Indian Journal of Biochemistry & Biophysics
Vol. 35, August 1998, pp. 224-228

Hormonal regulation of malate-aspartate shuttle enzymes during postnatal development of mice

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Received 3 November 1997; accepted 6 April 1998

The regulation of malate-aspartate shuttle enzymes by hydrocortisone was studied in the liver and kidney of 15-, 30- and 60-day old mice. It has been observed that adrenalectomy decreases and hydrocortisone treatment to adrenalectomized mice increases the activity of cytosolic and mitochondrial malate dehydrogenase and cytosolic aspartate aminotransferase in the liver of 15-, 30- and 60-day old mice. Per cent decrease following adrenalectomy remained almost similar in all the postnatal ages studied. However, hydrocortisone effect shows age-dependency. In the kidney, adrenalectomy decreases and hydrocortisone treatment increases the activity of these isoenzymes only in 30- and 60-day old mice. These findings entail that the same enzyme in different tissues of developing animals may be regulated differentially by the same physiological stimuli. Our findings on hormonal regulation of malate-aspartate shuttle enzymes show that they are subjected to different physiological control in various tissues during postnatal development of mice.

The malate-aspartate shuttle is considered to be the primary mechanism in transferring the reducing equivalents from cytosolic NADH to the mitochondria in many animal tissues[1,2]. It has been observed that the inner mitochondrial membrane is impermeable to NADH. The NADH formed during glycolysis in the cytosol must be regenerated to NAD^+ for glycolysis to operate. The shuttle involves an influx of malate and glutamate and efflux of aspartate and ketoglutarate from mitochondria[3,4]. The main enzymes of the shuttle are malate dehydrogenase (MDH) and aspartate aminotransferase (AsAT). Both these enzymes have two homologous and genetically independent isoenzymes: one in the cytosolic (c-) and the other in the mitochondrial (m-) fractions[5,6]. Endogenous activity expression of malate - aspartate shuttle enzymes during postnatal development of mice have already been reported by our group[7]. Shuttle enzymes as well as shuttle activity in a reconstituted system show an early expression in the liver as compared to the kidney of mice during postnatal development.

Glucocorticoids play a crucial role in development and aging of animals[8,9]. Kanungo and Gandhi[10] reported that c-MDH but not m-MDH is inducible by cortisone in the liver of old rats. The impairment of m-MDH induction in old animals can be repaired in regenerating liver after hepatectomy. Sharma and Patnaik[11] have earlier reported that the liver cytosolic but not the mitochondrial AsAT is regulated by hydrocortisone during aging of rats. Glucocorticoid effects on various processes depend on the level as well as on the physicochemical properties of its receptor. Age-dependent changes in the inducibility of enzymes by glucocorticoid have been reported to be influenced by the level of receptors and also by the post-receptor events[12,13].

The different metabolic adjustments that take place in developing animals are the adaptations to the changing demands made upon them. Study of all the enzymes of a particular metabolic pathway provides a complete profile of their biological function. Keeping in view the importance of such study, we planned to see the regulation of malate-aspartate shuttle enzymes by hydrocortisone during postnatal development of mice.

Materials and Methods

Male Swiss albino mice (Balb/ c strain) were used for the experiments. They were maintained

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under normal laboratory conditions at 24 ± 2°C and fed with standard pellet diet (Amrut Laboratory, Pune) and tap water *ad libitum*. Male mice of three different postnatal age groups (15-, 30- and 60- day) were used for the hormonal studies. All the chemicals used were of analytical grade, and the biochemicals were obtained from Sigma Chemical Co., USA.

Hormone treatments

Effects of adrenalectomy and the administration of hydrocortisone to adrenalectomized mice were studied on the activities of MDH and AsAT isoenzymes in the liver and kidney of mice at three different postnatal ages (day 15, 30 and 60). The mice of each age group were divided into three sets of 4-5 animals. Set I and set II were used for the study of adrenalectomy and adrenalectomy plus hydrocortisone, respectively and set III was used as a non-adrenalectomized control.

All the mice of set I and II were bilaterally adrenalectomized. In addition to normal pellet diet, these mice were given 0.9% NaCl instead of water for five days following adrenalectomy during which, endogenous glucocorticoid becomes negligible in the blood[12]. On the 6th day, mice of set II were administered intraperitoneally with hydrocortisone (1 mg/ 100 g body wt) in 0.5 ml of 0.9% NaCl having 10% ethanol. Pilot experiments showed that i.p. dose of 1 mg/ 100 g body wt of hydrocortisone to the mice for three consecutive days exhibited maximum effect on the activities of MDH and AsAT. The mice belonging to set I received the same amount of 0.5 ml suspension medium (10% ethanol and 0.9% NaCl) at a fixed time of the day for three days. All the mice were sacrificed after 6 hr of the final hormone injection and their tissues (liver and kidney) were taken out, washed in ice-cold saline, blotted and stored at –70°C till the assay of MDH and AsAT.

Preparation and assay of shuttle enzymes

Homogenates (10%, w/v) of the liver and kidney were prepared in ice-cold 0.25 *M* sucrose. Each homogenate was centrifuged at 800 × *g* for 10 min at 2°C to sediment nuclei. The resulting supernatant was further centrifuged at 14,000 × *g* for 30 min at 2°C to sediment mitochondria. The supernatant thus obtained was used for the assay of cytosolic MDH and AsAT. The mitochondrial pellet was washed twice, suspended in a solubilizing medium (0.25 *M* sucrose / 10 m*M* potassium phosphate buffer, *p*H 7.5/ 0.5% Triton X-100) for 3 hr, and used for the assay of mitochondrial MDH and AsAT.

Both the isoenzymes of MDH[14] and AsAT[15] were assayed spectrophotometrically as described earlier[7]. Protein contents of cytosolic and mitochondrial fractions were determined[16]. The activity of both the isoenzymes of MDH and AsAT was expressed as units (μmole NADH oxidized per min) per mg protein. The data were statistically analyzed and the level of significance (*p*) between two sets of data was calculated according to student's t-test.

Results

Regulation of malate dehydrogenase (MDH) isoenzymes

Adrenalectomy (A/d) causes a significant decrease (-20%, -30% and -25%, respectively) in the activity (units/ mg protein) of liver cytosolic MDH of mice at all the postnatal ages studied (day 15, 30 and 60). It also decreases (-26%, -23% and -26%, respectively) the activity level of liver mitochondrial MDH at those ages (Fig. 1A and B). Administration of hydrocortisone to adrenalectomized mice significantly increases (+49%, +46% and +27%, respectively) the activity of liver cytosolic malate dehydrogenase at all the three postnatal ages studied. It also increases (+32%, +33% and +21%, respectively) the activity level of mitochondrial malate dehydrogenase at all the three ages of mice , albeit to a lesser degree (Fig. 1A and B).

Adrenalectomy significantly decreases (-24% and -22% for cytosolic and -29% and -22% for mitochondrial) the activity of kidney malate dehydrogenase at 30- and 60- day of postnatal ages. However, it does not show any effect on the activity of this enzyme (cytosolic and mitochondrial) in preweaned mice (15-day old) (Fig. 2A and B). Administration of hydrocortisone increases (+29% and +33% for cytosolic and +41% and +30% for mitochondrial) the activity of both the isoenzymes of malate dehydrogenase at those postnatal ages (day 30 and 60) of mice (Fig. 2A and B).

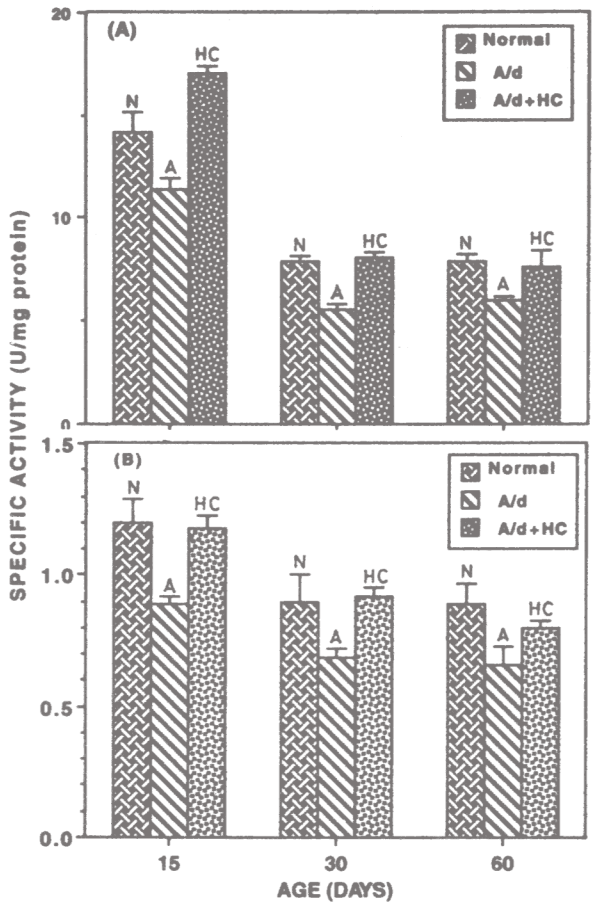

Fig. 1—Effects of adrenalectomy (A/d) and hydrocortisone (HC) on the activity of cytosolic (A) and mitochondrial (B) malate dehydrogenase isoenzymes in the liver of male mice at various postnatal ages. [N, Normal; A, adrenalectomised; HC, adrenalectomised and hydrocortisone treated. Hormonal treatments and other experimental conditions are described in methods section. Values are means for 4-5 mice of each age group. Bars represent standard deviation].

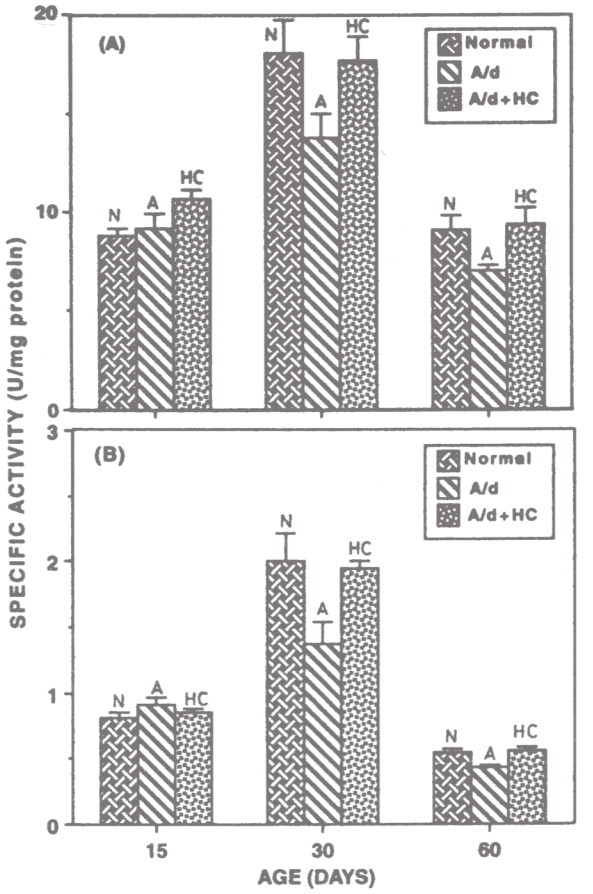

Fig. 2—Effects of adrenalectomy (A/d) and hydrocortisone (HC) on the activity of cytosolic (A) and mitochondrial (B) malate dehydrogenase isoenzymes in the kidney of male mice at various postnatal ages. [N, Normal; A, adrenalectomised; HC, adrenalectomised and hydrocortisone treated. Hormonal treatments and other experimental conditions are described in method section. Values are means for 4-5 mice of each age group. Bars represent standard deviation].

Regulation of aspartate aminotransferase (AsAT) isoenzymes

A significant decrease (-24%, -20% and -28%, respectively) in the activity of liver cytosolic AsAT of mice was observed following adrenalectomy in all the postnatal ages studied (Fig. 3A). However, it does not show any effect on the activity of mitochondrial AsAT in the liver of mice at any of the three postnatal ages studied (Fig. 3B). Administration of hydrocortisone causes a significant increase (+39%, +36% and +28%, respectively) in the activity of liver cytosolic AsAT at those postnatal ages of mice (Fig. 3A).

In kidney, adrenalectomy causes a decrease (–24% and –22%) in the activity of cytosolic AsAT in postweaned mice only (i.e. day 30 and 60) (Fig. 4A). Administration of hydrocortisone to adrenalectomized mice significantly increases (+21% and 22%) the activity of cytosolic AsAT in the kidney of only those mice (Fig. 4A). These treatments have no effect on the activity of mitochondrial AsAT in the kidney of mice at either of the postnatal ages studied (Fig. 4B).

Discussion

The malate-aspartate shuttle is primarily involved in the transfer of reducing equivalents from cytosolic NADH to the mitochondria in various tissues[2]. The cytosolic isoenzymes of both MDH and AsAT are also implicated in gluconeogenesis, since the former converts malate and the latter converts aspartate to oxaloacetate, which is then converted to phosphoenolpyruvate[6]. The functional

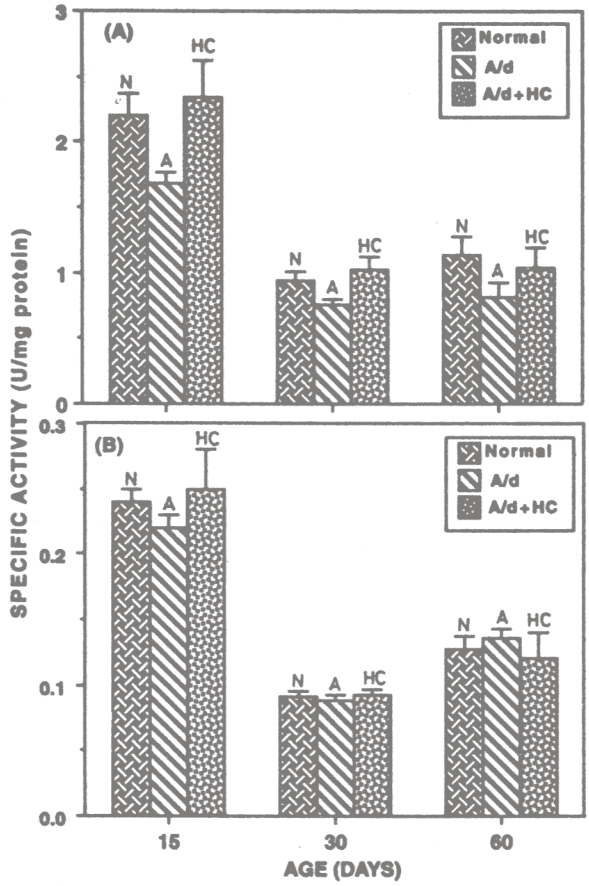

Fig. 3—Effects of adrenalectomy (A/d) and hydrocortisone (HC) on the activity of cytosolic (A) and mitochondrial (B) aspartate aminotransferase isoenzymes in the liver of male mice at various postnatal ages. [N, Normal; A, adrenalectomised; HC, adrenalectomised and hydrocortisone treated. Hormonal treatments and other experimental conditions are given in methods section. Values are means for 4-5 mice of each age group. Bars represent standard deviation].

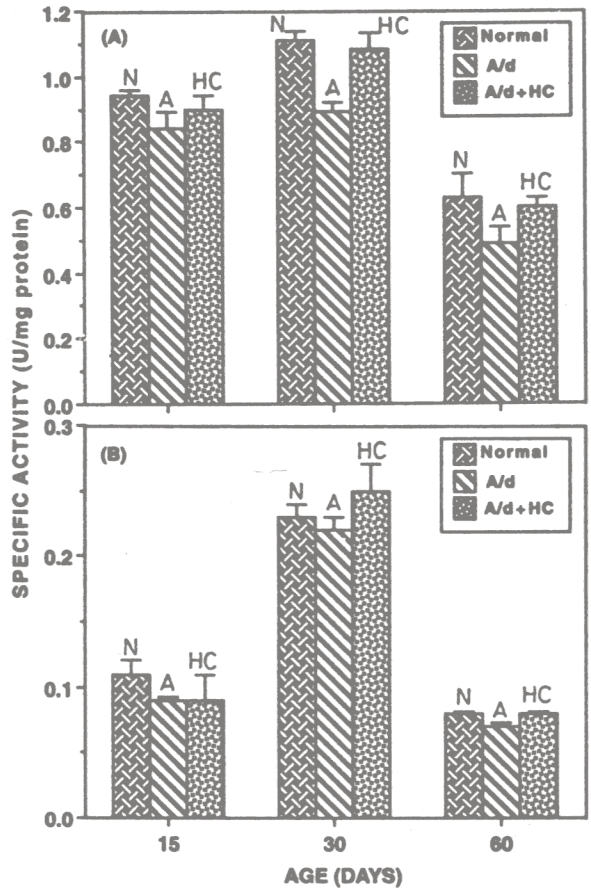

Fig. 4—Effects of adrenalectomy (A/d) and hydrocortisone (HC) on the activity of cytosolic (A) mitochondrial (B) aspartate aminotransferase isoenzymes in the kidney of male mice at various postnatal ages. [N, Normal; A, adrenalectomised; HC, adrenalectomised and hydrocortisone treated. Hormonal treatments and other experimental conditions are given in methods section. Values are means for 4-5 mice of each age group. Bars represent standard deviation].

significance of malate-aspartate shuttle also unfolds the degree of control points for glycolysis, gluconeogenesis and Krebs cycle. We have earlier reported a differential expression of shuttle enzymes as well as shuttle activity in the liver and kidney of mice during postnatal development[7].

It was observed that adrenalectomy decreases and administration of hydrocortisone increases the activity of cytosolic and mitochondrial malate dehydrogenase in the liver of 15-, 30- and 60-day old mice. The per cent decrease following adrenalectomy is almost similar in all the postnatal ages studied. However, the magnitude of increase of cytosolic MDH by hydrocortisone was higher in 15- and 30-day old mice as compared to 60-day. This indicates that glucocorticoids do play a role in the regulation of these isoenzymes. The higher magnitude of increase of cytosolic malate dehydrogenase in 15- and 30-day old mice may be because of the endogenous level of glucocorticoid receptors and/ or post receptor events at early postnatal ages[17]. Sharma and Patnaik[18] have also reported that the magnitude of induction of liver cytosolic malate dehydrogenase by hydrocortisone decreases in rats as a function of age.

Adrenalectomy decreases and administration of hydrocortisone to adrenalectomized mice increases the activity of kidney cytosolic and mitochondrial malate dehydrogenase only in 30- and 60-day old mice. They do not show any effect on the activity of kidney malate dehydrogenase (cytosolic and mitochondrial) in preweaned animals (15-day old). These findings corroborate[19] the observation that the same enzyme in different tissues of developing

animals might be regulated differentially by the same physiological stimuli. The gene responsible for the synthesis of cytosolic and mitochondrial malate dehydrogenase are reported to be different[20,21]. The inducibility of mitochondrial malate dehydrogenase by hydrocortisone is significantly lower than that of cytosolic MDH. This may be due to the differential responsiveness of genes of cytosolic and mitochondrial malate dehydrogenase isoenzymes towards hydrocortisone.

Results on the hormonal regulation of shuttle enzymes demonstrate that adrenalectomy decreases and hydrocortisone increases the activity of c-AsAT significantly in the liver of all the three postnatal ages (15-, 30- and 60-day). However, these treatments do not exhibit any significant effect on the activity of m-AsAT of the liver of mice at these postnatal ages. The results are in agreement with the earlier studies[11] wherein, m-AsAT of rat liver was also shown to be irresponsive to glucocorticoids.

Our findings also exhibit that adrenalectomy decreases and the administration of hydrocortisone increases the activity of kidney cytosolic aspartate aminotransferase only in the postweaned mice (30- and 60-day old). Since both the isoenzymes of AsAT are also genetically independent[6], they differ from one another even in different tissues for their responses towards hydrocortisone. Although both the isoenzymes of AsAT are involved in gluconeogenesis, it is the cytosolic isoenzyme whose activity is regulated by glucocorticoid[11,12,22]. Aggerbeck *et al.*[23] reported that both the activity as well as mRNA level of cytosolic AsAT are increased by glucocorticoid in hepatoma cell line,Fao. Adrenalectomy and hydrocortisone do not show any effect on kidney aspartate aminotransferase in 15-day old mice. It has recently been demonstrated that the translation of mRNA for both the isoenzymes of AsAT is subject to tissue-specific regulation in an age-related manner[24]. A similar phenomenon was observed in the case of kidney MDH isoenzymes at this postnatal age of mice. It may be due to the differential level of glucocorticoid receptors and/ or other trans-acting factors in the liver and kidney of mice during postnatal development[25-27]. These studies indicate that factors like hormones, their receptors and the tissue-specific trans-acting factors, needed for expression of specific genes are important for the maintenance of the level of adaptive response of enzymes[13,28].

Acknowledgement

We thank the University Grants Commission and the Department of Science and Technology, New Delhi and the North Eastern Hill University, Shillong for providing research facilities and financial assistance.

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