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The Creatine and Creatine Phosphate Contents of the Small Intestine Mucosa of the Rat before and during Food Ingestion

O. HÄNNINEN and K. HARTIALA

From the Department of Physiology, University of Turku, Finland

The total creatine and creatine phosphate contents of rat's small intestine mucosa were studied before and during food ingestion.

Before food ingestion it was found that both the total creatine and creatine phosphate contents were higher in the oral end of the small intestine mucosa than in the aboral end; these contents showed, however, an increase again in the last 10 cm segment. No statistically significant differences were found between the stages before and during food ingestion. During food ingestion, distribution of the contents of the compounds was, however, slightly different than before food ingestion.

The ratio of the creatine phosphate to the total creatine remained in both series approximately the same (*ca.* 40 %) and no striking differences were present in the mucosa of the different parts of the small intestine.

It was found that *p*-chloromercuribenzoate could not prevent reduction of the recovery values in the tissue extracts.

Creatine and creatine phosphate has been found in all rat tissues.[1] In muscular cells, in which the creatine phosphate content is very high — more than that of adenosine tri- and diphosphates [2] — this compound is considered to play an important role in the contraction mechanism. Creatine phosphate is regenerated in the reaction catalysed by adenosine triphosphate creatine transphosphorylase.[3] Possibly creatine can also be phosphorylated by other reactions.[4] The final role of creatine in the metabolism is not yet clear.

In our previous studies, we have been able to show variations in the distribution of adenosine triphosphate in the gastrointestinal mucosa so that in the oral part, where the most effective absorption of nutrients takes place,[6] the adenosine triphosphate content, is also higher than in the aboral part.[7] According to Kroeger and Edwards [5] different fourths of rat's small intestine show differences in their creatine phosphate contents. In the present study

systematic determinations were made of the distribution of creatine phosphate and creatine in the different segments of rat's small intestine mucosa both before and during food ingestion.

METHODS

Wistar male rats were used (weight range 205—225 g). The animals were kept on a mixed diet, fed at 9 a.m.[8] Specimens for study of the stage before food ingestion were taken one hour before feeding and for study of the stage during ingestion 3 h after feeding. The animals were anesthesized lightly with ether in a glass container, after which the abdominal cavity was exposed in a cold room at 4°. The appendix was lifted from the abdominal cavity, the small intestine was cut 5 mm above the ileocecal junction. In the ventral wall of the stomach about 5 mm above the pylorus a hole was cut, through which 50 ml of 0.3 N perchloric acid (4°C) and thereafter 50 ml of air was injected with a syringe.[9] The small intestine was then removed and transferred into distilled water (4°C), where the blood was rinsed away. The first and last 5 mm portions of the intestine were discarded and the actual specimens were taken as follows: No. 1: first 4.5 cm, Nos. 2—5: following successive 10 cm segments, and No. 6: the fifth last 10 cm, No.7: the third last 10 cm, and No. 8 the last 10 cm segment. The intestinal specimens were opened, the possible remainings of the intestinal contents were carefully removed with a moist brush. The mucosa was scraped from the segments with an ampoule file. Specimens were crushed with a glass rod and extracted with 4 ml of 0.3 N perchloric acid (4°C) for 15 min. After this the specimens were centrifuged (about 13 000 *g* for 10 min, 4°C). Supernatant was overneutralized with 2 ml of 0.8 N NH_4OH (4°C) and the obtained solution was immediately used for creatine and creatine phosphate determinations. The method of Ennor and Rosenberg[10] was modified as follows: 2.5 ml of H_2O was added to 0.5 ml of specimen and in the total creatine determinations, after a preincubation for 2 min in a 65°C water bath, 1.0 ml of 0.46 N HCl was added and 9 min after this 1.0 ml of 0.46 N NaOH, and the tubes were cooled with running water. In the free creatine determinations 2.0 ml of 0.23 N NaCl was used instead of the acid and base. The procedure of both total and free creatine determinations was then continued by adding 2.0 ml of α-naphthol (1 %) and *p*-chloromercuribenzoate (10 mM)-stock alkaline solution and the diacetyl reagent. The measurement was carried out 25 min after this at wave length 525 mμ with a Beckmann DU spectrophotometer. Three parallel determinations were made from each specimen; a known amount of creatine was added to one of these in order to check the recovery. Reference specimens of creatine and creatine phosphate were obtained from Merck AG., Darmstadt, and Sigma Chemical Co., St Louis, respectively.

Control specimens also from other tissues were occasionally taken from some animals.

The dry weight of the specimens was determined by adding 2 drops of 0.8 N NH_4OH to the tissue precipitates obtained by perchloric acid extraction and centrifugation and keeping the tared centrifuge tubes for 8 h at 92°C and weighing them after 45 min of standing at room temperature. The weights were corrected for NH_4ClO_4.

RESULTS

The possible decreasing effect of ether narcosis on the creatine phosphate content was studied by preparing two animals using local anesthesia (Xylocain Astra, 1 %). The amount of creatine phosphate to the total creatine content in different specimens was approximately the same as in animals after ether narcosis. The ether narcosis did not affect the colour of the mucous membranes of the animals; the whiskers vibrated still and the muscle relaxation was far from complete. If, however, apnoe was produced during the preparation, the animal was discarded, since the creatine phosphate content in such cases was found to decrease very rapidly.

Of the 50 ml of 0.3 N perchloric acid used for perfusion about 30—40 ml was recovered. The postprandial recovery was somewhat higher; the intestinal contents thus contributed to these values. The possible extraction effect of the perfused perchloric acid on the creatine and creatine phosphate contents in the intestinal mucosa was studied by determining the creatine and creatine phosphate contents in the perfusate, using Ringer solution as a control. From the normally treated animals as an average 1.7 μmoles of creatine was recovered in the perfused perchloric acid. Creatine was found to increase towards the last milliliters. Repeated perfusion still increased the amount of creatine per ml. In 150 ml used in one case *ca.* 6 μmoles of creatine was found in the perfusate. In this case, however, the total creatine content of the mucosa specimens was only somewhat lower than the usual average, and the creatine phosphate content of the mucosa was markedly higher than the average (55—60 % of the total creatine). The acid hydrolyzed perfusates gave regularly lower values than the unhydrolyzed specimens. In repeated perfusions with Ringer solution, material reacting like creatine was continuously recovered. Yet creatine determinations from the mucosa after Ringer solution perfusion gave markedly higher total creatine concentrations than after perchloric acid perfusion. The dry weight values of the mucous membrane were lower after the Ringer solution perfusion.

Minimal amounts of material reacting like creatine was found in the feces.

In order to study the possible movements of creatine and creatine phosphate in the mucosa during the perfusion, the perchloric acid was injected in some animals from the aboral end of the small intestine towards the oral end. No differences could be found.

The efficiency of the extraction of creatine and creatine phosphate was studied by repeated extractions. The first extraction gave as an average 90 % and the other *ca.* 9 % of the total creatine. The first extraction gave also 90 % of the creatine phosphate. The use of a Potter-Elvehjem type all glass homogenizer did not increase the efficiency of the extraction.

During the perchloric acid extraction it was found that creatine phosphate was hydrolyzed from the 0.5 μmole specimen at a rate of about 1 %/h. After

Table 1. Creatine and creatine phosphate contents of small intestine mucosa of rat before food ingestion. The mean contents (and standard errors) of ten animals were calculated per gram of dry weight.

	Creatine	Creatine phosphate
Sample	μmoles/g of dry weight	μmoles/g of dry weight
1	20.97 ± 1.61	7.34 ± 0.62
2	18.95 ± 1.80	8.14 ± 0.99
3	18.75 ± 1.59	7.97 ± 0.54
4	17.85 ± 1.67	7.33 ± 1.13
5	17.97 ± 1.41	7.65 ± 0.64
6	16.15 ± 1.36	6.70 ± 0.77
7	15.47 ± 1.14	6.04 ± 0.71
8	18.75 ± 1.99	6.57 ± 0.94

Table 2. Creatine and creatine phosphate contents of small intestine mucosa of rat during food ingestion. The mean contents (and standard errors) of nine animals were calculated per gram of dry weight.

	Creatine	Creatine phosphate
Sample	μmoles/g of dry weight	μmoles/g of dry weight
1	20.77 ± 2.12	8.15 ± 1.25
2	18.47 ± 2.23	7.51 ± 1.18
3	17.74 ± 1.73	7.70 ± 0.80
4	18.67 ± 1.95	8.13 ± 1.42
5	18.75 ± 1.88	7.80 ± 1.34
6	18.36 ± 1.47	7.32 ± 0.89
7	20.79 ± 2.92	7.22 ± 1.00
8	23.28 ± 2.77	9.84 ± 1.83

the neutralization with ammonia no further hydrolysis could be found during the following 3 h. Also in the strongly alkaline α-naphthol-*p*-chloromercuribenzoate solution the creatine phosphate remained unhydrolyzed for one hour.

In the creatine determination procedure used, arginine was found to interfere with the determination. By using ascending paper chromatography (phenol-ethanol-ammonia solvent [9]) about 0.028 μmoles/mg of tissue dry weight of an amino acid mixture was obtained corresponding to the R_F of arginine. This amount was the same in specimens 2, 5 and 7.

The recovery of the additions of known amounts of creatine during the creatine determinations was 60 %, with or without *p*-chloromercuribenzoate. The recovery was the same for the different specimens of the small intestine. Creatine recovery from the liver was slightly lower.

The results are collected in Tables 1 and 2. The decrease of the total creatine content found at the stage before food ingestion from specimens Nos. 1—7 is statistically significant; also the creatine phosphate contents of the gastrointestinal mucosa differ statistically significantly in the creatine phosphate-rich first part (specimen No. 3) as compared to specimen No. 7. In the postprandial studies, differences between the specimens are not significant, nor do they differ significantly from the values of the stage before ingestion.

The relative amount of creatine phosphate to the total creatine amount varied somewhat but was about 40 % of this.

The creatine contents in the other tissues of the gastrointestinal tract were approximately the same as in the small intestine: in the mucosa of glandular stomach *ca.* 20 μmoles/g, in the membranous part of stomach slightly less and in the mucosa of the appendix *ca.* 25 μmoles/s. Specimens containing all parts of the gastrointestinal wall revealed slightly higher creatine contents than those found in the mucosa. The total creatine content in the whole wall specimens began to decrease from the third specimen downwards. The total creatine content of the liver was found to be 3.2 μmoles/g. Control experiments of the creatine content of *musculus rectus abdominis* gave *ca.* 73 μmoles/g dry weight of creatine.

DISCUSSION

The injected perchloric acid extracts some of the mucosal creatine. This quantity, however, could not be exactly determined by measurement of the creatine-like reacting material in the perfusate, because the intestinal contents interfered with the determination. The smaller amount of creatine-like reacting material recovered after acid hydrolysis, reveals disappearance of one or several sources of error by the effect of heat and acid. The low content of creatine-like reacting material found in the feces, indicates that it is absorbed or destroyed in the aboral part of the intestine.

The arginine content present in the intestinal mucosa did not have a bearing on the creatine phosphate contents obtained, since the method is based on the determinations of the free and total creatine. The amino acid spots detected by paper chromatography and resembling arginine, indicated so large amounts that the creatine determinations surely would have been disturbed if arginine was the sole component. These spots however, did not, give the typical arginine reaction as shown by using Sakaguchi's oxine or α-naphthol reagents.[12]

The effect of *p*-chloromercuribenzoate on the determinations of creatine in the small intestine mucosa can be considered negligible, since its use did not improve the recovery in specimens containing about 20 μg. Also the recoveries obtained from the liver without *p*-chloromercuribenzoate did not differ from the corresponding values obtained from the intestinal mucosa (*cf.* Ref.[1]). On the other hand; if cysteine was added to the specimens, *e.g.* 2 μmoles/specimen, *p*-chloromercuribenzoate reduced the otherwise distinct thiol-inhibition. The component, contributing to the decrease of the recovery in the tissues, is thus not obviously the compound acting through thiol groups.

The lower dry weight values found in the Ringer-perfused control animals and the following increase in the creatine content per dry weight unit may be due to the precipitation of intestinal contents on the mucosa during the perchloric acid perfusion. The intestinal mucosa after Ringer solution perfusion before and during food ingestion is different; at the latter stage it is thicker and more solid. A similar difference was not found in connection with perchloric acid perfusion. The gastrointestinal tract wall is strongly hyperemic during digestion. Part of the blood is denaturated probably with the mucosa during perchloric acid perfusion and is a source of error in dry weight estimations. Elimination of blood by bleeding, however, is not possible due to the disturbance of metabolism and oxidative phosphorylation during oxygen lack.

The distribution of the total and phosphate-bound creatine before food ingestion in different segments of the intestine resembles somewhat the previous findings on the distribution of the adenosine triphosphate and ATP-hydrolyzing enzyme system, [7] the hexokinase activity during preabsorption,[8] active absorption capacities of various nutrients,[13-16] and the capacity to synthesize β-D-glucopyranosiduronic acids.[17]

In this study creatine phosphate was found to comprise some 40 % of the total creatine in the mucosa. It has usually been considered to count only some 20 % of the total. [10] The values obtained for the first and last specimens may be somewhat unreliable, because the perfusion holes probably disturbed the

blood circulation here more than elsewhere before the perchloric acid denatured the mucosa.

Kroeger and Edwards [5] found, by using acid hydrolysis and phosphate determinations, that the creatine phosphate content decreases significantly as a function of the length of the small intestine. The contents reported by them are markedly higher than those found in this work. However, their method based on the liberated phosphate is rather unspecific and many phosphate fractions may cause the increase of inorganic phosphate during the hydrolysis.[18] Ennor and Rosenberg[10] have given *ca.* 2 μmoles/g dry weight as the creatine phosphate content of the intestinal smooth muscle layer.

The creatine contents in the liver as well as the total creatine content in sceletal muscle was found to be approximately the same as those reported by Ennor and Rosenberg.[10]

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