The synergistic action of caffeine or adenosine on glucose stimulation of liver glycogen synthase phosphatase activity

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Recent studies indicate that glucose directly stimulates synthase phosphatase activity in vitro but only at high, non-physiological concentrations. Present results demonstrate that at a physiological concentration glucose can be stimulatory, provided that an appropriate second effector is present. Caffeine and adenosine are examples of such effectors which act synergistically with glucose to enhance synthase phosphatase activity. Caffeine but not adenosine enhances glucose stimulation of phosphorylase phosphatase activity. In the absence of glucose, caffeine but not adenosine stimulates both synthase and phosphorylase phosphatase reactions. Thus, glucose regulation of glycogen synthase activation in vivo could require a second effector. Neither the identity nor source of such an effector is known. The putative regulator could be a mediator for a hormone such as insulin. The present work suggests that the chemical nature of the effector might be that of a derivatized purine of which nucleosides are an example.

Caffeine *Glucose* *Adenosine* *Glycogen synthase* *Phosphatase* *Liver*

1. INTRODUCTION

Significantly greater rates of phosphorylase *a* inactivation have been reported in isolated hepatocytes incubated with caffeine and glucose rather than with glucose alone [1]. This result was ascribed to a synergistic action of caffeine and glucose of phosphorylase *a* [2,3] which promoted phosphorylase phosphatase activity. Activation of glycogen synthase also was accelerated by the combination compared to glucose alone [1]. Because a synergism between glucose and caffeine has been recognized only insofar as they are ligands of phosphorylase *a*, these results have been taken as support for a controlling role for phosphorylase *a* in the regulation of glycogen synthesis [4,5].

Recently, direct stimulation of synthase phosphatase activity by caffeine [6] and glucose [7] in a glycogen particle preparation was demonstrated. Each effect was independent of the phosphorylase *a* concentration [6,7]. These observations have the potential of obviating the regulatory mechanism described in [4,5]. Such a mechanism is not consistent with recent in vivo observations [8,9]. However, effective glucose stimulation was apparent only at very high, non-physiological concentrations [7]. Here, a synergistic stimulation of synthase phosphatase activity by caffeine or the nucleoside, adenosine, and a physiological concentration of glucose was demonstrated in a glycogen particle preparation. Neither the parent, adenine, nor the higher homologue, AMP, had effects similar to adenosine.

2. EXPERIMENTAL

Caffeine, adenosine and adenine were obtained from Sigma (St.Louis, MO) and were of the highest quality available. Preparation of a rat liver glycogen particle suspension has been described [7,10,11], as have the assays for synthase and phosphorylase phosphatase activities [7,10,11]. One unit of either synthase or phosphorylase is the amount of enzyme which incorporates 1 μmol glucose into gly-

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cogen in 1 min at 30°C under the assay conditions employed. Synthase phosphatase is expressed as the number of units of synthase I/g wet wt generated per min. Phosphorylase phosphatase activity is expressed as the decrease in units of phosphorylase *a*/ml reaction mixture per min.

3. RESULTS

Synthase phosphatase activity is increased only modestly (fig.1) and often not at all by glucose concentrations of 10 mM or less [7]. However, in combination with 0.5 mM caffeine, 10 mM glucose had a marked stimulatory effect (fig.1). At its maximum, the rate was more than twice that of the caffeine control. It is evident that the effect of glucose and caffeine is greater than the added individual effects. A saturating concentration of caffeine (2 mM) with glucose had a similar effect (fig.2). Qualitatively, glucose and caffeine, in combination, also increased phosphorylase phosphatase activity to an extent greater than the sum of their individual effects. A quantative estimate of enhanced phosphorylase phosphatase activity is not possible because true phosphorylase *a* concentrations are underestimated (compare zero time values, fig.1,2). The carryover of glucose and caffeine from the phosphatase reaction mixture is sufficient to partially reduce the measured phosphorylase *a* activity ([2,3], unpublished).

Caffeine competes for adenosine binding sites in various systems implying some important structural similarities between caffeine and adenosine [12–14]. We therefore studied the effect of adenosine on glucose stimulation of phosphatase activity. Adenosine at 0.5 mM has no stimulatory effect on either the synthase or phosphorylase phosphatase

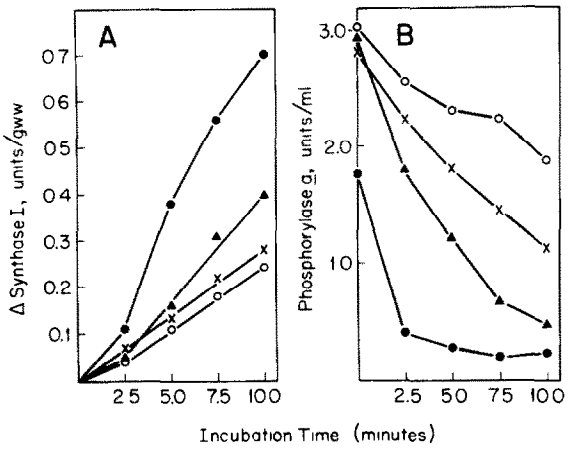

Fig.1. Glucose effects on liver synthase and phosphorylase phosphatase activities in the presence and absence of caffeine. Liver glycogen particles were prepared from animals treated with glucagon and were incubated at 25°C with 10 mM glucose (×—×). 0.5 mM caffeine (▲—▲) or 10 mM glucose with 0.5 mM caffeine (●—●). A control reaction mixture with no addition is also indicated (○—○). Aliquots were withdrawn at the times indicated and analyzed for both synthase and phosphorylase activities. Results represent the means of 4 separate experiments. (A) Synthase phosphatase activity. Total synthase activity averaged 0.97 units/g wet wt and synthase I was initially 12% of total. (B) Phosphorylase phosphatase activity. The initial phosphorylase *a* concentrations in all mixtures except the combination were not significantly different. Significant concentration differences ($p \geq 0.05$) were demonstrated at 2.5 min and thereafter (control *vs* caffeine) or at 5 min and thereafter (control *vs* glucose) as evidence of phosphorylase phosphatase stimulation by caffeine and glucose, respectively.

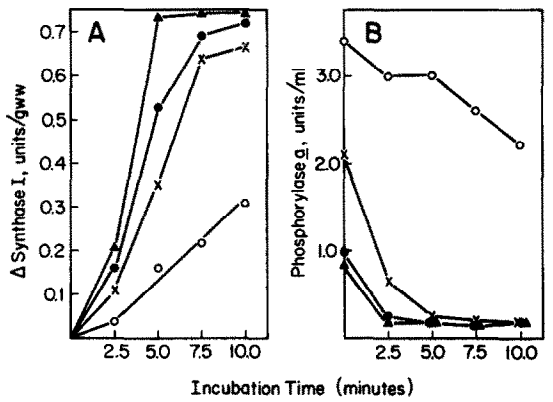

Fig.2. Glucose stimulation of synthase and phosphorylase phosphatase activities enhanced by a saturating concentration of caffeine. Glycogen particle preparations were made and incubated as in the legend for fig.1. Reaction mixtures contained either no addition (○—○), 2 mM caffeine (×—×), 10 mM glucose and 2 mM caffeine (●—●) or 80 mM glucose and 2 mM caffeine (▲—▲). Results represent the means of 3 separate determinations. (A) Synthase phosphatase activity. Total synthase activity averaged 1.00 unit/g wet wt and synthase I was initially 21% of total. (B) Phosphorylase phosphatase activity.

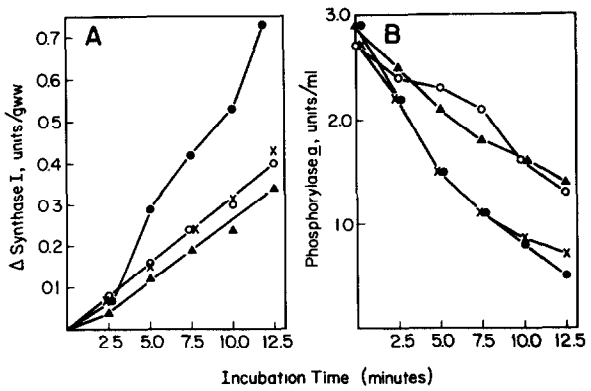

Fig.3. Glucose stimulation of liver synthase and phosphorylase phosphatase activities in the presence and absence of adenosine. Experiments with glycogen particle preparations were conducted and analyzed as described in the legend for fig.1. Reaction mixtures contained either no addition (○—○), 10 mM glucose (×—×), 0.5 mM adenosine (▲—▲) or 10 mM glucose and 0.5 mM adenosine (●—●). Results of 3 separate experiments have been combined. (A) Synthase phosphatase activity in experiments where total synthase averaged 0.94 units/g wet wt and the initial synthase I was 16%. (B) Phosphorylase phosphatase activity. All initial phosphorylase *a* concentrations were not significantly different. No evidence indicated that adenosine influenced glucose stimulation of phosphorylase phosphatase activity. At 5 min and thereafter, the concentrations of phosphorylase *a* in both the glucose and glucose plus adenosine reaction mixtures were significantly different from control ($p \geq 0.05$) but were not different from each other.

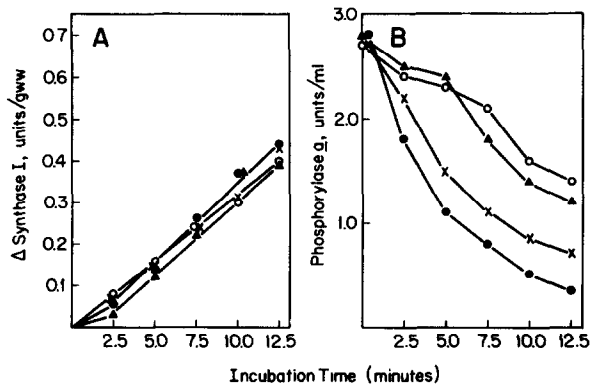

Fig.4. The effect of adenine on glucose stimulation of synthase and phosphorylase phosphatase activities. Experiments were conducted as described in the fig.1 legend except that reaction mixtures contained either no additions (○—○), 10 mM glucose (×—×), 0.5 mM adenine (▲—▲) or 10 mM glucose and 0.5 mM adenine (●—●). Results represent the means of 3 separate experiments. (A) Synthase phosphatase activity. Total synthase was 0.93 units/g wet wt and the initial synthase I was 16%. (B) Phosphorylase phosphatase activity. There was no significant difference in the initial phosphorylase *a* concentrations of all reaction mixtures. Although concentrations at 5 min and thereafter were significantly different ($p \geq 0.05$) when control and either glucose or glucose plus adenine mixtures were compared, there was no significant difference between glucose and the glucose–adenine combination.

reactions (fig.3). However, in combination with 10 mM glucose there was marked enhancement of glucose stimulation of synthase phosphatase activity. This was equivalent to that observed with caffeine. Since neither glucose nor adenosine had a significant stimulatory effect, individually, the effect of the combination is strongly synergistic. In contrast to caffeine, adenosine did not stimulate phosphorylase phosphatase activity nor did it enhance the stimulatory effect of glucose.

Adenine had no effect on the synthase phosphatase or phosphorylase phosphatase reactions either alone or in combination with glucose (fig.4). In preliminary studies, inosine at a 0.5 mM concentration also enhanced glucose stimulation of synthase phosphatase activity (unpublished).

4. DISCUSSION

Recent in vivo studies [8,9] do not support the role for phosphorylase *a* in the regulation of glycogen synthase activation by glucose advocated by others [4,5]. However, direct regulation of synthase activation by glucose, until recently, seemed untenable. New in vitro evidence showed that glucose stimulates the synthase phosphatase reaction [7]. This reaction is responsible for the activation of glycogen synthase. However, the concentration of glucose required exceeds that in normal liver. Nevertheless, as the present results indicate, glucose in the physiological concentration range can stimulate the synthase phosphatase reaction provided an appropriate second effector is present. Caffeine, itself a stimulator [6], is an example of such an effector, though not normally of physiological importance. Adenosine, intrinsic to the tissue, is also a second effector. Results with

adenosine not only verify those with caffeine but suggest possible physiological significance of enhanced glucose stimulation. The structural clues provided by caffeine and adenosine may aid in the search for a putative effector.

Caffeine and glucose are both ligands of phosphorylase *a* [2]. Individually, and especially in combination, they stabilize the 'T' conformer of phosphorylase *a*, the preferred phosphatase substrate [3]. Although the physiological significance is unclear, evidence that glucose and caffeine acting in synergy to promote phosphorylase phosphatase activity is also presented. However, effects of caffeine and glucose, individually, on synthase phosphatase activity are direct and independent of phosphorylase *a* [6,7] as are those of the combination (unpublished). Phosphorylase *a* as the exclusive glucose receptor in the regulation of glycogen metabolism is thus obviated. Direct glucose binding to either synthase phosphatase or its substrate, synthase D, enhanced by a second effector may be the mechanism of glucose stimulation of synthase activation. This is significant because it represents a mechanism, compatible with intracellular events following glucose administration, by which glucose could promote liver glycogen synthesis.

Adenosine is known to regulate an array of intracellular events including modification of adenine nucleotide concentrations as well as gluconeogenesis, lipogenesis and fatty acid oxidation in liver [15–17]. In addition, its effects on the vasculature in many tissues are well recognized [18,19]. These effects are all initiated when adenosine binds to an appropriate cell surface receptor. In the present studies a possible intracellular regulatory function for adenosine, or a compound it represents, has been recognized for the first time. The nucleoside structure must have importance because neither the higher nor lower homologue of adenosine, 5′-AMP [6] nor adenine, respectively, acts as a second effector with glucose. Preliminary results indicate that inosine, another nucleoside, may also be active.

The mediator of insulin action, a glycopeptide, described in [20], may be responsible for the activation of glycogen synthesis in adipose tissue and skeletal muscle [21]. In liver, however, glucose is the acute regulator but the role of insulin, though important, may be to potentiate glucose action. The effects of caffeine and more particularly, adenosine, in relation to glucose stimulation of glycogen synthase activation reflect one possible mechanism of insulin action in liver. There is uncertainty as to the liver concentration of adenosine which is reported variously as 10^{-6} M [22] to 10^{-4} M (personal communication, Dr A. Fischer, North Dakota State University). Using the assay system described here further work will be directed at identifying the putative effector, whether adenosine or a related compound. Special attention will be given to determining whether the effector concentration varies as a response to extracellular influences including insulin.

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