Induction of High Affinity Epidermal Growth Factor Binding in the Aorta of Dahl Hypertensive Rats Fed with High Salt Diet

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Dahl salt sensitive rats (DS) developed severe hypertension on four weeks of high salt feeding while the Dahl salt resistant rats (DR) remained normotensive under the same conditions. The specific maximal binding of epidermal growth factor (EGF) in the freshly prepared kidney membranes of high salt fed DS rats was higher than those from DR rats (5.3 ± 1.9 vs. 1.6 ± 0.62 fmoles/mg protein, $p < 0.001$). Scatchard analysis of EGF binding in the kidney showed one class of receptors in the DR ($K_d = 0.75 \pm 0.05$ nM) as well as in the DS rats ($K_d = 0.69 \pm 0.06$ nM). The EGF binding in the aortic membranes of DS rats was also high compared to DR rats (24.98 ± 5.52 vs. 13.20 ± 4.10 fmoles/mg protein, $p < 0.001$). Scatchard analysis of EGF binding in the aorta showed one class of receptors in the DR aorta with a K_d of 0.70 ± 0.06 nM. On the other hand, in the DS rat aorta two classes of receptors, a high affinity form ($K_d = 0.05 \pm 0.01$ nM) and a low affinity form ($K_d = 3.5 \pm 0.3$ nM) were noted. The induction of a high affinity species of EGF receptors in the aorta, appears to be a mechanism unique to the salt fed DS rats. (*Hypertens Res* 1996; 19: 65-68)

Key Words: Dahl rats, salt sensitivity, EGF receptor, vascular proliferative response

The development of hypertension in the Dahl salt-sensitive rat, when exposed to a diet of high NaCl content, is dependent on a combination of genetic and environmental factors. The Dahl salt-sensitive (DS) rat is genetically predisposed to become hypertensive, whereas Dahl salt-resistant (DR) rat, remains normotensive (1). The hypertension that develops in the DS rat is severe and is uniformly fatal if salt feeding is continued. Since the renin-angiotensin system is suppressed in this model of hypertension (1), several other mechanisms for the development of hypertension have been proposed including an impairment in the ability of the kidneys to handle salt load (2), alterations in immunological (3) or hormonal (4, 5) controls, or changes in peripheral noradrenergic function (6, 7).

We have previously reported that high salt feeding in the DS rats leads to an increased maximal epidermal growth factor (EGF) binding, in the aortic and kidney tissue membranes (8). In the same study, we had also examined changes in the mRNA expression of growth factors in several tissues. In the kidney tissue, the mRNA expression of EGF receptor (EGFR) was enhanced.

In the aortic tissue, however, no change in the mRNA expression of EGFR was found. Therefore the increased EGF binding observed in the aorta was attributed to a post-translational activation of

the EGFR protein. In order to explore the potential mechanisms of the post-translational activation, we have studied the kinetics of EGF binding in the aortic tissue of DR and DS rats following high salt feeding.

Methods

Dahl salt sensitive (DS) and salt resistant (DR) male rats of 7 weeks of age were obtained from HSD, Inc. The animals were fed for four weeks, with a high salt diet (8% NaCl) and were allowed to drink water *ad libitum*. Systolic blood pressures of the rats were determined by the tail cuff method. During the course of this study five out of 20 DS rats died of the well known effects of high salt feeding in this species (1). These animals were discarded and their tissues were not used for analysis. Chemicals were purchased from: Na 125 I (ICN); mouse EGF (Sigma); rat EGF (Bioproducts) and protein reagent (BioRad). Rat 125 I EGF was prepared by the chloramine-T method as described by Carpenter (9).

Isolation of Membranes

The tissues were sliced into small pieces and homogenized with 4 volumes of 50 mM Tris-HCl buffer, pH 7.4 containing 0.1 mM PMSF for 30

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seconds in a Polytron homogenizer at 0°C. The homogenate was spun at $1,000 \times g$ for 5 min and the pellet discarded. The supernatant was spun at $15,000 \times g$ for 20 min and the pellet was suspended in the homogenizing buffer. This suspension was used as a source of membranes for the binding studies.

EGF Binding Studies

The binding of ^{125}I labelled EGF to the tissue membranes was studied using a reaction mixture in a total volume of 200 μl containing 50 μl of membrane fraction (50 μg protein), 50 μl of Tris-HCl buffer, pH 7.4 with 1% bovine serum albumin and about 200,000 cpm of ^{125}I labelled EGF at a final concentration of 5 nM. After incubation at room temperature (25°C) for 2 hours, 1 ml of 10.4% PEG-8000 was added and centrifuged at $1,500 \times g$ for 10 min in a Sorvall HS-4 rotor. The supernatant was aspirated off and the pellet washed once with 1 ml PEG-8000 and then counted in a gamma counter. The non specific binding was measured in the presence of a large excess of unlabelled EGF and the values were subtracted from the total binding to derive specific binding. Initial studies established that the specific binding was linear from 10 to 150 μg membrane protein and that there was no degradation of EGF during the assay period.

Protein Estimation

The protein content of the membranes was determined according to the method of Lowry *et al.* (10).

Data Analysis

The competition binding curves were transformed to Scatchard plots and affinity (K_d) and maximal binding (B_{max}) were determined with the aid of LIGAND analysis (11). Statistical significance between the high and low affinity groups was first established by one way analysis of variance (ANOVA). Student's *t*-test (two-tailed evaluation) was used to compare differences between groups and compute significance. A *p*-value of 0.05 or less was considered significant.

Result

High salt feeding increased the blood pressure of DS rats. DS rats developed severe hypertension in four weeks of high salt feeding while DR rats remained normotensive (systolic BP, 201 ± 4 vs. 138 ± 4 mm of Hg, $n=6$, $p<0.001$). The body weight of either group was not affected significantly (248 ± 37 vs. 288 ± 19 g, $n=6$, NS).

Maximal specific EGF binding in the membranes from aorta, heart and kidney of DS and DR rats fed with normal and high salt diets is shown in Table 1. The binding is higher in DS rat aorta than in DR rat aorta. The kidney from DS rats fed with high salt also showed increased EGF binding. No significant change in EGF binding was noted either in DS or and DR rats in the heart tissue, following high salt feeding.

The effect of increasing EGF concentration on

Table 1. EGF Binding in High Salt Fed Dahl Rat Tissues

Strain	Tissue	<i>n</i>	EGF bound (fmol/mg protein)
DR	Kidney	5	1.60 ± 0.62
DS	Kidney	6	$5.30 \pm 1.90^*$
DR	Aorta	5	13.20 ± 4.10
DS	Aorta	6	$24.98 \pm 5.52^*$
DR	Heart	6	0.83 ± 0.13
DS	Heart	6	0.71 ± 0.09

Renal, aortic and cardiac tissue membranes from DS and DR rats fed with a high salt diet were analyzed for specific EGF binding. Values shown are mean $\pm$ standard deviation. * $p<0.01$.

EGF binding in aortic membranes is shown in Fig. 1A. The binding in DR increased exponentially with increasing concentration of EGF and saturated around 5 nM. This pattern of the saturation binding curve is consistent with a single class of receptors in DR aorta. This conclusion is supported and confirmed by the Scatchard analysis shown in Fig. 1B, wherein the plot of the binding is linear. ($K_d=0.7 \pm 0.06$ nM). In contrast the binding pattern in DS rats shown in Fig. 1A has several different noteworthy characteristics. Increased EGF binding can be observed at all added concentration of the agonist. This circumstance could result from an overall increased binding affinity of the receptors, in which case, the binding curve in DS should be approximately parallel and similar in shape to that of DR. On the other hand, the binding curve in DS shown in 1A is not smooth and has the appearance of two different components. Scatchard analysis (Fig. 1B) of aortic binding in DS shows two different classes of receptors exhibiting high and low affinity binding. The K_d of the high and low affinity receptors was 0.05 ± 0.01 nM and 3.5 ± 0.3 nM respectively, with a difference in affinity of almost two orders of magnitude. Scatchard analysis of the EGF binding data in the DR ($K_d=0.75 \pm 0.05$ nM) or DS ($K_d=0.69 \pm 0.06$ nM) kidney showed only one class of receptors (Fig. 2).

Discussion

Appearance of a new species of EGF receptors with high affinity in the salt fed Dahl rat aortae is very interesting. This can not be attributed to physical effects of salt feeding alone, since both DS and DR rats were exposed to high salt under the same conditions. The appearance of high affinity receptors only in DS rats suggests that the putative genetic abnormality in the DS rats being activated on exposure to high salt and resulting in the development of hypertension may somehow be related to the genesis of high affinity receptors. Previous studies, however, using spontaneously hypertensive rats (12) and showing increased binding in the aortic tissue have established that lowering of blood pressure of hypertensive animals by hydralazine treatment does

Fig. 1. EGF binding by Dahl rat aortic membranes. (A) EGF binding at increasing EGF concentration. The EGF concentration was varied from 0.1 to 5.0 nM. The specific binding (total binding minus the binding in the presence of an excess of unlabelled EGF) is shown. (DS, closed circles; DR, open circles). (B) Scatchard plot of the binding. The aortic membranes from the DR rats exhibit only one type of receptors and the membranes from the DS rat aorta exhibit two types of receptors.

Fig. 2. EGF binding by Dahl rat kidney membranes. (A) EGF binding at increasing EGF concentration. The EGF concentration was varied from 0.1 to 5.0 nM. The specific binding (total binding minus the binding in the presence of an excess of unlabelled EGF) is shown. (DS, closed circles; DR, open circles). (B) Scatchard plot of the binding. The kidney membranes from the DS or DR rats exhibit only one type of receptors.

not decrease the EGF binding, implying that the increased blood pressure is not a cause of increased EGF binding.

Furthermore the increased EGF binding in the SHR aortic tissue is observed in young rats before the onset of hypertension is clearly established (8).

The pattern of increased EGF binding in the aortic and renal tissues of DS rats, and the lack of change in the cardiac tissue (Table 1) is consistent with our previous observations in other models of genetic hypertension, using SHR (13, 14) and Lyon hypertensive rats (15). It is the aortic tissue however, that in our previous studies exhibited an important difference. In the SHR aorta, increased EGF binding was found to accompany increased mRNA expression of EGFR (13). In the DS rat on the other hand (8), the mRNA levels of EGFR were normal. Furthermore, the binding studies on the

aortae from SHR and Lyon hypertensive rats (13, 15) consistently showed only a linear scatchard plot indicating the existence of a single class of EGF receptors. Accordingly we suggest that the appearance of the new species of EGFR receptor with the high affinity in DS rats presumably results from a post translational event, rather than the transcription and synthesis of two different structural forms of the receptor. The present studies, however, do not establish the precise mechanism for the appearance of the high affinity receptors. There is abundant evidence that increased activity of EGFR can result from physical changes such as ligand induced oligomerization (16-19). Also subtle changes in the carbohydrate moiety of the receptors are known to induce significant alterations in its activity (20).

The present observations lend further support to the conclusion of our previous studies in other

genetic models of hypertension (12, 15) suggesting that a strong correlation exists between the development of hypertension and increased EGFR activity in the renal and vascular tissues. This association becomes all the more significant and intriguing with the recognition that the enhanced EGF receptor activity in the vascular tissue can come about in hypertensive models, not only through post transcriptional events resulting in increased mRNA expression and elevated receptor levels such as observed in the SHR as well as in the animals with angiotensin induced experimental hypertension (21), but presumably also as shown in the present study, potentially through post translational events. The mechanisms of an interaction between the appearance of vascular high affinity EGF receptors accompanied with increased EGFR activity in the kidney tissue and salt sensitivity in salt loaded Dahl rats, is a subject for future studies. The present results can support a postulate that such a relationship exists.

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