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Developmental Studies on Creatine Kinase. Isoenzyme in Rat Gastrointestinal Tract

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Creatine kinase activity and its isoenzymatic profile in rat intestinal mucose during normal development have been studied. Creatine kinase enzymatic activity increased stepwise during fetal development and the first week of life. An isoenzymatic pattern of exclusively CK-BB types occurred in all segments of the digestive tract during the early fetal stage. The isoenzyme profile of creatine kinase in the esophagic tissue with advancing maturation of the fetus shifted in the same way as in adults, with preferential concentration of CK-MM. However, CK-BB continued to be the main isoenzyme in the rest of the digestive tract. Our results show that rats are particularly suitable for experimental studies of intestinal creatine kinase isoenzymes.

Key Words: Creatine kinase, Digestive tract, Development.

Creatine Kinases (ATP: creatine phosphotransferases, EC 2.7.3.2) from vertebrate tissues are formed by two subunits which may be of muscular (M), cerebral (B) or mitochondrial (Mi) type. Creatine kinase is found both in the cytoplasm and mitochondria within the cell. Cytoplasmic creatine kinase isoenzymes are integrated by M and B subunits as indicated by their eponyms (CK-MM, CK-MB and CK-BB), while mitochondrial creatine kinase appears to be of only one type, containing two identical Mi subunits.

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Clinical interest in creatine kinase has mostly been focused on the cytoplasmic forms. CK-MM isoenzyme is specific for striated muscle, while CK-BB isoenzyme occurs in a variety of tissues including brain, gastrointestinal mucosa and embryonic tissues (4, 12, 14). A hybrid form of creatine kinase (CK-MB), whose properties appear somehow intermediate between those of CK-MM and CK-BB forms can exist within cells where both types of subunits are produced (7). Creatine kinase catalyzes the transfer of high energy posphate between creatine and ATP, and plays a crucial role in maintaining a readily available source of energy for cellular use, as well as a key role in intra-

cellular energy transport from mitochondria to sites of utilization (10). This enzyme undergoes striking changes in activity and isoenzyme composition during embryonic development and differentiation of muscle cells (13).

Data on creatine kinase activity and distribution in rat intestinal mucosa during physiological development are still missing.

As a part of continuing study on enzyme composition of different tissues (8, 9), we are exploring the activity and the isoenzyme pattern of creatine kinase in various segments in the rat digestive tract at the end of fetal development, in the neonatal period and after 90 days of life.

Materials and Methods

Rats of the Wistar strain fed a stock laboratory diet were used in the present studies. Six fetuses were examined on the 15th and the 18th day of gestation, as well as eight neonates on the 1st, 3rd, 10th, 20th and 30th day after birth and, then 90 day-old adult rats, weighing 250 to 300 g. An equal number of male and female rats were selected. Two fetuses were taken from each mother. After an overnight fasting, rats were anesthetized with ethyl ether and, the whole gastrointestinal tract from the pharynx to the rectum was removed as a single piece. Portions of the esophagus, stomach, duodenum, jejunum, distal 4 cm of the ileum and, distal 6 cm of the colon were isolated. Different areas of the gastrointestinal tract were identified by 50-tissue magnification when necessary. The whole intestine of the 15 day-old fetuses was used, since the different anatomical areas could not be identified. Whole gastrointestinal tract mucosa was scrapped off and immediately cooled on ice in the remaining cases. It was proved by means of specific tinction and studies under m/o that over 90 % of the scrapping from the esophagic mucosa and other digestive tract was composed of epithelial cells and that it contained no muscular cells.

Samples were homogenized with cold solution containing Pipes 30 mM, EDTA 3mM, DDT 1mM and Triton X-100, 0.5 % pH 7.4 in a glass-pestle homogenizer and were later centrifuged at 8.000 x g for 60 min, using supernatants for enzymatic analysis. Creatine kinase activity was determined by the SZASZ method (11), using a Beckman spectrophotometer at 37° C. Samples were diluted to such extent that linearity was obtained between activity and enzyme concentration. Separation of creatine kinase isoenzymes was carried out according to MERCER (6) modified (8). Activity was expressed as specific activity units (UI/mgr protein). Protein concentration was determined by the Bio-rad Laboratory method, using bovine serum albumin as the standard. We have used male rat liver as the reference tissue with results similar to those from other authors. Reactives were supplied by Boehringer Mannheim.

Results and Discussion

Creatine kinase activities in the gastrointestinal tract mucosa. — Changes of specific creatine kinase activity in rat gastrointestinal tract mucosa during fetal and extra-uterine development are shown in table I. Creatine kinase activity in the gastrointestinal tract mucosa of 15 day-old rat fetuses is quite low, whereas it increases stepwise during late fetal life (from the 16th to the 22nd day of gestation) and, the first postnatal week, reaching its highest value on the 10th day after birth.

After creatine kinase activity increase seems to be associated to cell division and morphological maturation (5). The above enzyme activity was highest in the esophagus (2.3 ± 0.5) and lowest in the adult ileum (0.7 ± 0.5), which might result

Table I. *Ontogenetic alteration of specific creatine kinase activity in the rat gastrointestinal tract.* Values are given as mean + SEM. Each group contains 6-9 values. Enzyme activity was expressed in terms of specific activity (number of enzyme units per milligram of protein).

	Days after fertilization			Birth	Days after birth				Adult
	15	18	21	3	10	20	30		90
Esophagus	0.3±0.1	1 ±0.1	1.3±0.3	2.6±0.3	3.9±0.5	2.9±0.5	3.1±0.8		2.3±0.5
Stomach	0.2±0.1	0.6±0.3	0.7±0.3	1.2±0.9	1.6±0.8	1.4±0.6	1.5±0.9		1.1±1
Duodenum	—	0.8±0.4	0.9±0.3	1.7±1	2 ±0.7	1.8±0.6	2 ±1		1.8±1.2
Jejunum	0.2±0.1	0.5±0.3	0.9±0.2	1.2±0.6	1.9±0.6	1.6±0.7	1.7±0.5		1.4±0.6
Ileum	—	0.2±0.1	0.4±0.1	0.8±0.4	1.2±0.7	1 ±0.5	0.9±0.5		0.7±0.5
Colon	0.3±0.1	0.6±0.4	0.7±0.4	1.2±0.5	1.3±0.8	1 ±0.8	1.1±0.7		0.8±0.7

from a higher muscle activity and energy expenditure in the proximal parts of the rat's digestive tract mucosa. Creatine kinase activity in the esophagus increased 13-fold from the 15th day of fetal development (0.3 ± 0.1) to day 10th of extrauterine life (3.9 ± 0.5) when higher values than those of adult rat were detected. However, the aforementioned rise in CK activity ranged between 3 and 10-fold during the same developmental intervals.

Isoenzymatic distribution of creatine kinase. — Creatine kinase isoenzyme patterns of rat gastrointestinal mucosa during fetal and extrauterine development are expressed in table II as percent of the total CK activity. CK-BB isoenzymes was the main form of the enzyme in the fetal and adult rat GI tract mucosa, except for the adult rat esophagus whose predominant isoenzyme was CK-MM, as has previously been shown (8). The latter is due to a progressive rise in CK-MM isoenzyme activity after the 15th day of rat fetal life. CK-MB isoenzyme activity of rat GI tract mucosa seems not to be relevant.

Developmental CK isoenzyme patterns have been reported by EPPENBERGER *et al.* (1), in the skeletic muscle of chicken as well as by HOMES (13) and KLOOSTERBOER *et al.* (11) in rat skeletal muscle, showing an increase of CK-MM activity during development, which seems to be associated to the augmentation of contractile elements. The role of creatine kinase and its isoenzyme profile in the gastrointestinal tract is still to be determined. It might be important to generate required energy for absorption, so that its activity

Table II. *Isoenzyme pattern of creatine kinase in rat gastrointestinal tract.* Values are given as mean of percentages.

	Days after fertilization									Days after birth												Adult		
	15			18			21			3			10			20			30			90		
Isoenzyme:	MM	MB	BB	MM	MB	BB	MM	MB	BB	MM	MB	BB	MM	MB	BB	MM	MB	BB	MM	MB	BB	MM	MB	BB
Esophagus	0	0	100	39	0	61	68	0	32	68	1	31	85	1	14	88	2	10	94	1	5	93	1	6
Stomach	0	0	100	5	1	94	7	1	92	12	1	87	10	1	89	12	1	87	12	4	84	11	5	84
Duodenum		—		0	0	100	5	3	92	11	2	87	10	1	89	10	2	88	9	3	88	9	3	88
Jejunum	0	0	100	5	0	95	11	1	88	24	2	74	19	3	78	21	4	75	22	4	74	19	5	76
Ileum		—		1	0	99	7	2	91	9	2	89	8	2	90	9	3	88	9	3	88	7	4	89
Colon	0	0	100	4	0	96	4	1	95	8	2	90	6	4	91	7	2	91	7	2	91	5	3	92

MM=CK-MM isoenzyme (muscular type).
MB=CK-MB isoenzyme (miocardial type).
BB=CK-BB isoenzyme (brain type).

is higher in the jejunum and duodenum than in the ileum and colon. Maximal activity levels of CK on the 10th day of extrauterine life might be due to the relevance of the absorptive function at the time of development.

It was very difficult to obtain fresh fetal gastrointestinal tissue in human beings while CK isoenzymes in devitalized and non refrigerated tissues (12) are worthless because of their inestability. Therefore extrapolation of animal studies to human beings is the only approximation to CK and CK-isoenzymes ontogenesis. Our studies showed that the gastrointestinal tract mucosa CK isoenzyme profile in adult human subjects is similar to that of adult rats and, as well as that the appearance of a malignant GI tract neoplasia in adult humans induces CK isoenzyme patterns which resemble those found in the fetal rat GI tract mucosa (2).

Resumen

Se determina la actividad de la creatin-kinasa y sus isoenzimas en la mucosa del tracto digestivo de la rata durante su desarrollo normal. La actividad de la creatin-kinasa aumenta de forma escalonada durante el desarrollo fetal y la primera semana de vida. Todos los tramos de la mucosa digestiva presentan en las dos primeras semanas de gestación un patrón isoenzimático compuesto exclusivamente por CK-BB. El perfil isoenzimático de la CK en la mucosa esofágica se modifica al avanzar la maduración fetal predominando como en el adulto la concentración CK-MM. En el resto del tracto digestivo, sin embargo, la CK-BB continúa siendo la isoenzima principal. Los resultados muestran que la mucosa digestiva de la rata es particularmente útil para el estudio de las isoenzimas de la CK.

Palabras clave: Creatin-kinasa, Aparato digestivo, Desarrollo.

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