10^{-11} to 10^{-4} M), or PD123177 (from 10^{-10} to 10^{-3} M), as described in Materials and Methods. Results are mean ± standard error of data from eight animals, measured individually.

	K_i		
	DuP 753	CGP42112A	PD123177
	M		
AT_1 receptors			
Subfornical organ	$2.8 \pm 0.5 \times 10^{-7}$	$2.3 \pm 0.4 \times 10^{-5}$	$6.7 \pm 0.6 \times 10^{-4}$
Paraventricular nucleus	$1.6 \pm 0.2 \times 10^{-7}$	$1.4 \pm 0.1 \times 10^{-5}$	$2.1 \pm 0.1 \times 10^{-4}$
Nucleus of the solitary tract	$1.2 \pm 0.2 \times 10^{-7}$	$0.7 \pm 0.1 \times 10^{-5}$	$2.3 \pm 0.4 \times 10^{-4}$
AT_2 receptors			
Ventral thalamic nuclei	$3.0 \pm 0.4 \times 10^{-4}$	$6.5 \pm 0.3 \times 10^{-10}$	$4.2 \pm 0.8 \times 10^{-7}$
Medial geniculate nucleus	$3.1 \pm 0.4 \times 10^{-4}$	$4.4 \pm 0.3 \times 10^{-10}$	$2.3 \pm 0.2 \times 10^{-7}$
Locus coeruleus	$2.3 \pm 0.1 \times 10^{-4}$	$4.1 \pm 0.5 \times 10^{-10}$	$3.6 \pm 0.3 \times 10^{-7}$
Inferior olive	$2.4 \pm 0.2 \times 10^{-4}$	$11.5 \pm 1.0 \times 10^{-10}$	$4.5 \pm 0.2 \times 10^{-7}$

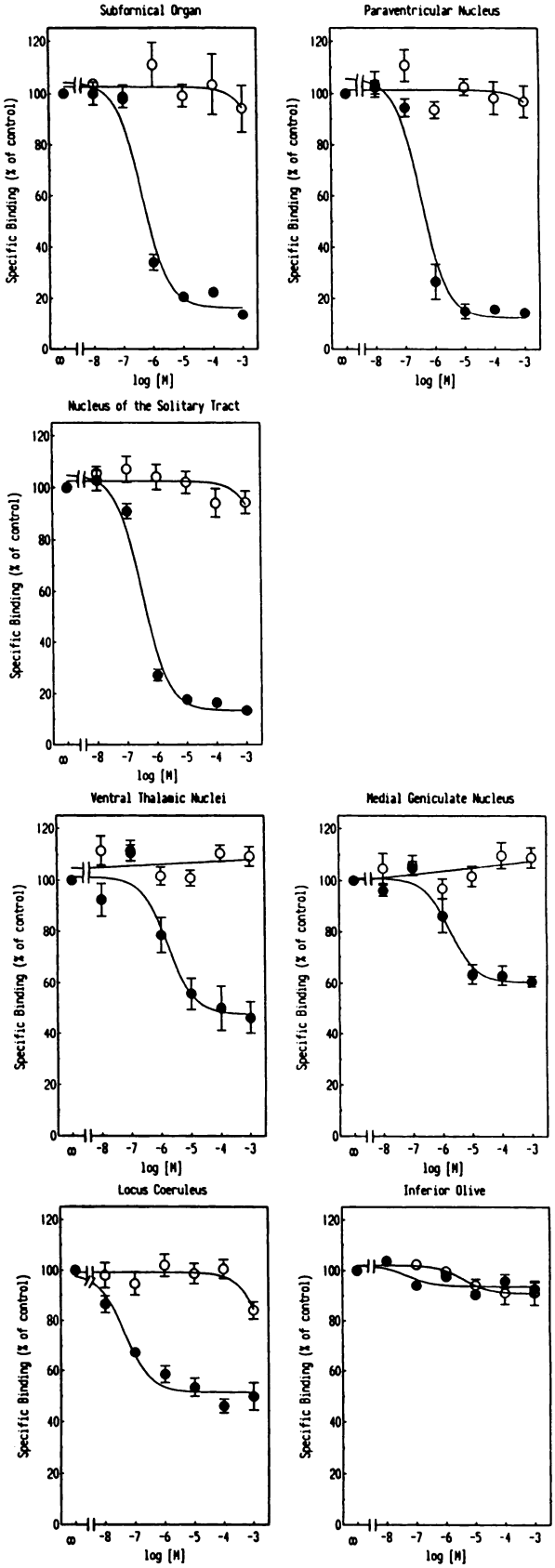

Fig. 2. Effects of nucleotides on AT binding. Consecutive sections from rat brains were incubated with 5×10^{-10} M ^{125}I-Sar^1-AT and increasing concentrations of GTPγS (●) or ATPγS (○). Results are expressed as means ± standard errors of groups of 10 rats measured individually, from two separate experiments.

regulation of the response to stress (7–9, 12–14, 27, 28). AT_2 receptors are concentrated in areas involved in sensory and motor control, where effects of AT have not yet been described (7–9, 12). The idea that the AT_1 and AT_2 receptors probably play different roles in the brain is emphasized by developmental differences. The expression of the AT_2 receptors is higher early in development, whereas AT_1 receptors are similarly expressed in young and adult rats (8, 9).

Earlier pharmacological and pathophysiological evidence, from studies on AT binding in areas that were later shown to contain only AT_1 receptors, indicated that the AT_1 receptors could be selectively regulated. AT receptors in the subfornical organ were up-regulated by water deprivation and by a reduction in the extracellular fluid volume; those in the paraventricular nucleus were not (29–33). Spontaneously hypertensive rats had higher numbers of AT receptors in the subfornical organ and the nucleus of the solitary tract, but no difference occurred in the paraventricular nucleus (34, 35). These earlier experiments did not address the question of possible biochemical heterogeneity of AT_1 receptors.

In turn, a recent developmental study (9) revealed not only a developmental difference between AT_1 and AT_2 receptors but also differences in development between anatomically selected groups of AT_2 receptors. Of particular interest were the extremely high concentrations of AT_2 receptors in the inferior olive of young (2-week-old) rats, which were severalfold higher than concentrations in any other AT_2-rich area (9).

Against this background, we attempted to address the question of the possible pharmacological heterogeneity of AT_1 and AT_2 receptors in the brain, circumscribing our study to a few, well characterized areas. First, a detailed competition study with AT antagonists was conducted. In agreement with previous reports, brain AT_1 and AT_2 receptors could be clearly differentiated by their affinity for a selective AT_1 antagonist (DuP 753) or for selective AT_2 antagonists (CGP42112A and PD123177) (4–9, 11, 12), and all brain areas studied contained only one AT receptor subtype. The relative potency of competition for AT antagonists was, for the AT_1 receptors, DuP 753 ≫ CGP42112A > PD123177 and, for the AT_2 receptors, CGP42112A > PD123177 ≫ DuP 753.

A further comparative analysis of the potency of the receptor antagonists suggested additional differences among the AT_2 receptors. AT_2 receptors in the inferior olive showed slightly (2–3 times) lower affinity for CGP42112A, compared with all other AT_2 areas studied. However, the physiological significance of these differences has not yet been clarified.

Our present results indicate that binding of the AT agonist ^{125}I-Sar^1-AT to all AT_1 receptors studied is sensitive to guanine nucleotides. Addition of a metabolically stable analogue of GTP, GTPγS, decreased agonist binding affinity. Inhibition of binding in the presence of guanine nucleotides has been reported earlier for AT (36) and other peptide receptors, such as those for substance P (37), somatostatin (38), growth hormone-releasing factor (39), and neuropeptide Y (40). The present results are consistent with recent studies demonstrating that binding to AT_1 receptors in fetal liver and lung parenchyma (16), adult liver (19), superior cervival ganglia (21), and aorta of the immature and adult rats (18), as well as to rat vascular AT receptors (41) and to vascular smooth muscle cells, which have been classified recently as AT_1 receptors (3), is also sensitive to guanine nucleotides. The inhibition of AT binding

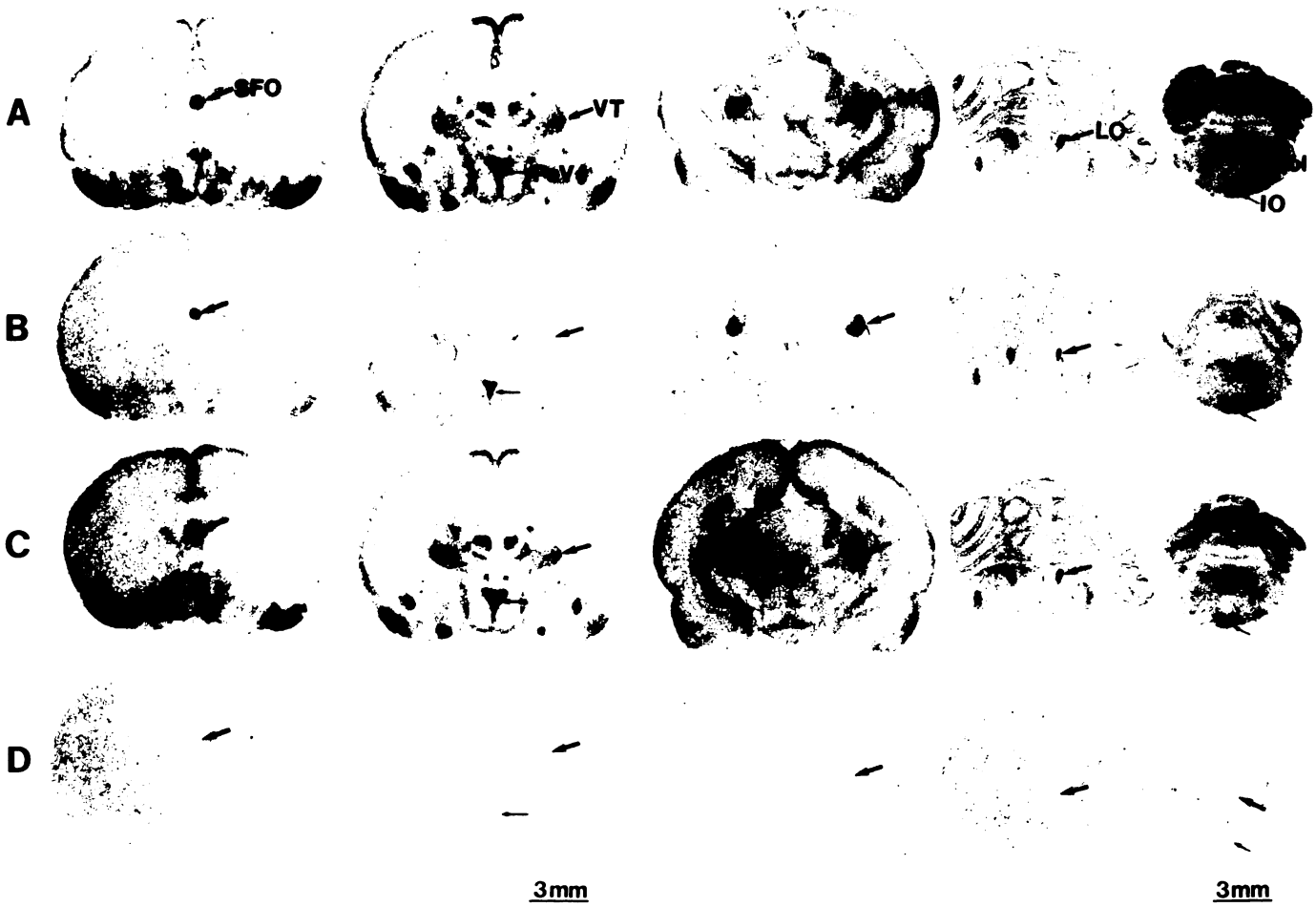

Fig. 3. Autoradiographic localization of nucleotide effects on AT binding. A, Total binding (^{125}I-Sar^1-AT concentration was 5×10^{-10} M). B, Consecutive section incubated as in A, in the presence of 10^{-4} M GTPγS. C, Consecutive section incubated as in A, in the presence of 10^{-4} M ATPγS. D, Consecutive section incubated as in A, in the presence of 5×10^{-6} M unlabeled AT. Nomenclature is from Paxinos and Watson (56). *SFO,* subfornical organ; *PaV,* paraventricular nucleus; *MG,* medial geniculate nucleus; *VT,* ventral thalamic nuclei; *LC,* locus coeruleus; *IO,* inferior olive; *Sol,* nucleus of the solitary tract.

to its AT_1 receptors by guanine nucleotides indicates that brain AT_1 receptors, like peripheral AT_1 receptors, may be coupled functionally to effectors by G proteins and that they belong to the superfamily of G protein-coupled receptors (42).

AT_2 receptors in peripheral tissues are currently believed to be insensitive to guanine nucleotides and not coupled to G proteins (16, 18–20). We have found that AT binding to AT_2 receptors in the rat inferior olive is not sensitive to GTPγS. These data are similar to those obtained for AT binding to AT_2 receptors in bovine cerebellar cortex (20) and do not support an association of inferior olive AT_2 receptors with G proteins. However, agonist binding to other brain AT_2 receptors (those located in the ventral thalamic and medial geniculate nuclei and the locus coeruleus) was indeed significantly decreased, and the affinity for the agonist was reduced, by incubation in the presence of GTPγS. This is the first report suggesting the presence of G protein-coupled AT_2 receptors. These guanine nucleotide-sensitive brain AT_2 receptors are possibly different from other guanine nucleotide-insensitive AT_2 receptors in brain (20) and peripheral tissues (16, 18–20). In addition, the sensitivity of AT_2 binding to guanine nucleotides was not uniform, with the locus coeruleus exhibiting a 2-order of magnitude higher sensitivity than the ventral thalamic nuclei or the medial geniculate nucleus. Thus, these guanine nucleotide-sensitive AT_2 receptors in the brain may be further heterogeneous with respect to their guanine nucleotide sensitivity, and possibly with respect to their functional linkage to effectors by G proteins.

Many different types of G proteins have been described (42). We have made a preliminary attempt to identify the G protein(s) that could be involved in the signal-transduction mechanism(s) of AT in selected brain areas. Pertussis toxin, the toxin of *Bordetella pertussis,* activates ADP-ribosylation of specific types of G proteins, such as G_i and G_o (42), and decreases the receptor affinity state (43–46) or binding density (47) for agonists, including AT (48), both *in vitro* and *in vivo.* We have observed that *in vivo* treatment with pertussis toxin differentially affects binding to some AT_1 and AT_2 receptors in the rat brain. The interval chosen after pertussis toxin administration was similar to that used by others in the evaluation of the effects of the toxin on AT binding to receptors in the adrenal zona glomerulosa (36, 43), on the affinity state for agonists of renal adrenoceptors (44), or on neuropeptide binding in the pituitary gland (47). We noticed that the sensitivity of AT binding to *in vivo* treatment with pertussis toxin was heterogeneous for both AT_1 and AT_2 receptors in rat brain.

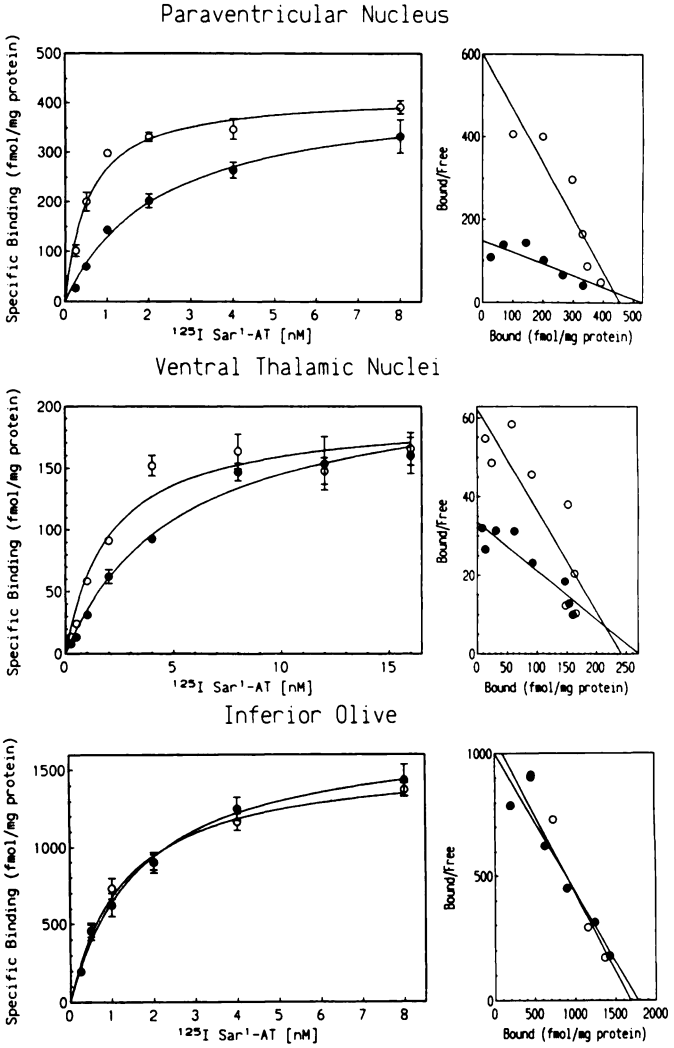

Fig. 4. Effect of guanine nucleotides on AT binding characteristics. The figure represents saturation curves (means ± standard errors of five rats measured individually) (*left*) and Scatchard plots (means of five rats) (*right*) derived from consecutive sections incubated without (○) or with (●) GTPγS.

TABLE 2

Effect of GTPγS on ^{125}I-SAR^1-AT binding

Data are means ± standard errors of 10 rats measured individually, from two separate experiments.

	IC_{50}
	M
AT_1 receptors	
Subfornical organ	$4.3 \pm 0.5 \times 10^{-7}$
Paraventricular nucleus	$3.4 \pm 0.3 \times 10^{-7}$
Nucleus of the solitary tract	$3.7 \pm 0.5 \times 10^{-7}$
AT_2 receptors	
Ventral thalamic nuclei	$1.6 \pm 0.5 \times 10^{-6}$
Medial geniculate nucleus	$2.4 \pm 0.6 \times 10^{-6}$
Locus coeruleus	$4.7 \pm 1.1 \times 10^{-8}$
Inferior olive	$>10^{-3}$[a]

[a] $>10^{-3}$, no significant inhibition occurred with concentrations of up to 10^{-3} M GTPγS.

Binding to AT_1 receptors was sensitive to pertussis toxin only in the paraventricular nucleus and not in the subfornical organ or the nucleus of the solitary tract. For AT_2 receptors, binding was sensitive to pertussis toxin in all areas showing guanine nucleotide sensitivity but not in the inferior olive, which contains guanine nucleotide-insensitive AT_2 receptors. These data suggest that AT_1 receptors in brain of young rats may be associated with both pertussis toxin sensitive- and -insensitive G proteins and that G proteins coupled to AT_2 receptors may be only pertussis toxin-sensitive types, such as G_i or G_o, which is a major pertussis toxin substrate in the central nervous system (42). However, at present, the direct effect of pertussis toxin on signal-transduction mechanisms of brain AT receptors cannot be totally clarified, because of the controversy regarding their second messenger system(s) (49–52).

TABLE 3

Effect of GTPγS on AT binding characteristics

Data are means ± standard errors of tissues from five rats measured individually.

AT_1 receptors	$K_d \times 10^{-9}$ *M*		B_{max}	
	Control	GTPγS (3×10^{-7} M)	Control	GTPγS (3×10^{-7} M)
			fmol/mg of protein	
Subfornical organ	1.6 ± 0.1	3.7 ± 0.7[a]	353 ± 34	340 ± 38
Paraventricular nucleus	0.8 ± 0.1	3.4 ± 0.5[b]	441 ± 16	488 ± 33
Nucleus of the solitary tract	1.2 ± 0.3	5.9 ± 0.5[b]	584 ± 46	725 ± 50
AT_2 receptors	$K_d \times 10^{-9}$ *M*		B_{max}	
	Control	GTPγS (10^{-5} M)	Control	GTPγS (10^{-5} M)
			fmol/mg of protein	
Ventral thalamic nuclei	4.5 ± 0.6	8.3 ± 0.6[b]	231 ± 30	273 ± 21
Medial geniculate nucleus	2.5 ± 0.2	3.3 ± 0.2[a]	262 ± 26	238 ± 33
Locus coeruleus	2.3 ± 0.3	5.4 ± 0.6[b]	257 ± 24	340 ± 16[a]
Inferior olive	1.6 ± 0.3	1.8 ± 0.3	1660 ± 52	1759 ± 119

[a] $p < 0.05$, control versus GTPγS-treated (unpaired *t* test).
[b] $p < 0.01$.

TABLE 4

Effect of pertussis toxin treatment on AT binding

^{125}I-Sar^1-AT concentration was 5×10^{-10} M. Data are means ± standard errors for groups of five rats, measured individually.

	Control	Pertussis toxin
	fmol/mg of protein	
AT_1 receptors		
Subfornical organ	128.4 ± 10.8	143.2 ± 6.8
Paraventricular nucleus	54.8 ± 7.4	27.7 ± 3.4[a]
Nucleus of the solitary tract	40.4 ± 0.8	38.3 ± 2.4
AT_2 receptors		
Ventral thalamic nuclei	11.2 ± 2.0	5.0 ± 0.5[a]
Medial geniculate nucleus	63.0 ± 4.1	40.6 ± 1.4[a]
Locus coeruleus	39.6 ± 2.4	17.8 ± 2.2[a]
Inferior olive	142.0 ± 8.2	149.2 ± 4.6

[a] $p < 0.01$, control versus pertussis toxin-treated group.

TABLE 5

Brain AT receptor subtypes

	AT_1 receptors		
	Subfornical organ	Nucleus of the solitary tract	Paraventricular nucleus
GTPγS sensitivity	Present	Present	Present
Pertussis toxin sensitivity	Absent	Absent	Present

	AT_2 receptors			
	AT_{2A}			AT_{2B}
	Ventral thalamic nuclei	Medial geniculate nucleus	Locus coeruleus	Inferior olive
GTPγS sensitivity	Present	Present	Present (higher)	Absent
Pertussis toxin sensitivity	Present	Present	Present	Absent

The present study on AT receptor antagonist affinities, guanine nucleotide effects, and sensitivity to pertussis toxin pretreatment indicates that the binding of AT is affected differently in selected brain areas and suggests that the recently described brain AT receptor subtypes may consist of disparate, biochemically distinct subgroups.

Brain AT_2 receptors could be further classified in two distinct subgroups. In the ventral thalamic and medial geniculate nuclei and in the locus coeruleus, binding to AT_2 receptors was sensitive to guanine nucleotides and to pertussis toxin treatment, suggesting an association to specific G protein subtype(s). These AT_2 receptors, although highly expressed early in development, do not reach a concentration comparable to that in the inferior olive (9). We propose to name these receptors as AT_{2A} receptors.

Conversely, AT_2 receptors located in the inferior olive were insensitive to guanine nucleotides and to pertussis toxin treatment. These receptors are very highly expressed early in development (9). We propose to name these receptors as AT_{2B} receptors.

The brain AT_1 receptors may also consist of different subtypes, probably all linked to G proteins, as indicated by their guanine nucleotide sensitivity. It is tempting to consider the AT_1 receptors in the paraventricular nucleus as different from those located in other brain areas, because the paraventricular nucleus is the only area studied so far where AT_1 binding is sensitive to pertussis toxin treatment. In addition, AT binding to the paraventricular nucleus is modulated by corticosteroids (28, 31) but not by alterations in fluid regulation or hypertension, which affect other AT_1 receptors (53).

The existence of AT_1 receptor heterogeneity was also implied by studies on their signal-transduction mechanisms. Fetal AT_1 receptors and AT receptors in peripheral tissues from adult animals that have been shown to contain only AT_1 receptors utilize phosphoinositide hydrolysis as one of their intracellular signal-transduction mechanisms (16, 21, 22). Pituitary cells show several signal-transduction mechanisms for AT. In pituitary cells, containing only AT_1 receptors, pertussis toxin blocks the effect of AT to inhibit adenylate cyclase, without blocking its ability to stimulate phosphoinositide hydrolysis and calcium mobilization (54). These data suggest that, in the pituitary, some of the AT receptors are coupled to phosphoinositide hydrolysis through G_p (pertussis toxin insensitive), whereas other AT_1 receptors are coupled in an inhibitory fashion to adenylate cyclase, through G_i (pertussis toxin sensitive) (54). However, little is known about signal-transduction mechanisms for brain AT, which has been reported to stimulate (49) or inhibit (50) brain phosphoinositide hydrolysis in brain slices, to decrease the level of cGMP (52), and to stimulate prostaglandin release (51) in neuronal and glial cell culture systems. In addition, a single receptor may activate more than one signal-transducing G protein (55). Thus, the evidence presented in the literature and here is only suggestive of AT_1 receptor heterogeneity. The present results, and the proposed nomenclature, are summarized in Table 5.

In conclusion, rat brain AT_1 receptors may be, similarly to the fetal AT_1 receptors and to those AT_1 receptors expressed in vascular smooth muscle cells of adult animals, associated with G proteins. Rat brain AT_2 receptors, on the basis of guanine nucleotide and pertussis toxin sensitivity, can be considered to belong to two different pharmacological subtypes, which we propose to call AT_{2A} and AT_{2B}. Further studies, including clarification of their signal-transduction mechanisms and development of more selective antagonists, could elucidate whether brain AT_1 and AT_2 receptors are biochemically heterogeneous. The nature of the second messenger(s) and the roles of the AT_{2A} and AT_{2B} receptors are presently unknown.

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