Biochemical Pharmacology, Vol. 30, No. 19, pp. 2673–2678, 1981.
Printed in Great Britain.

0006-2952/81/192673-06 $02.00/0

EFFECTS OF CHRONIC β-BLOCKERS TREATMENT ON CATECHOLAMINE SYNTHESIZING ENZYMES IN SPONTANEOUSLY HYPERTENSIVE RATS

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(*Received* 2 *February* 1981; *accepted* 15 *April* 1981)

Abstract—In the present work the effects of two β-blocking agents (Propranolol : 40 mg/kg/day and S 2395 : 20 mg/kg/day) on the activity of central and peripheral catecholaminergic (CA) structures were studied in SHR after 55 days of oral treatment. These effects were assessed by measuring the activity of tyrosine hydroxylase (TH), dopamine-β-hydroxylase (DBH) and phenylethanolamine-*N*-methyltransferase (PNMT) in different structures dissected out from treated and control SHR.

At the peripheral level, the only significant change was a decrease in the DBH activity of the adrenal medulla in propranolol-treated SHR. The plasma DBH activity was not altered. In the pons medulla, propranolol and S 2395 increased the TH activity of the A_6, C_1 and C_2 region, and propranolol decreased the DBH activity in the A_6 and the C_2 region. The PNMT activity of these 3 neuronal groups remained unchanged. In the hypothalamus, TH and DBH activity exhibited no consistent changes. On the other hand, the PNMT activity was significantly reduced by propranolol in the anterior hypothalamus and in the median eminence but not in the posterior hypothalamus.

The effects of β-blockers on the activity of central and peripheral CA structures were markedly different from those observed in similar conditions after chronic treatment with hydralazine, a peripheral vasodilator. Therefore, it was concluded that the enzymatic changes observed were not a consequence of the treatment induced decrease in blood pressure, but might more probably reflect a specific action of β-blockers on the central and peripheral CA structures of the SHR.

The β-blocking agents are widely used as antihypertensive agents, but their mechanism of action remains unclear. Among the numerous factors which could explain the fall in blood pressure induced by the blockade of β-adrenergic receptors, a decrease in the sympathetic tone resulting from an action on vasomotor centers in the brain has been proposed by several authors [1–3].

As central catecholaminergic (CA) neurons are involved in the control of blood pressure and in the development of various models of experimental hypertension [4–6], it could be hypothetized that if the β-blockers act centrally, they could alter the central CA systems controlling the blood pressure.

The aim of this work was to determine if a chronic treatment of spontaneously hypertensive rats (SHR) with β-blocking agents might modify the activity of the central CA neurons involved in blood pressure regulation as well as the activity of the peripheral sympathetic nervous system. For that purpose, a biochemical approach was used, i.e. the measurement in these tissues of the CA synthesizing enzymes activities : tyrosine hydroxylase (TH), the rate limiting enzyme in the biosynthesis of CA, the noradrenaline synthesizing enzyme, dopamine β-hydroxylase (DBH) and the adrenaline forming enzyme, phenylethanolamine-*N*-methyltransferase (PNMT). It is well accepted now that these enzymatic activities are closely related to the impulse traffic of the CA neurons [7].

MATERIAL AND METHODS

Animals and treatment. Four week-old male SHR (supplied by IFFA-Credo, Domaine des Oncins, 69 Saint Germain sur l'Arbresle, France) were used. The animals were housed under constant conditions of temperature (22 ± 2°) and lighting (8 a.m.–8 p.m.). They had free access to a standard laboratory diet (Charles Rivers) and tap water.

For 8 weeks, the animals received orally, between 9.00 and 10.00 a.m., 40 mg/kg/day of dl-propranolol hydrochloride (ICI Pharma) or 20 mg/kg/day of S 2395 (DL-hydroxy-2′-*t*-butylamino-3′-propyloxy)-8-thiochromane hydrochloride, Laboratoires Servier, Neuilly, France), a new β-blocker [8]. 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 20 mg was chosen to be equipotent with respect to its antihypertensive properties to that of propranolol 40 mg as indicated by previous pharmacological experiments [8] and Heimburger (personal communication)

Blood pressure, heart rate and body weight measurements. The systolic blood pressure was measured twice weekly by a tail-cuff plethysmographic

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method without anesthesia, using a pneumatic pulse transducer and a programmed electrosphygmomanometer (Narco Biosystems, model PE 300 and DMP4B Physiograph). The animals were prewarmed in a box at 36° for 15 min. Heart rate was determined on pulse recording. Body weight was also measured the same days as blood pressure.

Dissection. At the end of the treatment, the animals were killed between 9.00 and 11.00 a.m. by decapitation. The blood was collected, the adrenal glands and superior cervical ganglia were dissected out and quick-frozen on dry-ice. The brain was removed, sectioned in a vertical frontal plane at the mesencephalic level and the anterior and posterior parts were quick-frozen on dry-ice.

The posterior part of the brain was cut in 500 μm thick, frontal serial sections, and brain nuclei were removed with a hollow needle (0.9 mm i.d.) according to the following modifications of the technique of Palkovits [9] : Locus coeruleus (A_6 group, Dahlström and Fuxe 10) was punched out as previously described [11]; the areas referred as C_1 and C_2 regions [12], were punched from 5 consecutive sections using the posterior limit of the area postrema as a caudal landmark [13].

The anterior part of the brain was cut in 400 μm thick, frontal serial sections and hypothalamic regions were dissected out under a binocular microscope, using the atlas of Jacobowitz and Palkovits [14] as a reference. The region referred as median eminence was preleved with a hollow needle and included the median eminence and the nucleus arcuatus. The posterior hypothalamus was dissected out from the same coronal slices with a scalpel blade and using the dorsal limit of the third ventricle as a landmark : such a prelevement included principally the dorsomedialis, ventromedialis and hypothalamicus posterior nuclei. The anterior hypothalamus was dissected out from the slices rostral to those from which the posterior hypothalamus was dissected; the prelevement included the supraopticus, preopticus, suprachiasmaticus, hypothalamicus anterior, para- and periventricularis nuclei.

The tissues were stored at −80° until the biochemical assays.

Tissue homogenization. The tissues obtained in each rat were disrupted mechanically or by ultrasounds (20 kHz, 40 W, 10 sec) in 2 mM potassium phosphate buffer pH 6.0 containing 0.2 per cent Triton X100 (v/v). The homogenization volume was 300 μl for the C_1, C_2 and A_6 areas (left and right sides pooled) and 100 μl for the median eminence. The anterior and posterior hypothalamus were homogenized in 30 vol. of buffer, the superior cervical ganglia in 500 vol. and the adrenal glands in 200 vol. (diluted to 500 vol. for the DBH and PNMT assays). The homogenates were centrifuged (5000 × *g*, 15 min) at 4° and the supernatants used for the biochemical assays.

Enzymatic assays. The tyrosine hydroxylase activity was determined on 50 μl of supernatant by a modification [13] of the radiometric method of Nagatsu *et al.* [15] using 3,5[^{3}H] tyrosine as a substrate.

The dopamine-β-hydroxylase activity was measured on 10 μl of supernatant by a modification of the method of Molinoff *et al.* [16]. Five microlitres of $CuSO_4$ were added to 10 μl of supernatant (or 5 fold diluted plasma). The concentration of the $CuSO_4$ solution varied among the tissues assayed and was (expressed as 10^{-5} M) : 2.5 for the adrenal medulla, 5 for the superior cervical ganglia, 20 for the locus coeruleus, 25 for the C_1 or C_2 areas, 10 for the median eminence, 100 for the posterior and anterior hypothalamus and 25 for the plasma. Ten microlitres of an incubation mixture were added, containing : 2 μl of 0.5 M sodium fumarate pH 5.5, 2 μl of 22 mM ascorbic acid pH 5.5 (freshly prepared), 1 μl (260 units) of catalase (Boehringer), 1 μl of 8 mM Pargyline (Sigma), 2 μl of 0.4 M sodium acetate buffer pH 5.5 and 2 μl of 12.5 mM tyramine (Sigma). Blanks were made by boiling tissues. The incubation was carried out at 37° for 30 min for adrenal medulla, superior cervical ganglia and locus coeruleus, for 45 min for the C_1 and C_2 areas and 60 min for the hypothalamic structures. The incubation was stopped by cooling in an ice-water bath and 10 μl of a second reaction mixture were added. This reaction mixture contained : 5.5 μl of 1 M Tris–HCl buffer pH 8.6, 1 μl of bovine adrenal PNMT partially purified [17], 2.5 μl of 100 mM sodium ethylenediaminetetracetate and 1 μl (0.5 μCi) of *S*-adenosylmethionine methyl-^{3}H (specific radioactivity 10 Ci/mmole, New England Nuclear). The reaction was carried out at 20° for 5 min and was stopped by cooling in an ice-water bath and by addition of 65 μl of 0.7 M sodium borate buffer pH 10.0. Toluene (900 μl) containing isoamyl alcohol (3v/2v) was added. After shaking and low speed centrifugation, 600 μl of the organic phase was added to a scintillation vial and the liquid was evaporated at 65° under a stream of air. The remaining radioactivity was measured by liquid scintillation after the addition of 300 μl of absolute ethanol and 3 ml of toluene containing 4 per cent of diphenyl oxazole (PPO) and 0.1 per cent of diphenyl oxazolyl benzene (POPOP). The mean blank value was about 1500 cpm. The lowest activity (obtained in the C_1 and C_2 regions) was 4–5 times above the blank value.

The phenylethanolamine-*N*-methyltransferase activity was measured according to a modification of the technique of Saavedra *et al.* [18]. Ten microlitres of a reaction mixture were added to 10 μl of supernatant. This incubation mixture contained 4 μl of 1 M Tris–HCl buffer pH 8.6, 3 μl of bi-distilled water, 2 μl of 4 mM phenylethanolamine (DL-β-hydroxyphenylethanolamine, Sigma) and 1 μl (0.5 μCi) of *S*-adenosylmethionine methyl-^{3}H (specific radioactivity 10 Ci/mmole, New England Nuclear). Blanks were made by omitting phenylethanolamine. The incubation was carried out for 10 min for adrenal glands, 30 min for C_1, C_2 areas and locus coeruleus, and 60 min for the hypothalamic regions. The reaction was stopped by cooling in an ice-water bath and addition of 65 μl of 0.7 M borate buffer pH 10.0 Toluene (900 μl) containing 3 per cent of isoamyl alcohol (v/v) was added. After shaking and low speed centrifugation, 600 μl of the organic phase were drawn, evaporated at 40° under a stream of air and the remaining radioactivity was measured by liquid scintillation as in the DBH assay. The mean blank value was about 200 cpm. The low-

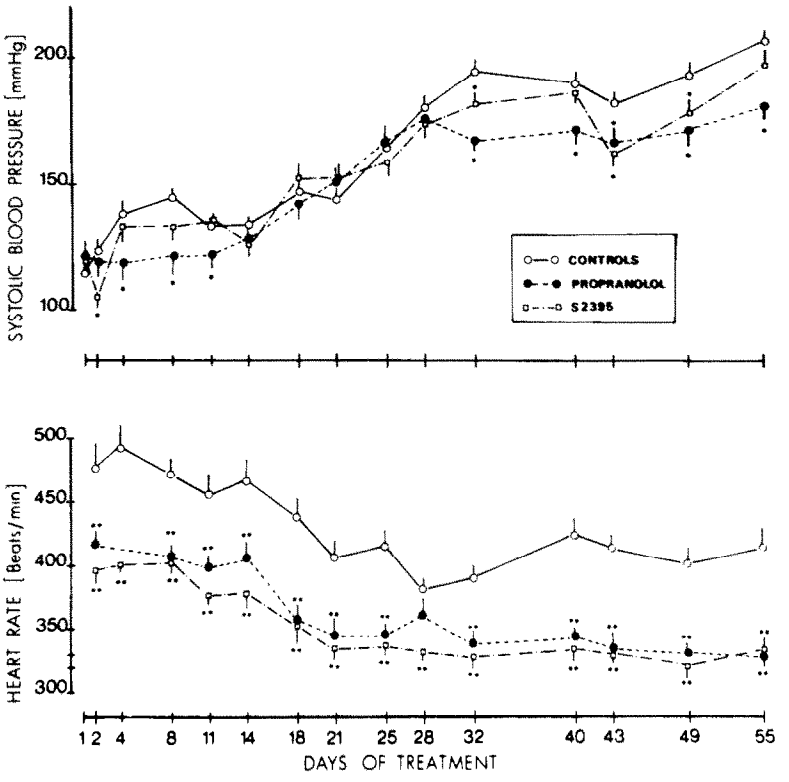

Fig. 1. Effects of chronic oral treatment with propranolol (40 mg/kg/day) and S 2395 (20 mg/kg/day) on systolic blood pressure and heart rate in SHR. Values are expressed as mean ± S.E.M. Significant differences from controls: *P < 0.05, **P < 0.01. The number of animals was: controls, 10; Propranolol, 9; S 2395, 10.

est activity (obtained in the locus coeruleus) was 2–3 times above the blank value.

Protein determination. Total proteins were estimated on an aliquot of supernatant with the Folin phenol reagent method of Lowry *et al.* [19] using bovine serum albumin (Sigma) as a standard.

Statistical analysis. Results were expressed as mean ± S.E.M. of absolute values. As treated and control rats were simultaneously assayed, the values obtained in treated rats were also expressed as percentage of those obtained in controls. Further statistical analysis used the Student's *t*-test.

RESULTS

Systolic blood pressure and heart rate

As shown in Fig. 1, the systolic blood pressure of both propranolol and S 2395 treated SHR became significantly lower than that of controls at the 32nd day of treatment. On the day that they were killed the blood pressure of S 2395-treated rats was not significantly higher than that of propranolol-treated rats. In contrast the heart rate was almost immediately reduced by propranolol and more markedly by 2395. Heart rate remained significantly ($P < 0.01$) lower than that of controls throughout the experiment. The body wt of the SHR was not altered by the treatments.

Enzymatic activities in peripheral tissues (see Fig. 2)

Superior cervical ganglion. No difference in TH or DBH activity was found between treated and control SHR.

Adrenal medulla. While there was no change in TH nor in PNMT activity, a significant decrease (−29 per cent $P < 0.01$) in DBH activity was found in propranolol but not in S 2395-treated SHR as compared to controls.

Plasma DBH activity. No change in plasma DBH activity was found. The activities, expressed as nmoles of octopamine formed/hr/ml of plasma, were : 3.70 ± 0.21 ($n = 10$) in control SHR, 4.15 ± 0.41 ($n = 9$) in S 2395-treated SHR and 3.55 ± 0,24 ($n = 9$) in propranolol-treated SHR.

Enzymatic activities in nuclei of the medulla oblongata (see Fig. 3) and of the hypothalamus (see Fig. 4)

C_1 region. While there was no change in DBH nor in PNMT activities, a significant increase in TH activity (+ 36.2 per cent, $P < 0.01$) was observed in propranolol- but not in S 2395-treated SHR.

C_2 region. The TH activity was found to be increased in both S 2395 and propranolol-treated SHR (respectively + 18.9 per cent, $P < 0.05$ and + 27.3 per cent $P < 0.01$). The DBH activity was found decreased only in propranolol-treated SHR (−16.3 per cent, $P < 0.01$). The PNMT activity was unchanged in this area.

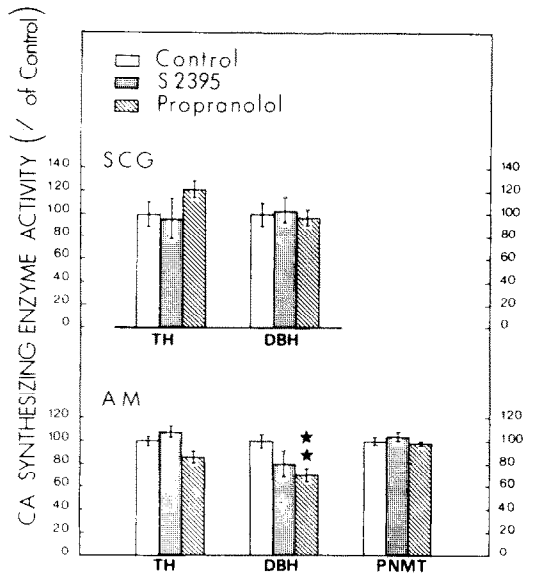

Fig. 2. Effects of a chronic oral administration of propranolol (40 mg/kg/day) or S 2395 (20 mg/kg/day) on catecholamine synthesizing enzymes activities in peripheral tissues of SHR. The activities (mean ± S.E.D.) are expressed as percentage of the control (untreated) SHR. Statistical difference from controls are indicated: $^{**}P < 0.01$. The number of animals was: controls, 10; S 2395-treated, 9; propranolol-treated, 9. The absolute activities in control SHR are indicated below and expressed per organ pair. Tyrosine hydroxylase (TH) activities (pmoles of DOPA formed/hr) were: (a) superior cervical ganglia (SCG), 999 ± 109; (b) adrenal medulla (AM), 26.523 ± 1205. Dopamine-hydroxylase (DBH) activities (nmoles of octopamine formed/hr) were (a) SCG, 30.3 ± 3.3; (b) AM, 72.7 ± 4.8. Phenylethanolamine-*N*-methyltransferase (PNMT) activities (pmoles of *N*-methylphenylethanolamine formed/hr) were: AM, 21,118 ± 584.

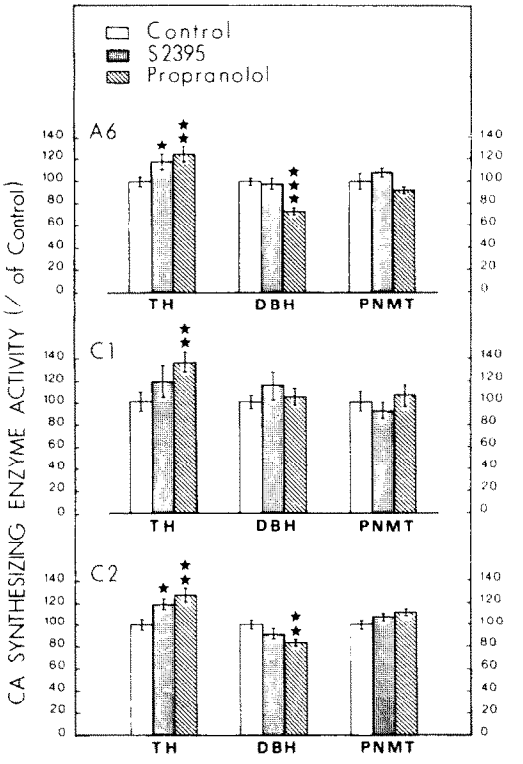

Fig. 3. Effects of chronic oral administration of propranolol (40 mg/kg/day) or S 2395 (20 mg/kg/day) on catecholamine synthesizing activities in pons-medulla nuclei of SHR. The activities (mean ± S.E.M.) are expressed as percentage of the control (untreated) SHR. Statistical difference from controls are indicated: $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. The number of animals in each group is the same as in Fig. 2. The absolute activities in control SHR are indicated below and expressed per mg of protein. Tyrosine hydroxylase (TH) activities (pmoles of DOPA formed/hr) were (a) locus coeruleus (A_6 group), 1281 ± 54; (b) C_1 region, 259 ± 21; (c) C_2 region, 260 ± 14. Dopamine-β-hydroxylase (DBH) activities (nmoles of octopamine formed/hr) were: (a) locus coeruleus, 45.36 ± 1.36; (b) C_1 region, 4.88 ± 0.29, (c) C_2 region, 7.92 ± 0.32. Phenylethanolamine-*N*-methyltransferase (PNMT) activities (pmoles of *N*-methylethanolamine formed/hr) were (a) locus coeruleus, 5.50 ± 0.38; (b) C_1 region, 64.66 ± 5.76; (c) C_2 region; 73.23 ± 2.93.

Locus coeruleus. An increase in TH activity was found in both propranolol and S 2395-treated SHR (respectively + 25.0 per cent, $P < 0.01$ and + 17.9 per cent, $P < 0.05$). A decrease in DBH activity was also observed but only in propranolol-treated SHR (− 27.2 per cent, $P < 0.001$). The PNMT activity was unaltered.

Median eminence. The TH activity was significantly decreased in S 2395 (− 28.1 per cent, $P < 0.05$), but not in propranolol-treated SHR. The DBH activity remained stable and a significant decrease in PNMT activity was observed in propranolol-(− 16.7 per cent, $P < 0.05$), but not in S 2395-treated SHR.

Anterior hypothalamus. The TH activity remained unchanged while the DBH activity was decreased in S 2395 (− 12.8 per cent, $P < 0.01$), but not in propranolol-treated SHR. The PNMT activity decreased in both S 2395- and propranolol-treated SHR (respectively − 14.6 per cent, $P < 0.05$ and − 19.6 per cent, $P < 0.01$).

Posterior hypothalamus. No change in enzymatic activities was found in this region.

DISCUSSION

As previously reported [20, 21] a chronic propranolol treatment (40 mg/kg/24 hr) of SHR induced a progressive and moderate decrease in systolic blood pressure, associated with a marked and sustained bradycardia. In similar conditions, S 2395 (20 mg/kg/24 hr), a new β-blocking agent, induced similar effects.

In treated SHR, changes in the CA synthesizing activities were obtained at both the central and the peripheral level. Generally these variations were more marked in the propranolol than in the S 2395-treated animals, i.e. in the SHR which exhibited the greatest decrease in systolic blood pressure.

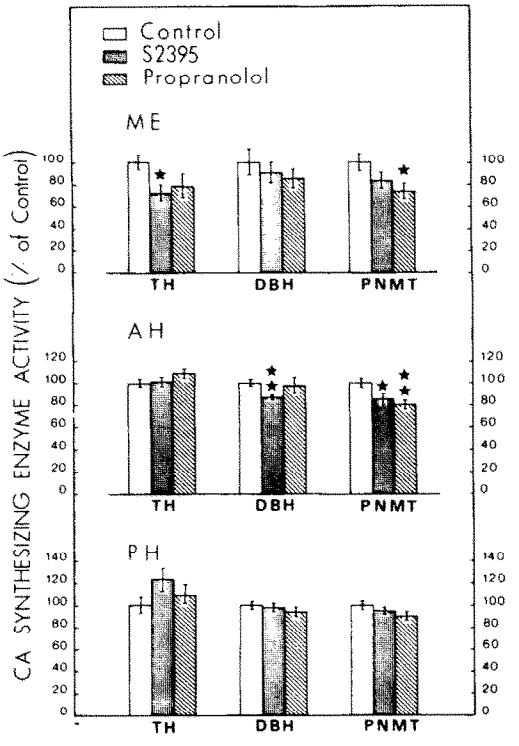

Fig. 4. Effects of chronic oral administration of propranolol (40 mg/kg/day) or S 2395 (20 mg/kg/day) on catecholamine synthesizing enzymes activities in hypothalamic regions of SHR. The activities (mean ± S.E.M.) are expressed as percentage of the control (untreated) SHR. Statistical difference from controls are indicated: $^{*}P < 0.05$, $^{**}P < 0.01$. The number of animals in each group is the same as in Fig. 2. The absolute activities in control SHR are indicated below and expressed per mg of protein. Tyrosine hydroxylase (TH) activities (pmoles of DOPA formed/hr) were (a) median eminence (ME), 1350 ± 97; (b) anterior hypothalamus (AH), 450 ± 15; (c) posterior hypothalamus (PH) 657 ± 46. Dopamine-β-hydroxylase (DBH) activities (nmoles of octopamine formed/hr) were (a) ME, 2.06 ± 0.26; (b) AH, 3.46 ± 0.11; (c) PH, 3.05 ± 0.10. Phenylethanolamine-*N*-methyltransferase (PNMT) activities (pmoles of *N*-methylphenylethanolamine formed/hr) were: ME, 10.44 ± 0.84; (b) AH, 6.86 ± 0.29; (c) PH, 6.07 ± 0.23.

At the peripheral level, the DBH activity was lowered in the adrenal medulla. This modification did not seem to be a consequence of the fall in blood pressure, since an hypotension induced by the peripheral vasodilator, dihydralazine, led to an increase in DBH activity in the adrenal medulla [22]. As a consequence, this lowered DBH activity might reflect a decrease in the adrenal medulla activity which could be one of the factors leading to the fall in blood pressure. Such a finding has previously been reported [1]. On the other hand no variation in CA synthesizing enzymes activities has been found in the superior cervical ganglia. This is consistent with the unchanged plasma DBH activity and may possibly indicate that the outflow from sympathetic fibers remained unaltered. These results are in contrast with previous works who reported a decreased electrical activity of renal sympathetic nerves [3] or a lowered TH and DBH activity in the superior cervical ganglia [23] of propranolol treated rabbits. Nevertheless, the fact that the sympatho-adrenal tone might be reduced by β-blockers favors the hypothesis of a central action of these drugs.

In the brain, propranolol and S 2395 induced an increase in the TH activity of the C_1, C_2 and A_6 areas. This increase does not seem to be a consequence of the decrease in blood pressure since the dihydralazine induced hypotension was associated with a decreased TH activity in the C_2 region [22]. As it has been reported that propranolol stimulates the TH activity of the striatum in the rat [24] it can be postulated that the increased activity of CA cell bodies of the brainstem might be due to a direct effect of β-blockers, related or not to their antihypertensive properties. Constrastingly, no important change in the TH activity could be found in the hypothalamic areas which are known to receive terminals originating from A_6, C_1 and C_2 regions. Although it could be hypothesized that the changes observed in the pons medulla occur in neurons devoid of important ascending projections, such a discrepancy remains to be explained. However similar differences between the enzymatic activity of the perikarya and of their postulated terminals have already been reported by Saavedra [25] and Denoroy [26].

DBH activity was decreased in the A_6 and the C_2 regions, a result which constrasts with their increased TH activity. As DBH activity remained stable in the C_1 group it appears that these modifications were not due to a general unspecific effect of β-blockers. The discrepancy between TH and DBH modifications might be better related to a differential effect of these drugs on the various types of neurons existing in these regions. Accordingly to Hökfelt [27] the possible presence of dopaminergic neurons in the A_2/C_2 region could explain the dissociation between TH and DBH activity reported here. Furthermore, a similar decrease in DBH activity in the C_2 area has been previously observed after a dihydralazine treatment [22]. As this region was punched out as containing the NTS, locus of the first synapse of the baro-reflex arch [4, 5], such a lowered capacity to synthetize noradrenaline could be due to a decrease in the activity of some neurons which normally facilitate the baroreflex and which are partially inactivated by the hypotension.

The results found in the hypothalamic areas contrast with those observed in the pons medulla. No important variations of TH or DBH activity could be detected in hypothalamic regions, except for slight changes in S 2395-treated but not in propranolol-treated SHR. Contrarily, the PNMT activity, which was unaltered in the pons medulla, decreased in the anterior hypothalamus and, to a lesser extent, in the median eminence of both S 2395- and propranolol-treated SHR. Thus, it appears that a chronic β-blocker treatment could lower the capacity to synthesize adrenaline in the terminals of the anterior part of the hypothalamus without modifying it in the cell bodies (C_1 and C_2 areas) and in other regions containing adrenergic terminals (posterior hypothalamus, locus coeruleus). As an increased catecholamine content has been found in the anterior

hypothalamus and median eminence of similarly treated SHR (Heimburger *et al.*, personal communication), it can be suggested that the association of a decreased synthesis capacity with an enhanced neurotransmitter content might reflect a decrease in the turnover of adrenaline in the anterior hypothalamus of β-blocker-treated SHR.

The anatomical specificity of this effect of β-blockers is interesting as it is established that the anterior and the posterior part of the hypothalamus play an opposite role in the control of blood pressure [28, 29]. The mechanisms which could account for such a specificity need further investigations in order to precise the central effects of β-blockers and their relationships with their antihypertensive properties.

Acknowledgements—This work was supported by grants from the CNRS, the Fondation de la Recherche Médicale Française and the Laboratoires Servier (Neuilly, France).

REFERENCES

1. B. D. Bhagat, in *Catecholamines: Basic and Clinical Frontiers* (Eds. E. Usdin, I. J. Kopin and J. Barchas) Vol II, p. 1446. Pergamon Press, New York (1979).
2. J. N. Sharma, B. B. Sandrew and S. C. Wang, *Neuropharmacology* **18**, 1 (1979).
3. P. I. Korner, P. K. Dorward, P. A. Blombery and G. J. Frean, *Circ. Res.* **46 II**, 26 (1980).
4. J. P. Chalmers, *Circ. Res.* **36**, 469 (1975).
5. D. J. Reis, T. H. Joh, M. A. Nathan, B. Renaud, D. W. Snyder and W. A. Talman. in *Nervous System and Hypertension* (Eds. P. Meyer and H. Schmitt) p. 147 Wiley–Flammarion, New York–Paris (1979).
6. W. de Jong, P. Zandberg, H. Wijnen, F. P. Nijkamp, B. Bohus and D. H. G. Versteeg, in *Nervous System and Hypertension* (Eds. P. Meyer and H. Schmitt) p. 165. Wiley–Flammarion, New York–Paris (1979).
7. E. Costa and J. L. Meek, *Ann. Rev. Pharmac.* **14**, 491 (1974).
8. M. Laubie, H. Schmitt, P. Mouille, G. Cheymol and J. C. Gilbert, *C. r. hebd. Séanc. Acad. Sci.* Paris, **273**, 1234 (1971).
9. M. Palkovits, *Brain Res.* **59**, 449 (1973).
10. A. Dahlström and K. Fuxe, *Acta Physiol. Scand.* **62**, Suppl. 232, 1 (1964).
11. M. Buda, B. Roussel, B. Renaud and J. F. Pujol, *Brain. Res.* **93**, 564 (1975).
12. T. Hokfelt, K. Fuxe, M. Goldstein and O. Johansson, *Brain. Res.* **66**, 235 (1974).
13. B. Renaud, S. Fourniere, L. Denoroy, M. Vincent, J. F. Pujol and J. Sassard, *Brain. Res* **159**, 149 (1978).
14. D. M. Jacobowitz and M. Palkovits, *J. Comp. Neurol.* **157**, 13 (1974).
15. T. Nagatsu, M. Levitt and S. Udenfriend, *Analyt. Biochem.* **9**, 122 (1964).
16. P. B. Molinoff, R. Weinshilboum and J. Axelrod, *J. Pharmac. exp. Ther.* **178**, 425 (1971).
17. J. K. Saelens, M. S. Schoen and G. B. Kovacsis, *Biochem. Pharmac.* **16**, 1043 (1967).
18. J. M. Saavedra, M. Palkovits, M. J. Brownstein and J. Axelrod, *Nature, Lond.* **248**, 695 (1974).
19. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
20. K. Kubo Esumi and K. Ennyu, *Arch. Int. Pharmacodyn.* **227**, 30 (1977).
21. C. Richer, N. Veniurini-Souto and J. F. Giudicelli, *Experientia* **35**, 656 (1979).
22. L. Denoroy, B. Renaud, M. Vincent, J. Sacquet and J. Sassard, *Eur. J. Pharmac.* **58**, 207 (1979).
23. A. E. G. Reine and I. W. Chubb, *Nature, Lond.* **267**, 265 (1977).
24. D. A. V. Peters and I. M. Mazuriewicz-Kwilecki, *J. Pharm. Pharmac.* **27**, 671 (1975).
25. J. M. Saavedra, H. Grobecker and J. Axelrod, *Circ. Res.* **42**, 529 (1978).
26. L. Denoroy, S. Fourniere, M. Vincent, B. Renaud, J. F. Pujol and J. Sassard, *Brain. Res.* **162**, 184 (1979).
27. T. Hökfelt, K. Fuxe, M. Goldstein, O. Johansson, A. Ljungdahl, J. Lundberg and M. Schultzberg, in *Catecholamines: Basic and Clinical Frontiers* (Eds. E. Usdin, I. J. Kopin and J. Barchas) Vol. II, p. 1007. Pergamon Press, New York (1979).
28. M. A. Nathan and D. J. Reis, *Circ. Res.* **37**, 226 (1975).
29. A. Philippu, H. Dietl, U. Ströhl and V. T. Truc, in *Catecholamines: Basic and Clinical Frontiers* (Eds. E. Usdin, I. J. Kopin and J. Barchas) Vol. II, p. 1428. Pergamon Press, New York (1979).