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Central lactate metabolism suppresses food intake via the hypothalamic AMP kinase/malonyl-CoA signaling pathway

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ABSTRACT

Previous studies showed that centrally administered glucose and fructose exert different effects on food intake – glucose decreasing and fructose increasing food intake. Because of the uncertainty of whether fructose can cross the blood–brain-barrier, the question is raised; can dietary fructose directly enter the CNS? Evidence is presented that fructose administered by intraperitoneal (ip) injection to mice is rapidly (<10 min) converted to lactate in the hypothalamus. Thus, fructose can cross the blood–brain-barrier to enter the CNS/hypothalamus for conversion to lactate without prior (slower) conversion to glucose in the liver. Fructose-derived hypothalamic lactate is not, however, responsible for the orexigenic effect of fructose. Ip lactate administered at a level equivalent to that of fructose generates a higher level of hypothalamic lactate, which rapidly triggers dephosphorylation/inactivation of AMP-kinase. Thereby, ACC — a substrate of AMP-kinase that catalyzes malonyl-CoA formation — is dephosphorylated and activated. Consistent with these findings, ip or centrally (icv) administered lactate rapidly increases (<10 min) hypothalamic malonyl-CoA. Increasing hypothalamic malonyl-CoA suppresses the expression of the orexigenic and increases the expression of the anorexigenic neuropeptides, which decrease food intake. All downstream effects of hypothalamic lactate are blocked by icv administered oxamate, a potent inhibitor of lactate dehydrogenase, thus verifying the central action of lactate.

Introduction

Centrally-metabolized glucose and fructose have inverse effects on the hypothalamic AMP kinase/malonyl-CoA signaling pathway that regulates feeding behavior [1–3]. Glucose metabolism in the hypothalamus suppresses, whereas fructose metabolism increases food intake [4,2]. The basis for this paradox is due to the fact that glucose and fructose enter the glycolytic pathway at different points — glucose entering at the level of glucose-6-phosphate and fructose entering at the triose phosphate stage (see Scheme 1A) and bypasses the slow regulatory step (catalyzed by PFK) through which glucose must pass. Central glucose metabolism increases hypothalamic ATP (see Fig. 1 in Ref. [2]), which decreases AMP prompting the inactivation of AMP kinase, activation ACC and up-regulation of hypothalamic malonyl-CoA. Increasing malonyl-CoA causes increased expression of the anorexigenic and a decreased expression of the orexigenic hypothalamic neuropeptides, thus a suppression of food intake. Because the initial ATP-consuming step of central fructose metabolism to F1P in the hypothalamus is more rapid than the ATP-consuming step of central glucose metabolism to G6P (see Scheme 1A), fructose causes rapid depletion of ATP and a compensatory rise of AMP [2]. The increase in AMP level caused by central (icv) fructose has an inverse effect (relative to the effect of glucose) on each step of the hypothalamic AMP kinase/malonyl-CoA signaling pathway and produces an increase in food intake.

This raises the question, however, does *dietary* fructose, which enters the bloodstream, cross the blood–brain-barrier (BBB) to enter the hypothalamus? This is an important issue as large amounts of high fructose sweeteners are now being consumed, particularly by young adolescents [5–7]. This issue is difficult to address experimentally, since dietary fructose entering the bloodstream is rapidly converted to glucose in the liver and only after a lag of >15 min begins to appear in the bloodstream [2]. Thus, there is only a short time window of ~15 min during which the effect of dietary or ip fructose on hypothalamic/CNS signaling can be distinguished from that evoked by glucose.

Many blood-borne molecules are excluded from the CNS by the BBB. While it is well known that glucose readily crosses the BBB, uncertainty still exists as to whether fructose crosses this barrier [8–10]. To address this issue, we investigated whether

Abbreviations: ACC, acetyl-CoA carboxylase; NPY, neuropeptide Y; AgRP, Aguoti regulated peptide; POMC, proopiomelanocortin; CART, cocaine and amphetamine-regulated transcript; G6P, glucose-6-phosphate; CNS, central nervous system; BBB, blood–brain barrier; 2-DG, 2-deoxyglucose; PFK, phosphofructokinase; LDH, lactate dehydrogenase; ip, intraperitoneal; icv, intracerebroventricular.

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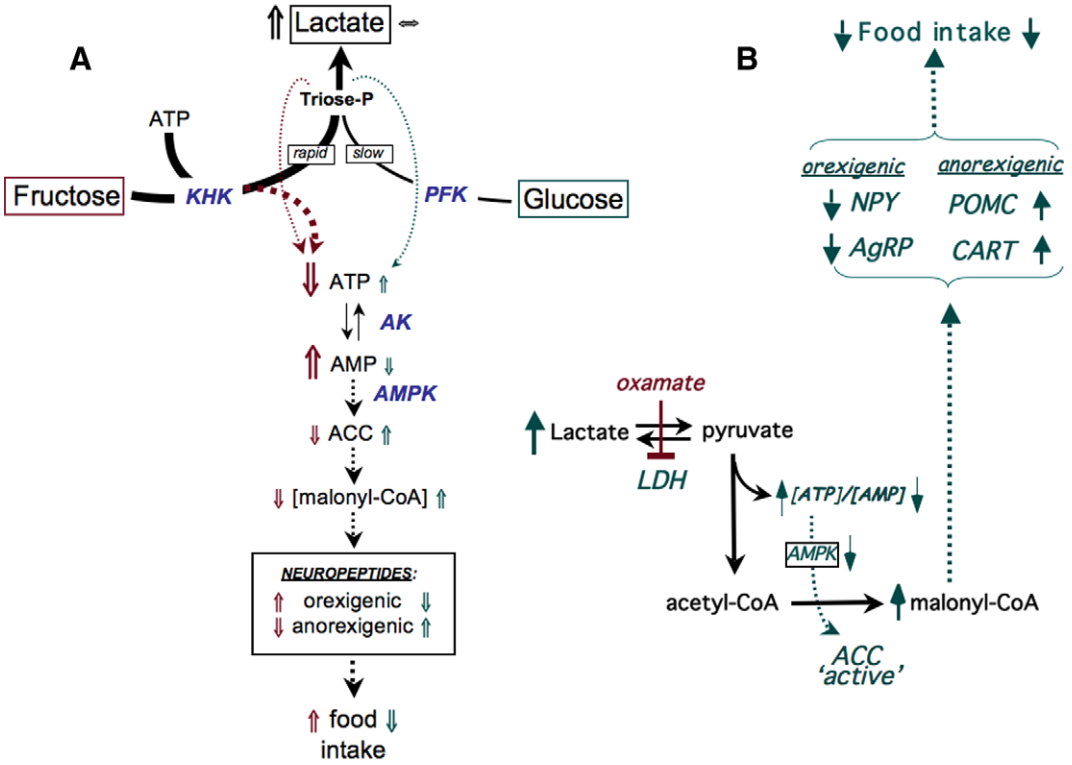

Scheme 1. (A) Conversion of glucose *versus* fructose to lactate and outline of effects on the hypothalamic AMP kinase/malonyl-CoA signaling pathway. Fructose is converted to lactate more rapidly than glucose as indicated by the breadth of the black curved arrows. Fructose bypasses the slow PFK step catalyzed by KHK which causes depletion of ATP thereby increasing AMP which activates the AMP kinase (AMPK)-malonyl-CoA signaling pathway giving rise to increased food intake. The red arrows (on the left) indicate the effect of hypothalamic fructose metabolism on the level or activity of intermediates in the pathway. The rate of glucose metabolism is slower than that of fructose because of the PFK-catalyzed rate-limiting step. The green arrows (on the right) indicate the effect on the level or activity of intermediates in the pathway, which lead to a decrease of food intake. (B) Metabolism of lactate in the hypothalamus and its effect on the AMP kinase/malonyl-CoA signaling pathway. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this paper.)

fructose administered ip can enter the hypothalamus and undergo conversion to lactate in the hypothalamus. Previous findings [2] showed that following ip injection, fructose rapidly appears in the blood its level peaking at <5 min after administration. Only after a lag of ~15 min does glucose (formed from fructose) appear in the blood being delayed by conversion of fructose to glucose in the liver [2]. By conducting short-term experiments within the 15-min time window before significant conversion of fructose to glucose occurs, it was possible to distinguish the effect of fructose on hypothalamic malonyl-CoA from that of glucose. These findings showed that ip fructose lowered hypothalamic malonyl-CoA in <15 min after ip fructose [2]. Moreover, the icv injection of 2-deoxyglucose (2-DG), which inhibits central glucose metabolism, prevented the conversion of ip glucose – but not ip fructose – to hypothalamic malonyl-CoA [1–3]. It was shown that 2-DG blocks the rise of hypothalamic malonyl-CoA following the ip glucose, but had no effect on hypothalamic malonyl-CoA, following ip administration of fructose. These findings did not, however, rule out the possibility that circulating fructose (of dietary origin) is excluded from the CNS by the BBB, and thus, did not undergo metabolism in the CNS.

The present investigation addresses this issue and shows that ip fructose crosses the BBB and reaches the hypothalamus as indicated by its rapid conversion to lactate in and accumulation in the hypothalamus. Ip glucose, however, at the same dosage did not lead to hypothalamic lactate accumulation. Evidence is also presented showing that peripheral (or central) lactate, at doses producing even higher levels of hypothalamic lactate accumulation, suppress food intake mediated by the hypothalamic AMP kinase/malonyl-CoA signaling system.

Materials and methods

With the exception of the quantification of lactate dehydrogenase, all methods have been described previously [2]. Tissue lactate levels were determined using the lactate assay kit (Megazyme, Wicklow, Ireland).

Results and discussion

Fructose crosses the blood–brain-barrier

An indirect approach was taken to determine if fructose can cross the BBB to enter and undergo metabolism by the hypothalamus. The effect of ip fructose on lactate concentration in the hypothalamus was assessed 10 min after administration, before it could have undergone conversion to glucose in the liver (see above). Previous studies [2] showed that blood fructose level peaks <5 min after ip injection, whereas blood glucose (arising from fructose in the liver) is delayed for >15 min after ip fructose injection (Fig. 1 in Ref. [2]). As shown in Fig. 1A ip fructose led to a substantial rise in hypothalamic lactate, whereas ip glucose did not. Note that the levels of lactate arising from these sugars represent 'steady state' concentrations – the net result of lactate formation *versus* lactate removal. This result is consistent with our previous findings [2], which showed that fructose uptake by the hypothalamus is more rapid than that by glucose, since fructose by-passes the rate-limiting phosphofructokinase (PFK) step of glycolysis that slows the rate of glucose metabolism to form lactate. As illustrated in Scheme 1A glucose enters glycolysis at the level of G6P, whereas fructose enters at the triose phosphate level bypassing the highly