Pharmacology Biochemistry & Behavior, Vol. 25, pp. 903–911, 1986. © Ankho International Inc. Printed in the U.S.A. 0091-3057/86 $3.00 + .00

Alpha-Adrenoceptors and Monoamine Contents in the Cerebral Cortex of the Rodent *Jaculus orientalis*: Effects of Acute Cold Exposure

NOURIA LAKHDAR-GHAZAL,[1] LOUISE GRONDIN, WAIL A. BENGELLOUN AND TOMÁS A. READER[2,3]

Département de Biologie, Unité de Recherche en Neurosciences, Université Mohammed V, Rabat, Morocco and Centre de Recherche en Sciences Neurologiques, Département de Physiologie, Faculté de Médecine Université de Montréal, Montréal, Québec H3C 3J7, Canada

Received 2 December 1985

LAKHDAR-GHAZAL, N., L. GRONDIN, W. A. BENGELLOUN AND T. A. READER. *Alpha-adrenoceptors and monoamine contents in the cerebral cortex of the rodent* Jaculus orientalis: *Effects of acute cold exposure.* PHARMACOL BIOCHEM BEHAV **25**(4) 903–911, 1986.—The tritiated adrenergic antagonists prazosin ([^{3}H]PRZ) and idazoxan ([^{3}H]IDA, or RX-781094) bind specifically and with high affinity to α_1- and α_2-adrenoceptors respectively, and were used to measure adrenoceptors in membrane preparations obtained from the cerebral cortex of *Jaculus orientalis*. Membrane preparations were also obtained from a group of cold exposed animals, to determine whether these adrenoceptors could be modified by a thermic stress. The density of receptors (B_{max}; maximum binding capacity) and the dissociation constant (K_d 25°C) were estimated by iterative modelling, and by using the procedure of Hill. After acute cold exposure (16 hr, 5°C) there was a decrease in the affinity of the α_1-adrenoceptors, as judged by the K_d 25°C for [^{3}H]PRZ, with no changes in the B_{max}. The α_2-sites did not show any significant changes, as revealed by [^{3}H]IDA binding. Pretreatment of the membrane preparations from control animals with the disulfide and sulfhydryl reactives DL-dithiothreitol, 5,5′-dithiobis-(2-nitrobenzoic acid) and N-ethylmaleimide decreased specific [^{3}H]PRZ and [^{3}H]IDA binding, with minor changes in non-specific counts, indicating that the fixation of these ligands was to the receptor proteins. The endogenous cortical monoamine contents were also determined in the frontal cerebral cortex of these same animals, using high performance liquid chromatography with electrochemical detection. The catecholamine levels and their major metabolites were found to be stable in the cortex after the acute thermic stress, but there was a marked reduction in serotonin with a normal content in 5-hydroxyindole-3-acetic acid.

Cortical alpha-adrenoceptors α_1-Sites α_2-Sites Prazosin Idazoxan Cortical monoamines

THERE are two types of α-adrenergic receptors in the cerebral cortex, and they have been classified as α_1- and α_2-adrenoceptors. Originally this subdivision contemplated their situation at post- or presynaptic locations respectively. However, with the possible existence of pre- as well as postsynaptic α_2-sites, the only valid criteria to differentiate α_1- from α_2-adrenoceptors are pharmacological and/or biochemical parameters [3, 17, 42–44]. The studies aimed at these characterizations benefit undoubtedly from the advent of radiolabelled drugs (agonists and antagonists) with relatively high specific activities. Using these markers, it is common practice to perform direct *in vitro* biochemical assays and investigate their pharmacological properties. Generally, simple saturation curves are performed to calculate the maximum binding capacity (B_{max}), while the affinity is derived from the estimated dissociation constant (K_d). The rodent *Jaculus orientalis* hibernates, and CNS monoamines, i.e., noradrenaline (NA), dopamine (DA) and serotonin (5-HT) have been proposed to play major roles in this state [22], thus constituting an interesting model to study neurotransmitter changes and/or interactions in response to thermic stress. The aim of this first study was to try to measure α-adrenoceptors in the cerebral cortex of control animals and in a group acutely exposed to cold with two α-adrenergic radioligands, i.e., [^{3}H]prazosin ([^{3}H]PRZ; for the α_1-sites) and [^{3}H]idazoxan ([^{3}H]IDA; for the α_2-sites). Since receptors

[1]Visiting Scientist, A.P.P.U.I., Agence Canadienne pour le Développement International.

[2]Chercheur-boursier Senior, Fonds de la Recherche en Santé du Québec (FRSQ).

[3]Requests for reprints should be addressed to Dr. Tomás A. Reader, Centre de Recherche en Sciences Neurologiques, Faculté de Médecine, Université de Montréal, C.P. 6128, Succ. A., Montréal, Québec, H3C 3J7, Canada.

ABBREVIATIONS

[^{3}H]PRZ	[^{3}H] prazosin
[^{3}H]IDA	[^{3}H]idazoxan
B_{max}	maximum binding capacity
K_d	dissociation constant
nH	Hill coefficient
CMC	coefficient of multiple correlation
fmol/mg p.	fentomoles per mg of protein
nM	nanomolar
pM	picomolar
DL-DDT	dithiothreitol
DTNB	5,5'-dithiobis-(2-nitrobenzoic acid)
NEM	N-ethylmaleimide

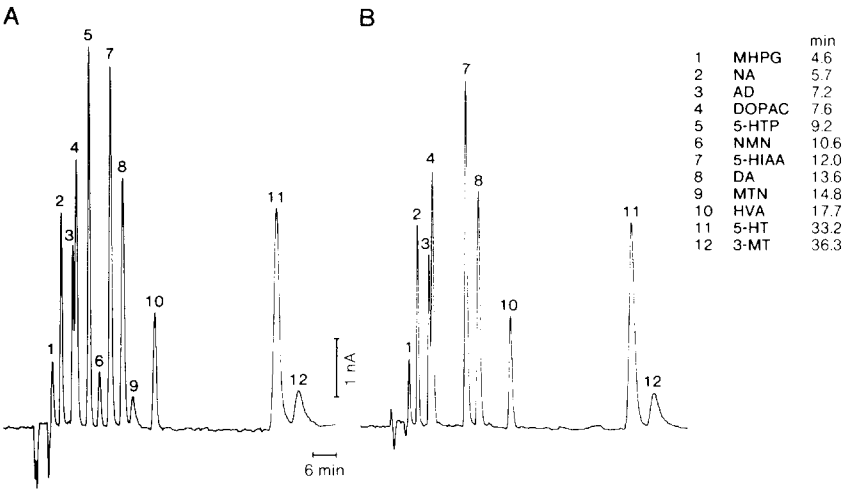

FIG. 1. Chromatograms of external standards of monoamines, using electrochemical detection to determine the peaks of the eluate. The mobile phase was monochloroacetic buffer 0.1 M at pH 3.35 containing 800 mg/l of Na_2EDTA, 280 mg/l of sodium octyl phosphate and 10% methanol (v/v). The flow rate was 1.2 ml/min and the column temperature 36°C. (A) The addition of 3.25 ng each of the oxidized and methylated metabolites of the CA, i.e., normetanephrine (NMN) and metanephrine (MTN), and of 5-hydroxy-2-tryptophan (5-HTP) did not interfere with the assay, since they had different retention times. (B) Chromatogram of the regular standard solution, which allows for the determination of 4-hydroxy-3-methoxyphenylglycol (MHPG), noradrenalin (NA), adrenaline (AD), 3,4-dihydroxyphenylacetic acid (DOPAC), 5-hydroxyindole-3-acetic acid (5-HIAA), dopamine (DA), 4-hydroxy-3-methoxyphenyl-acetic acid (homovanillic acid; HVA), serotonin (5-HT) and 3-methoxytyramine (3-MT). For these compounds the procedure was found to be linear, between 250 pg–250 ng.

are of protein nature, and therefore possess both sulfhydryl (-SH) and disulfide (-SS-) groups, we also looked into the possible role of these groups in the binding of [^{3}H]PRZ and [^{3}H]IDA. This approach could decide if the binding of the ligands was to protein receptors, or simply a fixation to or a partition into the lipid moiety of the membrane preparations. Previous studies performed with membrane preparations from rat cerebral cortex had shown that biochemical modifications of thiols decreased specific [^{3}H]PRZ and [^{3}H]IDA binding, with a certain degree of selectivity for the α_2-sites [28, 35, 36]. It was therefore of interest to determine if the α-adrenoceptors of *Jaculus orientalis* presented these biochemical properties as well. Finally, endogenous catecholamines (CA) and 5-HT, as well as some of their major metabolites, were measured by high performance liquid chromatography with electrochemical detection in samples of frontal cortex, to determine if changes in receptors could be the reflection of modifications of monoamine levels in this area.

METHOD

Eleven adult *Jaculus orientalis* from the Département de Biologie (Université Mohammed V, Rabat, Morocco) were used in this study. After arriving in Canada, they were divided in two groups; 5 rodents were used as controls, while

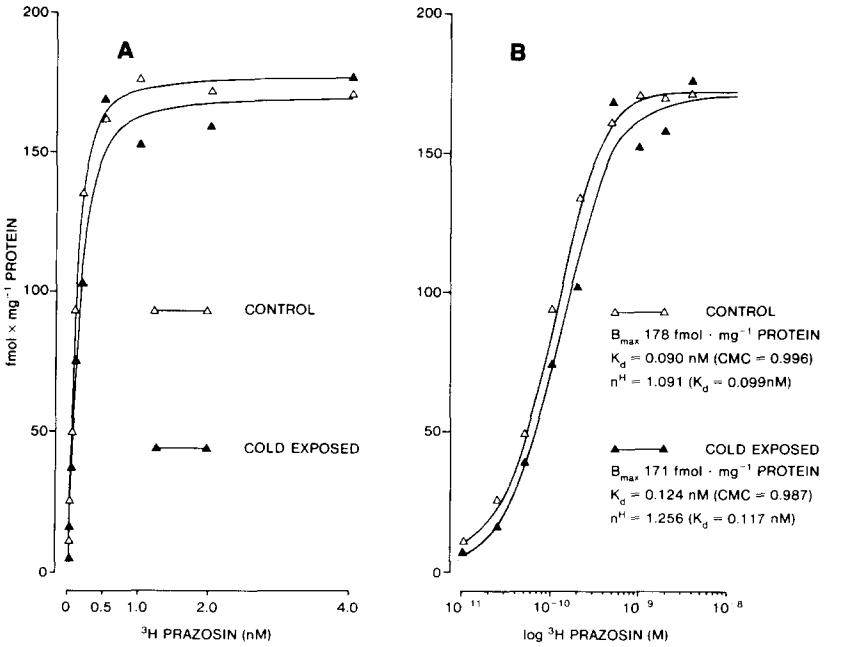

FIG. 2. Saturation curves of [^{3}H]prazosin ([^{3}H]PRZ; α_1-sites) binding to membrane preparations from the cerebral cortex of *Jaculus orientalis*, performed with 9 concentrations (0.01–4 nM). Data points represent means of duplicate determinations from a representative experiment. (A) The saturation curves represent the amounts specifically bound (in fmol/mg protein) as a function of ligand concentration in a membrane preparation from a control animal (△) or after an acute cold exposure (16 hr at 5°C) (▲). (B) Computer modelling of the saturation curves, using FIT [1,26] for a model with a single binding affinity state for [^{3}H]PRZ. The coefficients of multiple correlation (CMC) were never below 0.97, and attempts to fit these binding data to a two state model did not improve the fit. The values were: Control, B_{max}=178 fmol/mg protein, K_d=0.09 nM (CMC=0.996), Hill coefficient (nH) of 1.09 and a K_d by Hill-type analysis of 0.09 nM; Cold exposed, B_{max} 171 fmol/mg protein, K_d=0.12 nM (CMC=0.987), nH=1.26 with a K_d by Hill-type analysis of 0.12.

the six remaining animals were kept for 16 hr in a cold room (5°C) prior to sacrifice. One of the cold exposed rodents entered hibernation, and it was excluded from the data here reported. The rodents were swiftly beheaded with a scissors to avoid the decapitation stress induced by a guillotine, and their brains quickly removed and frozen on dry ice. The neocortex was dissected and white matter, blood clots and pia carefully removed. Equivalent samples of the frontal cerebral cortex were dissected out from every animal and destined for monoamine (MA) assays. For comparison purposes of the MA determinations, a series of assays were also performed on 12 adult male Sprague-Dawley rats (250–300 g) which were sacrificed by decapitation and their brains removed and dissected as above described. The samples were homogenzed in 0.1 N cold $HClO_4$ in a glass homogenizer with a Teflon pestle, centrifuged (12,500 rpm for 45 min, 4°C) and the supernatants used for the assay of monoamines by high performance liquid chromatography (HPLC), following established procedures [18, 38, 41]. The pellets were dissolved overnight in 1 N NaOH, for protein assay [23]. The remaining neocortex was destined to receptor binding assays. All the dissection procedures were performed early in the morning and completed by 0900 hr.

For the HPLC assays 200 μl aliquots of the $HClO_4$ extracts diluted with mobile phase (1:1, v/v) and filtered through a 0.2 μm mesh were injected into a reversed-phase column (5 μm particles, 250×4.6 mm Biophase ODS; Bioanalytical Systems, BAS; West Lafayette, IN). The mobile phase was made of 0.15 M monochloroacetic acid, containing 0.75–0.80 g/l of ethylenediaminotetraacetic acid (EDTA; Sigma Chemical Co., St. Louis, MO), 200–300 mg/l of octyl sodium sulphate (OSS; Kodak, Rochester, NY) and 10% (v/v) methanol, adjusted to pH 3.35 with 1 N OHNa and degassed under vacuum prior to use. The column effluent was detected at +850 mV using a glassy carbon against the indifferent Ag/AgCl electrode. By varying the rate of flow (0.7 to 1.2 ml/min), the amount of OSS and the temperature (32–36°C) of the column (temperature controller LC22A; BAS) separation of the compounds (Fig. 1) 4-hydroxy-3-methoxy-phenylglycol (MHPG), noradrenaline (NA), adrenaline (AD), 3,4-dihydroxyphenylacetic acid (DOPAC), 5-hydroxyindole-3-acetic acid (HIAA), 3-hydroxytyramine (DA, dopamine), 4-hydroxy-3-methoxy-phenylacetic acid (HVA, homovanillic acid), 5-hydroxytryptamine (5-HT, serotonin) and 3-methoxytyranine (3-MT) could be obtained within 30–60 min [19, 21, 38].

For the receptor binding assays the remaining neocortex was homogenized (Polytron, 15 sec) in 40 volumes (w/v) of cold sodium/potassium phosphate [27] buffer (50 mM, pH 7.4), centrifuged (40,000 × g, 15 min at 4°C), washed

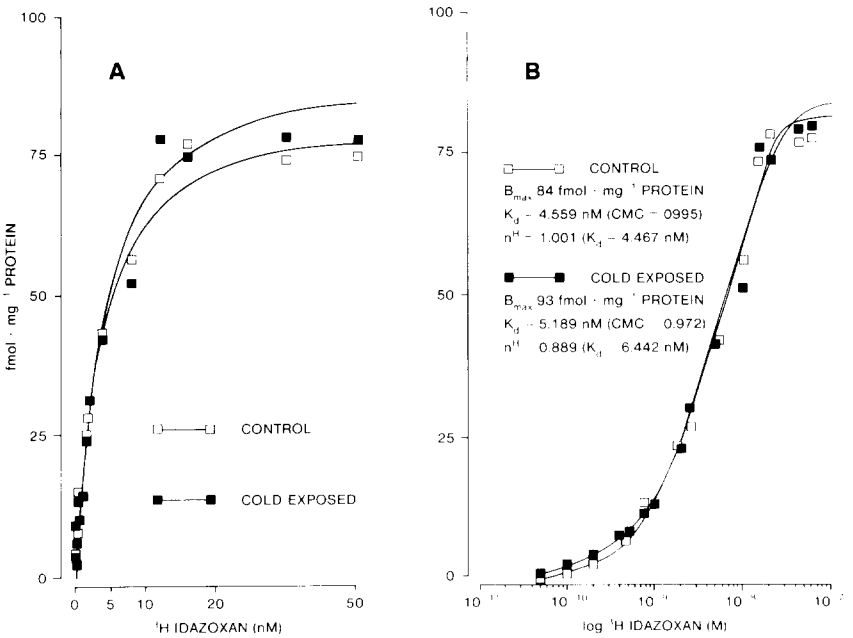

FIG. 3. Saturation curves of [^{3}H]idazoxan ([^{3}H]IDA; α_2-sites) binding to membrane preparations from the cerebral cortex of *Jaculus orientalis*, performed with 15 concentrations (0.025–50 nM). Data points represent means of duplicate determinations from a representative experiment. (A) The saturation curves represent the amounts specifically bound (in fmol/mg protein) as a function of ligand concentration in a membrane preparation from a control animal (□) or after an acute cold exposure (16 hr at 5°C) (■). (B) Computer modelling of the saturation curves, using FIT [1,26] for a model with a single binding affinity state for [^{3}H]IDA. The coefficients of multiple correlation (CMC) were never below 0.95, and attempts to fit these binding data to a two state model did not improve the fit. The values were: Control, B_{max}=84 fmol/mg protein, K_d=4.56 nM (CMC=0.995), Hill coefficient (nH) of 1.10 and a K_d by Hill-type analysis of 4.47 nM; Cold exposed, B_{max} 93 fmol/mg protein, K_d=5.20 nM (CMC=0.972), nH=0.89 with a K_d by Hill-type analysis of 6.44 nM.

twice, and after the final centrifugation, resuspended in cold incubation buffer. The membrane preparations were added in 140–180 μl aliquots to tubes already containing 10–20 μl of cold buffer, with or without phentolamine. The binding experiments were performed with [^{3}H]prazosin (prazosin, [furoyl-5-^{3}H]; [^{3}H]PRZ; Amersham; specific activity 11.3 Ci/mmol) for the α_1-sites [2, 4, 16, 25, 33, 34] and with [^{3}H]idazoxan ([^{3}H]RX-781094; (1,4-[6,7-^{3}H] benzodioxan-2-yl)-2-imidazoline; Amersham; specific activity 57 Ci/mmol) for the α_2-sites [5, 13, 14, 27, 36, 37]. The saturation experiments were performed at equilibrium [25,27] with 10–12 dilutions of [^{3}H]PRZ and 15 of [^{3}H]IDA (final volume of 300 μl). Every curve was done in duplicate (two tubes for total binding and two tubes for non-specific counts). After incubating with the radioactive ligands at 25°C for 45 min, specific binding (defined as total binding minus the non-specific counts determined in the presence of 10–30 μM phentolamine) was measured by rapid filtration (<5 sec) over 24 mm Whatman GF/C glass fiber filters and two washes (<10 sec) with 5 ml cold buffer. For the competition experiments, 160 μl aliquots of membrane preparations from rat cerebral cortex were pipetted into tubes containing (in 40 μl) the adrenergic drugs (20 concentrations, from 10^{-12} to 10^{-4} M) and after 30 min the ligand was pipetted (200 μl) at fixed concentrations of 0.1 nM for [^{3}H]PRZ and of 1.0 nM for [^{3}H]IDA [37]. The tubes were then incubated and the binding assay proceeded as above described. In subsequent experiments, the effects of thiol reagents were investigated as follows: 180 μl aliquots of membrane preparation were added to tubes already containing 20 μl of buffer (with and without phentolamine) and then increasing concentrations of the reagents DL-dithiothreitol (DL-DTT), N-ethylmaleimide (NEM) and 5,5′-dithiobis-(2-nitrobenzoic acid) were pipetted in 100 μl aliquots. The tubes were incubated for 30 min at 25°C and placed on crushed ice. The radioactive ligands were then added to give a final concentration of 0.25 nM for [^{3}H]PRZ and of 5 nM for [^{3}H]IDA (final volume 400 μl). The tubes were then incubated for 45 min, and the assay proceeded as above described. Radioactivity was counted by liquid scintillation (Econofluor, New England Nuclear) in a LKB Rackbetta II counter (efficiency 45–65%). Protein concentrations were assayed [23] in 100 μl aliquots of the membrane preparations and were usually between 1.5–2.0 mg/ml. The analyses of the saturation curves were performed by the methods of Hill [15] and using the iterative model FIT [1,26]. This last procedure gives a more precise estimate of both B_{max} and the K_d than the conventional Scatchard analysis [20, 36, 37, 45]. Competition curves and experiments with disulfide and sulfhydryl reactives were analysed with the iterative model INHIBITION [1].

TABLE 1

SPECIFIC [^{3}H]PRAZOSIN AND [^{3}H]IDAZOXAN BINDING TO MEMBRANE PREPARATIONS OF THE CEREBRAL CORTEX OF SPRAGUE-DAWLEY (SD) RATS AND OF THE RODENT JACULUS ORIENTALIS (JO) IN CONTROLS AND ANIMALS EXPOSED TO COLD (5° C) FOR 16 HOURS

	Iterative analysis		Hill-type analysis	
	B_{max} (fmol/mg p)	K_d (nM)	nH	K_d (nM)
[^{3}H] Prazosin				
SD rats (n=6)	182 ± 9	0.16 ± 0.01	1.31 ± 0.04	0.15 ± 0.01
JO rodents				
Controls (n=5)	159 ± 6	0.10 ± 0.005	1.11 ± 0.05	0.10 ± 0.003
Cold exposed (n=5)	164 ± 12	0.16 ± 0.02*	1.08 ± 0.05	0.17 ± 0.02*
[^{3}H] Idazoxan				
SD rats (n=6)	155 ± 10	1.71 ± 0.22	0.99 ± 0.05	1.72 ± 0.28
JO rodents				
Controls (n=5)	84 ± 12	4.25 ± 0.69	1.06 ± 0.04	4.14 ± 0.69
Cold exposed (n=5)	89 ± 10	4.48 ± 1.06	0.99 ± 0.03	4.59 ± 1.04

The values represent the means ± S.E.M. of five (or six) separate experiments for each group performed with 10–12 membrane preparations, and in duplicate.

The density of binding sites (B_{max}; maximum binding capacity) in fentomoles/mg protein and the dissociation constant (K_d 25° C) in nanomoles, were calculated by the iterative analysis FIT [1, 26]. The Hill-type plots [15] were the least-square linear regression of the log [Bound/(B_{max}-Bound] versus the log of radioligand concentration.

Statistical significance between the JO controls and the exposed was estimated by unpaired Student's t-test; *$p<0.05$.

RESULTS

A series of saturation curves were performed in duplicate with 9–15 concentrations, ranging from 0.01 to 4 nM for [^{3}H]PRZ, and from 0.05 to 50 nM for [^{3}H]IDA. The radioligands were added in 150 μl aliquots to tubes already containing 140 μl of membrane preparation and 10 μl of buffer (with or without phentolamine). The incubations then proceeded as described in the Method section. These experiments (Figs. 2 and 3) allowed us to determine both the receptor densities (B_{max}) as well as the dissociation constants (K_d), and the values obtained for rat cerebral cortex and from the cortex of control and cold exposed rodents, and for each ligand are given in Table 1. For the α_1-adrenoceptors the saturation curves with [^{3}H]PRZ (Fig. 2A) revealed a single class of binding sites (Fig. 2B). In control rodents, the mean B_{max} was of 159 fmol/mg protein, and did not differ from that obtained from membrane preparations of cold exposed animals (B_{max}=164 fmol/mg protein). However, cold exposure significantly decreased the affinity (controls, K_d=99 pM; cold exposed, K_d=161 pM). The Hill coefficients were about 1, thus further supporting a single class of receptors. In addition, we could verify by this procedure the decrease in affinity (controls, K_d=102 pM; cold exposed, K_d=169 pM). In the case of the α_2-adrenoceptors labelled by [^{3}H]IDA, the saturation curves (Fig. 3A) were also found to be compatible with a single class of binding sites (Fig. 3B), with no changes either in receptor density or in the dissociation constants after the acute cold exposure. The Hill-type of analysis supported the existence of a single class of [^{3}H]IDA binding sites, and with no changes in the affinities. Throughout this study the selectivity of [^{3}H]PRZ and [^{3}H]IDA binding was examined in competition experiments with membrane preparations from rat cerebral cortex (Table 2).

In order to determine the protein nature of the binding sites, membrane preparations of control rodents were pretreated with reactives known to modify disulfide and sulfhydryl groups, i.e., DL-DTT, NEM and DTNB. For each reagent the tissues were incubated at 25°C for 30 min, and after this preincubation ended, either [^{3}H]PRZ or [^{3}H]IDA were added and the binding experiments proceeded for another 45 min, as described in the Method section. Twelve concentrations of reagents were used, from 0.001 to 100 mM, and all three reactives markedly decreased specific [^{3}H]PRZ and [^{3}H]IDA binding with negligible changes in the non-specific counts. These results indicate that specific binding is to protein receptors, while the non-specific counts are due to partition or to adsorption of the ligands to the lipid moiety of the membrane preparations, although a negligible fixation to proteins not containing -SS- or -SH groups cannot be entirely ruled out. The reductions in specific binding were dose-dependent (Fig. 4), and outline a particular role for these bonds when adrenergic antagonists are used in the biochemical assay of α-adrenoceptors.

The endogenous MA and some of their major metabolites in the frontal cortex were determined by HPLC with electrochemical detection. Samples obtained from equivalent cortical regions of Sprague Dawley rats were also assayed for comparison purposes (Table 3). The major catecholamine CA from a biochemical aspect was NA, i.e., in the control rodents it amounted to 3.4 ng/mg protein, and remained unchanged in the samples from the cold exposed animals. The other two CA (i.e., and AD) were also detected and measured, but there were no significant differences between the

TABLE 2

ANTAGONIST AND AGONIST INHIBITION OF [^{3}H] PRAZOSIN AND [^{3}H] IDAZOXAN BINDING TO MEMBRANE FROM RAT CEREBRAL CORTEX

	[^{3}H] Prazosin		[^{3}H] Idazoxan	
	Ic_{50} (nM)	K_i (nM)	Ic_{50} (nM)	K_i (nM)
Adrenergic Antagonists				
Prazosin	0.22	0.13	744	469
Phentolamine	66	28	28	18
Corynanthine	527	323	16,013	10,104
Pyrextramine	566	346	133	84
Yohimbine	802	491	43	27
Piperoxan	927	568	47	29
Benextramine	1,000	612	583	368
Idazoxan	1,092	669	5	3
(−) Propranolol	29,808	18,254	7,846	4,950
Adrenergic Agonists				
Oxymetazoline	126	77	677	427
Clonidine	641	392	127	80
(−) Noradrenaline	1,598	978	6,916	4,364
(−) Adrenaline	4,670	2,860	4,502	2,841
Phenylephrine	9,647	5,908	4,160	2,625
(−) Isoproterenol	119,882	73,416	>125,000	n.d.

The inhibition of specific alpha-adrenoceptor binding was determined by incubating the membrane preparations from rat cerebral cortex with 0.1 nM of [^{3}H] prazosin or 1.0 nM of [^{3}H] Idazoxan in the presence of the corresponding unlabelled drug. Non-specific binding was defined by the counts obtained in the presence of 10 μm phentolamine, or after thermal (80° C for 15 min) denaturation of the receptor protein.

The concentrations of unlabelled ligand that caused 50 percent inhibition of specific binding (Ic_{50}) were calculated by the iterative analysis inhibition [1, 26, 36] and then used to estimate the inhibition-dissociation constants (K_i) by the method of Cheng and Prusoff [6].

two groups (Table 3). There were no significant changes documented for the endogenous levels in MHPEG, DOPAC and HVA. The 5-HT content in the control rodents was of 3.4 ng/mg protein, and after cold exposure it was significantly reduced to 0.5 ng/mg protein. Interestingly, the major metabolite of serotonin, i.e., 5-HIAA remained unchanged, giving an increased 5-HIAA/5-HT ratio in the cold exposed rodents, suggestive of an increased turnover rate or an accrued utilization rate. The DA metabolite 3-methoxytyramine (3-MT) was not detected in the samples of *J. orientalis* but measurable levels were found in rat cortex, possibly due to the decapitation procedure.

DISCUSSION

The monoamine innervations of the cerebral cortex have been fairly well characterized for the mammalian CNS, in studies performed mainly in the rat brain, using histofluorescence, microchemical, radioautographic and immunocytochemical methodologies (for a review see [32]). Biochemical determinations of endogenous MA levels performed in restricted CNS areas provide the initial elements to map out the innervation in localized brain regions [18, 29–31]. In the control rodents, the most abundant CA measured was NA, with absolute values similar to those determined in equivalent regions of the rat brain either with radioenzymatic [30, 31, 39] or with HPLC [38] methodologies, and represented 62% of the total catecholamine contents (TCC) (Table 3). The endogenous content in DA was also in the same range as the levels documented for the rat neocortex [30], amounting to about 12% of TCC. Since two of the metabolites (DOPAC and HVA) were also measured in relatively great amounts in this cortex, it can be concluded that the DA levels represent the neurotransmitter contained in a dopaminergic pathway, in addition to the amounts normally expected to be present as a precursor in the NA fibers. The values of AD in this series of assays were higher than those reported [30, 31, 38] or measured for the rat brain, i.e., they amounted to more than 1 ng/mg protein, and represented about 25% of TCC. This higher content in AD was an unexpected finding, and could be attributed to the presence in this species of a specific adrenergic projection to the neocortex. Possible contaminants of the adrenaline were considered, i.e., 5-hydroxy-L-tryptophan (5-HTP); normetanephrine (NMN) and metanephrine (NTM), but were excluded since their retention times differed from that of AD with the present HPLC conditions (Fig. 1A). An immunocytochemical examination of the distribution in this rodent of phenylethanolamine-N-methyltransferase (i.e., PNMT, the enzyme that synthesizes adrenaline from noradrenaline) and/or a mass spectrometric analysis is required to settle this issue. The indoleamine 5-HT and its metabolite 5-HIAA were found for the control animals in amounts simi-

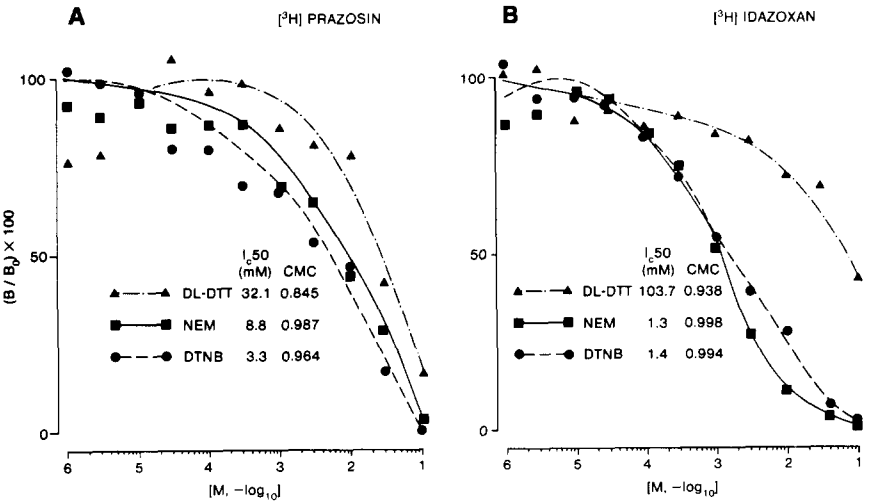

FIG. 4. Effect of sulfhydryl and disulfide reactives on [^{3}H]prazosin ([^{3}H]PRZ; α_1-sites) and [^{3}H]idazoxan ([^{3}H]IDA; α_2-sites) binding to membrane preparations from the cerebral cortex of *Jaculus orientalis*. Immediately before the incubation with fixed concentrations of the radioligands, membrane preparations were pretreated for 30 min at 28°C with increasing concentrations of the reactives DL-dithiothreitol (DL-DTT; ▲), N-ethylmaleimide (NEM; ■) and 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB; ●). The binding assays then proceeded as described in the Method section. Data points are the means of determinations performed in triplicate from representative membrane preparations of control animals. The lines are theoretical and were drawn by computer modelling, using INHIBITION [1,26] to estimate the concentrations of reactives that decrease specific binding by 50% (Ic50). The inhibition constants (K_i) were calculated by the method of Cheng and Prussoff [6,7]. (A) Specific binding of [^{3}H]PRZ (0.25 nM) was affected by oxidation (DTNB; Ic50=3.3 mM, CMC=0.964; K_i=0.9 mM), alkylation (NEM; Ic50=8.8 mM, CMC=0.987; K_i=2.5 mM) and reduction (DL-DTT; Ic50=32.1 mM, CMC=0.845; K_i=9.1 mM). (B) Specific binding of [^{3}H]IDA (5.0 nM) was very sensitive to NEM (Ic50=1.3 mM, CMC=0.998; K_i=0.61 mM) and DTNB (Ic50=1.4 mM, CMC=0.994; K_i=0.62 mM), and less affected by DL-DTT (Ic50=103.7 mM, CMC=0.938; K_i=47.7 mM).

lar to those found for the rat neocortex using radioenzymatic [29–31, 39] and HPLC methods [38] (Table 3). After the acute cold exposure there was an important reduction in 5-HT contents, and since 5-HIAA levels remained constant the 5-HIAA/5-HT ratio increased, suggesting an accrued utilization or an increased turnover rate. Interestingly, when 5-HT synthesis is inhibited by p-chlorophenylalanine both 5-HT and 5-HIAA levels are decreased in the cerebral cortex [38]. The significance of the decrease in 5-HT with no changes in 5-HIAA after an acute cold exposure remains speculative at present, essentially because the cerebral cortex may not be the CNS region where the major temperature adaptations and/or regulations take place.

The adrenergic receptors of the α_1- and α_2-subtype [42–44] were assayed using two antagonists that have the pharmacological profiles characteristic of these sites [11, 13, 14, 24] and are selective in *in vitro* competition studies [3, 9, 10, 16, 17, 36, 37].

The α-adrenoceptors were determined in saturation curves, analysed with the iterative model FIT [1,26] for one single class of binding sites. Attempts to fit the data to two-site models did not improve it, as judged by the coefficients of multiple correlation. The Hill-type plots [15,45] showed coefficients of about one, for both [^{3}H]PRZ as well as for [^{3}H]IDA, thus supporting the single-site models for both α_1- and α_2-binding sites. This analysis was also used to calculate the dissociation constant, i.e., at the ligand concentration at which half the available receptors are occupied by the ligand, the log [Bound/B_{max} −Bound)] equals 0, so that the antilog of the x-intercept in the K_d [34, 36, 45].

The use of chemical reactives that modify -SS- and -SH groups can rule out the participation of lipids in receptor assays, and determine some of the properties of the binding sites. Although the three reactives here investigated decreased specific [^{3}H]PRZ and [^{3}H]IDA binding, there were quantitative differences in the effects induced by NEM (alkylation) and DTNB (oxidation), as judged by the Ic50 (i.e., the concentration of reactive that decreased by 50% specific binding) [6,7]. These two agents were quite potent on the [^{3}H]IDA binding site, pointing out to an important role for dithiols and reduced monothiols in the recognition site (Fig. 3). The reducing agent DL-DTT [8], although less potent, also decreased specific binding. The α_1-site labelled by [^{3}H]PRZ was affected by oxidation, alkylation and reduction as well, but the Ic50 values for NEM and DTNB were higher and that of DL-DTT lower than the ones estimated for the α_2-site labelled by [^{3}H]IDA. One may conclude that the binding of [^{3}H]PRZ and [^{3}H]IDA was to proteins, since lipids are devoid of -SS- and -SH groups [28, 35, 36].

After an acute cold exposure, there were no changes in the B_{max} of α_1- or α_2-receptor sites, nor in the K_d for [^{3}H]IDA. [^{3}H]PRZ binding however, was decreased after cold exposure, i.e., there was a slight but significant increase in the K_d values (Fig. 1B and Table 1). Since the NA con-

TABLE 3

ENDOGENOUS MONOAMINE CONTENT IN THE FRONTAL CORTEX OF SPRAGUE-DAWLEY (SD) RATS AND OF THE RODENT JACULUS ORIENTALIS (JO) IN CONTROLS AND ANIMALS EXPOSED TO COLD (5° C) FOR 16 HOURS

	SD rats		JD rodents		
			Controls	Cold exposed	Change %
	REA (n=12)	HPLC (n=6)	HPLC (n=5)	HPLC (n=5)	
NA	2.8 ± 0.3	3.7 ± 0.2	3.4 ± 1.0	3.5 ± 0.5	+3.6
AD	0.2 ± 0.006	0.2 ± 0.04	1.4 ± 0.2	1.8 ± 0.6	+34.6
MHPG	n.d.	0.5 ± 0.1	1.4 ± 0.1	1.2 ± 0.2	−10.1
DA	0.6 ± 0.1	0.5 ± 0.1	0.7 ± 0.2	0.2 ± 0.4	−14.6
DOPAC	n.d.	0.7 ± 0.1	1.1 ± 0.3	1.1 ± 0.3	+0.1
HVA	n.d.	1.3 ± 0.1	2.8 ± 1.0	1.0 ± 0.4	−63.4
3-MT	n.d.	5.6 ± 0.2	n.d.	n.d.	—
5-HT	3.0 ± 0.04	3.5 ± 0.3	3.4 ± 0.8	0.5 ± 0.2	−85.1*
5-HIAA	n.d.	4.5 ± 0.4	3.2 ± 0.6	3.2 ± 0.3	+0.2

The results are expressed as the mean content in ng/mg protein ± SEM. The data from the radioenzymatic assays (REA) is from [31].

Statistical significance between the control and the cold exposed JO rodents was determined by unpaired Student's *t*-test; $*p<0.05$.

NA=noradrenaline; AD=adrenaline; MHPG=4-hydroxy-3-methoxyphenylglycol; DA=dopamine; DOPAC=3,4-dihydroxy-phenylacetic acid; HVA=homovanillic acid; 3-MT=3-methoxytyramine; 5-HT=serotonin; 5-HIAA=5-hydroxyindole-3-acetic acid.

n.d.=not determined.

tent measured by HPLC was unchanged in the cold exposed animals, we did not expect major changes in the binding parameters. In fact, a decrease in NA content could have been accompanied by an increase in the receptor numbers of "up-regulation," as has been documented in deafferentation experiments [24, 33, 34]. This was not the case in this study, in which the endogenous cortical CA content and both α_1- and α_2-receptor densities remained unchanged. Therefore, the relatively small modifications in the K_d for [^{3}H]PRZ cannot be explained solely in relation to NA content. Interactions between the NA and 5-HT systems in the cerebral cortex [40] have been already documented in correlative biochemical and electrophysiological studies [12,38] but the exact nature of some of these regulations will require an analysis of the possible modifications of CA and 5-HT receptors in relation to endogenous MA levels.

The present study is the first biochemical report on the CA and 5-HT innervations, and some of the binding properties of α-adrenergic receptors in the cerebral cortex of the rodent *J. orientalis*. Because this animal hibernates and the MA are implied in the behavioural and metabolic modifications required for this state [22], it constitutes an interesting model to further examine overall and general physiological adaptative changes in relation to neurotransmitter content and the properties of specific receptor proteins.

ACKNOWLEDGEMENTS

This work was supported by grants from the Medical Research Council of Canada (MT-6967) and the Quebec Heart Foundation. Dr. T. A. Reader is a member of Equipe de Recherche sur les Neurotransmetteurs du Cerveau (Fonds de la Recherche en Santé du Québec). Phentolamine was a generous gift from Ciba-Pharmaceuticals, Ontario. The help of Miss Hélène Dussault in typing the manuscript and the graphic work of Mrs. Giovanni Battista-Filosi and Daniel Cyr are greatly appreciated.

REFERENCES

1. Barlow, R. B. *Biodata Handling With Microcomputers*. Amsterdam: Elsevier Science Publishers, 1983.
2. Brière, R., A. L. Sherwin, Y. Robitaille, A. Olivier, L. F. Quesney and T. A. Reader. α-1 Adrenoceptors are decreased in human epileptic foci. *Ann Neurol* **19:** 26–30, 1986.
3. Bylund, B. D. and D. C. U'Prichard. Characterization of α1- and α2-adrenergic receptors. *Int Rev Neurobiol* **24:** 343–431, 1983.
4. Cavero, I. and A. G. Roach. The pharmacology of prazosin, a novel anti-hypertensive agent. *Life Sci* **27:** 1525–1540, 1980.

5. Chapleo, C. B., J. C. Doxey, P. L. Myers and A. G. Roach. RX 781094, a new potent, selective antagonist at α2-adrenoceptors. *Br J Pharmacol* **74:** 942p, 1981.
6. Cheng, Y. C. and W. H. Prussoff. Relationship between the inhibition constant (Ki) and the concentration of inhibitor which causes 50 per cent inhibition (150) of an enzymatic reaction. *Biochem Pharmacol* **22:** 3099–3108, 1973.
7. Chou, T.-C. Relationships between inhibition constants and fractional inhibition in enzyme-catalized reactions with different numbers of reactants, different reaction mechanisms, and different types and mechanisms of inhibition. *Mol Pharmacol* **10:** 235–247, 1974.
8. Cleland, W. W. Dithiothreitol, a new protective reagent for SH groups. *Biochemistry* **3:** 480–482, 1964.
9. Dausse, J. P., P. Guicheney, L. Diop and P. Meyer. Caractérisation biochimique des récepteurs α-adrénergiques centraux. *J Pharmacol* **15:** Suppl I, 23–33, 1984.
10. Diop, L., J.-P. Dausse and P. Meyer. Specific binding of [^{3}H]rauwolscine to α_2-adrenoceptors in rat cerebral cortex: comparison between crude and synaptosomal plasma membranes. *J Neurochem* **41:** 710–715, 1983.
11. Drew, G. M. Effects of α-adrenoceptor agonists and antagonists on pre- and postsynaptically located α-adrenoceptors. *Eur J Pharmacol* **36:** 313–320, 1976.
12. Ferron, A., L. Descarries and T. A. Reader. Altered responsiveness to biogenic amines in rat cerebral cortex after serotonin denervation or depletion. *Brain Res* **231:** 93–108, 1982.
13. Freedman, J. E. and G. K. Aghajanian. Idazoxan (RX 781094) selectively antagonizes α_2-adrenoceptors on rat central neurons. *Eur J Pharmacol* **105:** 265–272, 1984.
14. Gadie, B., A. C. Lane, P. S. McCarthy, I. F. Tulloch and D. S. Walter. 2-Alkyl analogues of idazoxan (RX781094) with enhanced antagonist potency and selectivity at central α_2-adrenoceptors in the rat. *Br J Pharmacol* **83:** 707–712, 1984.
15. Hill, A. V. The possible effects of the aggregation of the molecules of hemoglobin on its dissociation curves. *J Physiol (Lond)* **40:** iv–vii, 1910.
16. Hornung, R., P. Presek and H. Glossmann. Alpha adrenoceptors in rat brain: direct identification with prazosin. *Naunyn Schmiedebergs Arch Pharmacol* **308:** 223–230, 1979.
17. Howlett, D. R., P. Taylor and D. S. Walter. α-Adrenoceptor selectivity studies with RX 781094 using radioligand binding to cerebral cortex. *Br J Pharmacol* **76:** 294p, 1982.
18. Keller, R., A. Oke, I. Mefford and R. N. Adams. Liquid chromatographic analysis of catecholamines. Routine assay for regional brain mapping. *Life Sci* **19:** 995–1004, 1976.
19. Kempf, E. and P. Mandel. Reverse-phase high-performance liquid chromatographic separation and electrochemical detection of norepinephrine, dopamine, serotonin and related major metabolites. *Anal Biochem* **112:** 223–231, 1981.
20. Klotz, I. M. Numbers of receptor sites from Scatchard graphs: facts and fantasies. *Science* **217:** 1247–1249, 1982.
21. Kontur, P., R. Dawson and A. Monjan. Manipulation of mobile phase parameters for the HPLC separation of endogenous monoamines in rat brain tissue. *J Neurosci Methods* **11:** 5–18, 1984.
22. Lakdhar-Ghazal, N., A. Calas and W. B. Bengelloun. Effects of centrally applied monoamines on energy metabolism of *Jaculus orientalis*. In: *Homeostasis and Thermal Stress: Experimental and Therapeutic Advances*, edited by K. E. Cooper, P. Lumar, E. Schonbaum and W. L. Veale. Basel: Karger, 1985, pp. 129–132.
23. Lowry, O. H., N. J. Rosebrough, A. L. Farr and R. J. Randall. Protein measurements with Folin phenol reagent. *J Biol Chem* **193:** 265–275, 1951.
24. Menkes, D. B., D. W. Gallager, J. F. Reinhard, Jr. and G. K. Aghajanian. α_1-Adrenoceptor denervation supersensitivity in brain: physiological and receptor binding studies. *Brain Res* **272:** 1–9, 1983.
25. Miach, P. J., J.-P. Dausse, A. Cardot and P. Meyer. ^{3}H-Prazosin binds specifically to α_1-adrenoceptors in rat brain. *Naunyn Schmiedebergs Arch Pharmacol* **312:** 23–26, 1980.
26. Parker, R. B. and D. R. Waud. Pharmacological estimation of drug-receptor dissociation constants. Statistical evaluation. I. Agonists. *J Pharmacol Exp Ther* **177:** 1–12, 1971.
27. Pimoule, C., B. Scatton and S. Z. Langer. RX 781094: a new antagonist ligand labels α_2-adrenoceptors in the rat brain cortex. *Eur J Pharmacol* **95:** 79–85, 1983.
28. Quennedy, M.-C., J. Bockaert and B. Ruout. Direct and indirect effects of sulfhydryl blocking agents on agonist and antagonist binding to central α_1- and α_2-adrenoceptors. *Biochem Pharmacol* **33:** 3923–3928, 1984.
29. Reader, T. A. Serotonin distribution in rat cerebral cortex; Radioenzymatic assays with thin-layer chromatography. *Brain Res Bull* **5:** 609–613, 1980.
30. Reader, T. A. Distribution of catecholamines and serotonin in the rat cerebral cortex: Absolute levels and relative proportions. *J Neural Transm* **50:** 13–27, 1981.
31. Reader, T. A. Catecholamines and serotonin in rat frontal cortex after PCPA and 6-OHDA: Absolute amounts and ratios. *Brain Res Bull* **8:** 527–534, 1982.
32. Reader, T. A. The role of catecholamines in neuronal excitability. In: *Basic Mechanisms of Neuronal Hyperexcitability*, edited by H. H. Jasper and N. M. VanGelder. New York: Alan R. Liss, Inc., 1983, pp. 281–321.
33. Reader, T. A. and R. Brière. Selective noradrenergic denervation and ^{3}H-prazosin binding sites in rat neocortex. *Brain Res Bull* **10:** 155–158, 1983.
34. Reader, T. A. and R. Brière. Long-term unilateral noradrenergic denervation: Monoamine content and ^{3}H-prazosin binding sites in rat neocortex. *Brain Res Bull* **11:** 687–692, 1983.
35. Reader, T. A. and R. Brière. Evidence for the participation of disulfide and sulfhydryl groups in the specific binding of [^{3}H]prazosin in cerebral cortex. *Neurochem Res* **10:** 107–122, 1985.
36. Reader, T. A., R. Brière and L. Grondin. Alpha-1 and alpha-2 adrenoceptor binding in cerebral cortex: role of disulfide and sulfhydryl groups. *Neurochem Res* **11:** 9–27, 1986.
37. Reader, T. A., R. Brière and L. Grondin. Alpha-1 and alpha-2 adrenoceptor binding in cerebral cortex: competition studies with [^{3}H] prazosin and [^{3}H] idazoxan. *J Neural Transm*, in press, 1986.
38. Reader, T. A., R. Brière, L. Grondin and A. Ferron. Effects of p-chlorophenylalanine on cortical monoamines and on the activity of noradrenergic neurons. *Neurochem Res* **11:** 1025–1035, 1986.
39. Reader, T. A. and P. Gauthier. Catecholamines and serotonin in the rat central nervous system after 6-OHDA, 5-7-DHT and p-CPA. *J Neural Transm* **59:** 207–227, 1984.
40. Reader, T. A. and H. H. Jasper. Interactions between monoamines and other transmitters in cerebral cortex. In: *Monoamine Innervation of Cerebral Cortex*, edited by L. Descarries, T. A. Reader and H. H. Jasper. New York: Alan R. Liss, Inc., 1984, pp. 195–225.
41. Saller, C. F. and A. I. Salama. Rapid automated analysis of biogenic amines and their metabolites using reversed-phase high-performance liquid chromatography with electrochemical detection. *J Chromatogr* **309:** 287–298, 1984.
42. Starke, K. α-Adrenoceptor subclassification. *Rev Physiol Biochem Pharmacol* **88:** 199–236, 1981.
43. Summers, R. J., B. Jarrott and W. J. Louis. Selectivity of a series of clonidine-like drugs for α_1 and α_2 adrenoceptors in rat brain. *Neurosci Lett* **20:** 347–350, 1980.
44. Wood, C. L., C. D. Arnett, W. R. Clarke, B. S. Tsai and R. J. Lefkowitz. Subclassification of alpha-adrenergic receptors by direct binding studies. *Biochem Pharmacol* **28:** 1277–1282, 1979.
45. Zitwin, J. A. and D. R. Waud. How to analyse binding enzyme and uptake data: the simplest case, a single phase. *Life Sci* **30:** 1407–1422, 1982.