Evidence of an increase in brain postsynaptic α_1-receptors in spontaneously hypertensive rats

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Brain catecholaminergic neurons have been implicated in the development and maintenance of genetic hypertension in the rat, and brain catecholaminergic metabolic pathways in genetically hypertensive rats have been extensively investigated (De Champlain et al 1967; Chalmers & Wurtman 1971; Saavedra et al 1976; Renaud et al 1978). However, possible changes in postsynaptic receptors have yet to be described, even though many antihypertensive agents like clonidine, α-methyldopa or β-blockers affect the α- and β-receptors of noradrenergic neurons.

Since direct labelling of α- and β- central postsynaptic receptors has been reported (Greenberg et al 1976; Bylund & Snyder 1976) in the present study we have compared the regional distribution of (−)-[^{3}H]dihydroalprenolol (DHA) and [^{3}H]WB 4101 binding in 7 week and 16 week old hypertensive and normotensive rats. We used the Okamoto Aoki strain of spontaneously hypertensive rats (SH rats) (Okamoto & Aoki 1963), which represents one of the most widely accepted animal models, essentially because the WKY (normotensive) rats can be used as controls since they were selected from the same parent strain.

[^{3}H]-2-[(2′,6′-Dimethoxy) phenoxyethylamino] methylbenzodioxan ([^{3}H]WB 4101 specific activity 25·4 Ci mmol^{-1}) and [^{3}H]dihydroalprenolol ([^{3}H]DHA specific activity 41·3 Ci mmol^{-1}) were purchased from N.E.N.

Male SH and WKY rats (Charles River, France) had their systolic blood pressure measured by the tail cuff method without anaesthesia by a B.P. recorder 8002 after prewarming at 30°C (Gerold & Tschirky 1968) and 24 h later they were decapitated, the brains rapidly removed and the following regions were quickly dissected: cortex, cerebellum, medulla, pons, hypothalamus, striatum, midbrain, hippocampus. The brain regions from 2 rats were pooled. [^{3}H]WB 4101 binding was determined according to Greenberg et al (1976). The [^{3}H]WB 4101 concentration was 1 nM, incubation time 15 min at 25 °C and (−)-noradrenaline (100 μM) was used for the non-specific binding. [^{3}H]DHA binding was determined according to Banerjee et al (1977). The [^{3}H]DHA concentration was 4 nM, incubation time 20 min at 25°C and (±)-propranolol (100 μM) was used for the non-specific binding.

Results and discussion. The two age groups selected represent different stages in the development of hypertension: the seven-week age group represents the period before the onset of hypertension which is established at 16 weeks. Systolic blood pressure of SH rats was 154 ± 3 mm Hg at 7 weeks and 214 ± 4 mm Hg at 16 weeks. At the same ages that of their control (WKY) was 147 ± 4 mm Hg and 135 ± 2 mm Hg respectively.

Specific [^{3}H]DHA binding to brain membranes from SH and WKY rats is shown in Table 1. No difference was observed between strains at 16 weeks. We have also compared [^{3}H]DHA binding in 7-week old SH and WKY rats because β-blockers delayed the development of hypertension in young SH rats but had little effect in adult SH rats (Kubo et al 1977; Ruskoaho & Karppanen 1977). Again no difference was observed. Moreover no variation was seen for each strain as a function of time. However, marked differences existed from one brain region to another. The highest density of binding sites occurred in the striatum and cerebral cortex and the lowest in the medulla and pons. These results agree with

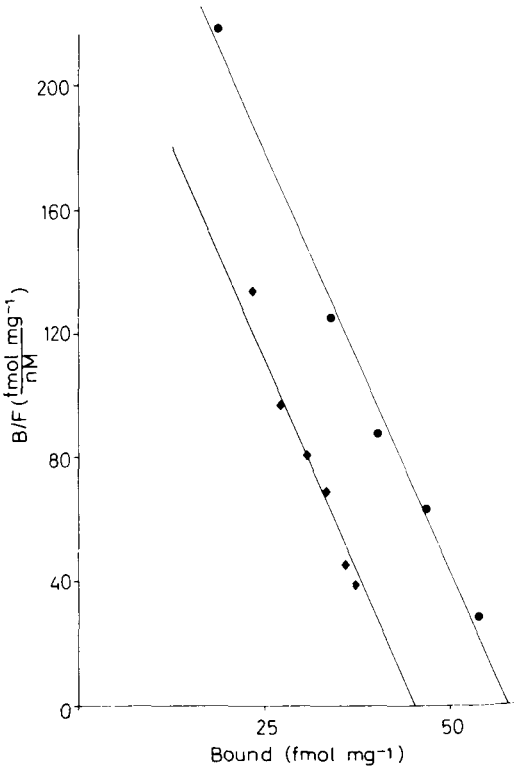

FIG. 1. Scatchard plot of the specific binding of [^{3}H]WB 4101 in midbrain membranes of SH (●) and WKY (◆) rats of 16 weeks. Each saturation curve was determined from pooled midbrain of 15 rats, concentrations of [^{3}H]WB 4101 were from 0·1 to 2 nM.

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Table 1. Comparative regional distribution of specific (−) [^{3}H]dihydroalprenolol binding to brain membranes from SH and WKY rats. Results are expressed as [^{3}H]DHA bound in fmol mg^{-1} protein. Each determination was carried out on the pool of 2 rats and values represent the mean of 8 to 10 determinations in triplicate.

Age		Cortex	Cerebellum	Medulla	Pons	Hypothalamus	Striatum	Midbrain	Hippocampus
16 weeks	SHR	69±4	67±3	30±2	33±3	39±4	76±4	34±2	39±2
	WKY	73±3	63±4	26±2·5	33±2	35±3	77±5	35±3	39±2
7 weeks	SHR	65±5	57±3	29±2·8	31±2	37±2	80±2	32±2	37±2
	WKY	64±5	59±5·8	28±1	28±1	37±2	78±4	31±2	34±3

those found by Bylund & Snyder (1976) in Sprague-Dawley rat brain: Scatchard analysis of saturation curves of [^{3}H]DHA binding indicated a single binding component in the whole brain of SH and WKY rats. No variation of dissociation constant (K_D) or of the maximum specific binding (B_{max}) was observed. At 16 weeks K_D were 2·2 ± 0·2 nM, 2·2 ± 0·2 nM and B_{max} 104 ± 6 fmol mg^{-1} protein, 106 ± 4 fmol mg^{-1} protein for SH and WKY rats respectively. At 7 weeks K_D were 2·3–0·2 nM, 2·6 ± 1 nM and B_{max} 139 ± 7 fmol mg^{-1} protein, 146 ± 8 fmol mg^{-1} protein for SH and WKY rats respectively.

The brain regional distribution of [^{3}H]WB 4101 specific binding was very different from that of [^{3}H]DHA (Table 2). For instance, hypothalamus which contained (with cerebral cortex and hippocampus) the highest density of [^{3}H] WB 4101 binding sites, had one of the lowest densities of [^{3}H]DHA binding sites. At 16 weeks a significant increase in [^{3}H]WB 4101 binding sites in three parts of the brain was observed in SH versus WKY rats (Table 2). The specific binding of [^{3}H]WB 4101 increased by 31% in pons, 22% in midbrain and 21% in hippocampus. In order to determine whether the increased binding of [^{3}H]WB 4101 in SH versus WKY rats was due to an increased number of binding sites or to a change in affinity of the binding sites, midbrain samples of rats were incubated with concentrations of [^{3}H]WB 4101 ranging from 0·1 to 2 nM. The data were analysed by the method of Scatchard (1949); a representative analysis is shown in Fig. 1. The analysis of the saturation curve indicated a single binding component. No significant difference between K_D values from SH and WKY rats occurred (K_D = 0·17 nM). In contrast the B_{max} (i.e. the X intercept on a Scatchard plot) increased in SH rats (B_{max} = 58 fmol mg^{-1} protein) compared with the value obtained in WKY rats (B_{max} = 43 fmol mg^{-1} protein). Thus the increase in [^{3}H]WB 4101 binding appears to be due to an increased number of α-adrenoceptors (35%) in this area rather than a change in binding affinity. To test for cooperativity we plotted these data according to the Hill equation. A straight line was obtained with a Hill coefficient of 1·11 and 1·06 for SH and WKY rats respectively suggesting the absence of positive or negative cooperative effects within the concentration range studied.

Two types of postsynaptic α-adrenoceptors have been found by binding studies (Berthelsen & Pettinger 1977; U'Prichard & Snyder 1978). α_1 receptors are labelled by [^{3}H]WB 4101, α_2 by [^{3}H]clonidine. No variation in the number of α_2-receptors (i.e. specific [^{3}H]clonidine binding sites) in SH compared with WKY rats has been detected (unpublished results).

In this study we have observed an increase in the number of α_1-postsynaptic adrenoceptors in the brain of SH rats but not of α_2- or of β-postsynaptic adrenoceptors.

Such results could suggest that selective interaction with α_1-receptors by drugs like prazosin (U'Prichard et al 1978) could constitute a progress in the treatment of hypertension. Since the concentrations of noradrenaline required for stimulation of postsynaptic α-adrenoceptors are about 100 times higher than those necessary to stimulate the postsynaptic β-receptors (Alder-Graschinsky & Langer 1975) the increase in α_1-receptors in SH rats could not be attributed to presynaptic events leading to a decreased noradrenaline concentration in the synaptic cleft. However it is not clear if the increase in the number of α_1-receptors in SH rats is involved in the etiology of the hypertension or only a consequence of other neurochemical events.

Table 2. Comparative regional distribution of specific [^{3}H]WB 4101 binding to brain membranes from SH and WKY rats of 16 weeks. Results were expressed as [^{3}H]WB 4101 bound in fmol mg^{-1} protein. Each determination was carried out on the pool of 2 rats and values represent the mean of 10 determinations in triplicate. *$P < 0.05$ **$P < 0.001$.

	Cortex	Cerebellum	Medulla	Pons	Hypothalamus	Striatum	Midbrain	Hippocampus
SHR	85±5	45±5	41±3	38±2**	63±2	43±3	50±1**	64±4*
WKY	82±7	38±5	40±3	29±1	60±6	45±3	41±1	53±2

We wish to thank Boehringer for a donation of [^{3}H]clonidine.

November 9, 1979

Note added in proof: During the preparation of the manuscript U'Prichard, Greenberg and Snyder published an increase in B_{max} of [^{3}H]WB 4101 but not of [^{3}H]clonidine in the whole brain of SHR. (Nervous System and Hypertension, P. Meyer and H. Schmidt, ed., Wiley Flammarion, Paris, 1979, pp. 31–48).

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Effects of chronic heat exposure on drug metabolism in the rat

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Chronic exposure of rats to high ambient temperature may cause a variety of pathophysiological changes such as lowering basal metabolism (Chaffee & Roberts 1971) and diminution of liver weight (Ray et al 1968). Little is known, however, about the possible effects of chronic heat exposure on the disposition of drugs in animals or man. A few studies have shown increased drug toxicity following heat exposure (Hovevy-Sion & Kaplanski 1979; Keplinger et al 1959). A possible cause for the increased toxicity, may be the reduced rate of drug metabolism. We have found that in vitro hepatic *N*-demethylation of *p*-chloro-*N*-methylaniline and aniline hydroxylase activities in chronically heat exposed rats are substantially reduced (Kaplanski & Ben-Zvi 1980). The present study was therefore undertaken to investigate the rate of oxidative drug metabolism in vivo in chronically heat exposed rats.

Materials and methods. Male Charles River rats, 8 weeks old, were housed two per cage for at least 30 days in one of two rooms: a control room kept at 22±2 °C and 40% relative humidity and a hot room kept at 35± 2 °C and 20–30% R.H. Both rooms were illuminated from 5 a.m. until 7 p.m. Rat chow and water were freely available.

Hexobarbitone (Sigma, St. Louis, Missouri, U.S.A.) was dissolved by dropwise addition of 1M NaOH and injected intraperitoneally at a dose of 100 mg kg^{-1} (20 mg ml^{-1}). On day 31 of heat exposure, sleeping time was recorded as the time during which the righting reflex was absent.

[^{14}C] Antipyrine (50 Ci mmol^{-1}, The Radiochemical Centre Amersham, U.K.) was diluted with antipyrine (BDH Chemicals Ltd, Poole, U.K.) and injected intraperitoneally at a dose of 15 mg kg^{-1} (12·53 Ci mmol^{-1}) on days 32 and 34 of heat exposure. Samples of blood were withdrawn from the tail of each rat under light ether anaesthesia. Antipyrine blood concentration was determined after extraction according to Bakke et al (1974). Antipyrine half-life time ($t_{\frac{1}{2}}$) was estimated from least squares regression analysis of the log blood concentration of antipyrine versus time in the elimination phase. Apparent volume of distribution (aVd) was calculated according to Aarbakke et al (1978), assuming complete absorption of antipyrine after an intraperitoneal injection. Metabolic clearance rate (MCR) was calculated according to the formula

$$MCR = \frac{aVd \times \ln 2}{t_{\frac{1}{2}}} \quad .. \quad .. \quad .. \quad (1)$$

Rectal temperatures were recorded just before injection of hexobarbitone or antipyrine in the respective experiments.

All drugs were injected between 8 and 10 a.m. to avoid possible variation related to circadian rhythm. Statistical evaluation of the data was carried out using the Students *t*-test.

Results. Table 1 shows that the biological half-life time of antipyrine was almost doubled in heat exposed rats

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