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INCREASED CENTRAL α-2 ADRENERGIC RECEPTORS MEASURED WITH [^{3}H]YOHIMBINE IN THE PRESENCE OF SODIUM ION AND GUANYLNUCLEOTIDES

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The concentration of cerebral cortical α-2 adrenergic receptors measured using the antagonist ligand [^{3}H]yohimbine was increased by the addition of sodium chloride or guanylnucleotides. The effects of the two factors together were additive, the total increase in receptor concentration being from 55 ± 5.8 to 106 ± 5.8 fmol/mg protein in the presence of both 200 mM NaCl and 10^{-5}M guanylyl-5'-imidodiphosphate. Similar increases were produced by sodium acetate and GTP. Neither sodium ion nor guanylnucleotides caused any change in the affinity of the receptors for [^{3}H]yohimbine but caused an additive decrease in the average affinity of agonists. Similar effects of sodium ion and guanylnucleotides on receptor concentration were observed in hypothalamus but not kidney.

INTRODUCTION

Central α-adrenergic receptors were first measured with the non-selective α-antagonist ligand [^{3}H]dihydroergocrytptine ([^{3}H]DHE)[1]. Biphasic displacement of bound [^{3}H]DHE by the α-1 and α-2 selective compounds prazosin and yohimbine indicated the presence of both receptor subclasses in the central nervous system [2]. More recent studies have used selective radioligands such as the antagonists [^{3}H]WB4101 [3] or [^{3}H]prazosin [4] for α-1 receptors and the agonist ligands [^{3}H]clonidine [5] or [^{3}H]para-amino-clonidine [6] for α-2 receptors. However the binding of agonist compounds to both central andperipheral α-2 receptors is complex. Agonists but not antagonists bind to sites of more than one affinity. Moreover, agonist but not antagonist affinity is modulated by cations and guanylnucleotides [5,7,8].

The introduction of [^{3}H]yohimbine has provided a selective antagonist ligand for α-2 receptors. In membranes prepared from peripheral tissues the binding of [^{3}H]yohimbine is unaffected by sodium ion and guanylnucleotides in contrast to the previously used agonist

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.igands [9,10,11]. In the course of experiments attempting to characterize [^{3}H]yohimbine)inding to membranes from the central nervous system, it was found that [^{3}H]yohimbine)inding was increased in the presence of sodium ion and GPP(NH)P. This paper reports ;tudies investigating this observation.

IETHODS

l. Materials

Yohimbine [methyl ^{3}H] specific activity 75-90 Ci/mmol was obtained from Searle Nucleonics.

The following compounds were obtained from the Sigma Chemical Company, St. Louis - 'ohimbine hydrochloride, tris (hydroxymethyl) aminomethane, l-epinephrine bitartrate, .-arterenol bitartrate, l-isoproterenol HCl, guanosine-5'-triphosphate (GTP), guanylyl-5'-imidodiphosphate (GPP(NH)P), adenosine-5'-triphosphate (ATP), guanosine-5'-monophosphate (GMP). d-Epinephrine bitartrate was obtained from KEK Fine Chemicals. Prazosin was supplied by Pfizer and α-methylnorepinephrine by Sterling Pharmaceuticals.

2. Preparation of membranes.

Adult Sprague-Dawley rats (150-250g) were killed by decapitation. Cerebral cortex and hypothalamus were dissected [12]. Tissue was homogenized in 50 mM tris HCl pH 7.7, 10 mM $MgSO_4$, 0.25M sucrose, using a Potter-Elvejhem homogeniser. Homogenates were filtered through two layers of gauze and centrifuged at 30,000 g for 15 min. The pellets were washed three times with the above sucrose buffer. The final pellets were resuspended in 50 mM tris HCl, pH 7.7, 10 mM $MgSO_4$, 1 mM mercaptoethanol at a protein concentration of 3-6mg/ml. Protein concentration was measured by the method of Lowry et al. [13] using bovine serum albumin as standard. Kidney membranes were prepared as described previously [14].

3. [^{3}H]Yohimbine Binding

Incubations contained in a final volume of 200 μλ - [^{3}H]yohimbine (1-50 nM), 50 mM tris HCl pH 7.7, 10 mM $MgSO_4$, 1 mM mercaptoethanol, membranes 1-3 mg/ml protein and other additions as indicated. Incubation was at 25°C for 20 mins and was terminated by filtration through Whatman GF/C glass fibre filters. The filters were washed with 10 ml of ice cold buffer. Non-specific binding was defined as binding not displaceable by 10^{-4}M l-epinephrine. Non-specific binding represented 30% of total binding at 10 nM [^{3}H]yohimbine in brain membranes in the presence of sodium ion and guanylnucleotides. In the absence of these factors non-specific binding to central membranes was 50% of total at 10 nM [^{3}H]yohimbine (see Results) and 30% of total when renal membranes were used.

RESULTS

1. Effects of sodium ion and guanylnucleotides on [^{3}H]yohimbine binding to cerebral cortical membranes.

The addition of increasing concentrations of NaCl between 10 and 300 mM produced a concentration dependent increase in specific binding of [^{3}H]yohimbine to cerebral cortical membranes. No effect on non-specific binding was observed. GTP and its analogue GPP(NH)P (10^{-8}-10^{-4}M) also increased specific binding with no effect on non-specific binding (Figure 1). Scatchard analysis of binding in the presence of NaCl and

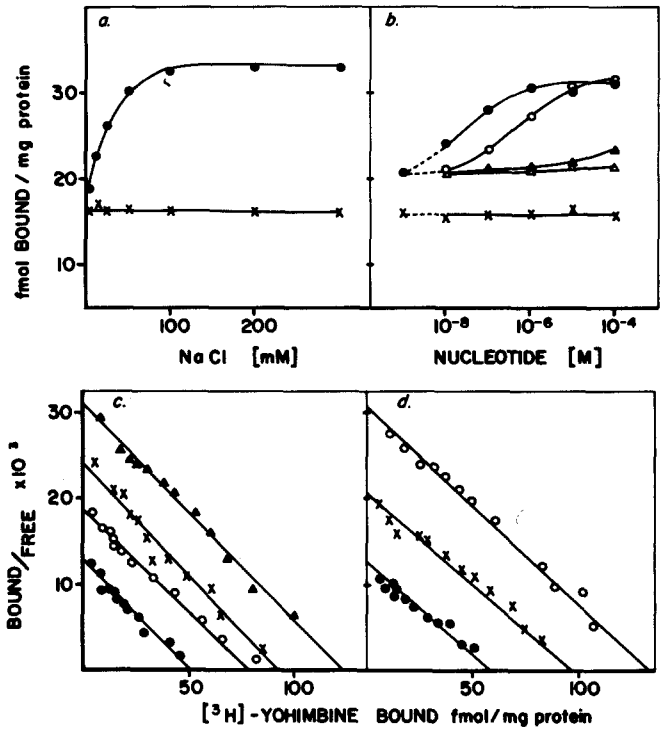

Figure 1: Effects of sodium chloride and guanylnucleotides on [^{3}H]yohimbine binding to cerebral cortical membranes.

a) Effect of NaCl on specific and non-specific binding at 10 nM [^{3}H]yohimbine.
●–● specific binding; X-X non-specific binding

b) Effect of nucleotides at 10 nM [^{3}H]yohimbine.
●–● guanylyl-5'-imidodiphosphate (specific binding)
X-X guanylyl-5'-imidodiphosphate (non-specific binding)
O-O GTP specific binding; Δ-Δ ATP specific binding
Δ-Δ GMP specific binding

c) & d) Scatchard analysis of [^{3}H]yohimbine binding
c) ●–● no additions
O-O 10^{-5}M guanylyl-5'-imidiodiphosphate
X-X 200 mM NaCl
▲–▲ 200 mM NaCl plus 10^{-5}M guanylyl-5'-imidiodiphosphate

d) ●–● 200 mM KCl
X-X 200 mM sodium acetate
O-O 200 mM sodium chloride plus 10^{-5}M GTP

guanylnucleotides indicated that the increase in binding was due to an increase in available sites and not to a change in affinity. The increases in sites produced by NaCl and guanylnucleotides were additive (Figure 1; Table 1).

2. Specificity of the NaCl-guanylnucleotide effect.

KCl at 200 mM was ineffective in increasing [^{3}H]yohimbine binding. However 200 mM sodium acetate produced a similar increase to that produced by 200 mM NaCl (Figure 1;

TABLE 1:
Increase in cerebral cortical α-2 receptors in the presence of sodium ion and guanylnucleotides.

	NO ADDITIONS	NaCl 200mM	GPP(NH)P 10^{-5}M	NaCl + GPP(NH)P	Na ACETATE + GPP(NH)P	NaCl+GTP (10^{-4}M)**
Kd [nM]	10.3±2.5	10.9±1.6	9.8±1.2	8.8±1.3	9.5±2.1	8.8±2.3
α-2 Receptor conc. (fmol/mg)	55±5.8	78*±5.2	73*±5.4	106+±5.8	101+±6.1	114++±7

Results are mean ± SE of 4 or more experiments.
* $p<0.01$ relative to no additions.
+ $p<0.01$ relative to GPP(NH)P
++ $p<0.01$ relative to NaCl.
** plus 20 mM phosphorylcreatine and 10 μg/ml creatine kinase.

ble 1). These results indicated that the causative agent was Na^+ ion and not Cl^- ion. P (in the presence of a generating system 20 mM phosphoryl creatine plus 10 μg/ml eatine kinase) and GPP(NH)P produced similar maximum increases in [^{3}H]yohimbine binding EC_{50} values for the two were respectively 2 ± $1x10^{-7}$ (SE) and 1.5 ± $1x10^{-6}$ (SE) (n=3) . ATP and GMP at concentrations up to 10^{-4}M were ineffective (Figure 1).

A similar relative increase in receptor sites was caused by the addition of NaCl (200) and GPP(NH)P (10^{-5}M) to hypothalamic membranes. However these factors failed to cause y detectable increase in [^{3}H]yohimbine binding to renal cortical α-2 receptors. reover the addition of NaCl and GPP(NH)P did not affect [^{3}H]prazosin binding to cerebral rtical membranes. Thus the effect of NaCl and guanylnucleotides was specific for α-2 renergic receptors of the central nervous system (Figure 2).

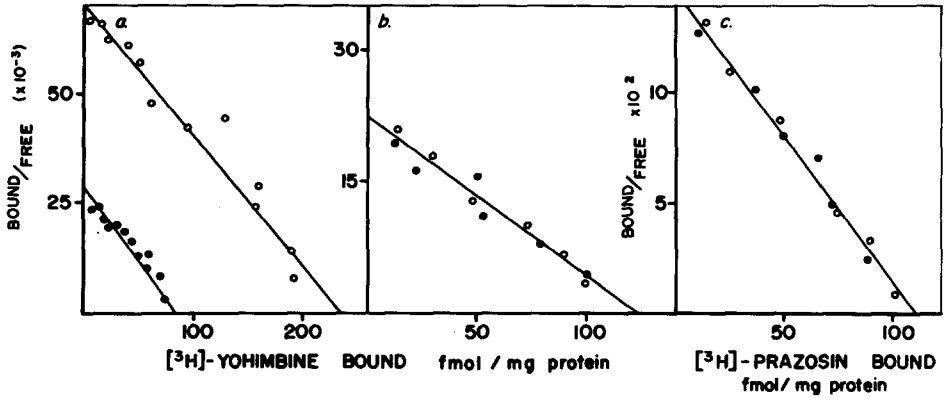

Figure 2: Effect of sodium chloride and guanylyl-5'-imidodiphosphate GPP(NH)P oncentral and peripheral α-receptor binding. Scatchard analysis of binding data using [^{3}H]yohimbine and [^{3}H]prazosin. All experiments were performed at least 3 times. ●–● no additions; ○–○ 200 mM NaCl plus 10^{-5}M GPP(NH)P.
a) Hypothalamus [^{3}H]yohimbine binding
b) Renal cortex [^{3}H]yohimbine binding
c) Cerebral cortex [^{3}H]prazosin binding

TABLE 2:

Affinities of α-adrenergic agonists and antagonists for cerebral cortical α-2 receptors in the presence and absence of 200 mM NaCl and 10^{-5}M GPP(NH)P.

COMPOUND	NO ADDITIONS	NaCl + GPP(NH)P
l-epinephrine	$8.8 \pm 0.2 \times 10^{-8}$	$4.3 \pm 0.3 \times 10^{-6}$
d-epinephrine	$1.2 \pm 0.3 \times 10^{-6}$	$>10^{-4}$
l-norepinephrine	$1.7 \pm 0.3 \times 10^{-7}$	$6.2 \pm 0.4 \times 10^{-6}$
α-methylnorepinephrine	$9.8 \pm 0.3 \times 10^{-8}$	$3.1 \pm 0.5 \times 10^{-6}$
yohimbine	$1.4 \pm 0.2 \times 10^{-8}$	$9.7 \pm 0.3 \times 10^{-9}$
prazosin	$2.5 \pm 0.5 \times 10^{-5}$	$2.7 \pm 0.4 \times 10^{-5}$
phentolamine	$8.5 \pm 0.6 \times 10^{-9}$	$1.1 \pm 0.8 \times 10^{-8}$

Results are $K_{I\ av}$ [I] values [M] ± SE of 3 experiments.

3. Effects of NaCl and GPP(NH)P on the binding affinities of α-adrenergic agonists and antagonists.

There was no effect of NaCl and GPP(NH)P on the affinities of the antagonist compounds prazosin, phentolamine and yohimbine for cerebral cortical α-2 receptors (Table 2). The binding affinities of α-2 adrenergic agonists to cerebral cortical α-2 receptors was decreased in the presence of NaCl (200 mM) and GPP(NH)P (10^{-5}M). The binding of l-epinephrine was further examined in the presence of the two factors separately as well as in combination. NaCl (200 mM) or GPP(NH)P (10^{-5}) both caused a 10-20 fold decrease in the average affinity of epinephrine. Together a 100-200 fold decrease was observed. The effects of NaCl and GPP(NH)P on the binding of l-epinephrine were similar in membranes from cerebral cortex and renal cortex (Figure 3).

DISCUSSION

In this paper we have demonstrated a specific increase in the concentration of central α-2 adrenergic receptors measured in the presence of sodium ion and guanylnucleotides.

While the combination of sodium ion and guanylnucleotides produced a 100% increase in the number of α-2 receptors, no change in the affinity of the receptors for yohimbine or the other antagonists tested (phentolamine and prazosin) could be detected. In contrast, the average binding affinities of agonist compounds were decreased in the presence of sodium ion and guanylnucleotides. Both of these factors separately decreased agonist

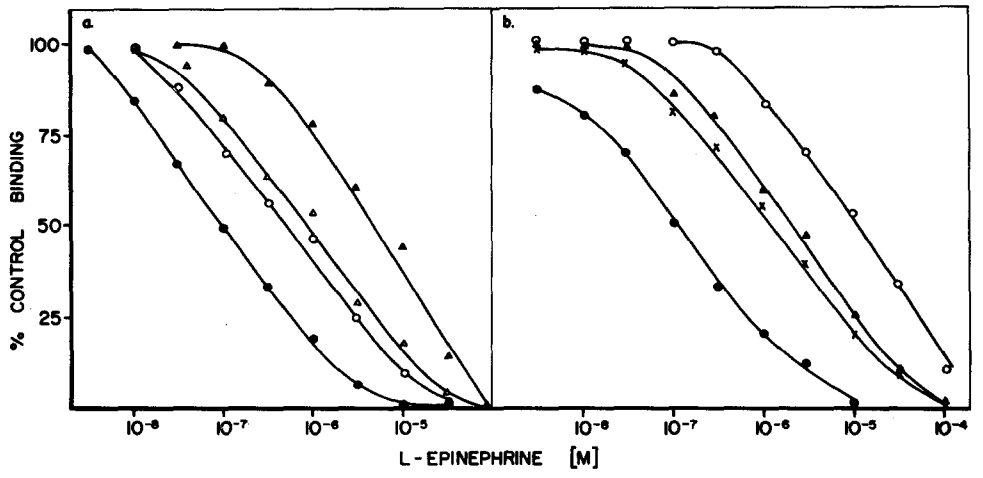

Figure 3: Effects of sodium chloride and guanylyl-5'-imidodiphosphate (GPP(NH)P)on the affinity of l-epinephrine for α-2 receptors in cerebral cortex and renal cortex. The experiments were performed 3 times.
●-● no additions; ○-○ 10^{-5}M GPP(NH)P; Δ-Δ 200 mM NaCl; ▲-▲ 200 mM NaClplus 10^{-5}M GPP(NH)P.
a) Cerebral cortex
b) Renal cortex

finity and the effects of the two factors together were additive. This effect, on onist affinity at central α-2 receptors, was similar to that observed in the kidney ere α-2 receptors are coupled to the inhibition of adenylate cyclase [18]. Similar idings have also been reported in platelets [7] and liver [8]. Despite the similarities the effects of these factors on agonist binding in cerebral cortex and renal cortex eir effects on receptor concentration were clearly tissue specific.

Monovalent cations and GTP are both required for maximum inhibition of adenylate clase via α-2 receptors in platelets, adipocytes, liver and kidney [15-18]. Similar ecificity applies to modulation of α-2 agonist affinity indicating a relationship tween the binding properties of agonist compounds and adenylate cyclase coupling in ese tissues [7-9]. The similar effects of these factors on agonist affinity at cerebral tical α-2 receptors suggest that these are also coupled to adenylate cyclase nibition. Moreover the requirement for sodium ion and GTP to increase available eptor sites in cerebral cortex membranes suggests that this is also a function of enylate cyclase coupling. Since there was no apparent change in the characteristics of e receptors it appears that the effect of sodium ion and GTP has been to unmask further eptors which were not otherwise available for [^{3}H]yohimbine binding.

The mechanism of the observed increase in central α-2 receptors and its relationship to receptor function cannot be ascertained without further purification of the components However the practicalconsequences are more obvious. Our results demonstrate that the measured concentration of central α-2 receptors depends on the incubation concentration o sodium ion and guanylnucleotides. The use of an antagonist ligand has enabled this relationship to be clearly demonstrated because, with the previously used agonist ligands the increase in sites would be masked by the simultaneous decrease in ligand affinity in the presence of sodium ion and guanylnucleotides.

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