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CREATINE SALTS PROVIDE NEUROPROTECTION EVEN AFTER PARTIAL IMPAIRMENT OF THE CREATINE TRANSPORTER

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Abstract—Creatine, a compound that is critical for energy metabolism of nervous cells, crosses the blood-brain barrier (BBB) and the neuronal plasma membrane with difficulty, and only using its specific transporter. In the hereditary condition where the creatine transporter is defective (creatine transporter deficiency) there is no creatine in the brain, and administration of creatine is useless lacking the transporter. The disease is severe and incurable. Creatine-derived molecules that could cross BBB and plasma membrane independently of the transporter might be useful to cure this condition. Moreover, such molecules could be useful also in stroke and other brain ischemic conditions. In this paper, we investigated three creatine salts, creatine ascorbate, creatine gluconate and creatine glucose. Of these, creatine glucose was ineffective after transporter block with guanidine acetic acid (GPA) administration. Creatine ascorbate was not superior to creatine in increasing tissue creatine and phosphocreatine content after transporter impairment, however even after such impairment it delayed synaptic failure during anoxia. Finally, creatine gluconate was superior to creatine in increasing tissue content of creatine after transporter block and slowed down PS disappearance during anoxia, an effect that creatine did not have. These findings suggest that coupling creatine to molecules having a specific transporter may be a useful strategy in creatine transporter deficiency. In particular, creatine ascorbate has effects comparable to those of creatine in normal conditions, while being superior to it under conditions of missing or impaired creatine transporter.

Key words: creatine, creatine gluconate, creatine ascorbate, creatine glucose, neuroprotection, creatine transporter deficiency.

INTRODUCTION

Creatine or Methyl Guanidino-Acetic Acid is an amino acid that is central to the energetic metabolism of the cells, particularly those with high energy requirements like neurons (Béard and Braissant, 2010). Inside the cells creatine is reversibly phosphorylated to phosphocreatine, with which it is in constant equilibrium. The functions of this creatine/phosphocreatine system are twofold. Under physiological conditions it moves ATP from the site where it is synthesized (the mitochondrion) to the cytoplasmic sites where it is utilized (mainly the plasma Na/K-ATPase). Under pathological conditions of energy deprivation, it provides rapidly available energy to replenish failing energy reserves. The body, even the brain, can synthesize creatine but is also takes it up with the diet. To reach the brain, creatine synthesized by the body or taken up with the diet must cross the blood-brain barrier (BBB), and to do so it needs a specific transporter (creatine transporter or CrT) codified by the gene SLC6A8 (Christie, 2007). Once across the BBB, it needs again the same transporter to cross the cell plasma membrane and enter brain neurons (Lunardi et al., 2006). Exogenous creatine may be useful for human therapy in two main disease groups:

1. Cerebral ischemia and stroke (Balestrino et al., 2002; Prass et al., 2006; Perasso et al., 2013).
2. Hereditary diseases with primary deficiency of brain creatine (Stockler, 1997). Among them, creatine can be used to restore its brain content in those conditions where its synthesis is impaired (GAMT or AGAT deficiency), but it is not useful in the hereditary creatine transporter deficiency (OMIM 300352) where cerebral synthesis of creatine is not sufficient to provide normal brain content. In the latter case, lacking the transporter it cannot be taken up by the BBB nor by cerebral cells. Thus, creatine transporter deficiency is currently incurable (Nasrallah et al., 2010).

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Abbreviations: BBB, blood-brain barrier; CrT, creatine transporter; GPA, guanidine acetic acid; TLC, thin layer chromatography.

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A possible way of administering creatine in creatine transporter deficiency could be to modify the creatine molecule in such a way as to create a molecular structure that can cross the BBB and at the same time maintain a biological activity similar to that of creatine (Balestrino et al., 2002). While such molecules have indeed been researched, even by our laboratory (Perasso et al., 2008, 2009; Adriano et al., 2011; Enrico et al., 2013), no fully satisfactory treatment is available. For this reason, we shifted our attention to the formation of creatine salts formed by two moieties, one of which is a molecule that has its own transporter. In this way, the salt may be transported across both BBB and cell plasma membrane by the latter transporter, that is unimpaired by creatine transporter deficiency. Once in the cells, the salts should be split and free creatine should remain. These compounds may therefore be useful for the treatment of creatine transporter deficiency. Furthermore, they could also be used to treat cerebral ischemia and stroke (Perasso et al., 2013). Specifically, we tested three different creatine derivatives: (1) a salt formed from creatine and ascorbate (a molecule that has been before suggested as a vector across the BBB – Dalpiaz et al., 2005); (2) a salt formed from creatine and glucose; and (3) a salt from creatine and D-gluconic acid (this latter salt, commercially available, should use the glucose transporter to cross the BBB).

As a model, we used mouse hippocampal slices. Although no BBB obviously exists in this *in vitro* model, creatine still needs its transporter to cross the neuronal plasma membrane and enter cells, thus compounds' efficacy under conditions of creatine transporter impairment may be tested in this model (Lunardi et al., 2006). As a gauge of efficacy, we tested the ability of creatine and of creatine-derived compounds to:

1. Delay the disappearance of evoked electrical potentials during anoxia, a well-known effect of creatine that we used before in our research (Perasso et al., 2008) and
2. Increase the tissue concentrations of creatine and phosphocreatine.

We carried out the above investigations both under control conditions and after pharmacological block of the creatine transporter.

RESULTS

Creatine-derived compounds

Creatine gluconate (Fig. 1a) and creatine glucose (Fig. 1c) are two salts formed by creatine and D-gluconic acid and by creatine and glucose, respectively. Creatine ascorbate (Fig. 1b) is a salt formed by creatine and ascorbic acid (Vitamin C). The creatine-ascorbate and creatine glucose were synthesized following the method described in the Experimental procedure. Creatine gluconate is commercially available (see "Experimental procedure" section).

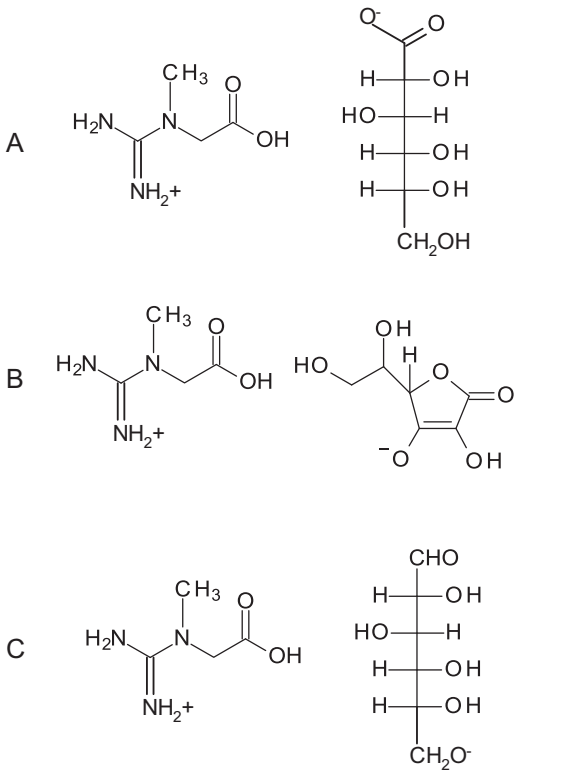

Fig. 1. (a) Creatine gluconate, (b) Creatine ascorbate, (c) Creatine glucose.

We analyzed these creatine derivatives in HPLC-MS to certify the purity of the final products. We also investigated the stability of these salts in saline solution. We found that creatine glucose content is reduced to about half after about 75 min (Fig. 2A). Creatine ascorbate is reduced to about half after 40 min (Fig. 2B). Creatine glucose is the most stable of the three compounds, its content being reduced to half after 180 min. (Fig. 2C). Creatine monohydrate by contrast is rather stable in an aqueous solution, at least for a few hours (Adriano et al., 2011).

Effects on tissue concentrations of creatine and of phosphocreatine

In these experiments, we tested creatine, creatine-gluconate, creatine-ascorbate and creatine-glucose under normal conditions and after block of the creatine transporter to assess the tissue creatine and phosphocreatine concentration.

Effects under normal conditions. Under control conditions, i.e. in slices where the creatine transporter is normally working, all compounds were able to increase tissue creatine concentration, with no differences among them (Fig. 3A). Moreover, all of them were also able to increase phosphocreatine concentration except creatine itself, which showed only a not significant trend toward increase (Fig. 3B).