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BIPHASIC STIMULATION OF AMINO ACID UPTAKE BY GLUCAGON IN HEPATOCYTES

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SUMMARY: Glucagon, 1.4 μM, increased the steady-state distribution ratios of alanine and α-aminoisobutyric acid in rat hepatocytes. As a function of time, glucagon stimulated the one-min rates of uptake of alanine and α-aminoisobutyric acid in two distinct phases. In the first phase both valinomycin and glucagon rapidly increased the rate of alanine uptake in a medium containing 6 mEq/l K^+ but not in one containing 12 mEq/l K^+. Cycloheximide did not alter the first but completely abolished the second phase of stimulation. These results indicate that the biphasic stimulation of alanine uptake in liver produced by glucagon depends upon cell membrane hyperpolarization in the first phase and on protein synthesis in the second.

INTRODUCTION: By use of AIB[1] as a model amino acid, prior studies have demonstrated that the stimulation of the hepatic uptake of amino acids by glucagon is dependent on protein and RNA synthesis (1-4). In the present communication, we report studies in freshly isolated rat hepatic parenchymal cells showing that the stimulation of uptake of alanine and AIB by glucagon is biphasic and mediated by more than one process. The first phase appears to depend on cell membrane hyperpolarization and the second phase on protein synthesis.

MATERIALS AND METHODS: Hepatic parenchymal cells were isolated from fasted male Sprague-Dawley rats as reported previously (5). Transport studies were performed at 37°C in Krebs-Henseleit medium containing 20 mg/ml bovine serum albumin, 15 mM lactate and 1 mM [^{14}C]alanine or [^{14}C]AIB. To measure alanine uptake, 0.2 mM aminooxyacetate was added to the medium in order to inhibit the degradation of alanine. One-min rates of alanine and AIB uptake were measured as described previously (5), except that 0.2 mM aminooxyacetate was added one min before the addition of alanine. This modification was made to minimize any inhibitory effect of aminooxyacetate on protein synthesis (6) and to permit examination of the effect of cycloheximide on basal and glucagon-stimulated alanine uptake. Studies with AIB were performed in the

[1]The abbreviations used are: AIB, α-aminoisobutyric acid; System A, Alanine-preferring system of neutral amino acid transport; System ASC, alanine, serine, cysteine system; System L, leucine system.

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absence of aminooxyacetate. Valinomycin was dissolved in absolute ethanol before addition to the cell incubations. Appropriate radioactive markers were used to identify intra- and extracellular distribution spaces (5).

RESULTS AND DISCUSSION: The effect of glucagon on the time course of accumulation of [^{14}C]alanine and [^{14}C]AIB is shown in Fig. 1. In agreement with our earlier observations (5), isolated hepatocytes accumulated alanine rapidly from the medium containing 1 mM alanine. The steady-state distribution ratio reached 8 within 15 to 30 min. When 1.4 μM glucagon and alanine were added together, this maximally effective concentration of hormone increased alanine accumulation as early as 5 min after hormone addition and raised the steady-state distribution ratio to about 12 in 60 min. By comparison, AIB was accumulated more slowly: 90 to 120 min were required to attain a steady-state distribution ratio of 8. Addition of 1.4 μM glucagon also increased the distribution ratio of this amino acid, but, in contrast to alanine, a significant increase in AIB accumulation was evident only after 15 min of incubation.

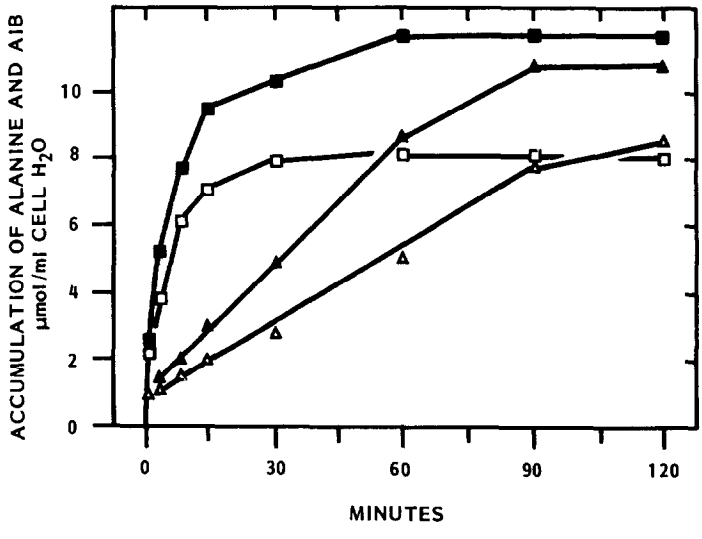

Figure 1. Effect of glucagon on the time course of uptake of alanine and AIB. Hepatocytes, 5 to 9 million cells per ml, were incubated in a medium containing 1 mM alanine (squares) or 1 mM AIB (triangles). Glucagon, 1.4 μM, (filled symbols) was added at the start of incubation. Results are the means of 3 experiments.

In order to examine the effect of glucagon on alanine uptake under conditions that minimize simultaneous amino acid efflux, one-min rates of uptake were measured as a function of time of exposure of hepatocytes to glucagon (Fig. 2). It has been previously validated that such measurements can be employed to estimate initial rates of uptake (5). Following 5 min of glucagon exposure, the one-min rate of alanine uptake was increased 40%. With increasing duration of exposure to glucagon, a biphasic effect was observed. This response was characterized by an early peak at 5-15 min, a trough at 30 min and a second, sustained rise in rate thereafter. Although several studies have shown that glucagon can stimulate the uptake by AIB with little time delay (7-10), none of them has reported an onset of action as fast as that demonstrated here with alanine. Additionally, to our knowledge, the biphasic effect here described has never been reported.

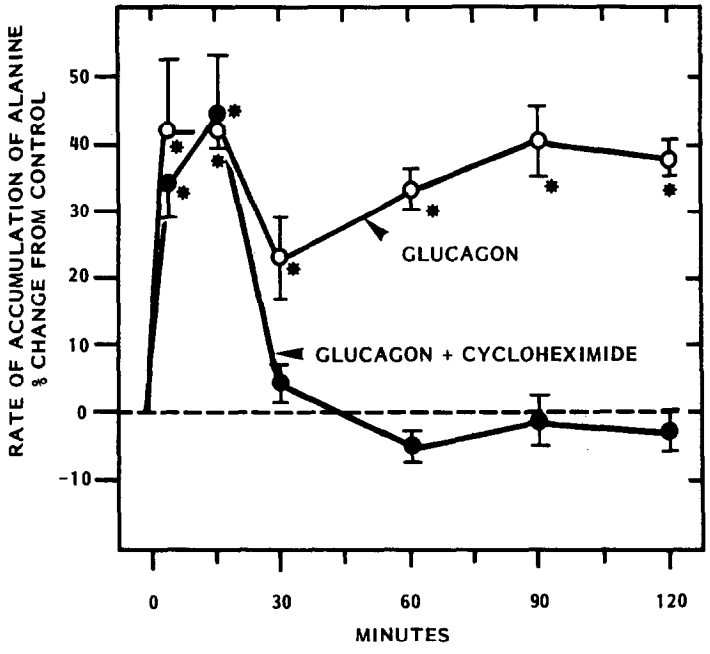

Figure 2. Effect of duration of glucagon preincubation on the rate of uptake of alanine. Hepatocytes were incubated with 1.4 μM glucagon for the time periods shown and after which, 1 mM alanine was added for determination of the one-min rate of uptake. Where indicated 50 μg/ml cycloheximide was added with glucagon. Results are mean ± SE of 5 experiments. Asterisks indicate significant ($P<0.05$ by paired t test) increases in the rate of alanine uptake when compared with control.

To ascertain the contribution of protein synthesis to this effect of glucagon, cycloheximide, 50 μg/ml, was added together with glucagon during preincubation with the cells. Cycloheximide had no effect on the first phase of stimulation but completely abolished the second phase (Fig. 2). Identical results were obtained when cycloheximide was added 30 min before the addition of glucagon during preincubation. Cycloheximide also did not alter the basal rate of accumulation of alanine. It is, therefore, apparent that stimulation of the rate of alanine uptake occurring after 30 min of exposure to glucagon is dependent on protein synthesis, whereas the initial phase of stimulation is not.

We have previously shown that alanine accumulation by isolated hepatocytes is mediated by Systems A, ASC and L and that System A is the predominant transport channel (5). Since AIB is also primarily transported by System A (5) and its accumulation in isolated hepatocytes is also stimulated

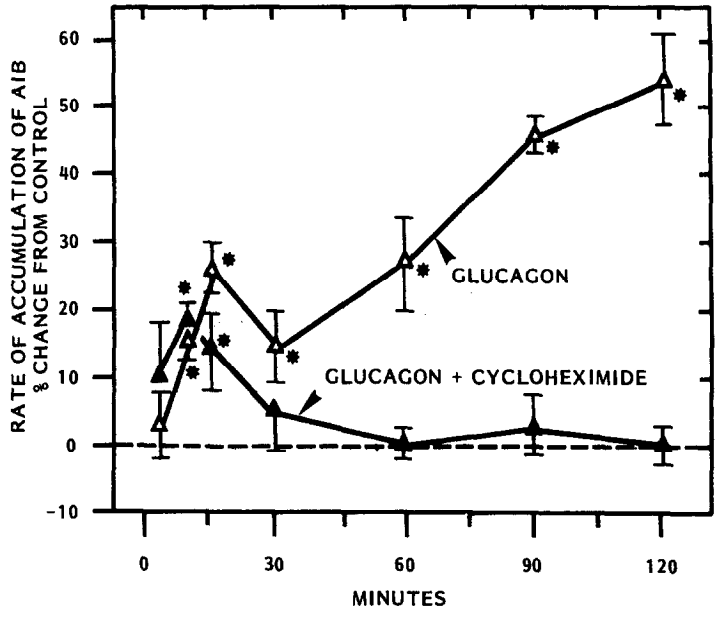

Figure 3. Effect of duration of glucagon preincubation on the rate of accumulation of AIB. Experiments were performed as in Fig. 2 except one-min rates of AIB uptake were determined. Results are the mean ± SE of 5 experiments. Asterisks indicate $P<0.05$ by paired t test.

by glucagon (Fig. 1), one would expect the rates of uptake of AIB and alanine to exhibit a similar pattern of response to glucagon. Figure 3 shows the effect of exposing hepatocytes to 1.4 μM glucagon on the one-min rates of AIB uptake. As with alanine, the stimulation of the rate of uptake of AIB was biphasic but quantitative differences were noted. The first phase of stimulation was less pronounced with AIB, and a significant increase was observed only after 10 min of incubation. Furthermore, the second phase of stimulation was slower in attaining a maximal effect. These differences may relate to the lower basal rate of AIB uptake as compared with that for alanine, 0.73 ± 0.03 (mean ± SE, n=8) vs 2.15 ± 0.05 μmol/ml cell H_2O per min, respectively. As with alanine, addition of cycloheximide 30 min prior to addition of glucagon failed to alter the first phase of stimulation of AIB uptake but completely abolished the effect of glucagon after 30 min (Figure 3). Cycloheximide also did not alter the basal rate of accumulation of AIB even after preincubation for as long as 150 min. Hence, it appears that glucagon produces qualitatively similar effects on the rates of accumulation of alanine and AIB.

One early effect of glucagon on liver which might alter amino acid uptake is cell membrane hyperpolarization (11-12). This effect is mediated by selective efflux of potassium ion from liver (11). Since accumulation of neutral amino acids is an electrogenic process, cell hyperpolarization would be expected to enhance sodium-dependent amino acid uptake (13). Studies were therefore undertaken to examine the effect of hyperpolarization on alanine uptake by hepatocytes. Figure 4 shows the effect of valinomycin on the one-min rate of alanine uptake in a medium containing either 6 or 12 mEq/l potassium. When 1 μM valinomycin was added to the medium containing 6 mEq/l potassium, the rate of alanine uptake increased rapidly, attaining nearly 20 percent stimulation at 5 and 15 min of ionophore exposure. Thereafter alanine uptake returned to the basal level. When valinomycin was added to the medium containing 12 mEq/l potassium, the rate of alanine uptake remained

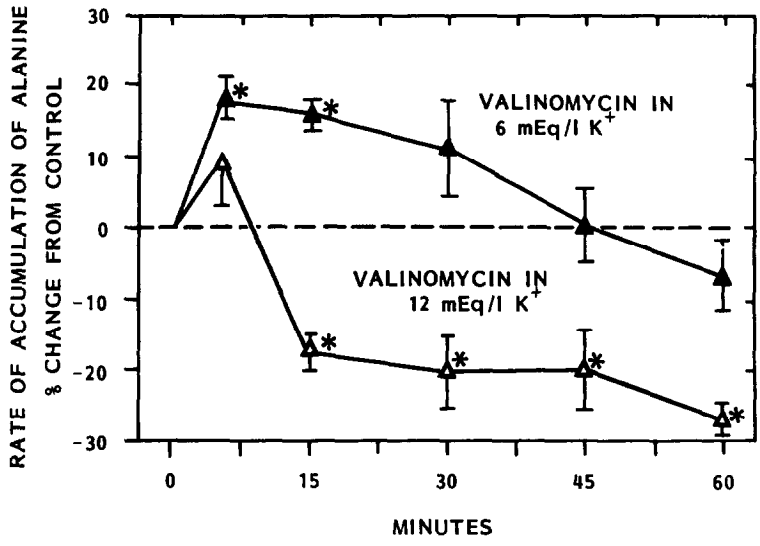

Figure 4. Effect of preincubation with valinomycin on the rate of accumulation of alanine. Experiments were performed as in Fig. 2, except that valinomycin 1 μM was added instead of glucagon and the potassium concentration of the medium was increased to 12 mEq/l by partial substitution of KCl for NaCl. In the medium containing 6 mEq/l K choline chloride was added instead of KCl to lower medium Na+ concentration to a degree comparable to that in the 12 mEq/l K^+ medium. Results are mean ± SE of 3 experiments. Asterisks indicate $P<0.05$ by paired t test.

at or below the basal rate of uptake for the entire period of observation. This observation therefore indicates that hyperpolarization can rapidly augment the rate of accumulation of alanine by hepatocytes.

Figure 5 shows studies of the effect of preincubation of hepatocytes with glucagon in a medium containing either 6 or 12 mEq/l potassium. As noted in Figure 2, glucagon produced biphasic stimulation of the rate of alanine uptake in the medium containing 6 mEq/l potassium. By contrast, the initial phase of stimulation produced by glucagon was abolished when hepatocytes were incubated in the medium containing 12 mEq/l potassium. The later phase of hormonal stimulation, however, was little altered by incubation of cells in this medium.

These studies indicate that glucagon produces biphasic stimulation of hepatic alanine and AIB uptake. The initial phase of stimulation is transient and appears to be related to hormone-dependent hyperpolarization of the

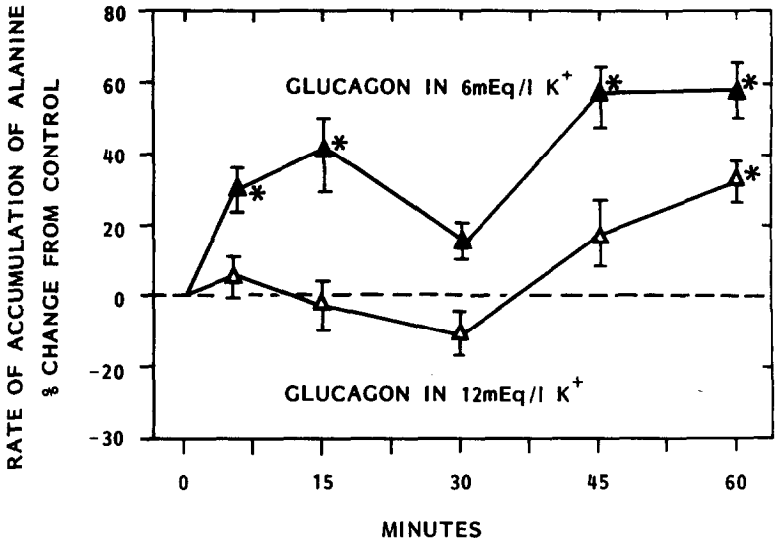

Figure 5. Effect of a high K^+ medium on the the biphasic stimulatory effect of glucagon on the rate of alanine uptake. Experiments were performed as in Figs. 2 and 4. Results are mean ± SE of 4 experiments. Asterisks indicate $P<0.05$ by paired t test.

hepatocyte plasma membrane. The second phase is dependent upon protein synthesis and appears identical to the hormone-dependent effect on hepatic amino acid uptake described previously (1-4).

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