α₁-Adrenergic Receptor Binding in the Spontaneously Hypertensive Rat

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SUMMARY Increased sympathetic outflow from the central nervous system to the periphery may contribute to the initiation of hypertension in spontaneously hypertensive rats (SHR). As this alteration in sympathetic activity may be mediated in part by α₁-adrenergic receptors in the central nervous system, the current study examined α₁-adrenergic receptors in various brain areas of SHR and normotensive Wistar-Kyoto control rats (WKY). The α₁-adrenergic receptor number and apparent affinity constants of brain sections of both young prehypertensive animals (4 weeks old) and mature hypertensive animals (12 weeks old) were studied with the α₁-adrenergic receptor antagonist [³H]WB-4101 to label the α₁-adrenergic receptor. Five brain regions were studied: rostral hypothalamus, caudal hypothalamus, locus ceruleus, nucleus tractus solitarius, and frontal cortical poles. In comparison to normotensive controls, mature hypertensive rats had a significantly greater density (p < 0.05) of the α₁-adrenergic receptors in the rostral hypothalamus (+11%), caudal hypothalamus (+25%), and frontal cortical poles (+20%). Significantly greater (p < 0.05) α₁-adrenergic receptor density was found in the rostral hypothalamus (+27%), caudal hypothalamus (+60%), and locus ceruleus (+39%) of the young prehypertensive SHR compared with age-matched WKY. These results indicate the presence of altered adrenergic receptor systems in the brains of genetically hypertensive animals and suggest that changes in the receptor systems take place during establishment of the hypertension. (Hypertension 7: 333-339, 1985)

KEY WORDS • α₁-adrenergic receptors • spontaneously hypertensive rats • central nervous system • receptor binding

RECENT evidence indicates that alterations in central nervous system (CNS) catecholaminergic mechanisms may be related to the development of hypertension in Okamoto-Aoki-derived spontaneously hypertensive rats (SHR). This suggestion is supported by several lines of evidence: (1) Manipulation of CNS areas rich in catecholamines can modify blood pressure more in SHR than in normotensive Wistar-Kyoto control rats (WKY). In this respect, posterior hypothalamic lesions yield larger blood pressure decrements in SHR than in WKY, while electrical stimulation of the posterior hypothalamus yields larger blood pressure increases in SHR than in WKY. (2) Intraventricular administration of the catecholamine neurotoxin 6-hydroxydopamine to young SHR can prevent or delay the development of hypertension. (3) On a cellular level there are several neurochemical differences between the adrenergic systems of the SHR and WKY. In comparison to the WKY, the SHR demonstrate lower hypothalamic norepinephrine (NE) levels, altered hypothalamic tyrosine hydroxylase and dopamine β-hydroxylase activities, and enhanced uptake of NE by hypothalamic vesicles. Thus, there are several abnormalities in the catecholaminergic system of the SHR.

The CNS adrenergic receptors also have been measured in SHR and WKY, but the results thus far have been conflicting. Although the exact reasons for the conflicting results are not clear, in most studies the receptors have been measured in either whole brain or relatively large tissue sections. It is known, however, that receptors often are localized to relatively discrete areas of the CNS. Thus, differences in receptor number may well be lost in large tissue sections. In addition, many investigators have relied primarily on single-point determination for most of their comparisons, giving limited information as to receptor number and affinity. For example, Gheyouche and colleagues both measured α₁-adrenergic receptors in areas of the brain of SHR and WKY but came up with opposite results using single-point analysis of receptor number. In the present study we have measured the number and affinity of α₁-adren-
ergic receptors in discrete areas of brains of SHR and WKY using small tissue sections and multipoint (saturation isotherm) analysis. In addition to multipoint analysis, a careful choice of tests was made for the statistical analysis to increase the precision of parameter estimation (B_{max} and K_{a}). The tissue sections studied were chosen because of the evidence linking each of the areas to blood pressure control mechanisms.\textsuperscript{12-15}

Materials and Methods

Two separate experiments were performed. The α-adrenergic receptors were examined in discrete brain areas of mature SHR and WKY with established hypertension. With the establishment of differences between mature SHR and WKY in receptor number in some areas, similar measurements were done on young prehypertensive SHR and age-matched WKY to explore the developmental importance of the changes.

Animals

Mature (11-week-old) and young (3-week-old) male SHR and age-matched male control WKY were obtained from Charles River Breeding Laboratories (Wilmington, MA). Animals were maintained on a 12-hour light–12-hour-dark lighting cycle (lights on at 0600) with standard laboratory chow and tap water available ad libitum. Room temperature was maintained at 24 ± 2.8°C. All rats were allowed to adjust to these living conditions for at least 1 week before they were killed.

Blood Pressure Measurements

Systolic blood pressure was measured with tail cuff plethysmography under mild restraint in unanesthetized, prewarmed (35°C for 20 minutes) animals on the day before they were killed. The same method was used for both mature and young animals, although a smaller restraining cage and tail cuff were necessary for young animals. The average of three pressure recordings was taken as the systolic tail artery pressure for a given rat. These data confirmed that at 4 weeks of age both the SHR and WKY had tail artery systolic pressures well within the normotensive range (SHR, 134 ± 8 mm Hg versus WKY, 124 ± 4 mm Hg; mean ± SEM). The difference in blood pressure between strains was not statistically significant at this age (p > 0.05). As expected, however, the 12-week-old SHR were distinctly hypertensive (183 ± 6 mm Hg) and had significantly higher pressures (p < 0.01) than did the WKY of the same age (137 ± 13 mm Hg).

Dissection Procedure

Animals were killed by decapitation between 0900 and 1100 hours on the day of the experiment, and five brain areas — frontal cortex, rostral hypothalamus, caudal hypothalamus, locus ceruleus area, and nucleus tractus solitarius area — were immediately removed, weighed, and placed on ice. The cortical section consisted of both frontal poles of the telencephalon anterior to the genu of the corpus callosum. The initial hypothalamic section consisted of tissue from the preoptic areas to the mamillary bodies in the rostral-caudal direction and extended laterally for 2 mm on either side of the midline and 2 mm deep to the surface. This initial section was further divided into rostral and caudal hypothalamic halves by a coronal cut through the middle of the median eminence. The locus ceruleus section consisted of a 3- x 2- x 2-mm block of tissue obtained from the midline at the rostral border of the floor of the fourth ventricle. The fifth section, containing the nucleus tractus solitarius, also consisted of a 3- x 2- x 2-mm piece of tissue dissected from the midline at the caudal border of the fourth ventricle just below the obex. The general anatomical locations of these tissue sections are illustrated in Figure 1.

![Figure 1](http://hyper.ahajournals.org/)

**Figure 1.** Sagittal section of rat brain, 0.5 mm lateral to the midline (modified from Pellegrino et al.\textsuperscript{16}), illustrating tissue sections dissected for binding analysis. Sections include the areas of the nucleus tractus solitarius (NTS), locus ceruleus (LC), caudal hypothalamus (CH), rostral hypothalamus (RH), and frontal cortex (CTX).
cause of the small quantity of tissue available from each rat, it was necessary to pool the tissue from 10 to 15 rats each time the experiment was performed. Consistency of dissection was evaluated by weighing each tissue segment immediately after removal. The variance of tissue weights within a group and the differences in mean tissue weights between strains were compared to assess variability in dissection and differences in brain size with a two-tailed Student’s t test.

Receptor Analysis

The α₁-adrenergic receptors were studied with the α₁-adrenergic receptor antagonist [³H]WB-4101 (³H)2(2,6-dimethoxy) phenoxylethylamino] methylbenzodioxan; specific activity = 24.7 Ci/mmol; New England Nuclear Corp., Boston, MA) according to the procedure of Greenberg and colleagues¹⁷ with minor modifications. In brief, tissue was homogenized in 20 volumes of ice-cold Tris-hydrochloride buffer (50 mM, pH 7.7, at 25°C). The homogenate was then centrifuged for 15 minutes (48,000 g at 0°C), the supernatant discarded, and the tissue rewashed and re-centrifuged as described above. The pellet was resuspended in 20 volumes of Tris buffer, and 3-mg aliquots of tissue were incubated in varying concentrations of [³H]WB-4101. Tissue suspensions were analyzed for protein concentration with a modification of the Lowry method.¹⁸ Specific binding was determined by the addition of phentolamine (10⁻⁶ M, phentolamine hydrochloride, Ciba-Geigy, Newark, NJ). Each experimental preparation was done in duplicate and replicated three or four times, which resulted in some day-to-day variability in estimates of receptor number.

Specific binding data were analyzed in two phases. First, specific binding, expressed as amount of binding per milligram of protein, was analyzed with LIGAND (Biomedical Computing Technology Information Center, Nashville, TN), a computerized, iterative non-linear curve-fitting program.¹⁹ Both single- and multiple-binding-site models were tested with each tissue area to determine which model provided the best fit to the data. In all cases a single-site analysis provided the best fit to the data. Estimates of receptor affinity (Kᵦ) were generated from this program and compared for differences between strains with the two-tailed Student’s t test.

Estimates of receptor number were generated from a second calculation with the use of regression analysis on a reverse reciprocal plot²⁰,²¹ of combined experiments. All data points (both specific binding and bound/free ratio) from a given experiment were transformed to standard scores.²² Normalization of the data in this way permits combination of data from separate runs of the same experiment while accounting for differences in mean and variance of data between different replications. The normalized pooled data were then arrayed in a single reverse reciprocal plot and mathematically fit by a regression line with the method of least squares. Finally the z score of the y-intercept of each regression line was converted to receptor number.²² Such a regression analysis is valid only when a single-binding-site model is used and when the dissociation constants are not different between strains. These criteria were independently verified with LIGAND. Statistical comparison of y-intercepts between strains was performed with t tests.²² Because previous studies suggested adrenergic abnormalities that are likely to result in increased adrenergic receptor number in SHR as compared with WKY, comparisons between strains were made with a one-tailed t test, with p < 0.05 as the criterion for statistical significance.

Results

Central Nervous System α₁-Adrenergic Receptors in Mature (12-week-old) Rats

A comparison of tissue weights in the various tissue sections failed to show any significant difference between strains for any tissue section (Table 1; see p. 336). The small variance within groups is supportive of consistent dissection procedures. There were no significant differences between strains in protein concentration in any of the sections. The receptor binding data for each tissue area from the four experimental replications were combined as described in the Methods section and plotted on a reverse reciprocal plot (bound versus bound/free). An illustrative example of this analysis is presented in Figure 2 for the data from the frontal cortex. The results of the receptor analysis
of all sections are presented in Table 1. No significant differences were found between SHR and WKY in the $K_a$ values as estimated by LIGAND for any of the tissue areas studied. In addition, attempts to fit the data with multiple-site models resulted in no statistical improvement in fit, which suggests that WB-4101 binds to a single site in these tissues.

In contrast, however, there were significantly greater numbers of $\alpha_1$-adrenergic receptors per milligram of protein in the frontal cortex ($p < 0.01$), the rostral half of the hypothalamus ($p < 0.05$), and the caudal half of the hypothalamus ($p < 0.01$) of the SHR when compared with WKY. No differences were seen between SHR and WKY in receptor concentration in either the locus ceruleus or nucleus tractus solitarius areas.

Central Nervous System $\alpha_1$-Adrenergic Receptors in Prehypertensive (4-week-old) Rats

The results of the receptor analysis in the mature SHR and WKY prompted an analysis of receptor status in young prehypertensive SHR and age-matched control WKY. Once again there were no significant differences between SHR and WKY in the tissue weights or protein concentrations in the various sections (Table 2). An example of the binding data from the young animals (frontal cortex) is illustrated in Figure 3. LIGAND analyses again revealed that the data were adequately described by a single-site model.

The results of the receptor analysis for all sections are presented in Table 2. A significant difference ($p < 0.05$) in $K_a$ values between SHR and WKY was found only in the nucleus tractus solitarius area. In contrast,
the receptor density in the rostral hypothalamus, caudal hypothalamus, and locus ceruleus area was significantly greater in SHR than in WKY controls (p < 0.05). As there is a statistically significant strain difference in $K_a$ in the nucleus tractus solitarius area, comparison of the receptor density between strains is not valid.

**Discussion**

The results in the older (12-week-old) animals with established hypertension confirm and extend the previous findings of Cantor and co-workers $^9$ and Hellstrand and Engel $^{11}$. In the present study a significant increase in the density of $\alpha_1$-adrenergic receptors in the SHR was found in both caudal and rostral sections of the hypothalamus, as well as in frontal cortex, based on a saturation analysis. Cantor and colleagues $^9$ previously reported similar findings for whole hypothalamus using both single-point and saturation analysis, while Hellstrand and Engel $^{11}$ reported a trend toward an increase (p < 0.10) in whole hypothalamus using only single-point analysis. Hellstrand and Engel $^{11}$ also found a significant increase (p < 0.05) in $\alpha_1$-adrenergic receptors in the cortex, a result replicated in this study using saturation analysis.

In contrast to these findings, Gheyouche and colleagues, $^{10}$ using only single-point analysis, did not find any change in the number of $\alpha_1$-adrenergic receptors in the hypothalamus but did report an increase in the density of $\alpha_1$-adrenergic receptors in the pons and midbrain of hypertensive 16-week-old animals. These latter findings were not confirmed in this study in hypertensive animals in specific lower brain areas involved in the regulation of blood pressure, such as the nucleus tractus solitarius and locus ceruleus, nor were they confirmed by either Cantor and associates $^9$ or Hellstrand and Engel $^{11}$ in pons-midbrain or brain stem analyses respectively. These conflicting results may be due to subject variability related to the sources of the animals used in the various studies, as no two studies obtained animals from the same source. Alternatively, circadian fluctuations of $\alpha_1$-adrenergic receptor number $^{23}$ may have contributed to the inconsistencies. Unfortunately, previous studies have not identified the point in the light-dark cycle at which the animals were killed. In any case, the preponderance of the evidence from this and other studies supports the finding of an increase in $\alpha_1$-adrenergic receptors in both rostral and caudal areas of the hypothalamus and in the cortex of the SHR with established hypertension.

In young (4-week-old) prehypertensive animals a significant increase in $\alpha_1$-adrenergic receptors was found in the caudal and rostral hypothalamic sections (as in the older rats), as well as in the locus ceruleus area (all areas with dense catecholamine innervation and demonstrated roles in blood pressure regulation), $^7$ $^9$ $^{11}$ but not in the frontal cortex. Using a single-point analysis, Cantor and associates $^9$ had previously reported a significant increase (p < 0.05) in $\alpha_1$-adrenergic receptors in whole hypothalamus of the prehypertensive SHR, while Morris and co-workers $^{24}$ using
a multipoint saturation analysis, did not find an increase in $\alpha_1$-adrenergic receptor number in whole hypothalamus, brain stem, or cortex of the prehypertensive SHR. It is possible that $\alpha_1$-adrenergic receptor changes are highly localized in the hypothalamic and brain stem regions of the prehypertensive SHR and that analysis of larger tissue sections obscures the differences.

A comparison of the results between prehypertensive and hypertensive animals suggests that relative changes occur in receptor density in specific areas during the development of the hypertension. Compared with control WKY, prehypertensive SHR had increased $\alpha_1$-adrenergic receptor number in the locus ceruleus area as well as rostral and caudal hypothalamus, whereas SHR with established hypertension differed from control WKY in both rostral and caudal hypothalamus as well as cortex, but not in locus ceruleus or nucleus tractus solitarius. The importance of such changes is not entirely clear, but the data suggest several possibilities. A change in receptor number in the frontal cortex may be secondary to the development of hypertension, as it appears only after blood pressure has increased. Increases in receptor number in locus ceruleus and nucleus tractus solitarius areas may be part of the development of hypertension but probably do not play a role in its maintenance, as the strain differences are not apparent in older animals. Finally, strain differences in $\alpha_1$-adrenergic receptor number in the hypothalamus may be important to both generation and maintenance of high blood pressure because they are seen in both prehypertensive and chronic phases. Such suggestions are speculative but worth pursuing, although, of course, it is possible that the differences found at different ages merely reflect sampling fluctuations.

The mechanisms behind the observed strain differences in $\alpha_1$-adrenergic receptor number are also of interest because the results are consistent with the hypothesis that functional abnormalities of central catecholaminergic systems may contribute to the development of hypertension in the SHR. There are at least two possibilities to explain the regional increases in $\alpha_1$-adrenergic receptor number in SHR. The rate of synthesis of $\alpha_1$-adrenergic receptors may be one of the primary genetic mechanisms altered in the SHR. Alternatively, the changes in receptor number may be secondary to changes in function of the catecholamine synapse, which are also, ultimately, genetic. Specifically, the increases in $\alpha_1$-adrenergic receptor number seen in hypothalamic tissue may reflect an "up-regulation" of receptor number secondary to decreased NE levels in the synapse. In this respect, Saavedra and coworkers have reported that NE levels in rostral hypothalamic areas (anterior, periventricular, and paraventricular nuclei) are decreased in both prehypertensive (4-week-old) and hypertensive (14-week-old) SHR. As dopamine beta-hydroxylase activity is also decreased in these areas, Saavedra and associates suggested that activity of NE neurons in rostral hypothalamic areas of the SHR is lower than in WKY.

Therefore, increased numbers of $\alpha_1$-adrenergic receptors in this area may represent a compensation for decreased noradrenergic activity. This concept is further supported by evidence indicating that, when stimulated by microinjection of NE, rostral hypothalamic areas have a depressor effect on blood pressure. Thus, hypoactivity of the area is consistent with hypertension in the SHR. Interestingly, in locus ceruleus and nucleus tractus solitarius of mature SHR — where we found $\alpha_1$-adrenergic receptor number not to differ from WKY — Saavedra and colleagues also found NE levels to be equivalent between the two strains.

Evidence of elevated numbers of $\alpha_1$-adrenergic receptors in cortex is not limited to the spontaneously hypertensive rat model of hypertension. Using Scatchard analysis Yamada and co-workers found increased $\alpha_1$-adrenergic receptor number in the cortex of rats with established deoxycorticosterone acetate (DOCA) hypertension. Using single-point analysis they found no significant difference in $\alpha_1$-adrenergic receptor concentration in such large tissue areas as hypothalamus/thalamus, midbrain, cerebellum, or brain stem, but did find alterations in both $\alpha_1$-adrenergic and $\beta$-adrenergic receptor numbers in peripheral tissues. As they compared only DOCA rats with established hypertension with normotensive controls, little can be said about the developmental role of the cortical changes. The finding of changes in cerebral cortical $\alpha_1$-adrenergic receptor number in the nongenetic DOCA model of hypertension, along with the results of the present study in which cortical $\alpha_1$-adrenergic receptor numbers were increased after but not before the onset of hypertension, suggest that the cortical receptor changes do not have a direct genetic basis but may be secondary to other factors. On the other hand, changes in the SHR hypothalamus seem less likely to be secondary effects, as they are apparent both before and after the development of hypertension.

In addition, some controversy exists concerning the specificity of WB-4101 for the $\alpha_1$-adrenergic receptor. This controversy is based primarily on the observation that in rabbit uterine tissue WB-4101 apparently binds indiscriminately to both $\alpha_1$- and $\alpha_2$-adrenergic receptors. This lack of selectivity does not appear to extend to neural tissue, for which the evidence indicates that WB-4101 is specific for the $\alpha_1$-adrenergic receptor both in various neural structures as well as in tissue from several species. This evidence is consistent with our failure to detect multiple-binding sites in the current analysis and indicates that WB-4101 binds to a single $\alpha_1$-adrenergic receptor site.

In summary, the results of this study suggest a greater density of $\alpha_1$-adrenergic receptors in several areas of the CNS of SHR as compared with their normotensive controls. As elevated $\alpha_1$-adrenergic receptor number is also found in some CNS areas of young prehypertensive SHR, the changes do not appear to be secondary to high blood pressure. Greater $\alpha_1$-adrenergic receptor density, considered in relation to other reports of decreased catecholamine transmitter levels and decreased activity of related synthetic enzymes, suggests
decreased catecholaminergic function in areas of the CNS of the SHR.

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