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Levels of cytochromes in rat-brain mitochondria during post-natal development

Several aspects of mitochondrial metabolism during brain maturation have been described[1–3], however the pattern of changes of the different components of the respiratory chain have not yet been studied.

In the present communication the changes in the levels of cytochromes and ubiquinone in purified mitochondria at different stages of development are reported.

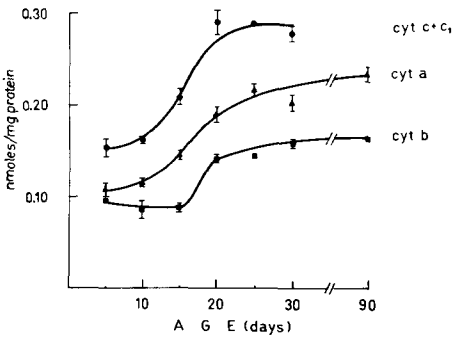
For the preparation of brain mitochondria groups of 15–45 Wistar rats of either sex at various ages were used. Purified mitochondria were isolated from whole brain by the method of LØVTRUP AND ZELANDER[4]. Cytochrome measurements were made by using a Chance wavelength scanning recording spectrophotometer[5] constructed in the Johnson Foundation, Philadelphia, Pa. For the assay of cytochromes $c + c_1$ and a, the cuvettes contained in final concentration: 10 mM phosphate buffer (pH 7.4), 0.22 M sucrose and rat-brain mitochondria (1.5–3 mg protein per ml); to one of the cuvettes, succinate was added to achieve 10 mM and the difference spectrum was recorded after becoming anaerobic. A similar schedule was followed for cytochrome b except that together with succinate, antimycin A (Sigma Chem. Co., Type III) was added to achieve a final concentration of 0.44 μg/ml; mitochondrial protein was 3–4 mg/ml. The millimolar extinction coefficients used were: 24 for cytochrome a[6] (605–630 nm); 19.1 for cytochrome $c + c_1$[5] (551–540 nm) and 20 for cytochrome b[7] (563–573 nm). The results were expressed as nmoles/mg protein. Each experiment was done in duplicate with 3–4 different mitochondrial preparations. No differences in the levels of cytochromes were found in the mitochondria coming from either fresh or frozen preparations. The extraction and assay of ubiquinone were performed as described by PUMPHREY AND REDFEARN[8], however, higher amounts of mitochondrial protein had to be extracted in the experiments with the youngest rats. The absorption between 230 and 320 nm was measured in a PMQ II Zeiss spectrophotometer, before and after reduction with BH_4K. The results were expressed as nmoles/mg protein by considering the difference in the spectrum of ubiquinone and ubiquinol at 272 nm and an extinction coefficient of 12.25 mM^{-1} (ref. 8). Protein was determined by the method of LOWRY *et al.*[9] using crystalline bovine serum albumin as a standard.

Fig. 1 shows the pattern of changes of the levels of cytochromes during maturation. The greater increase of cytochromes $c + c_1$ and a was observed between 10 and 20 days; thereafter cytochromes $c + c_1$ remained constant, while some increase seemed to occur for cytochrome a. For cytochrome b, practically no changes were observed between 5 and 15 days and a considerable increase between 15 and 20 days was found; thereafter the increase was very slight and remained constant up to 90 days.

In Fig. 2 are presented the levels of ubiquinone during maturation. An increase was observed between 10 and 20 days, with no significant increase thereafter.

Our results on the content of the different cytochromes in the 30-day-old rat are in general agreement with those reported by SACKTOR AND PACKER[10] and by WILLIAMS[11], and for ubiquinone with those of KADENBACH[12], which concerned older animals. These findings indicate that brain mitochondria attain the adult characteristics at about 30 days.

GREGSON AND WILLIAMS[2] described an increase in the content of cytochromes per mitochondrion in adult rats with respect to the new-born ones. Our findings also show that during development of the brain there is an increase in the concentration of cytochromes and of ubiquinone in mitochondria. These observations may be explained by an increase in the surface area of the inner mitochondrial membrane.



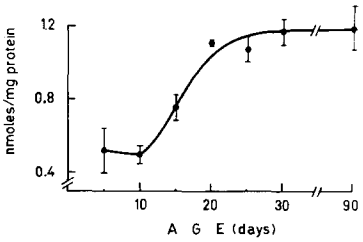


Fig. 1. Levels of cytochromes *b*, $c + c_1$ and *a* during post-natal development. Each point represents the mean of 3–4 brain mitochondria preparations, except in those lacking S.E. which come from a single one.

Fig. 2. Ubiquinone levels during development. Each point is the average of 2–3 individual mitochondrial preparations; the S.E. is indicated.

Table I shows the ratios of cytochromes $c + c_1$ and *b* to cytochrome *a*, at the different stages of development. It was observed that the ratio $c + c_1/a$ almost remained constant; on the other hand, the ratio b/a showed a significant difference between 10 and 15 days ($P < 0.05$) and between 15 and 20 days ($P < 0.001$). It has been described that there exist different cytochrome ratios either organ-wise or species-wise[11]; from the results presented here, this difference could be extended to rat-brain mitochondria during maturation.

TABLE I

RATIOS OF MITOCHONDRIAL CYTOCHROMES $c + c_1$ AND *b* TO *a* IN RAT BRAIN DURING DEVELOPMENT

Age (days)	$c + c_1/a$	b/a
5	1.42	0.88
10	1.40	0.75
15	1.43	0.59
20	1.53	0.75
25	1.33	0.66
30	1.36	0.79

Since the changes in the levels of cytochromes do not follow a unique pattern, it is conceivable that the composition of the respiratory assembly of brain mitochondria varies during development. Due to the fact that our mitochondrial preparation contains mitochondria coming from neurons and glial cells, the possibility that the changes reported here could be attributed to one or both types of mitochondria cannot be excluded.

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