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ONTOGENY REGULATES CREATINE METABOLISM IN RAT SMALL AND LARGE INTESTINE

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The ontogeny of intestinal CRT, AGAT and GAMT was investigated in foetuses, newborn, suckling, weaning and adult rats. In the colon, CRT mediates creatine transport because it was Na^+- and Cl^- dependent and inhibited by creatine and GPA. In addition, Northern assays showed two CRT transcripts (2.7-kb and 4.2-kb) and the *in situ* hybridisation revealed that CRT mRNA is restricted to the colon epithelial cells. The immunohistochemistry revealed that CRT protein was at the apical membrane of colon epithelia. Maturation decreased colonic CRT activity to undetectable levels and increased CRT mRNA abundance. Western assays revealed 57-, 65-, 80- and 116-kDa polypeptides at the intestinal apical membrane. The abundance of the 65-, 80- and 116-kDa polypeptides decreased with age, and that of 57-kDa was only observed in adult rats. The small and large intestine express AGAT and GAMT mRNAs. Maturation decreased AGAT mRNA abundance without affecting that of GAMT. For comparison, renal AGAT mRNA levels were measured and they were increased with age. The study reports for the first time that: i) the apical membrane of rat colon have an active CRT, ii) development down-regulates CRT activity *via* post-transcriptional mechanism(s), iii) the intestine might synthesize creatine and iv) intestinal and renal creatine synthesis is ontogenically regulated at the level of AGAT gene expression.

Key words: *creatine transport, L-arginine : glycine amidinotransferase, guanidinoacetate methyltransferase, intestine, development*

Abbreviations: *CRT, Na^+/Cl^-/creatine transporter; AGAT, L-arginine: glycine amidinotransferase; GAMT, guanidinoacetate methyltransferase; GPA, guanidinopropionic acid.*

INTRODUCTION

Creatine acts as an energy storage mechanism and transports/shuttles high-energy phosphate in tissues with high metabolic demands such as the heart, brain, retina, testes and skeletal muscle. Deficiencies in the enzymes involved in creatine synthesis, guanidinoacetate-methyltransferase (GAMT) and L-arginine : glycine amidinotransferase (AGAT), and in the $2Na^+$/$1Cl^-$/creatine transporter (CRT) (2) caused myopathies, mental retardation and other diseases (for reviews see 3-5). These observations strengthened the physiological relevance of creatine.

Plasma creatine levels depend on creatine biosynthesis, intestinal creatine absorption and renal reabsorption of filtered creatine.

Creatine is synthesized from arginine and glycine in two steps. The first reaction is catalyzed by AGAT and the second by GAMT. AGAT activity is under nutritional and hormonal control (6-12). Creatine represses AGAT mRNA expression and activity, thus regulating its own biosynthesis (7, 9, 12). The biosynthesis of creatine appears to be an inter-organ process, which mainly involves the pancreas, liver and kidneys. The synthesized creatine is exported to the blood and is taken up by creatine-requiring tissues *via* the CRT transporter (for reviews see 3, 5).

Oral creatine supplementation is widely used by athletes to improve performance (13) and in the medical field it may be useful for the treatment of neuromuscular, cardiovascular, and mitochondrial diseases as well as Parkinson's and Huntington's; muscle rehabilitation; hypoxia and energy-related brain pathologies; as an antitumoral, antiviral, neuro-protective agent and in organ transplantation (3, 14, 15).

Human and animal individuals have been used to investigate the benefits of oral creatine supplementation. CRT mediates the first step of small intestinal creatine absorption (16, 17) and ontogeny regulates intestinal CRT activity (18). However, information on whether: i) the intestine contributes to creatine synthesis, ii) the colon transports creatine or iii) ontogeny regulates intestinal creatine metabolism is not available. The current study investigates creatine transport activity in the colon and the intestinal abundance of CRT, AGAT and GAMT mRNAs and that of CRT protein in E20 foetuses, newborn, suckling, weaning and adult rats.

The results revealed that in the early stages of life the colon transports creatine by a specific NaCl-dependent process; that the intestine expresses AGAT and GAMT mRNAs and that both, creatine transport and synthesis are ontogenically regulated.

MATERIALS AND METHODS

Substances

[^{14}C]-creatine was purchased from American Radiolabeled Chemicals Inc. and [α^{32}P]-UTP from GE Healthcare Europe GmbH. The other reagents used in the current study were obtained from Sigma Chemical Co, Spain.

Animals

20 day gestation foetuses (E-20), newborn (less than 1 day-postpartum), suckling (12 and 15 day-old), weaning (30 day-old) and adult (2 and 5 month-old) Wistar rats were used. Rats were humanely handled and sacrificed in accordance with the European Council legislation 86/609/EEC concerning the protection of experimental animals. Mothers and adult rats were fed with a rat chow diet (Panlab 04) *ad libitum* and had free access to water. The experiments were performed in accordance with national/local ethical guidelines.

Creatine uptake experiments

The whole colon was removed, opened longitudinally and rinsed clean with ice-cold saline solution (0.9% NaCl). 2 cm pieces of the whole colon were incubated at 37°C in a thermostatic bath, with continuous shaking, in Ringer's solution containing of, in mM: 140 NaCl, 1.2 $CaCl_2$, 2.4 K_2HPO_4, 0.4 KH_2PO_4, 10 $KHCO_3$, 1.2 $MgCl_2$, pH 7.4, and 0.012 [^{14}C]-creatine, and it was continuously bubbled with 95%O_2/ 5%CO_2. When required, NaCl was isosmotically substituted by mannitol. At the end of the incubation time period the tissues were washed, weighed wet and extracted with $Ba(OH)_2$ and $ZnSO_4$. Samples were taken from the bathing solution and from the tissues extracts for radioactivity counting.

RNA preparation and Northern blotting

Total RNA was extracted as described (19). Northern hybridisation was carried out using Poly(A)$^+$RNA obtained from duodenum, jejunum, ileum and colon of 30 day-old rats, as reported (20). [α^{32}P]-UTP-labelled antisense riboprobe of CRT was generated from a rat cDNA fragment as described (16). Relative quantification of mRNA was determined with a phosphoimager system (Fuji Photo Film Co., LTD) using the PCBAS program (Raytest GmbH). Expression of CRT mRNA was normalised using cyclophilin mRNA.

In situ hybridisation

Floating 30 µm-sections of colon obtained from 15 day-old rats were hybridised with either antisense or sense digoxigenin-UTP-labeled riboprobes, generated from rat cDNA fragments as described (16). *In situ* hybridisation was performed as described (21), except that the sections were washed to high stringency conditions at 56°C. The sections were photographed with a Zeiss Axioskop 40 microscope.

Immunohistochemistry

Immunohistochemistry was carried out on 10 µm-sections of colon obtained from 15 day-old rats as described (16). The primary antibody used was a polyclonal anti-CRT from Alpha Diagnostic. Controls were carried out without primary antibody. The sections were photographed with a Leica TCS-SP2 confocal fluorescence microscope.

Western blotting

Protein samples of small intestinal mucosa homogenate and enterocyte apical membranes were used. Brush-border membranes were isolated as described (22). SDS-PAGE was performed according to Laemmli (23) as described (16). The blots were probed with a polyclonal anti-CRT antibody from Alpha Diagnostic and subsequently incubated with an anti-β-actin antibody to normalize CRT density values.

Relative quantification of real-time PCR

Total RNA was extracted from rat small and large intestine of different ages using RNeasy® kit (Qiagen). Real-time PCR was performed as described (24). Primers for the genes tested are given in *Table 1*. β-actin served as reference gene for samples normalisation.

Analysis of data

Data are presented as mean ± SEM for (n) separated animals. In the figures, the vertical bars that represent the SEM are absent when they are less the symbol height. Comparison between different experimental groups was evaluated by the two-tailed Student's t-test. Two-way ANOVA followed by Newman-Keuls´ test ($p< 0.05$) was used for multiple comparisons in *Figs 1*, *5* and *7* (GraphPad Prism Program).

RESULTS

Creatine uptake into the colon

30 min (^{14}C)-creatine uptake into the colon isolated from newborn, suckling and weaning rats was measured in the presence and nominal absence of NaCl in the incubation buffer. *Fig. 1 A* shows that NaCl-dependent creatine uptake was maximal at birth. Thereafter it decreased and it was no longer measurable in 30 day-old rats. NaCl-independent creatine uptake was not affected by age.

Creatine uptake into the colon isolated from newborn and suckling rats was inhibited by cold creatine and by guanidinopropionic acid (GPA), a high-affinity substrate of CRT (*Fig. 1B*).

Northern blots and in situ hybridisation

CRT mRNA expression in the colon of 30 day-old rats was examined by Northern assays using poly (A^+) RNA. The

Table 1. Oligonucleotides used in relative quantification of real-time PCR

Gene	Accesion number	Antisense (5' …3')	Sense (5' …3')
CRT	NM017348	TTCTATTACCTGGTCAAGTCCT	GCCTCAAGACTTTGTTCTCC
AGAT	NM031031	CCTGTCTCCTCTTACAACGA	TTCATATGTATTGGCCTTCACC
GAMT	NM012793	GCCTATGATACGTCTGACAC	CCATCGTTGCATTCAATAATCC
β-Actin	NM031144	ACCCACACTGTGCCCATCTA	CGGAACCGCTCATTGCC

Oligonucleotides were chosen according to the rat cDNA sequences entered in Genbank and designed using PerlPrimer program.

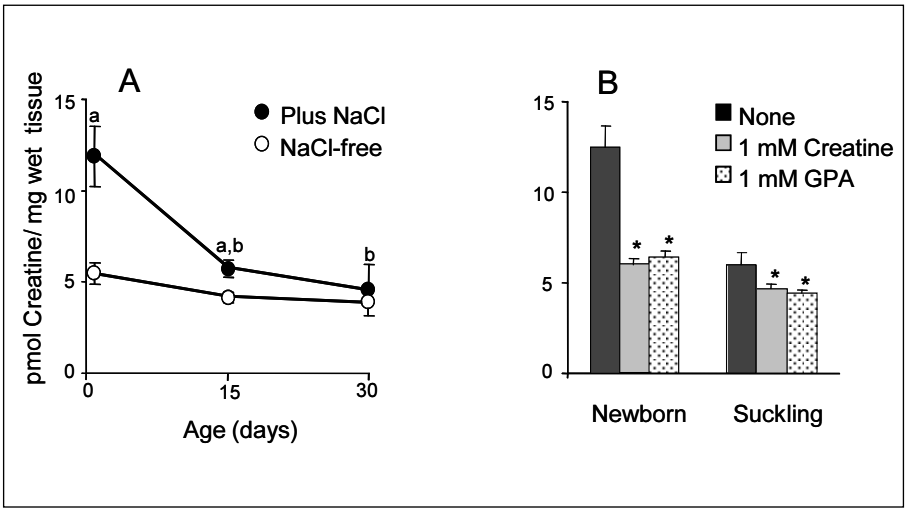

Fig. 1. Creatine uptake in rat colon. A: 30 min (^{14}C)-creatine uptake was measured in the whole colon isolated from newborn, suckling and weaning rats, in the presence (●) and absence (○) of extracellular NaCl. NaCl was isosmotically substituted by mannitol. B: The effect of either 1 mM guanidinopropionic acid (GPA) or 1 mM unlabeled creatine in the incubation buffer. Data are means ± SEM of 5 independent experiments. Two-way ANOVA showed an effect of maturation ($p<0.001$). Newman-Keuls test: "a", as compared with nominally NaCl-free conditions. "b", as compared with newborn rats. *$p<0.001$ as compared with absence of modifiers.

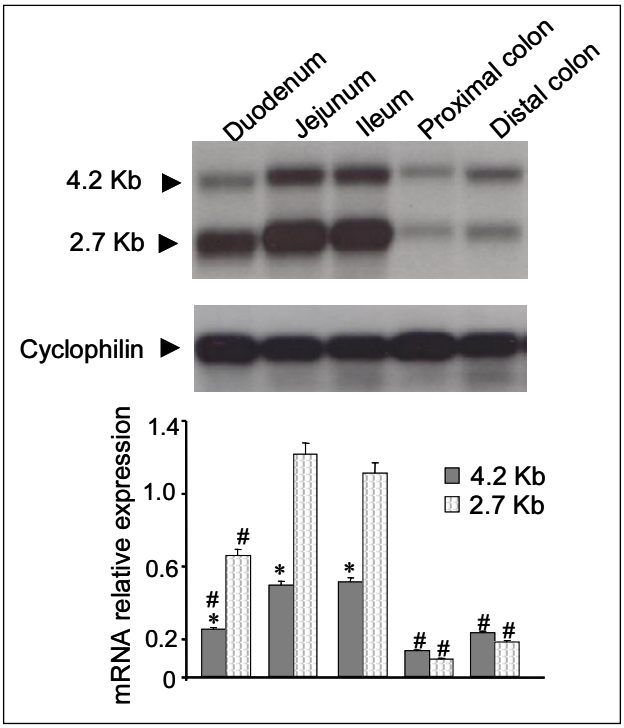

Fig. 2. Northern blots of CRT in small and large intestine of 30 day-old rats. 10 µg of poly(A$^+$) RNA were loaded onto the gel per lane. The size of the transcripts was determined by ribosomal RNA. *$p<0.001$, 2.7 kb vs 4.2 kb. #$p<0.001$, as compared with ileum.

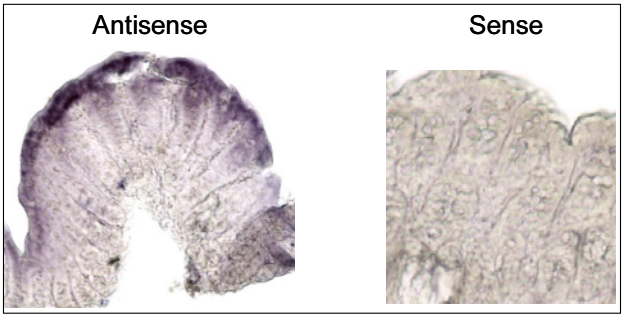

Fig. 3. In situ CRT mRNA expression. Panels are bright field photomicrographs of 15 day-old rats colon, which have been *in situ* hybridised with either antisense or sense digoxigenin-UTP-labeled riboprobes. Magnification: x 100.

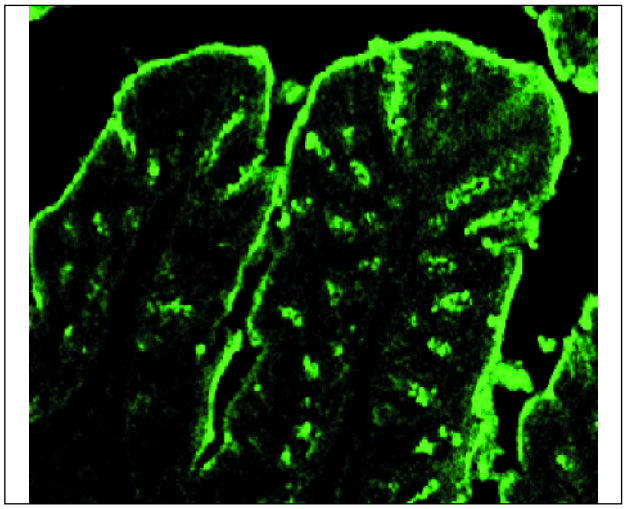

Fig. 4. Immunolocalization of CRT in rat colon. Sections of 15 day-old rats colon were immunostained with the polyclonal anti-N-terminal CRT antibody, as indicated in Methods. Magnification: x 50.

antisense riboprobe hybridised to two transcripts of 2.7-kb and 4.2-kb (*Fig. 2*). For comparison, the expression of CRT mRNA in the small intestine is given. The abundance of the two transcripts was greater in the small intestine than in the large intestine. In the former, the 2.7-kb transcript was significantly more abundant than that of 4.2-kb. In the colon the abundance of the two transcripts was of similar magnitude.

Northern blots do not inform on the type of cells expressing CRT mRNA within the epithelia as do the *in situ* hybridisation. The latter studies revealed that CRT mRNA expression was restricted to the cells lining the surface of the colonic mucosa (*Fig. 3*, antisense panel). The background labelling with the sense riboprobe on adjacent sections of the tissue was low (*Fig. 3*, sense panel).

Immunohistochemistry

The localisation by immunofluorescence of CRT protein in rat colon at the confocal microscopy level is given in *Fig. 4*. There was a strong specific signal at the apical membrane of cells lining the colonic mucosa. No immunofluorescence signal was detected when the intestine was incubated without the primary antibody (data not shown)

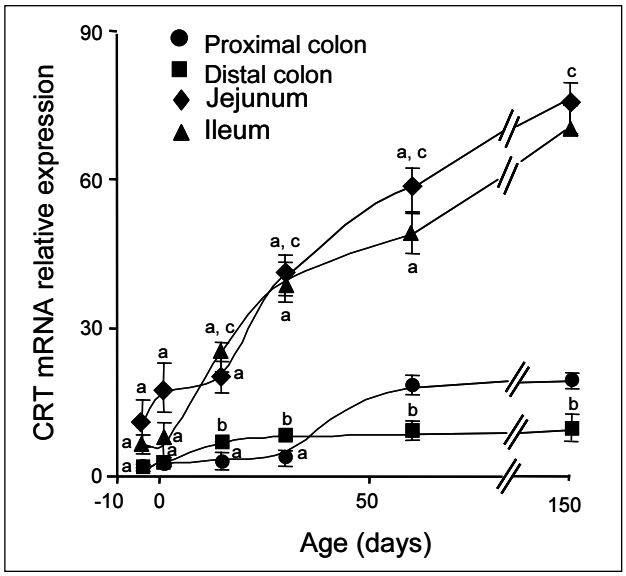

Fig. 5. Expression of rat CRT mRNA in the small and large intestine during development. CTR mRNA expression was evaluated by real time PCR analysis. Total RNA was isolated from small and large intestine of rats of different ages. The mRNA relative expression values were normalised in each sample using the β-actin as reference gene. The mRNA levels measured in the distal colon of rat foetuses were set at 1. Means ± SEM of 4 animals in each experimental condition. Two-way ANOVA showed an effect of maturation ($p<0001$). Newman-Keuls test: "a", as compared with adult rats (5 months old). "b", distal colon vs proximal colon. "c" jejunum and ileum vs. colon.

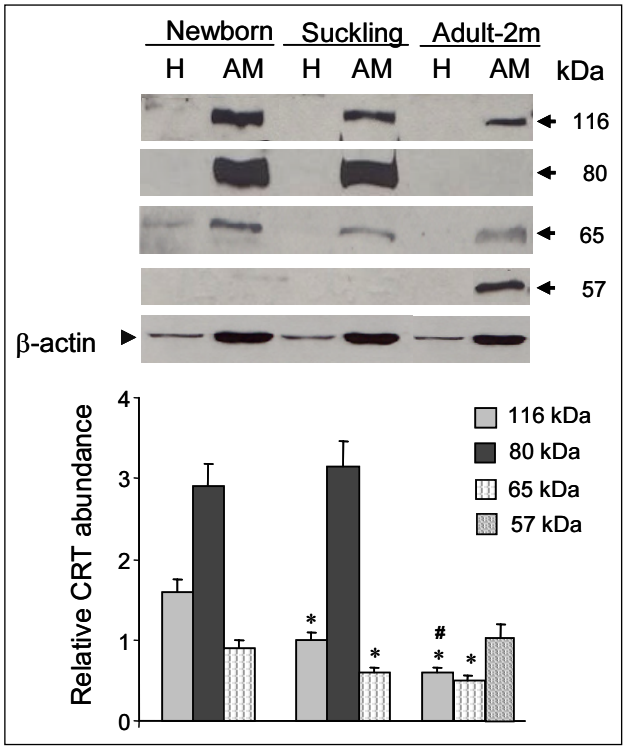

Fig. 6. Western blots of small intestine homogenate (H) and apical (AM) membranes. 70 µg protein were loaded to each lane. The blots were probed with the polyclonal anti-N-terminal anti-CRT antibody. Histograms represent the relative abundance of CRT protein in apical (AM) membrane. Means ± SEM, n= 3. *$p< 0.001$, as compared with newborn. #$p< 0.001$, as compared with suckling.

CRT mRNA expression versus age

The effect of ontogeny on CRT mRNA abundance in small and large rat intestine was examined by the real-time PCR technique. *Fig. 5* shows that in both small and large intestine CRT mRNA abundance increases from the foetus stage to adult life. At all the ages tested, CRT mRNA abundance was significantly higher in the small intestine in comparison to the large intestine.

Small intestinal CRT protein expression versus age

The antibody used detected 57-, 65-, 80- and 116-kDa polypeptides in the apical membrane of the enterocytes (*Fig. 6*). The intensity of all the bands was very faint in the homogenate (H) but highly enriched in the apical membranes (AM). Maturation decreased the intensity of the 65- and 116-kDa bands and that of 80-kDa was undetectable in 2 month-old rats. The 57-kDa polypeptide was only observed in the apical membrane of 2 month-old rats.

Intestinal mRNA levels of AGAT and GAMT versus age

The expression of AGAT and GAMT mRNAs in the small and large intestine was examined by the real-time PCR technique. The results (*Fig. 7*) revealed that at all the ages tested, AGAT mRNA abundance was higher in the colon than in the small intestine. In both intestinal regions the maximal abundance occurred in the foetuses and it decreased with age. In the foetuses, AGAT mRNA levels were significantly higher in the proximal colon than in the distal, and higher in the jejunum than in the ileum.

Compared to AGAT, intestinal GAMT mRNA abundance remains at a low level and it was affected by neither intestinal region nor development.

For comparison, AGAT and GAMT mRNA levels were measured in the renal cortex (*Fig. 7*). Maturation significantly increased the abundance of AGAT mRNA, without significantly modifying that of GAMT. At all the ages tested, the renal abundance of AGAT and GAMT mRNAs was higher than in the intestine. In the colon of foetuses, AGAT mRNA levels were approx 25% of those measured in the foetal renal cortex.

DISCUSSION

Body creatine homeostasis depends on endogenous synthesis, absorption from diet and renal handling. In rats, the first step of renal (25) and intestinal (16, 17) transepithelial creatine transport occurs *via* an apical Na^+/Cl^-/creatine transporter (CRT).

Traditionally, the upper part of the gut is the focus of studies on nutrient transport because it is the place where most nutrients are digested and absorbed. Here for the first time, we give evidence that in the early stages of life, the colon transports creatine *via* a Na^+/Cl^-/creatine transporter: creatine uptake into rat colon was NaCl-dependent and inhibited by either GPA or cold creatine.

The molecular studies indicate that an apical-membrane CRT mediates the NaCl/creatine transport activity measured in the colon. Thus, the rat colon expresses the same two CRT mRNA transcripts (ca 4.2- and 2.7-kb) previously observed in the rat small intestine (16). The *in situ* hybridisation studies corroborated the presence of CRT mRNA in the colon and revealed that this expression is restricted to the cells lining the surface of the colon mucosa. The crypt cells do not express CRT mRNA. Finally, the immunohistochemistry experiments show that fluorescence mostly appears along the apical membrane of

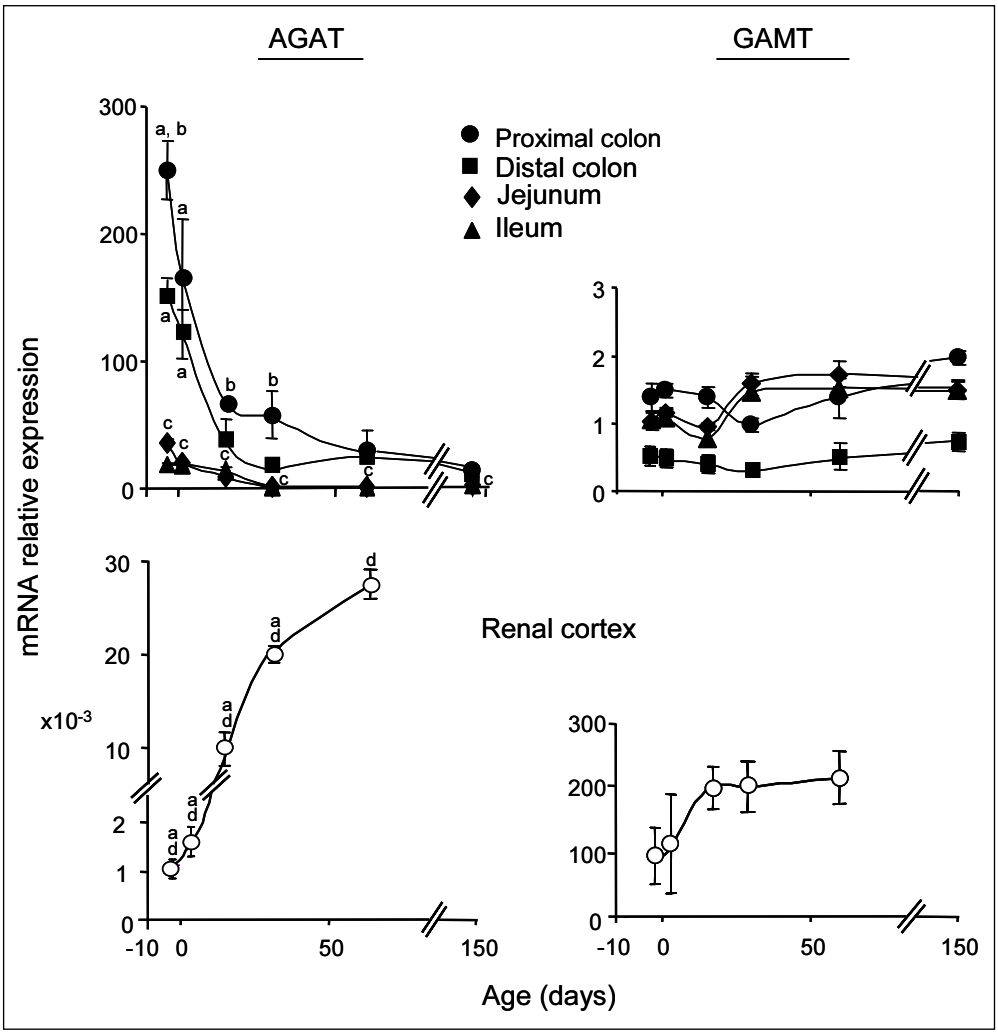

Fig. 7. Expression of rat GAMT and AGAT mRNAs in small and large intestine and renal cortex during development. The mRNA levels measured in ileum of rat foetuses were set at 1. Other details as in *Fig. 5*. Means ± SEM of 4 animals in each experimental condition. Two-way ANOVA showed an effect of maturation ($p<0001$). Newman-Keuls test: "a", as compared with adult rats. "b", proximal colon vs. distal colon. "c" small intestine vs. colon. "d" renal cortex vs. colon.

the cells lining the surface area of the colon mucosa, which agree with the *in situ* hybridisation results.

As observed in the small intestine (18), colonic NaCl/creatine transport activity is regulated by ontogeny, showing its highest value at birth and being no longer evident after weaning.

The Western assays detected four polypeptides in the apical membrane of the intestine but the results do not allow us to determine which corresponds to the CRT transporter. The 57-kDa polypeptide could be dismissed since it was only detected in 2 month-old rats, when CRT activity is not longer measurable. The 80-kDa polypeptide might be also ruled out because it was equally abundant in newborn and suckling rats, whilst CRT activity decreased. The intensity of the 116-kDa and 65-kDa polypeptides decreased with age and it could be hypothesise that the 65-kDa polypeptide represents the plasma membrane CRT and that of 116-kDa corresponds to a dimer form of CRT.

Studies on the size of CRT protein have yielded conflicting results. Estimates of its molecular weight vary from 40-kDa to 210-kDa. Immunoreactive bands of >100-kDa may represent aggregates of CRT (26). Tran *et al.* (27) concluded that the 55-kDa polypeptide is the plasma membrane CRT. West *et al.* (26) purified bovine CRT and the glycosilated transporter run as an approx 70-kDa band. Speer *et al.* (28) considered that the CRT polypeptide of 60–65 kDa (depending on the tissue and the species) very likely constitutes the CRT protein. They concluded that the conflicting results on the molecular size of CRT may arise from the cross-reaction of the anti-CRT antibodies with non-CRT polypeptides. More recently, Straumann *et al.* (29) suggest that the plasma membrane CRT has an apparent monomer molecular mass of 58-kDa.

Whereas brush-border membrane CRT abundance and activity decreased with maturation, CRT mRNA levels increased with age. This opposite developmental pattern suggests that post-transcriptional mechanisms may regulate CRT protein synthesis and/or insertion in the apical membrane. The CRT protein presents consensus sequences for phosphorylation that may be potentially phosphorylated by cAMP-dependent protein kinase A and by protein kinase C, affecting CRT transport activity (see 4, for a review). Our own unpublished observations revealed that in the chickens, intestinal NaCl/creatine transport activity is increased by phorbol 12-myristate 13-acetate, a PKC activator, and by forskolin, an adenylate cyclase activator. Other reports, however, showed that phorbol esters reduced CRT activity in cultured muscle cells and that PKA activation did not affect creatine transport rates (30, 31).

As previously observed (32), rat embryos express AGAT and GAMT mRNAs, the two enzymes involved in creatine synthesis. AGAT mRNA abundance was higher in the colon than in the small intestine and in both tissues it was down-regulated by ontogeny. The abundance of GAMT mRNA was not affected by intestinal region or by development. These observations agree with the view that the reaction catalyzed by AGAT is a regulated step in the biosynthesis of creatine (for reviews see 3, 5).

The presence of AGAT and GAMT mRNAs in the intestine suggests that this organ may have the capacity to synthesize creatine. In the early stages of life, the colon might contribute significantly to the biosynthesis of creatine since AGAT mRNA abundance is 25% of that in the renal cortex. Later on, the contribution of the kidney to creatine synthesis is much larger than that of the intestine, since AGAT mRNA abundance is around 3,000 times higher than that in the colon.

In conclusion, the results reveal that the rat colon takes up creatine *via* the CRT transporter. Ontogeny regulates colonic creatine transport activity, intestinal expression of CRT and AGAT, and renal AGAT expression. In the late gestation period and early stages of life the colon may significantly contribute to creatine homeostasis.

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Conflict of interests: None declared.

REFERENCES

1. Garcia-Miranda P, Peral MJ, Cano M, Calonge ML, Ilundain AA. Creatine transport in rat colon: developmental maturation. *J Physiol Biochem* 2007; 63: 32.
2. Salomons GS, Van Dooren SJM, Verhoeven NM, Cecil KM, Ball WS, Degrauw TJ, Jakobs C. X-linked creatine transporter gene SLC6A8 defect, a new creatine-deficiency syndrome. *Am J Human Genetics* 2001; 68: 1497-1500.
3. Wyss M, Kaddurah-Daouk R. Creatine, and creatine metabolism. *Physiol Rev* 2000; 80: 1107-1213.
4. Snow RJ, Murphy RM. Creatine and the creatine transporter: a review. *Mol Cell Biochem* 2001; 224: 169-181.
5. Brosnan JT, Brosnan ME. Creatine: endogenous metabolite, dietary, and therapeutic supplement. *Annu Rev Nutr* 2007; 27: 241-261.
6. Carlson M, Van Pilsum JF. S-adenosylmethionine: guanidinoacetate N-methyltransferase activities in livers from rats with hormonal deficiencies or excesses. *Proc Soc Exp Biol Med* 1973; 143: 1256-1259.
7. McGuire DM, Gross MD, Van Pilsum JF, Towle HC. Repression of rat kidney L-arginine:glycine amidinotransferase synthesis by creatine at a pretranslational level. *J Biol Chem* 1984; 259: 12034-12038.
8. McGuire DM, Tormanen CD, Segal IS, Van Pilsum JF. The effect of growth hormone and thyroxine on the amount of L-arginine:glycine amidinotransferase in kidneys of hypophysectomized rats Purification and some properties of rat kidney transamidinase. *J Biol Chem* 1980; 255: 1152-1159.
9. Walker, J B. Repression of arginine-glycine transamidinase activity by dietary creatine. *Biochim Biophys Acta* 1959; 36: 574-575.
10. Van Pilsum JF, Carlson M, Boen JR, Taylor D, Zakis B. A bioassay for thyroxine based on rat kidney transamidinase activities. *Endocrinology* 1970; 87: 1237-1244.
11. Ungar F, Van Pilsum JF. Hormonal regulation of rat kidney transamidinase; effect of growth hormone in the hypophysectomized rat. *Endocrinology* 1966; 78: 1238-1247.
12. Guthmiller P, Van Pilsum JF, Boen JR, McGuire DM. Cloning and sequencing of rat kidney L-arginine: glycine amidinotransferase. Studies on the mechanism of regulation by growth hormone and creatine. *J Biol Chem* 1994; 269: 17556-17560.
13. Terjung RL, Clarkson P, Eichner ER, *et al.* American College of Sports Medicine roundtable. The physiological, and health effects of oral creatine supplementation. *Med Sci Sports Exerc* 2000; 32: 706-717.
14. Guerrero-Ontiveros L, Wallimann T. Creatine supplementation in health, and disease. Effects of chronic creatine ingestion in vivo, down-regulation of the expression of creatine transporter isoforms in skeletal muscle. *Mol Cell Biochem* 1998; 184: 427-437.
15. Wyss M, Schulze A. Health implications of creatine, can oral creatine supplementation protect against neurological, and atherosclerotic disease? *Neuroscience* 2002; 112: 243-260.
16. Peral MJ, Garcia-Delgado M, Calonge ML, *et al.* Human, rat, and chicken small intestinal Na^+/Cl^-/creatine transporter, functional, molecular characterization, and localization. *J Physiol* 2002; 545: 133-44.
17. Tosco M, Faelli A, Sironi C, Gastaldi G, Orsenigo MN. A creatine transporter is operative at the brush border level of the rat jejunal enterocyte. *J Membr Biol* 2004; 202: 85-95.
18. Peral MJ, Galvez M, Soria ML, Ilundain AA. Developmental decrease in rat small intestinal creatine uptake. *Mech Ageing Develop* 2005; 126: 523-530.
19. Chomczynski P, Sacchi N. Single step method of RNA isolation by acid guanidium thiocyanate-phenol-chloroform extraction. *Anal Biochem* 1987; 162: 156-159.
20. Murillo-Carretero MI, Ilundain AA, Echevarria M. Regulation of aquaporins mRNA expression in rat kidney by water intake. *J Am Soc Nephrol* 1999; 10: 696-703.
21. Duran JM, Peral MJ, Calonge ML, Ilundain AA. Functional characterization of intestinal L-carnitine transport. *J Membr Biol* 2002; 185: 65-74.
22. De la Horra MC, Cano M, Peral MJ, *et al.* Na^+-dependent D-mannose transport at the apical membrane of rat small intestine and kidney cortex. *Biochim Biophys Acta* 2001; 1512: 225-230.
23. Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 1970; 227: 680-685.
24. Garcia-Delgado M, Garcia-Miranda P, Peral MJ, Calonge ML, Ilundain AA. Ontogeny up-regulates renal Na^+/Cl^-/creatine transporter in rat. *Biochim Biophys Acta* 2007; 11: 2841-2848.
25. Garcia-Delgado M, Peral MJ, Cano M, Calonge ML, Ilundain AA. Creatine transport into brush-border membrane vesicles isolated from rat kidney cortex. *J Am Soc Nephrol* 2001; 12: 1819-1825.
26. West M, Park D, Dodd JR, Kistler J, Christie DL. Purification and characterization of the creatine transporter expressed at high levels in HEK293 cells. *Protein Expr Purif* 2005; 41: 393-401.
27. Tran TT, Dai W, Sarkar HK. Cyclosporin A inhibits creatine uptake by altering surface expression of the creatine transporter. *J Biol Chem* 2000; 275: 35708-35714.
28. Speer O, Neukomm LJ, Murphy RM, *et al.* Creatine transporters: a reappraisal. *Mol Cell Biochem* 2004; 256-257: 407-424.
29. Straumann N, Wind A, Leuenberger T, Wallimann T. Effects of N-linked glycosylation on the creatine transporter. *Biochem J* 2006; 393: 459-469.
30. Dai W, Vinnakota S, Qian X, Kunze DL, Sarkar HK. Molecular characterization of the human CRT-1 creatine transporter expressed in Xenopus oocytes. *Arch Biochem Biophys* 1999; 361: 75-84.
31. Nash SR, Giros B, Kingsmore SF, *et al.* Cloning, pharmacological characterization, and genomic localization

of the human creatine transporter. *Recept Channels* 1994; 2: 165-174.
32. Braissant O, Henry H, Villard AM, Speer O, Wallimann T, Bachmann C. Creatine synthesis and transport during rat embryogenesis: spatiotemporal expression of AGAT, GAMT and CT1. *BMC Dev Biol* 2005; 5: 9.

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