Effect of Ascorbate and Dehydroascorbate on Tissue Uptake of Glucose

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SUMMARY

In vitro studies have suggested that ascorbate or dehydroascorbate share with glucose the same tissue-transport carrier. To determine if ascorbic acid or its oxidized form can inhibit tissue uptake of glucose, the brain uptake index (BUI) and muscle uptake index of glucose were determined by single arterial injection tissue-sampling technique. The injectate was either buffered Ringer's solution with varying concentrations of ascorbate, dehydroascorbate (pH 7.4), or 70% serum from individuals on vitamin C supplements. Ascorbic acid over a wide range of concentrations (0–10,000 mg/L) did not reduce the BUI. Ascorbic acid reduced BUI from the control value of 33 ± 3.2 to 20.1 ± 2.2% ($P < .01$) only at 100,000 mg/L; this effect was probably secondary to osmotic disruption of blood-brain barrier. In contrast, dehydroascorbate inhibited the BUI of glucose from baseline value of 32.8 ± 1.1 to 10.7 ± 0.67%, with an estimated K_i of 13.0 mM. Masseter muscle glucose uptake was not significantly altered over a wide range of ascorbate or dehydroascorbate concentrations in the injectate. Dehydroascorbate (7500 mg/L) did not significantly reduce the BUI of [^{14}C]phenylalanine (55.2 ± 4.4 vs. 62.1 ± 4.2% in controls). When serum from six individuals on calcium ascorbate (3–5 g/day) was compared with that of nine controls, the BUI was not different (19.3 ± 1.7 vs. 19.3 ± 1.1%). Similarly, supplementing the diet of eight healthy volunteers with 1 g calcium ascorbate for 8 days did not alter the BUI of glucose. We conclude that dehydroascorbic acid, but not ascorbic acid, inhibits in vivo brain but not muscle uptake of glucose, suggesting a common carrier system at the level of the blood-brain barrier. The low serum levels of ascorbate even after vitamin C supplementation are not sufficient to impair brain glucose uptake. *Diabetes* 36:1001–1004, 1987

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Studies of the tissue distribution of ascorbic acid indicate that the brain has a high retention capacity of ascorbate and it is a target tissue for vitamin C (1,2). The form of the vitamin available for transport to the tissues is still controversial. Martin and Mecca (1,3) concluded that the oxidized form of the vitamin, dehydroascorbic acid (DHA), is taken up preferentially by the tissues. Similarly, Bigley and co-workers (4,5), studying human leukocyte and fibroblast uptake of ascorbic acid, concluded that DHA, an electrically neutral molecule (unlike reduced ascorbate, which is a charged molecule), shares the glucose-transport carrier, and its uptake by the fibroblasts (like glucose) is insulin sensitive. In contrast, Hornig (2), employing a whole-body autoradiographic method, concluded that ascorbic acid rather than DHA is the form of the vitamin available for transport. A similar conclusion was reached by Davis et al. (6). They found that lymphocyte uptake of ascorbic acid is a carrier-mediated energy-dependent process and can be inhibited by D-glucose but is insulin insensitive. This observation suggests that the ascorbic acid carrier is similar but not identical to the glucose-transport carrier.

It is not known whether the blood-brain barrier (BBB) glucose carrier, an insulin-insensitive system, is identical to the BBB ascorbic acid or DHA carrier. Because the central nervous system has high retention capacity for ascorbate (1,2) and the BBB glucose-transport system may be involved in the transport of ascorbate, it is of interest to determine if ascorbic acid can competitively inhibit brain glucose uptake and predispose those who are on vitamin C supplements to neuroglycopenia.

MATERIALS AND METHODS

Male Fisher 344 rats (Harlan, Indianapolis, IN) at age 10–12 wk and a weight range of 170–210 g were used. [^{14}C]-D-glucose (sp act 359 mCi/mmol) and [^{3}H]H_2O (1 μCi/μl)

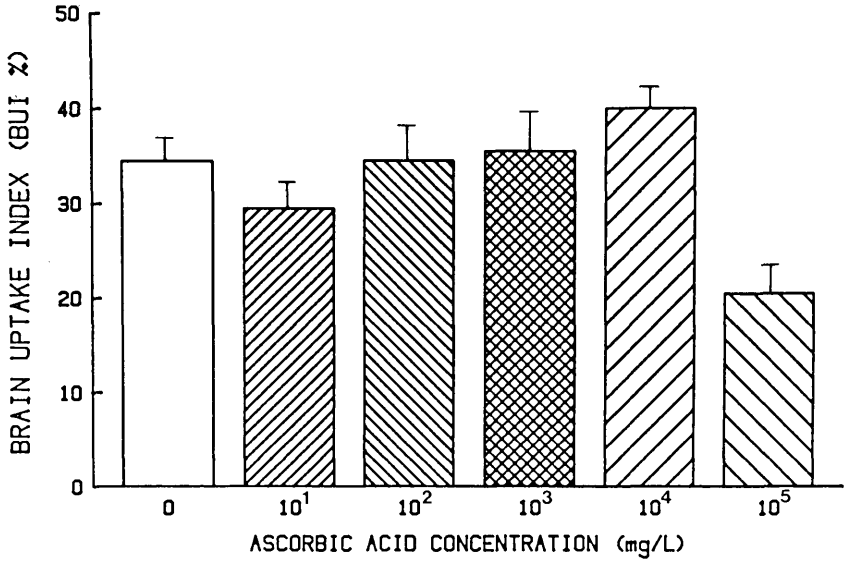

FIG. 1. Brain uptake index of glucose at different concentrations of ascorbate in injectate. Data are means ± SE of 3–6 measurements.

were purchased from New England Nuclear (Boston, MA). L-Ascorbic acid was purchased from Sigma (St. Louis, MO), and dehydroascorbic acid was purchased from Aldrich (Milwaukee, WI).

In vivo brain uptake of glucose was measured by the single arterial injection tissue-sampling technique of Oldendorf (7). Under pentobarbital sodium anesthesia (50 mg/kg i.p.), 0.2 ml of 10 mM HEPES-buffered Ringer's saline (pH 7.4) containing [^{14}C]-D-glucose (0.5 μCi/ml) and [^{3}H]H_2O (5 μCi/ml) was rapidly injected into the right carotid artery, and the animal was decapitated 15 s after injection. The ipsilateral forebrain was removed, and ~0.2 g of tissue was solubilized in 1.5 ml of Soluene (Packard, Downers Grove, IL) with shaking in a water bath for 2 h at 60°C. Ten milliliters of scintillation cocktail (Instagel, Packard) were added, and the radioactivity of ^{14}C and ^{3}H were simultaneously counted in a Beckman liquid scintillation counter. After correcting for ^{14}C spillover in a ^{3}H window, counts per minute were converted to true disintegrations per minute. The brain uptake index (BUI) of glucose was determined from the equation

$$\frac{(^{14}\text{C dpm}/^{3}\text{H dpm})\text{brain} \times 100}{(^{14}\text{C dpm}/^{3}\text{H dpm})\text{injectate}}$$

In some experiments the masseter muscle was also sampled and processed, and the muscle uptake index of glucose was calculated as described for BUI. The effect of adding various concentrations of ascorbate and dehydroascorbate to the injectate on brain and muscle uptake indices of glucose was evaluated.

To determine if the serum of individuals ingesting vitamin C can reduce rat brain uptake of glucose, BUI measurements in which the injectate was 70% serum from individuals who were either on 3–5 g of vitamin C daily for at least 2 mo (range 2 mo to 5 yr) or not taking any vitamin C supplements were performed.

Last, eight healthy volunteers were given 1 g calcium ascorbate daily for 8 days. The fasting serum glucose and in vitro BUI of glucose were determined before and after vitamin C supplementation. All results are presented as means ± SE, and statistical analysis was done with Student's *t* test.

RESULTS

Figure 1 shows that the BUI of glucose was not decreased with the addition of various concentrations of ascorbic acid in the injectate except for the highest concentration (100,000

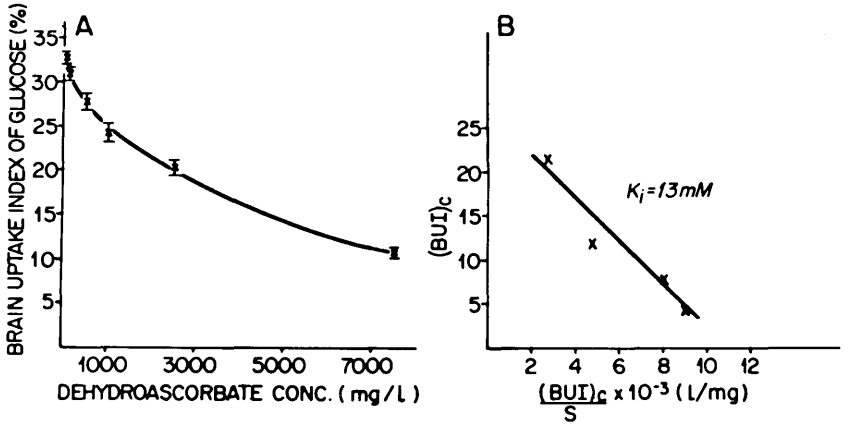

FIG. 2. *A*: brain uptake index (BUI) of glucose at different concentrations of dehydroascorbate (mg/L). Each *point* represents mean ± SE of 3–6 rats. *B*: Eadie-Hofstee transformation of saturation of BUI of glucose with dehydroascorbate. BUI_c = BUI_o − BUI_s, where BUI_o is in absence of dehydroascorbate and BUI_s is in presence of various concentrations of dehydroascorbate. S is concentration of dehydroascorbate in injection mixture.

mg/L), when the BUI decreased from a control value of 33 ± 3.2 to 20.1 ± 2.2% ($P < .01$). This is not a specific effect of ascorbic acid, because the injectate is hyperosmolar and can disrupt the BBB (8). Figure 2*A* illustrates the dose-dependent inhibitory effect of dehydroascorbate on BUI. Figure 2*B* shows the linear transformation of glucose-uptake inhibition data; it is analogous to an Eadie-Hofstee plot. In the presence of dehydroascorbate the BUI of glucose decreased from a baseline value of 32.8 ± 1.1 to 10.7 ± 0.67%, with an estimated K_i of 13.0 mM. Neither ascorbate nor dehydroascorbate had any significant effect on masseter muscle glucose uptake (Fig. 3).

To determine the specificity of the DHA effect on BBB glucose transport, the BUI of [^{14}C]phenylalanine was determined. The addition of 7500 mg/L of DHA in the injectate did not significantly alter the BUI of phenylalanine (55.2 ± 4.4 vs. 62.1 ± 4.2% in controls; $n = 5$). The BUI measured in the presence of 70% human serum was similar for those who were taking 3–5 g of ascorbate compared with those who did not use ascorbate supplements. The BUI for the control subjects ($n = 9$) was 19.3 ± 1.1%; for those on ascorbate ($n = 6$) the BUI was 19.3 ± 1.7%. The serum glucose levels in the two groups were similar (96.5 ± 4.9 vs. 104 ± 4.0 mg/dl, control vs. experimental). Similarly, supplementation of the diet of eight healthy volunteers with 1 g of calcium ascorbate daily did not influence the brain glucose uptake. The mean serum glucose level before ascorbic acid supplementation (96.5 ± 4.8 mg/dl) was similar to the level after 8 days of ascorbate intake (92.9 ± 3.2 mg/dl). The mean BUI before ascorbate supplementation (19.5 ± 1.2%) was similar to the value at the 8th day of supplementation (21.1 ± 1.7%).

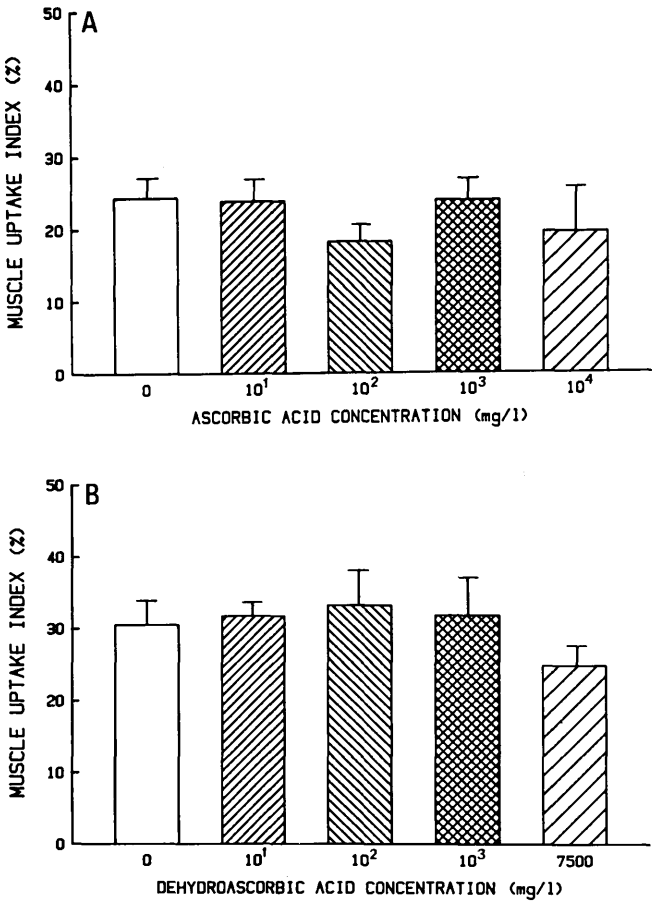

FIG. 3. Masseter muscle uptake index of glucose in presence of various concentrations of ascorbic acid (*A*) and dehydroascorbic acid (*B*) in injection mixture.

DISCUSSION

In vitro studies have suggested that ascorbic acid or its oxidized metabolite DHA is transported to the tissues, possibly via the same carrier as glucose is transported (4–6). The suggestion is based on the observation that glucose can inhibit ascorbic acid but not DHA uptake in lymphocytes (6), and in this model, ascorbate uptake was insensitive to insulin. In contrast, a study in human neutrophils and fibroblasts suggested that DHA is the form that is transported by the glucose carrier and that the process appears to be sensitive to ambient insulin concentrations (5). Differences in the cell type studied may account for the reported discrepancies. However, there is no evidence in vivo whether ascorbic acid or its oxidized metabolite would impair glucose transport in tissues.

Our data favors DHA as the form of the vitamin that shares with glucose the same transport system. Ascorbic acid was a poor competitor of glucose uptake in both insulin-sensitive (muscle) and -insensitive (brain) tissues. The BUI of glucose substantially decreased only at the highest concentration of ascorbic acid used. This is probably secondary to the osmotic disruption of the BBB with hyperosmolar solution in the injectate and may not be physiologically significant (8). In contrast, DHA inhibited BUI of glucose in a dose-dependent fashion. Unphysiologic concentrations of ascorbate and DHA were used to determine if these compounds share with glucose the same transport carrier. Note that the estimated K_i for the inhibition of glucose transport (13.0 mM) is close to the K_m of BBB glucose transport (9.0 mM) reported previously (9), suggesting that BBB transport of DHA is mediated by the glucose-transport system. Similarly, the BUI of glucose could be reduced to the same nonspecific levels with increasing amounts of either glucose (9) or DHA. This implies that no carrier sites are unique to either nutrient. Unlike phloretin, which can inhibit both glucose and amino acid transport (9,10), DHA could not inhibit BBB amino acid transport, suggesting that the DHA effect is specific for the glucose carrier.

The effect of DHA on glucose uptake appears to be tissue specific, because the muscle uptake index of glucose was not altered in the presence of DHA. It is possible that DHA may alter glucose uptake in other types of skeletal muscles with different glucose-uptake and insulin-binding capacities (11). The reduction of the BUI of glucose in the presence of DHA cannot be attributed to a nonspecific interaction between ^{14}C-labeled glucose and DHA. This sort of interaction is not tissue specific and should have affected the muscle glucose-uptake measurements. However, these observations do not exclude the possibility that ascorbic acid may also share with glucose the same carrier in the BBB, because the technique used in this study did not allow measurements of brain uptake of substrates with very slow BBB transport rates. Nevertheless, this technique is specially suited to determine in vivo if a putative competitor of glucose transport

is capable of altering tissue uptake of glucose. Our data indicate that ascorbic acid or DHA, at concentrations achievable with vitamin C supplements, does not reduce tissue glucose uptake. Therefore, diabetic patients or the elderly who are on vitamin C supplements are not more predisposed to developing neuroglycopenia with its attendant clinical sequelae. However, hyperglycemia could reduce the cellular uptake of ascorbate (12), and a subgroup of diabetic patients may have decreased leukocyte vitamin C content (see ref. 13 for review). The lack of vitamin C effect on tissue glucose uptake at physiologic concentrations of serum ascorbate is predictable from the kinetic parameters of DHA and glucose transport. Nevertheless, the documentation of the lack of a potential adverse effect of vitamin C intake on brain glucose uptake is reassuring for diabetic patients and their physicians.

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