Plasma branched-chain amino acids in cold- and heat-acclimatised rats

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Summary. The concentrations of plasma branched-chain amino acids, valine, isoleucine and leucine, were significantly elevated in cold-acclimatised rats, while these values were significantly reduced in heat-acclimatised rats, in both 2-week and 4-week temperature acclimatisation.

Involvement of amino acids in metabolic temperature acclimatisation has been scarecely investigated, possibly due to their complex metabolic fates. Recently, the importance of branched amino acids as metabolic fuels during the stressful situations of caloric deprivation (starvation) and increased caloric need (exercise) has been shown[1]. Therefore, the plasma concentration of branched-chain amino acids is expected to be affected more prominently than that of other amino acids in the altered metabolic states. In fact, the increased plasma concentration of branched-chain amino acids was reported in fasting men[1] and rats[2]. In the present study, we observed the changes in the plasma concentrations of branched-chain amino acids in cold- and heat-acclimatised rats in order to assess roles of these amino acids in temperature acclimatisation.

Adult male rats of the Wistar strain, weighing about 220 g, were divided into 3 groups; warm-acclimatised control group at 25 °C and 50% relative humidity, cold-acclimatised one maintained at 5 °C, and heat-acclimatised one maintained at 34 °C and 40–45% relative humidity. They had free access to food (Oriental MF rat biscuit, Oriental Yeast Co., Ltd., Tokyo) and tap water under the artificial lightning from 07.00 h to 19.00 h. After 2- or 4-week acclimatisation to respective temperatures blood specimen was obtained by decapitation at 09.00–11.00 h and the separated plasma was frozen at −30 °C until analyzed. The plasma concentrations of branched-chain amino acids were measured by the use of Hitachi Amino Acid Analyzer KLA-5 after the plasma was deproteinized by sulfosalycilic acid.

The results are summarized in the table. The plasma levels of branched-chain amino acids in warm-acclimatised control rats were comparable to those at 10.00 h reported by Fernstrom et al.[3]. The branched-chain amino acids in plasma have been reported to exhibit relatively great daily fluctuations, probably under the influences of dietary consumption and hormonal factors[3]. The values at 10.00 h approximately correspond to the daily average levels in the rats[3]. Accordingly, the present study was concerned with the plasma concentrations of these amino acids between 09.00 h and 11.00 h. Comparing to the warm-acclimatised control animals, the plasma levels of all branched-chain amino acids; valine, isoleucine and leucine, were significantly elevated in the cold-acclimatised rats, while those values were significantly reduced in the heat-acclimatised rats, in both 2-week and 4-week acclimatisation.

Animals such as rats have been previously reported to adapt to cold and heat possibly through regulating lipid and carbohydrate utilization, as conjectured from the changes in blood metabolites[4]. The present findings offer further evidence that the amino acids, especially branched-chain amino acids, regarded as essential nutrients for the organism, are involved in the metabolic temperature acclimatisation. However, the physiological significance of the changes in plasma branched-chain amino acids due to temperature acclimatisation awaits further study. Since the branched-chain amino acids lack or possess only weak potency as glucose precursors, oxidative degradation as metabolic fuels appears to be the main pathway for their utilization. Moreover, it is assumed that during fasting the pools of free branched-chain amino acids expand uniquely in the tissues, mainly in the muscle and liver despite their enhanced oxidation[1]. Despite the loss of skeletal muscle mass during fasting, a 45–95% increase in the pool size of branched-chain amino acids has been reported in this tissue[2]. In this context, it is interesting to note that cold acclimatisation depressed DNA synthesis in the skeletal muscle of rats, resulting in the reduced growth of this tissue and probably an increased availability of energy substrates, branched-chain amino acids, for nonshivering thermogenesis[5]. Therefore, it is likely that an elevated level of plasma branched-chain amino acids in the cold-acclimatised rats could result from an expanded pool of these amino acids in addition to their increased utilization. The branched-chain amino acids are only the essential amino acids which are

Effect of cold (5 °C) and heat (34 °C) acclimatisation on the plasma concentrations of branched-chain amino acids in rats

	Plasma amino acids (μm/ml plasma) Valine	Isoleucine	Leucine
I 2-week acclimatisation groups			
Warm-acclimatised control rats (10)	0.261 ± 0.0074	0.110 ± 0.0033	0.194 ± 0.0060
Cold-acclimatised rats (9)	0.310 ± 0.0125	0.125 ± 0.0062	0.225 ± 0.0103
p vs control rats	< 0.01	< 0.05	< 0.02
Heat-acclimatised rats (14)	0.202 ± 0.0113	0.083 ± 0.0046	0.150 ± 0.0075
p vs control rats	< 0.001	< 0.001	< 0.001
II 4-week acclimatisation groups			
Warm-acclimatised control rats (10)	0.273 ± 0.0175	0.128 ± 0.0023	0.222 ± 0.0017
Cold-acclimatised rats (9)	0.349 ± 0.0138	0.153 ± 0.0055	0.270 ± 0.0088
p vs control rats	< 0.01	< 0.01	< 0.001
Heat-acclimatised rats (4)	0.180 ± 0.0069	0.083 ± 0.0031	0.149 ± 0.0049
p vs control rats	< 0.001	< 0.001	< 0.001

Mean ± SEM. Number in the parenthesis denotes the number of samples analyzed. In some cases the plasma samples from 2 or 3 animals were pooled in order to obtain necessary amount of plasma for amino acid analysis.

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.

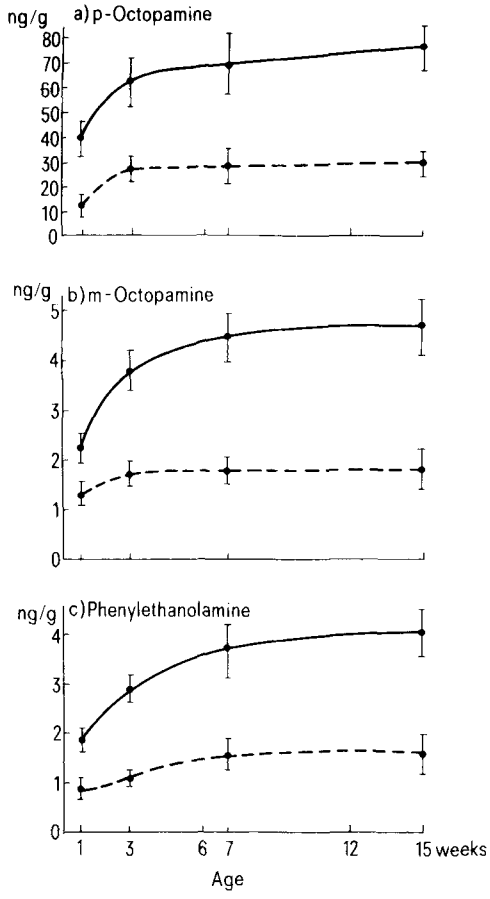

Fig. 1. p-Octopamine (A), m-octopamine (B), and phenylethanolamine (C) contents in the hypothalamus of SH rats (—) and controls Wistar-Kyoto rats (...). Values are given in ng of amines per g of wet tissue as a function of age (in weeks). 6 determinations were made at 3 weeks and 4 for the other ages.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