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

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A R T I C L E   I N F O

A B S T R A C T

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.

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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.
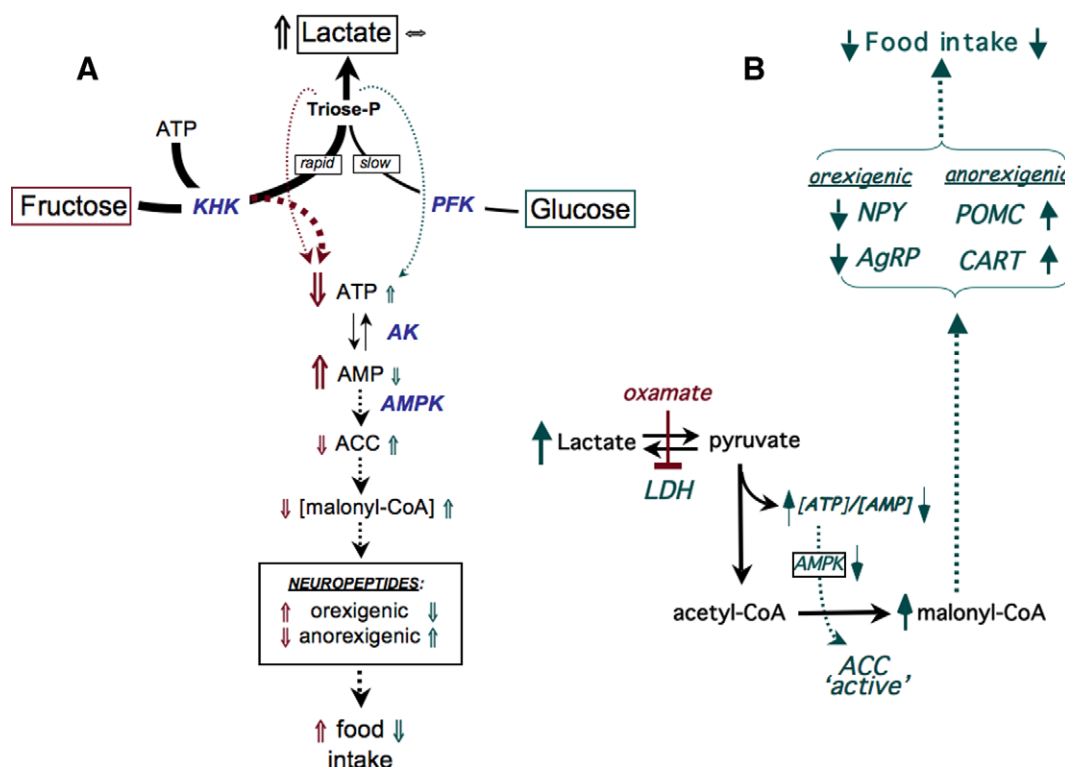
This raises the question, however, does dietary fructose, which enters the bloodstream, cross the blood–brain-barrier (BBB) to enter the hypothalamus? This is an important issue as large amounts of high fructose sweeteners are now being consumed, particularly by young adolescents [5–7]. This issue is 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

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
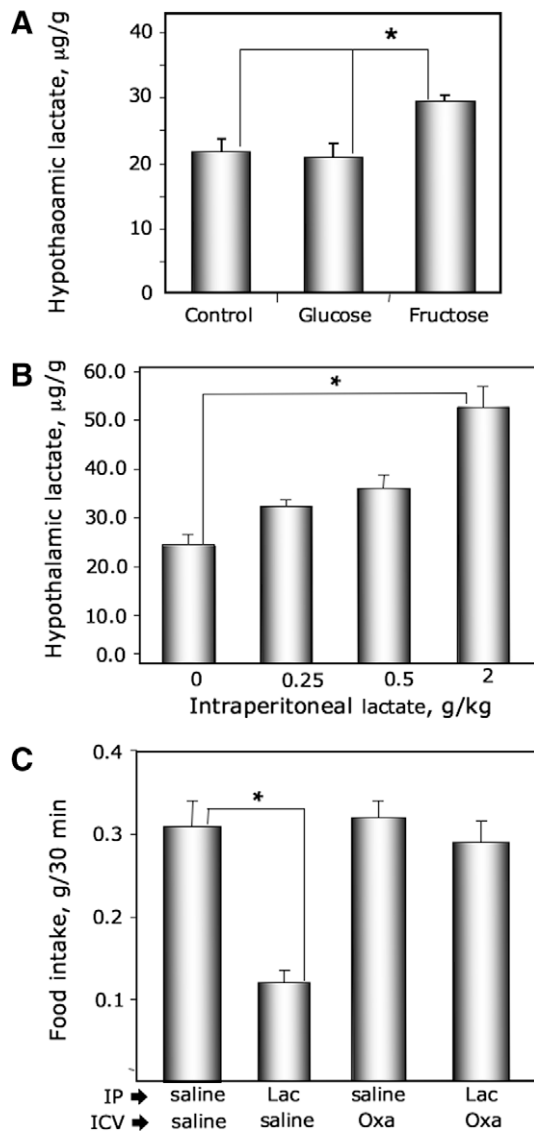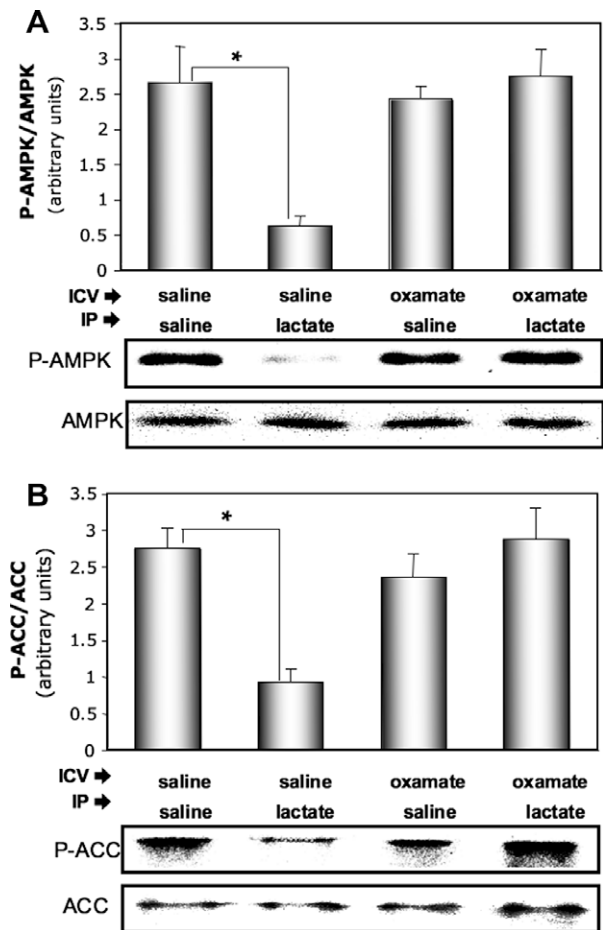
Fig. 1. (A) Effect of ip glucose or fructose (2 g/kg body weight) on hypothalamic lactate level. Hypothalamic lactate levels were determined 10 min after glucose *or* fructose injection; (B) Effect of ip lactate (0.5 g/kg body weight) with or without icv oxamate (10 µg in 2 µl) on hypothalamic lactate 30 min after lactate injection; and (C) food intake 30 min after lactate injection. $^*P < 0.001$.

Fig. 2. Effect of ip lactate (0.5 g/kg body weight) with or without icv oxamate (10 µg in 2 µl) on the phosphorylation state of hypothalamic AMP kinase (A) and acetyl-CoA carboxylase (B). Ten min after administration of lactate ± oxamate hypothalami were extracted and subjected to SDS/PAGE, immunoblotting with antibodies to AMPK, P-AMPK, ACC and P-ACC and quantification. $^*P < 0.001$.

Fig. 3. Effect of lactate and oxamate on hypothalamic malonyl-CoA. (A) ip lactate, (B) icv lactate and (C) ip lactate with or without icv oxamate. Ip lactate was 0.5 g /kg body weight; icv lactate was 10 µg in 2 µl and icv oxamate was at 10 µg in 2 µl. Hypothalamic malonyl-CoA was determined 5 min after ip or icv injection. $^*P < 0.001$ control *versus* saline/lactate.

regulated PFK step. These findings show that fructose can cross the BBB to undergo glycolytic metabolism to produce lactate.

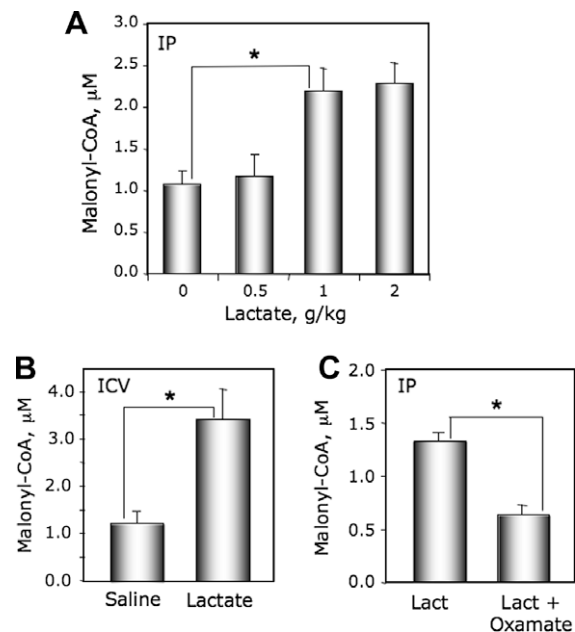*Peripheral administration of lactate increases hypothalamic lactate level and suppresses food intake*

Lactate is a major product of cerebral glucose metabolism, most notably of astrocytes [11]. While glucose is the primary physiological fuel for neurons of the CNS [12,13], abundant evidence indicates that lactate, derived from glucose metabolism by astrocytes, also serves as a fuel for neurons supplied by the 'glucose/lactate shuttle' [11]. Translocation of lactate across the plasma membrane occurs via monocarboxylic acid transporters present in both cell types.
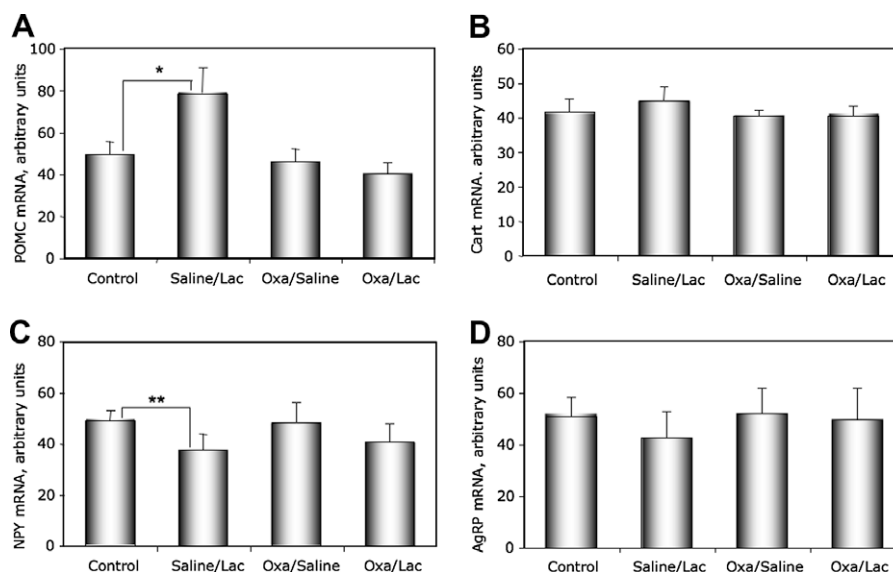
Lactate administered by ip injection is rapidly taken up by the hypothalamus (Fig. 1B) – its level being dependent on the dose administered. It should be noted that ip lactate reaches a much higher level than by a comparable dose of ip fructose (compare Fig. 1B and A). Peripherally administered (ip injection) lactate, like

Fig. 4. Effect of ip lactate and/or icv oxamate on hypothalamic neuropeptide mRNA expression. Ip lactate was 0.5 g/kg body weight and icv oxamate was at 10 μg in 2 μl. Hypothalamic neuropeptide mRNA levels were determined 5 min after ip or icv injection. $^{*}P < 0.001$; $^{**}P < 0.005$.

glucose, suppresses food intake (Fig. 1C). That the metabolism of hypothalamic lactate is required for the food intake effect is indicated by the fact that the lactate-induced suppression of food intake by lactate is blocked by centrally administered (icv) oxamate (Fig. 1C), a potent inhibitor of lactate dehydrogenase (LDH; [13]). Central oxamate alone has no effect on food intake lending credence to the specificity of lactate action.

Central lactate metabolism modulates the hypothalamic AMP kinase/ malonyl-CoA signaling system

Our previous studies showed that the central metabolism of glucose increases hypothalamic malonyl-CoA [1], which alters the expression of neuropeptides that control feeding behavior. To determine whether the suppression of food intake by central lactate utilizes the same hypothalamic signaling pathway as central glucose, the effect of lactate on intermediates in this pathway were assessed. As shown in Fig. 2A ip lactate rapidly (<5 min) decreased phosphorylation and thereby the activity of hypothalamic AMP kinase. Concurrently the phosphorylation state of ACC was reduced (Fig. 2B) increasing its catalytic activity. That both of these phosphorylation events are initiated by central lactate metabolism is indicated by the fact that icv oxamate reversed these effects (Fig. 2A and B). As expected the activation of ACC by ip or icv lactate led to an increase in the level of malonyl-CoA in the hypothalamus (Fig. 3A and B, respectively). That this effect is specific to central lactate metabolism is verified by the blockade of the lactate effect by icv oxamate (Fig. 3C).

Central lactate metabolism alters expression of hypothalamic anorexigenic and orexigenic neuropeptides

It is firmly established that physiological, genetic or pharmacological perturbations of hypothalamic malonyl-CoA provoke changes in the expression of the orexigenic and anorexigenic neuropeptides that control feeding behavior [1,2,14–17]. Thus, increasing hypothalamic malonyl-CoA lowers expression of key orexigenic neuropeptides and increases expression of anorexigenic neuropeptides, whose combined actions suppress food intake. As illustrated in Fig. 4A and C, respectively, ip lactate significantly increases the expression of POMC mRNA, which encodes αMSH, an

anorexigenic neuropeptide and significantly decreases the expression of NPY, an orexigenic neuropeptide. Moreover, icv oxamate – an LDH inhibitor – reverses these effects indicating that the central metabolism of lactate is causal. Although the effects on the expression of the mRNA encoding CART, an anorexigenic neuropeptide, and that encoding AgRP, an orexigenic neuropeptide, were not statistically significant, the trend was in the expected direction (Fig. 4B and D). These results are consistent with the lactate-induced changes in feeding behavior. Taken together our findings indicate that the central lactate metabolism initiates the sequence of events illustrated in Scheme 1B that result in the suppression of food intake.

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