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EFFECTS OF CHRONIC β-BLOCKER TREATMENT ON CATECHOLAMINE LEVELS IN SPONTANEOUSLY HYPERTENSIVE RATS

MICHÈLE HEIMBURGER, LUC DENOROY,*‡ BERNARD RENAUD,† JEAN SASSARD,* YVES COHEN and JACQUES WEPIERRE

Laboratoire de Pharmacologie, ERA CNRS No. 627, Faculté de Pharmacie, 92290-Chatenay-Malabry, France; *Département de Physiologie et Pharmacologie Clinique, ERA CNRS No. 894, Faculté de Pharmacie, 8 Avenue Rockefeller, 69008-Lyon, France; † Laboratoire de Neuropharmacologie, Faculté de Pharmacie, 69008-Lyon, France

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Abstract—In the present work the effects of a 55-day oral treatment with two β-blocking agents (propranolol 40 mg/kg per day and S 2395 20 mg/kg per day) on the catecholamine (CA) content of central and peripheral structures were studied in spontaneously hypertensive rats (SHR). The concentrations of dopamine (DA), noradrenaline (NA) and adrenaline (A) in different structures dissected out from treated and control SHR were measured by a radioenzymatic method.

At the peripheral level, no change in the concentration of NA (in the heart) or A (in the adrenal medulla) was observed. Propranolol increased the DA concentration in the C1 and C2 regions of the medulla oblongata and S 2395 increased the DA concentration only in the C2 region. In these two areas, the NA and A levels were unchanged. Both propranolol and S 2395 increased the DA, NA and A content in the locus coeruleus and in the anterior hypothalamus. On the contrary, there was no modification in the posterior hypothalamus.

The anatomical specificity of these alterations of the CA levels suggests that they could be related to a specific action of β-blockers on central catecholaminergic structures in SHR which might be linked to the antihypertensive effects of these drugs.

It has been suggested from several experimental studies that the antihypertensive effect of the β-blocking agents could be due to a decrease in the sympathetic tone resulting from an action on vasomotor centres in the brain [1–4]. As central catecholaminergic neurons are involved in the cardiovascular control and in the development of various models of experimental hypertension [5–8], one can hypothesize that if β-blockers act centrally they could alter the central catecholaminergic systems controlling the blood pressure. Recently we have reported that chronic treatment of young spontaneously hypertensive rats (SHR) with the β-blocking agents propranolol or S 2395 leads to a decrease in blood pressure and to alterations in the activities of catecholamine-synthesizing enzymes both in brain areas involved in the control of blood pressure and in peripheral structures of the sympathetic nervous system [9]. The aim of the present work was to determine if the levels of endogenous catecholamines (CAs) were altered in SHR treated by the same β-blocking drugs under the same conditions as those used previously [9].

MATERIALS AND METHODS

Animals and treatments. Four-week-old male SHR (supplied by IFFA-Credo, Domaine des Oncins, 69 Saint-Germain sur l'Arbresle, France) were used and housed 4–5 per cage in a thermally controlled room (22 ± 2°). They received a standard laboratory diet (Charles Rivers) and tap water *ad libitum*. For 8 weeks, the animals received orally, between 9.00 and 10.00 a.m., 40 mg/kg per day of *dl*-propranolol hydrochloride (ICI Pharma) or 20 mg/kg per day of S 2395 [*dl*-(hydroxy-2′-*t*-butylamino-3′-propyloxy)-8-thiochromane hydrochloride, Institut de Recherches Internationales Servier, Neuilly, France]. Control animals received saline. The drugs were administered through oesophageal intubation, under a volume of 0.5 ml/100 g. The dosage of S 2395 was chosen to be equipotent with respect to its antihypertensive properties to that of propranolol (40 mg) as indicated by previous pharmacological studies [10] and preliminary experiments.

Blood pressure, heart rate and body weight measurements. The systolic blood pressure was measured weekly between 2 and 4 hr following administration of the drugs, by a tail cuff plethysmographic method without anaesthesia (Narco Biosystems). Rats were prewarmed in a box at 38 ± 1° for 10 min. Heart rate was calculated from the pulse record. Body weight was also measured the same day as blood pressure.

Dissection. At the end of the treatment, the animals were killed by decapitation. The heart and the adrenal glands were dissected out. The brain was removed and quickly frozen as previously described [9]. The following brain areas—locus coeruleus (containing mainly the A6 group, Dahlström and Fuxe [11]), C1 and C2 regions [12]—were dissected out according to previously described modifications [13,

‡ To whom correspondence should be addressed.

14] of the method of Palkovits [15]. The hypothalamus was dissected out in three regions—median eminence, anterior hypothalamus and posterior hypothalamus—as described by Denoroy *et al.* [9].

Determination of catecholamine levels. Heart and adrenal glands: The heart or the adrenal glands were homogenized in 10 ml of ice-cold 0.4 M $HClO_4$ containing 10 μM ethylenediamine tetraacetic acid (EDTA) and 21 mM $Na_2S_2O_5$. The catecholamines were absorbed on alumina [16] and the noradrenaline (NA) of the heart and the adrenaline (A) of the adrenal glands were determined fluorimetrically [17]. *Brain regions:* The tissues were homogenized in 850 μl of ice-cold 0.2 M $HClO_4$ containing 5.4 mM EDTA and 10 mM $Na_2S_2O_5$. After centrifugation (20,000 *g*; 20 min), the catecholamines were assayed by a modification of a radioenzymatic procedure [18, 19]. Homogenate (350 μl) was added to 100 μl of the following mixture: 50 μl of 1.8 M Tris–HCl buffer, pH 9.5; 10 μl of 200 mM $MgCl_2$; 10 μl of 0.1 mM dithiothreitol; 25 μl of rat liver catechol-*O*-methyltransferase, partially purified [20]; and 5 μl (2.5 μCi) of *S*-adenosyl methionine methyl [^{3}H] (specific radioactivity 10 Ci/mmole, New England Nuclear). The incubation was carried out at 37° for 60 min and stopped by the addition of 150 μl of 1 M borate buffer, pH 8.0. Fifty μl of 44 mM sodium tetraphenylboron, 50 μl of a mixture of cold methoxytyramine, normetanephrine and metanephrine (0.05% w/v in 0.01 N HCl) and 10 ml of diethyl ether were then added. After shaking and centrifugation, 9 ml of the organic layer was removed and added to 0.5 ml of 0.1 N HCl. After shaking and centrifugation the organic layer was discarded. The aqueous layer was washed with 5 ml of *n*-butylacetate and evaporated to dryness. The residue was redissolved in 2 × 40 μl of a mixture of methanol and 1 mM HCl (4v/1v) and laid on a silica gel fluorescent thin-layer chromatography plate (Whatman LK 5DF). The chromatography was run with the following solvent: *t*-amyl alcohol–benzene–35% methylamine aqueous solution (6v/2v/3v). After drying, the spots containing [^{3}H]methoxytyramine, [^{3}H]normetanephrine or [^{3}H]metanephrine were localized under UV light and scraped. The [^{3}H]methoxytyramine was eluted by adding 1 ml of 0.5 M HCl and its radioactivity determined by scintillation counting after addition of 14 ml of Picofluor (Packard). The [^{3}H]metanephrine and the [^{3}H]normetanephrine were eluted by adding 1 ml of 0.05 M NH_4OH and then converted to [^{3}H]vanillin by addition of 50 μl of 4% $NaIO_4$, followed 5 min later by the addition of 50 μl of 10% glycerol (v/v). After acidification with 1 ml of 0.1 M acetic acid, addition of 10 ml of toluene containing 0.5% (w/v) of Permablend (Packard) and vigorous shaking, the radioactivity was determined by scintillation counting.

Protein determination. Total proteins were estimated on an aliquot of the redissolved protein precipitate according to the method of Lowry *et al.* [21] using ovalbumin (Merck) as a standard.

Statistical analysis. Results are expressed as mean ± S.E.M. of absolute values. Values obtained in treated rats are also expressed as percentage of those observed in control rats. Further statistical analysis used the Student's *t*-test for unpaired data.

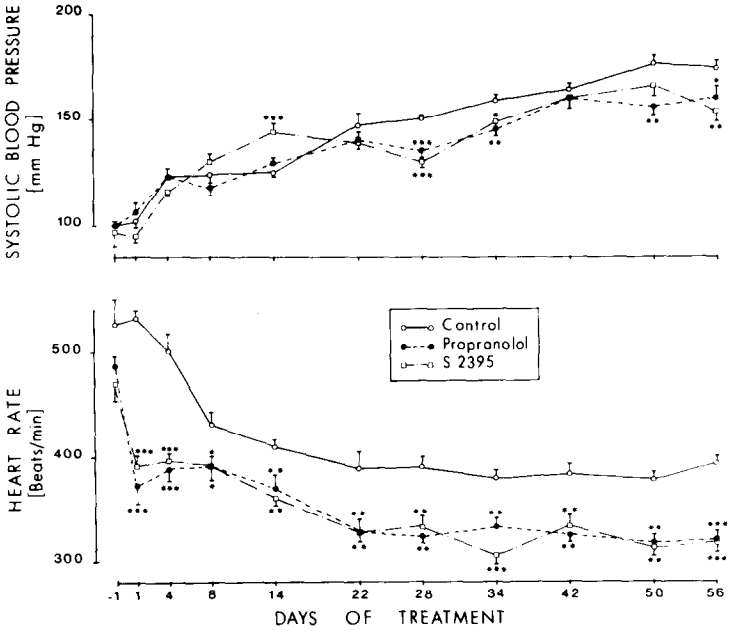

Fig. 1. Effects of chronic oral administration of S 2395 (20 mg/kg per day) or of propranolol (40 mg/kg per day) on systolic blood pressure and heart rate in SHR. The values at day '-1' correspond to a pretreatment measurement. The values are expressed as mean ± S.E.M. Significant differences from controls: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. The number of animals was: controls, 9; S 2395, 10; propranolol, 8.

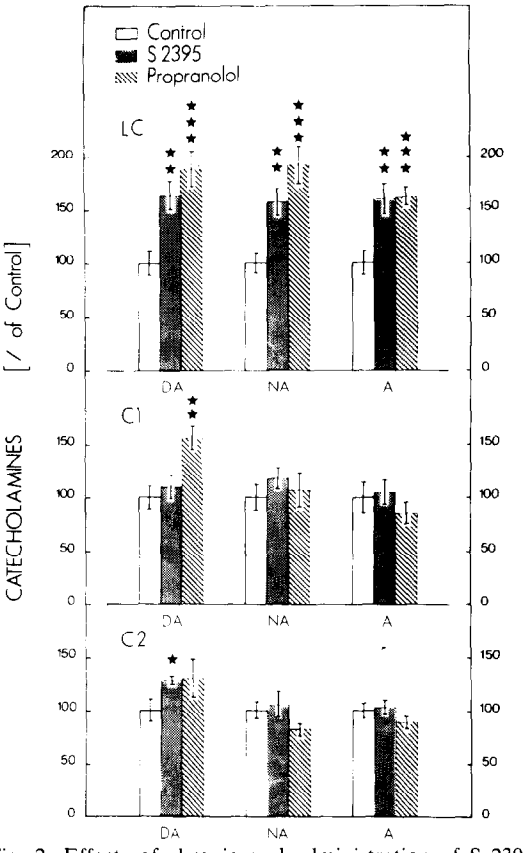

Fig. 2. Effects of chronic oral administration of S 2395 (20 mg/kg per day) or of propranolol (40 mg/kg per day) on catecholamine levels in pons-medulla nuclei of SHR. The levels are expressed as percentage (mean ± S.E.M.) of the control (untreated) SHR. Statistical differences from controls are indicated: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. The number of animals in each group is the same as in Fig. 1. The absolute values in control SHR are indicated below and expressed as ng per mg of protein. Dopamine (DA) levels were (a) locus coeruleus (LC), 1.05 ± 0.12; (b) C1 region, 0.48 ± 0.05; (c) C2 region, 1.33 ± 0.15. Noradrenaline (NA) levels were: (a) locus coeruleus, 9.50 ± 0.89; (b) C1 region, 3.48 ± 0.43; (c) C2 region, 10.19 ± 0.80. Adrenaline (A) levels were: (a) locus coeruleus, 0.190 ± 0.022; (b) C1 region, 1.850 ± 0.260; (c) C2 region, 0.380 ± 0.027.

RESULTS

Systolic blood pressure and heart rate

As shown in Fig. 1, the systolic blood pressure of both propranolol and S 2395-treated SHR did not differ from that of control SHR until the 22nd day of treatment, except for a significant increase in S 2395-treated SHR on the 14th day. On the contrary, at the 28th day of treatment the systolic blood pressure of both propranolol and S 2395-treated SHR became significantly lower than that of controls. This difference was still present on the day the animals were killed.

The heart rate was immediately reduced by propranolol or S 2395 and remained significantly lower than that of controls throughout the experiment (Fig. 1). The body weight of the SHR was not altered by either treatment.

Catecholamine levels in peripheral tissues

Noradrenaline level in the heart. No significant difference was found between S 2395 or propranolol-treated SHR (respectively 524 ± 24 and 529 ± 20 ng/g of tissue) and control SHR (493 ± 21 ng/g).

Adrenaline content of the adrenal medulla. No difference was found between S 2395 or propranolol-treated and control SHR (respectively 24.7 ±0.8, 25.1 ± 1.1 and 25.0 + 0.8 μg/organ pair).

Catecholamine levels in nuclei of the pons-medulla (see Fig. 2) and of the hypothalamus (see Fig. 3)

C1 region. While there was no change in the NA or A level, a significant increase in the dopamine (DA) level (+57%, $P < 0.01$) was observed in propranolol, but not in S 2395-treated SHR as compared to controls.

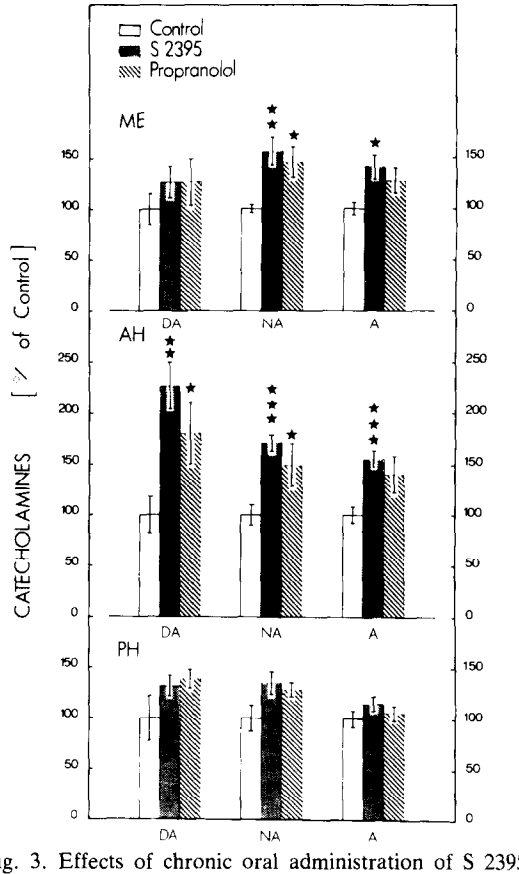

Fig. 3. Effects of chronic oral administration of S 2395 (20 mg/kg per day) or propranolol (40 mg/kg per day) on catecholamine levels in hypothalamic regions of SHR. The levels are expressed as percentage (mean ± S.E.M.) of the control (untreated) SHR. Statistical differences from controls are indicated: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. The number of animals in each group is the same as in Fig. 1. The absolute values in control SHR are indicated below and expressed as ng per mg of protein. Dopamine (DA) levels were: (a) median eminence (ME), 5.54 ± 0.82; (b) anterior hypothalamus (AH), 0.76 ± 0.14; (c) posterior hypothalamus (PH), 1.36 ± 0.30. Noradrenaline (NA) levels were: (a) ME, 11.19 ± 0.49; (b) AH, 9.09 ± 0.94; (c) PH, 11.00 ± 1.50. Adrenaline (A) levels were: (a) ME, 0.919 ± 0.059; AH, 0.417 ± 0.036; (c) PH, 0.619 ± 0.051.

C2 region. The DA levels were increased in both groups of treated SHR, but the difference was only significant in S 2395-treated SHR (+29%, $P < 0.05$). The NA and A levels were unchanged in this area.

Locus coeruleus. An increase in the DA levels was found in both S 2395 and propranolol-treated SHR (respectively +65%, $P < 0.01$ and +89%, $P < 0.001$). The NA levels were also increased in both S 2395 and propranolol-treated SHR (respectively +57%, $P < 0.01$ and +93%, $P < 0.001$) like the A levels (S 2395 +60%, $P < 0.01$, propranolol +62%, $P < 0.001$).

Median eminence. While no significant change was found in the DA level, the NA levels were increased in both S 2395 and propranolol-treated SHR (respectively +56%, $P < 0.01$ and +46%, $P < 0.05$). The A level was found to be higher in S 2395 (+40%, $P < 0.05$) but not in propranolol-treated SHR.

Anterior hypothalamus. The DA levels increased in both S 2395 and propranolol-treated SHR (respectively +125%, $P < 0.01$ and +80%, $P < 0.05$). The NA levels increased also in both S 2395 and propranolol-treated SHR (respectively +71%, $P < 0.001$ and +50%, $P < 0.05$). Likewise the A levels were higher in both groups of treated SHR, but the difference was significant only in S 2395-treated SHR (+55%, $P < 0.001$).

Posterior hypothalamus. No significant change in the DA, NA or A level was found in this region.

DISCUSSION

As previously reported [9], a chronic propranolol (40 mg/kg per day) or S 2395 (20 mg/kg per day) treatment induced in SHR a progressive and moderate decrease in systolic blood pressure, and an immediate and sustained bradycardia. This result is in agreement with other work about long-term treatment of SHR by β-blockers [22–24]. In addition, the S 2395 elicited a transient increase in blood pressure at day 14; no explanation has been found for this effect, which was not observed in a previous experiment using similarly treated SHR [9].

At the peripheral level, the A content of the adrenal glands as well as the NA content of the heart was unchanged in S 2395- or propranolol-treated SHR. At first these results do not seem to favour any alteration in the sympathetic tone after a long-term administration of β-blocking agents in SHR. However, it is difficult to interpret an absence of change in the levels of catecholamines in terms of unchanged neuronal activity. Moreover, the present results contrast with other work reporting a decrease in the activity of the sympathetic system in β-blocker-treated rats or rabbits [1, 2, 25–27]. However, in these studies, the functional state of the sympathetic system was assessed by measuring the activity of the catecholamine-synthesizing enzymes or by recording the electrical activity of sympathetic nerves. Therefore, our results reporting CA levels cannot be compared accurately with these earlier studies. In addition, in a previous experiment using similarly treated SHR we found that the activities of the catecholamine-synthesizing enzymes were generally unaltered, except for a significant decrease in dopamine-β-hydroxylase activity within the adrenal medulla, a result which suggested a reduction in the sympathoadrenal tone [9].

At the central level, the changes induced by propranolol could not be explained by its inhibitory effect on the monoamine oxidase [28] since propranolol treatment did not increase the level of the three CAs to the same extent, and exhibited effects restricted to some of the areas studied.

In the C1 and C2 areas of the medulla oblongata, propranolol increased the DA but not the NA or A levels, a result which is consistent with the elevated tyrosine hydroxylase activity that we observed in these regions in similarly treated SHR [9]. In the locus coeruleus, treatment by S 2395 or propranolol induced an increase in the concentrations of DA, NA and A. This region does not contain cell bodies of adrenaline neurons, but only terminals originating from the C2 group of the medulla oblongata. As no change in A concentration was found in the C2 group, this could suggest that propranolol has different effects on the A content of adrenaline neurons at the terminal and at the cell body levels.

The same discrepancy in changes in A concentration between the terminals and the perikarya was also observed for the anterior hypothalamus and the median eminence which contain terminals of adrenaline neurons originating from the C1 and C2 groups and where the A (and DA and NA) concentration was found to be increased in β-blocker-treated rats. On the contrary, no change in A concentration was found in the posterior hypothalamus, another region receiving an innervation by adrenaline neurons. The anatomical specificity of this effect is interesting since it is known that the anterior and the posterior parts of the hypothalamus play an opposite role in the control of blood pressure [29–31]. In addition, we have reported a decrease in phenylethanolamine-*N*-methyltransferase activity in the anterior part (and in the median eminence), but not in the posterior part of the hypothalamus of similarly treated SHR [9]. It can be suggested that the association of a decreased synthesis capacity with an increased neurotransmitter content might reflect a decrease in the turnover of adrenaline in the anterior hypothalamus of β-blocker-treated SHR.

A chronic treatment of SHR with β-blocking agents leads to alterations in the content of CA in brain areas involved in the blood pressure regulation. These modifications could be compensatory changes due to the blockade of central β-adrenoreceptors, may be without relation to the antihypertensive properties of S 2395 or propranolol. On the other hand, the functional significance of these changes could correspond to: (1) an alteration in the activity of some CA neurons due either to the fall in blood pressure or to a modification of the characteristics of the baroreflex induced by the long-term administration of β-blockers; (2) a primary alteration of CA neurons in the vasomotor centres by β-blockers leading to a decreased sympathetic tone contributing to the fall in blood pressure. It is beyond the scope of this paper to argue for or against one of these possibilities. Further studies are needed to determine precisely the relationships between the central effects of β-blockers and their antihypertensive properties.

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