metabolized primarily extrahepatically, the principal site in the rat being skeletal muscle, and the oxidation of these amino acids is known to be stimulated by catecholamines[6], which has been shown to be a main mediator of nonshivering thermogenesis in cold acclimatisation[7], and glucagon[6], which has also been suggested to be involved in temperature acclimatisation[4]. Considering these studies, the present study would appear to indicate that the changes in the plasma branched-chain amino acids reflex an increased utilization of these amino acids in cold acclimatisation and a decreased utilization in heat acclimatisation through the altered hormonal secretions.

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Age variation in the increase of hypothalamic and brain stem contents of phenylethanolamine m-octopamine and p-octopamine in spontaneously hypertensive rats (SH Kyoto)[1]

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Summary. Phenylethanolamine, m-octopamine and p-octopamine contents were determined as a function of age in the hypothalamus and brain stem of spontaneously hypertensive rats and controls Wistar Kyoto. In hypothalamus, the content of the 3 amines was 2–4-fold greater for the SH rats. In the brain stem, the phenylethanolamine and p-octopamine contents were 2–3-fold greater in SH rats but 5–6-fold higher in the case of m-octopamine. The difference appears at 3 weeks and correlates the blood pressure with the increase of age. The significance of these findings is discussed.

For several years there has been an increasing awareness of the involvement of the sympathetic nervous system in the regulation of blood pressure. The possible implication of an inhibiting central noradrenergic center in the spontaneously hypertensive rats (SH Kyoto) has been postulated[2]. Nevertheless, a direct correlation between hypertension and catecholamine contents of parts of the rat brain is still controvertial[3]. However, Nagatsu et al.[3] have shown that the hypothalamic tyrosine hydroxylase activity is increased at 3 weeks of age in the SH rats. The apparent discrepancy between these 2 results has made us aware of a possible modification in the content of other amines biosynthesized from tyrosine through the tyrosine hydroxylase pathway[4]. Phenylethanolamine, m- and p-octopamines are present in the rat brain[5,6] and can be seperated and determined with great sensitivity[6,7].

Methods. Female rats were used for this study. Either SHR Kyoto (genetically hypertensive) or Wistar Kyoto (used as controls) were raised under the same conditions. At 1, 3, 7 and 15 weeks the animals were killed by rapid decapitation after injection (75 mg/kg i.p.) of pargyline. Hypothalamus, brain stem and the 'rest' of the brain (without cerebellum) were dissected according to Glowinski and Iversen[8], rapidly frozen and kept at −70 °C. Tissues were tested within a week for their octopamines and phenylethanolamine content according to the method described by Molinoff et al.[9]. Tissues were homogenized with a Potter in 5 vol. of Tris-HCl buffer 0.05 M (pH 8.6) containing 1 mM pargyline. Extracts were centrifuged 5 min at 20,000 × g and the supernatants were kept in a boiling water bath for 5 min and recentrifuged for 5 min at 20,000 × g. 150 µl of supernatant were incubated at 37 °C with 37.5 µl of PNMT (phenyl ethanolamine N methyltransferase) partially purified according to Saelens et al.[10] and 0.04 nmoles of [^{3}H] SAM ([^{3}H] methyl S adenosyl methionine) from CEA Saclay 13.5 Ci/mmole in 60 µl of Tris-HCl buffer 0.05 M (pH 8.6). After 45 min incubation, the reaction was stopped by the addition of 200 µl of 0.5 M borate buffer (pH 11) saturated with sodium chloride and containing p-synephrine, norphenylephrine and N-methylphenylethanolamine (1 µg of each). N-Methylated amines were extracted with 5 ml of ethylacetate and centrifuged for 5 min at 10,000 × g.

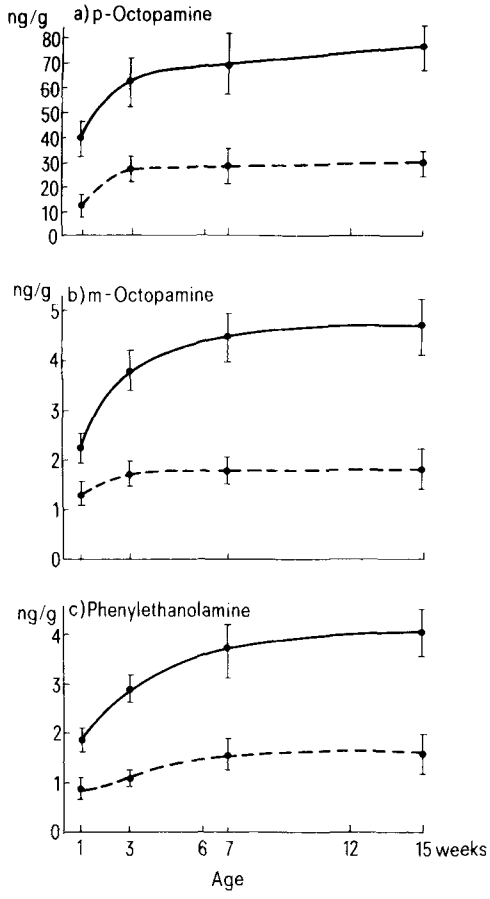

Fig. 1. p-Octopamine (A), m-octopamine (B), and phenylethanolamine (C) contents in the hypothalamus of SH rats (—) and controls Wistar-Kyoto rats (...). Values are given in ng of amines per g of wet tissue as a function of age (in weeks). 6 determinations were made at 3 weeks and 4 for the other ages. Indicated are the means ± SEM.

The organic phase was completely evaporated under nitrogen and the residue allowed to react with dansylchloride (1 dimethylamino naphtalene 5-sulfonylchloride 4 mg/ml in acetone) overnight in the dark. The 3 methylated amines were separated using the 3 consecutive solvent systems as described by Danielson et al.[6].

Results. Phenylethanolamine, m-octopamine and p-octopamine concentrations were determined in the brain stem and the hypothalamus. For SH rats and control rats, 6 animals were used at 3 weeks and 4 animals for the other ages: the average ± SEM of the blood pressure at 1 week was 116 ± 1 mm Hg, at 3 weeks 122 ± 2 mm Hg, at 7 weeks 153 ± 3 mm Hg and 182 ± 4 mm Hg at 15 weeks. For the corresponding controls it was 107 ± 1 mm Hg at 1 week, 113 ± 1.5 mm Hg at 3 weeks, 121 ± 1 mm Hg at 7 weeks and 122 ± 1 mm Hg at 15 weeks.

Depicted in figures 1 and 2 is the evolution of p-octopamine, m-octopamine and phenylethanolamine hypothalamic and brain stem content respectively, as a function of age, in hypertensive (SH) rats and control (Wistar Kyoto) rats. As shown in figure 1, the 3 amine hypothalamic contents are always higher in the hypertensive animals as compared to controls for each amine, the larger difference appears at 3 weeks of age where it represents a factor 2 for p-octopamine (figure 1, A), 1.72 for m-octopamine (figure 1, B), and 2.63 for phenylethanolamine (figure 1, C). At 15 weeks, this difference in the hypothalamus reaches 2.2 for p-octopamine, 2.05 for m-octopamine and 3.72 for phenylethanolamine, thereby correlating the increase in blood pressure, whereas the blood pressure of controls remains stable. The same kind of conclusion can be reached in the brain stem. As compared to control, there is twice as much p-octopamine at 3 weeks and 2.32-fold at 15 weeks of age for phenylethanolamine. A more drastic difference appears in the brain stem for m-octopamine since its content is 2.93 higher than the control at 1 week, 5.76 at 3 weeks, 6.5 at 15 weeks. No detectable amounts of any of the 3 amines were found in the 'rest' of the brain.

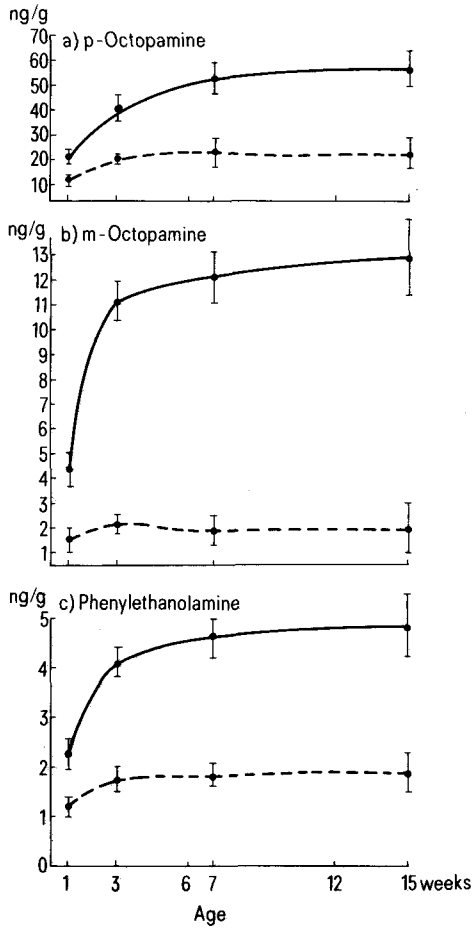

Fig. 2. p-Octopamine (A), m-octopamine (B) and phenylethanolamine (C) contents in the brain stem of SH rats (—) and controls Wistar Kyoto (...). Values are given in ng of amines per g of wet tissue as a function of age (in weeks) as indicated under figure 1.

Discussion. The phenylethanolamine, p-octopamine and m-octopamine hypothalamic and brain stem contents were found to be higher in SH-rats as compared to normotensive Wistar Kyoto. It seems to be well established that tyrosine hydroxylase activity is greater in the hypothalamus of SH-rats after 3 weeks of age[11]. This increased activity is correlated to a higher activity of the serum dopamine β-hydroxylase in such a way that increased catecholamines contents could be expected in hypothalamus and/or brain stem. These increased levels have not been formally established[3]. We have reported an important increase of the 3 amines content of SH-rats hypothalamus and brain stem at 3 weeks of age, and this observation has to be correlated to the increase in central tyrosine hydroxylase and serum dopamine β-hydroxylase if these enzymes are involved in the metabolism of phenylethanolamine and octopamine[12,13]. More striking is the very importantly increased content in m-octopamine in brain stem and hypothalamus. The use of specific techniques of determination[14,15] has allowed the demonstration of m-octopamine in the central[6] and peripheric[14] nervous system. This isomer of octopamine is believed to be biosynthesized by different pathways as compared to those leading to p-octopamine[15], and this observation appears as a strong support for the special involvement of m-octopamine in the central mediation of hypertension. Further support for this hypothesis are the more potent α-agonist properties of m-octopamine as compared to p-octopamine. Such an hypothesis deserves more complete investigations.

Moreover, the increase of phenylethanolamine, p-octopamine and m-octopamine hypothalamic and brain stem contents appears to be well correlated to the increase of blood pressure with the increase of age of the SH-rats.

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