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SODIUM REGULATION OF α_2-ADRENORECEPTORS IN DAHL RATS

EFFECT OF FEEDING A LOW OR HIGH SALT DIET

SANKARIDRUG M. PERIYASAMY* and JOHN P. RAPP
Department of Pharmacology and Department of Medicine, Medical College of Ohio, Toledo, OH 43699, U.S.A.

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Abstract—Sodium regulation of α_2-adrenoreceptors was investigated in inbred salt-sensitive (S) and inbred salt-resistant (R) rats fed a high or low salt diet. The systolic blood pressure was higher in S rats than in R rats, and this difference was obviously greater on a high salt diet. In rats fed a low or high salt diet, S rats had higher α_2-adrenoreceptor density in the kidneys compared with R rats as measured by [^{3}H]yohimbine binding and Scatchard analysis. The affinity of the receptors in the kidney for the antagonist, yohimbine, was nearly the same in these two strains either on a low or high salt diet. In the brain, the affinities or the numbers of receptors were not significantly different whether these two strains were fed a low or high salt diet. Inclusion of NaCl up to 80 mM in the assay medium did not alter the *in vitro* binding of [^{3}H]yohimbine in the kidney or brain. On the other hand, inclusion of NaCl in the assay medium reduced the ability of epinephrine in competing with [^{3}H]yohimbine for the receptor sites in the kidney and in the brain, and this effect of NaCl was the same in a given tissue between S and R rats, whether they were fed a low or high salt diet. These results suggest that: (1) in the kidneys, the receptor density and not the receptor affinity was different between S and R strains whether they were fed a low or high salt diet; (2) in the brain, the receptor density and affinity were the same between S and R rats regardless of the diet (low or high salt), indicating that the sodium salt diet modulates the peripheral but not the central α_2-adrenoreceptors; and this modulatory effect was observed only in S rats; (3) Na^+ was able to reduce the affinity of the agonist (epinephrine) for the receptors in both S and R rats, and this effect of Na^+ on central and peripheral α_2-adrenoreceptors was similar in prehypertensive rats and rats with salt-induced hypertension; and (4) the resistance of R rats to salt-induced hypertension was not due to the absence of Na^+ binding component involved in the regulation of α_2-adrenoreceptor–adenylate cyclase complex.

The idea that high salt intake plays a key role in the development of essential hypertension was promulgated over eight decades ago [1]. Since then, numerous epidemiological and clinical studies have provided support for the view that Na^+ plays a role in the etiology of hypertension [1]. The mechanism(s) by which Na^+ actually leads to the elevation of blood pressure is not well understood and is currently under intensive investigation.

The role of Na^+ in the modulation of hormones or neurotransmitter–receptor interactions has been studied extensively in various systems such as α_2-adrenoreceptors in kidneys [2], platelets [3, 4] and brain [5]; α_1-adrenoreceptors in kidneys [2] and brain [6]; opiate receptors in neuronal cells [7]; and muscarinic cholinergic receptors in the heart [8]. The modulation in the affinity of various receptors for the agonists by Na^+ is attributed to an increased dissociation rate of the agonists [9]. Also, the hormonal- or neurotransmitter-induced inhibition of adenylate cyclase in various membrane systems is regulated by Na^+ [10] and the regulatory effect of Na^+ on the receptor–adenylate cyclase complex is exerted through binding to the inner aspect of the plasma membrane [11]. In light of these findings, Insel and Motulsky [12] hypothesized that elevated intracellular Na^+ in some unspecified way leads to an enhanced response at membrane receptors to the adrenergic neurotransmitters and other hormones which could contribute to the development and maintenance of hypertension since a variety of membrane receptors are Na^+ sensitive.

Several experimental animal models of hypertension such as Dahl, Sabra, Milan, and SHR rats have been used to study the molecular mechanisms of Na^+-induced hypertension [13]. The Dahl salt-sensitive rats develop hypertension with age whether they are fed a low salt diet or a high salt diet [14–16]; however, feeding these rats a high salt diet accelerates the development of hypertension. In contrast, Dahl salt-resistant rats do not develop hypertension on either a low or a high salt diet [15, 16]. It is well documented that central and peripheral α_2-adrenoreceptors play a pivotal role in the regulation of blood pressure [17, 18]. Since these receptors are involved in the regulation of blood pressure and are modulated by Na^+, we investigated the possibility of a difference in the modulation of α_2-adrenoreceptors by Na^+ between Dahl salt-sensitive and Dahl salt-resistant rats using membrane-bound receptor preparations from whole kidneys, cerebral cortex and medulla oblongata.

MATERIALS AND METHODS

Inbred female Dahl sat-sensitive (SS/Jr) and

* Correspondence: S. M. Periyasamy, Ph.D., Department of Pharmacology, Medical College of Ohio, Toledo, OH 43699.

Table 1. Comparison of systolic blood pressure, body weight, affinity and binding sites in S and R rats fed a low or high salt diet

Diet	Blood pressure (mm Hg)	Body wt (g)	Kidney K_d (nM)	Kidney B_{max} (fmol/mg protein)	Cerebral cortex K_d (nM)	Cerebral cortex B_{max} (fmol/mg protein)
Low salt						
S	129 ± 3[a]	166 ± 4[c]	8.33 ± 0.64[c]	328 ± 17[a]	10.2 ± 0.83[c]	251 ± 12[c]
	*	NS	NS	†	NS	NS
R	118 ± 2[b]	160 ± 2[b]	7.69 ± 0.55[b]	256 ± 12[b]	10.1 ± 0.76[b]	243 ± 9[b]
High salt						
S	175 ± 4[a]	166 ± 5[c]	6.94 ± 0.61[c]	406 ± 12[a]	10.2 ± 0.4[c]	267 ± 8[c]
	‡	NS	NS	*	NS	NS
R	124 ± 4[b]	162 ± 3[b]	7.09 ± 0.84[b]	290 ± 10[b]	9.7 ± 0.46[b]	260 ± 6[b]

Values are means ± SE. Blood pressure and body weight values are the means of ten rats from each group. K_d and B_{max} values are the means of three experiments and each experiment was conducted in duplicate by pooling the tissues from two rats from each group. The data were analyzed by Student's unpaired *t*-test. K_d = dissociation constant; B_{max} = maximal binding sites; S = salt-sensitive rats; R = salt-resistant rats.

*–‡ Significant differences at * $P < 0.01$. † $P < 0.05$ and ‡ $P < 0.001$, respectively, and NS indicates not significant, for comparisons between S and R strains with a given diet.

[a] Significant difference at $P < 0.05$ between S rats on a high compared to a low salt diet.

[b,c] Values are not significant, i.e. $P > 0.05$, for comparisons between diets within a strain.

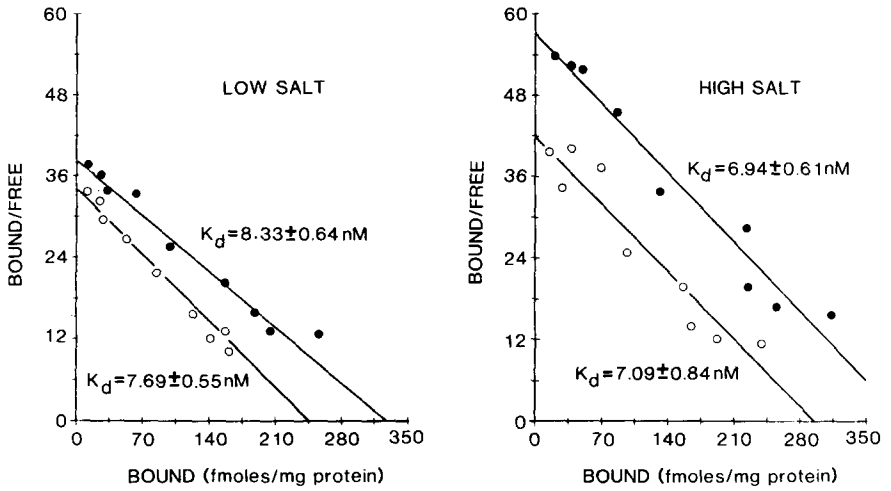

Fig. 1. Scatchard analysis of [^{3}H]yohimbine binding to rat kidney membranes prepared from S (●) and R (○) rats. Membranes were incubated with various concentrations of [^{3}H]yohimbine (0.5 to 20 nM), and specific binding was determined as described in Materials and Methods. Each point on the plot is the mean of three experiments, and each experiment was conducted in duplicate using the tissues pooled from two rats from each group.

inbred Dahl salt-resistant (SR/Jr) rats were used [16]. These inbred strains will be referred to hereafter in the text by their generic designations of S (salt-sensitive) and R (salt-resistant). The rats were weaned at 4 weeks of age and fed a standard rat chow containing 1% NaCl for 2 weeks (Wayne Rodent Blox, Continental Grain Co., Chicago, IL). Rats were then fed whole grain diets containing either 0.15% NaCl (low salt) or 8% NaCl (high salt) for 3 weeks (Teklad, Madison, WI). Systolic blood pressure was measured under ether anesthesia by the tail cuff method of Friedman and Freed [19] 5 days prior to killing the rats. The rats were decapitated and the kidneys and brains were removed. The kidneys were used immediately or stored at −70° until use. Cerebral cortex and medulla oblongata were dissected from the rest of the brain and used immediately or stored at −70° until use.

Preparation of rat renal membranes. The kidneys were thawed, minced, and homogenized (1:10, w/v) in 50 mM Tris–HCl, 1 mM EDTA, pH 7.5, with a glass homogenizer. The crude renal membrane preparations were obtained by differential centrifugation. Briefly, the homogenate was passed through four layers of cheese cloth and centrifuged at 3,000 *g* for 10 min; the resultant supernatant fraction was centrifuged at 40,000 *g* for 25 min. The pellet was washed once by resuspension and recentrifugation. The final pellet was suspended in the homogenization buffer (5 ml buffer/g wet tissue).

Preparation of rat brain membranes. Brain tissue was homogenized (1:10, w/v) in 50 mM Tris–HCl, 1 mM EDTA, pH 7.5, using a glass homogenizer and a Teflon pestle (10 strokes, 1500 rpm). The homogenate was centrifuged at 40,000 *g* for 15 min at 2–4°, and the supernatant fraction was discarded. The

Table 2. Effect of *in vitro* NaCl concentrations on specific [^{3}H]yohimbine binding to alpha$_2$-adrenoreceptors from renal membranes of S and R rats

	Specific activity (fmol/mg protein)			
	Low salt diet		High salt diet	
Treatment	S	R	S	R
Control	124 ± 5	107 ± 3*	184 ± 6	114 ± 5†
Control + 10 mM NaCl	125 ± 6	100 ± 4*	184 ± 5	116 ± 6†
Control + 20 mM NaCl	131 ± 7	103 ± 3*	183 ± 7	107 ± 8†
Control + 40 mM NaCl	130 ± 7	101 ± 7*	174 ± 6	110 ± 4†
Control + 80 mM NaCl	122 ± 5	102 ± 3*	176 ± 7	108 ± 3†

Rat kidney membranes were incubated for 40 min at 23° with 8 nM [^{3}H]yohimbine (final concentration) in the absence and presence of various concentrations of NaCl. Specific binding was determined as described in Materials and Methods. Values are the means ± SE for the specific activity, which represents the number of binding sites, from three experiments where each experiment was conducted in duplicate by pooling the tissues from two rats from each group. The data were analyzed by Student's unpaired *t*-test.

*,† Significantly different from the S strain on a given diet: * $P < 0.05$, and † $P < 0.01$.

membrane pellet was washed once by resuspension and recentrifugation. The final pellet was suspended in buffer containing 50 mM Tris–HCl and 1 mM EDTA (5 ml buffer/g wet tissue).

Ligand binding assay. Membranes (400–480 μg protein) were incubated with [^{3}H]yohimbine for 40 min at 23° in 20 mM Tris–HCl, 0.5 mM EDTA buffer in a total volume of 500 μl. Ascorbic acid (1 mM final concentration) was used as an antioxidant in experiments in which catecholamines were included. This concentration of ascorbic acid had no effect on the binding of [^{3}H]yohimbine. The binding was initiated by adding the membranes to the incubation medium and was terminated by the addition of 5 ml of ice-cold assay buffer followed by vacuum filtration over Whatman GF/C filters. The filter was then rapidly washed twice with 5 ml of ice-cold assay buffer. The whole process of filtration and washing was completed within 20 sec. The radioactivity bound to the membrane trapped by the filter was extracted into an Aqueous Counting Scintillant (Amersham) and counted in a Beckman Scintillation Counter with an efficiency of 40%. Nonspecific binding was determined in each assay by running parallel samples containing 10^{-5} M unlabeled phentolamine. Specific binding was obtained by subtracting nonspecific from total binding. The number of binding sites (B_{max}) and the affinity for antagonist (K_d) were calculated from Scatchard analysis of the binding data [20]. Membrane protein was determined according to the procedure of Lowry *et al.* [21] using bovine serum as the standard.

Effect of NaCl *on the epinephrine-induced displacement of* [^{3}H]*yohimbine from the binding sites.* To study the effect of various concentrations of NaCl on the displacement of [^{3}H]yohimbine by epinephrine, the following protocol was used. Membranes were incubated with 8 nM [^{3}H]yohimbine + 1 mM ascorbic acid or 8 nM [^{3}H]yohimbine + 0.3 μM epinephrine + 1 mM ascorbic acid. Simultaneously, membranes were also incubated with 8 nM [^{3}H]yohimbine + NaCl (10–80 mM) + 1 mM ascorbic acid or 8 nM [^{3}H]yohimbine + 0.3 μM epinephrine + NaCl (10–80 mM) + 1 mM ascorbic acid. The ligand binding was done at 23° for 40 min and was terminated by the addition of 5 ml of ice-cold buffer followed by vacuum filtration over Whatman GF/C filters. The filter was then rapidly washed twice with 5 ml of ice-cold buffer. The radioactivity bound to the membrane trapped by the filter was counted as described under ligand binding assay. Specific binding in this study refers to the fraction of bound radioligand displaced by 10 μM unlabeled phentolamine. The percentage of specific [^{3}H]yohimbine binding in the presence of 0.3 μM epinephrine + 1 mM ascorbic acid was calculated taking the specific binding in the presence of [^{3}H]yohimbine + 1 mM ascorbic acid as 100%. The percentage of [^{3}H]yohimbine binding in the presence of 0.3 μM epinephrine + NaCl + 1 mM ascorbic acid was calculated taking the specific binding in the presence of [^{3}H]yohimbine + NaCl + 1 mM ascorbic acid as 100%. The concentrations of [^{3}H]yohimbine, epinephrine and NaCl represent the final concentration in the assay medium.

Data analysis. Saturation binding data were analyzed by using a computer program [22]. The data were analyzed either by Student's unpaired *t*-tests or by three-way analysis of variance. A probability value of less than 0.05 was considered statistically significant. The data are expressed as means ± SE. Vertical lines in the figures indicate standard error of the mean.

RESULTS

Blood pressure and body weight. Although the systolic blood pressure was in the normal range, it was significantly higher in S rats than R rats when they were on a low salt diet. Feeding the rats a high salt diet produced a significant increase in blood pressure in S rats (from 129 to 175 mm Hg); on the other hand, such feeding caused very little change in blood pressure in R rats (from 118 to 124 mm Hg). However, the body weight of these animals was not changed significantly under these dietary conditions (Table 1).

[^{3}H]*Yohimbine binding to rat kidney membranes*

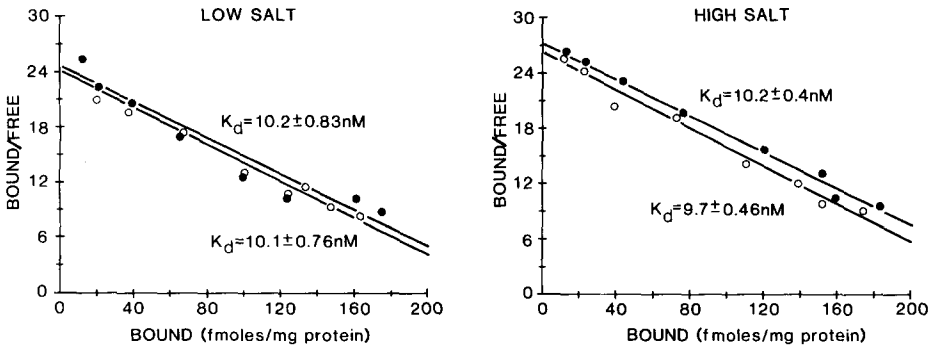

Fig. 2. Scatchard analysis of [^{3}H]yohimbine binding to rat cerebral cortex membranes prepared from S (●) and R (○) rats. Membranes were incubated with various concentrations of [^{3}H]yohimbine (0.5 to 20 nM), and specific binding was determined as described in Materials and Methods. Each point on the plot is the mean of three experiments, and each experiment was conducted in duplicate using the tissues pooled from two rats from each group.

as a function of [^{3}H]*yohimbine concentration*. Figure 1 shows the Scatchard plot of specific [^{3}H]yohimbine binding to rat kidney membranes obtained from S and R rats fed a low or high salt diet. As shown in the figure, the Scatchard plots were linear, suggesting the existence of a homogeneous population of noninteracting binding sites. In rats fed a low salt diet, S rats had a greater number of binding sites than the R rats. However, the affinity of the receptors for the [^{3}H]yohimbine was not significantly different between S and R rats (Fig. 1 and Table 1). In rats fed a high salt diet, the number of [^{3}H]yohimbine binding sites was much higher in S rats than in R rats but the affinity of the receptors remained the same in both S and R rats (Fig. 1 and Table 1). Further, changing the diet from low salt to high salt produced a significant increase in the receptor number in S rats, but there was no significant increase in that of R rats (Table 1).

[^{3}H]*Yohimbine binding to rat cerebral cortex membranes as a function of* [^{3}H]*yohimbine binding*. As observed with kidney membranes, the Scatchard plots of specific [^{3}H]yohimbine binding to rat cerebral cortex membranes obtained from S and R rats fed a low or high salt diet were linear, indicating the presence of single class of binding sites with no cooperativity (Fig. 2). In rats fed a low salt diet, the number of binding sites and the affinity of the receptors were not significantly different between S and R rats. A similar observation was made when S and R rats were fed a high salt diet. Unlike in the kidney, changing the diet containing low salt to high salt did not increase the binding sites in the cerebral cortex membranes of S rats (Fig. 2 and Table 1).

Effect of various concentrations of NaCl *on specific* [^{3}H]*yohimbine binding*. The effects of *in vitro* concentrations of NaCl on specific [^{3}H]yohimbine binding to membranes prepared from rat kidney, cerebral cortex and medulla oblongata are shown in Tables 2–4. As observed earlier, the specific [^{3}H]yohimbine binding was higher in rat kidney membranes of S rats than R rats, which were fed either a low or a high salt diet. However, there was no significant difference in the specific [^{3}H]yohimbine binding sites in either rat cerebral cortex or the medulla oblongata between S and R rats. NaCl up to 80 mM did not alter significantly the specific [^{3}H]yohimbine binding sites in membranes prepared from kidney, cerebral cortex and medulla oblongata of either strains. Similar results were obtained when we studied the effect

Table 3. Effect of *in vitro* NaCl concentrations on specific [^{3}H]yohimbine binding to alpha$_2$-adrenoreceptors from cerebral cortex membranes of S and R rats

	Specific activity (fmol/mg protein)			
	Low salt diet		High salt diet	
Treatment	S	R	S	R
Control	106 ± 2	108 ± 6	104 ± 9	110 ± 8
Control + 10 mM NaCl	107 ± 10	105 ± 7	103 ± 12	109 ± 7
Control + 20 mM NaCl	100 ± 4	108 ± 11	107 ± 14	117 ± 6
Control + 40 mM NaCl	98 ± 11	103 ± 10	98 ± 12	107 ± 8
Control + 80 mM NaCl	96 ± 4	96 ± 4	99 ± 7	104 ± 5

Rat cerebral cortex membranes were incubated for 40 min at 23° with 8 nM [^{3}H]yohimbine (final concentration) in the absence and presence of various concentrations of NaCl. Specific binding was determined as described in Materials and Methods. Values are the means ± SE for specific activity, which represents the number of binding sites, from three experiments where each experiment was performed in duplicate by pooling the tissues from two rats from each group. The data were analyzed by Student's unpaired *t*-test. There was no significant difference, i.e. $P > 0.05$, between the S and R strains on a given diet.

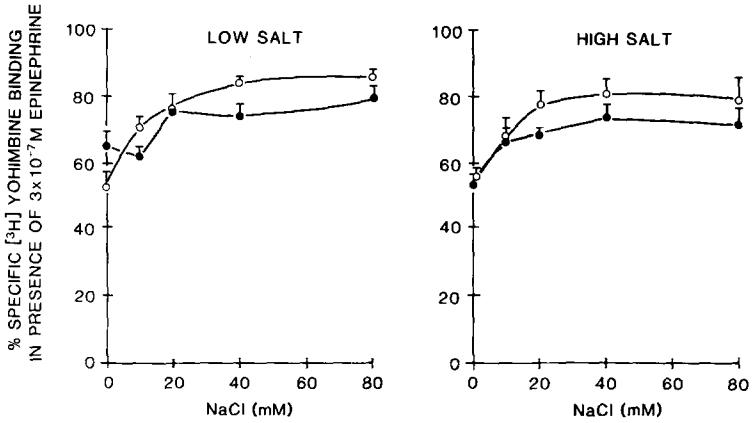

Fig. 3. Effect of various concentrations of NaCl on epinephrine-induced displacement of [^{3}H]yohimbine binding to rat kidney membranes from S (●) and R (○) rats. The experiments were carried out as described in Materials and Methods. Each point on the plot is the mean (±SE) of five separate experiments, and each experiment was conducted in triplicate using the tissues pooled from two rats from each group. The data were analyzed by three-way analysis of variance. The probability value was greater than 0.05 (S vs R rats) at all concentrations of NaCl used.

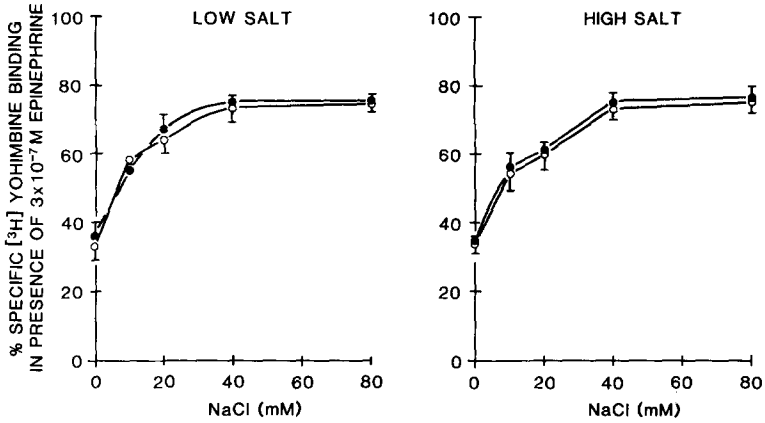

Fig. 4. Effect of various concentrations of NaCl on epinephrine-induced displacement of [^{3}H]yohimbine binding to rat cerebral cortex membranes from S (●) and R (○) rats. The experimental protocol is described in Materials and Methods. Each point on the plot is the mean (±SE) of five separate experiments, and each experiment was conducted in triplicate using the tissues pooled from two rats from each group. The data were analyzed by three-way analysis of variance. The probability value was greater than 0.05 (S vs R rats) at all concentrations of NaCl used.

Table 4. Effect of *in vitro* NaCl concentrations on specific [^{3}H]yohimbine binding to alpha$_2$-adrenoreceptors from medulla oblongata membranes of S and R rats

	Specific activity (fmol/mg protein)			
	Low salt diet		High salt diet	
Treatment	S	R	S	R
Control	54 ± 7	57 ± 6	54 ± 11	53 ± 8
Control + 10 mM NaCl	56 ± 8	56 ± 5	50 ± 12	52 ± 10
Control + 20 mM NaCl	59 ± 13	51 ± 8	55 ± 13	57 ± 11
Control + 40 mM NaCl	49 ± 4	53 ± 11	61 ± 9	57 ± 9
Control + 80 mM NaCl	49 ± 11	49 ± 3	54 ± 9	54 ± 13

Membranes were incubated for 40 min at 23° with 8 nM [^{3}H]yohimbine (final concentration) in the absence and presence of various concentrations of NaCl. Specific binding was determined as described in Materials and Methods. Values are means ± SE for specific activity, which represents the number of binding sites, from three experiments where each experiment was done in duplicate using the tissues collected from two rats from each group. The data were analyzed by Student's unpaired *t*-test. There was no significant difference, i.e. $P > 0.05$, between the S and R strains on a given diet.

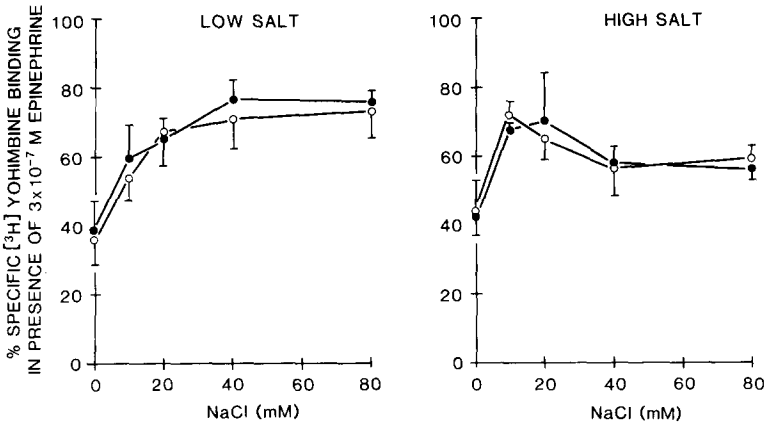

Fig. 5. Effect of various concentrations of NaCl on epinephrine-induced displacement of [^{3}H]yohimbine binding to rat medulla oblongata membranes from S (●) and R (○) rats. Each point on the plot is the mean (±SE) of five separate experiments, and each experiment was performed in triplicate using the tissues pooled from three rats from each group. The data were analyzed by three-way analysis of variance. The probability value was greater than 0.05 (S vs R rats) at all concentrations of NaCl used.

of NaCl on specific [^{3}H]rauwolscine binding in membranes prepared from kidney, cerebral cortex and medulla oblongata of S and R rats (data not shown).

Na^+ *regulation of* α_2*-adrenoreceptor–agonist interactions*. We investigated the effect of various concentrations of NaCl on epinephrine-induced displacement of [^{3}H]yohimbine binding to the membranes prepared from kidney, cerebral cortex and medulla oblongata of S and R rats fed a low or high salt diet. These results (Figs 3–5) show that in all the membranes the presence of 0.3 μM epinephrine in the assay medium reduced the [^{3}H]yohimbine binding as indicated by the fact that specific [^{3}H]yohimbine binding in the presence of epinephrine was less than 100%, where 100% is the specific [^{3}H]yohimbine binding in the absence of epinephrine. The extent of reduction in the [^{3}H]yohimbine binding produced by 0.3 μM epinephrine was different for different membrane preparations (compare Figs 3, 4 and 5). Inclusion of NaCl in the assay medium reduced the ability of epinephrine to displace [^{3}H]yohimbine from the binding sites. The effect of NaCl in attenuating the effect of epinephrine on [^{3}H]yohimbine binding was concentration dependent, as evidenced from the increase in [^{3}H]yohimbine binding as NaCl concentration was increased (Figs 3–5). This effect of NaCl was not significantly different in a given tissue between S and R rats. However, it is intriguing to note that NaCl, at a concentration of 10 mM, produced a maximal effect in reducing the affinity of epinephrine for α_2-adrenoreceptors of membranes prepared from medulla oblongata of S and R rats fed a high salt diet, and that this effect of NaCl was reduced at higher concentrations in all preparations (Fig. 5).

DISCUSSION

The mechanism(s) by which dietary NaCl contributes to the etiology of hypertension in the S rats remains a topic of considerable interest. Insel and Motulsky [12] postulated a hypothesis that salt loading increases intracellular Na^+ which, in turn, leads to an enhanced response to hormones and neurotransmitters at membrane receptors and this enhancement in response could then contribute to the development and maintenance of hypertension because a variety of membrane receptors are regulated by Na^+. Their hypothesis is vague as to the mechanisms involved and hard to comprehend since increased Na^+ concentration decreases agonist binding to receptors. On the other hand, Gavras [23] proposed the comprehensible theory that Na^+ induces hypertension by decreasing the affinity of neurotransmitters for α_2-adrenoreceptors in the central nervous system which results in disinhibition of sympathoinhibitory neurons that leads to a hyperadrenergic state characteristic of salt-induced hypertension [23].

The present study was conducted to test these hypotheses and also to determine whether S and R rats are affected, either similarly or differentially, by being fed a low or high salt diet after weaning. The present study shows that the S rats fed a low salt diet had a greater number of α_2-adrenoreceptors in the kidneys than their R counterparts, and that there was no significant difference in the affinity of the receptors for the antagonist in the kidney or in the brain. Further, the densities of the α_2-adrenoreceptors in the brain were similar in both strains maintained on a low salt diet (Table 1).

In a similar study, Pettinger *et al.* [24] reported that, in rats fed a low salt diet, the renal α_2-adrenoreceptors were higher in S than in R rats but the systolic blood pressure was nearly the same in both strains. In view of these findings, they suggested that the higher α_2-adrenoreceptor density in the kidney preceded the increase in the systolic blood pressure and, therefore, the higher renal α_2-adrenoreceptor density may be responsible for the development of hypertension in S rats. However, these authors did not study the effect of dietary Na^+ on central α_2-adrenoreceptors in S and R rats. The results of the present study confirm the findings of Pettinger *et al.* [24] as far as renal α_2-adrenoreceptors of S and R rats are concerned (higher renal α_2-adrenoreceptors

in S than R rats). Unlike the kidney, however, the central α_2-adrenoreceptors from cerebral cortex and medulla oblongata were nearly the same in both S and R rats, suggesting that the aberrations in the number of α_2-adrenoreceptors were probably limited to peripheral α_2-adrenoreceptors.

When S and R rats were fed a high salt diet, two changes were noticed. The number of renal α_2-adrenoreceptors was increased much more in S rats than in R rats and the systolic blood pressure was increased in S rats but not in R rats (see Table 1). There were, however, no changes in the affinity of the α_2-adrenoreceptors for the antagonist in the kidney or in the brain, or in the density of the receptors in the brain. It is interesting to note that recently Parini *et al.* [25] reported that there was no difference in the α_2-adrenoreceptor density and affinity in the cerebral cortex of Milan rats in the prehypertensive and hypertensive state.

Racz *et al.* [26] observed a greater increase in the synthesis of norepinephrine in the adrenal gland of S rats than R rats when they were fed a high salt diet. They proposed that the abnormal catecholamine synthesis of S rats to high salt intake could produce Na^+ retention and hypertension in these rats. These salt-induced changes in norepinephrine synthesis coupled with the increase in the number of α_2-adrenoreceptors could act synergistically in producing hypertension. Further, our data show that feeding the rats a high salt increased the number of receptors but did not alter the affinity of the receptors for the antagonist, and this increase in the receptor number was observed only in the kidneys of S rats suggesting that intake of high salt differentially regulated the central and peripheral α_2-adrenoreceptors of S rats. Whether or not feeding the S rats a high salt diet increases α_2-adrenoreceptors from vascular smooth muscle and other peripheral tissues remains to be investigated. Therefore, our findings do not support the hypothesis of Insel and Motulsky [12] or of Gavras [23]. However, it is possible that there may be differences in these strains subsequent to the ligand–receptor interaction either at the level of coupling between the receptor protein and G_i-protein or at the level of second messenger or both.

Parini *et al.* [27, 28] showed that cerebral α_2-adrenoreceptor densities as measured with [^{3}H]rauwolscine are lower in Sabra DOCA-salt-sensitive (SBH) than Sabra DOCA-salt-resistant (SBN) rats. Further, with *in vitro* studies Na^+ causes a marked increase in cerebral and renal high affinity α_2-adrenoreceptor densities in SBH rats, whereas the density of α_2-adrenoreceptors is weakly affected by Na^+ in SBN rats. Based on these studies they suggested that the absence of sodium regulation on cerebral cortex α_2-adrenoreceptors in SBN rats may be responsible for the resistance to salt-induced hypertension.

In the present study we used several concentrations of NaCl to investigate the effect of Na^+ on *in vitro* binding of [^{3}H]yohimbine to the membranes from kidney, cerebral cortex and medulla oblongata. Our results show that, unlike in the Sabra rats, the cerebral α_2-adrenoreceptor densities were similar in S and R rats. Also, the number of α_2-adrenoreceptors from medulla oblongata was nearly the same in S and R rats. Further, in our *in vitro* binding studies, NaCl up to 80 mM did not affect the number of α_2-adrenoreceptors from cerebral cortex, medulla oblongata and renal cortex as measured by [^{3}H]yohimbine binding in both strains fed a low or high salt diet. Similar results were obtained when the stereoisomer of [^{3}H]yohimbine, namely [^{3}H]rauwolscine, was used (data not shown). This is in contrast to the results of Parini *et al.* [27, 28]. The disparity between our findings and those of Parini *et al.* [27, 28] may be due to a difference in the strain of rats or in the experimental conditions or in the preparation of membranes.

It has been shown that the affinity of the agonist for the central and peripheral α_2-adrenoreceptors is decreased in the presence of NaCl [2, 6]. We therefore examined the effect of various concentrations of NaCl on the affinity of epinephrine (agonist) for central and renal α_2-adrenoreceptors. Our results show that NaCl reduced the affinity of the agonist for α_2-adrenoreceptors from kidney, cerebral cortex and medulla oblongata. These findings suggest that both S and R rats had a Na^+ binding component as a part of the receptor–adenylate cyclase complex. The ability of Na^+ to reduce the affinity of the agonist for the receptors was the same in S and R rats while ingesting a low or high salt diet. Therefore, the resistance of R rats to salt-induced hypertension cannot be attributed to the absence of a Na^+ binding component or the poor response to Na^+. These observations suggest that the mechanism of salt-induced hypertension in Sabra rats is somewhat different from Dahl salt-sensitive rats. Further, the present observations do not lend support to the hypothesis postulated by Gavras [23].

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