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Hormonal Regulation of Hepatic Amino Acid Transport

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The transport of 2-aminoisobutyric acid (AIB) into liver tissue was increased by both insulin and glucagon. We have now shown that these hormones do not stimulate the same transport system. Glucagon, possibly via cAMP, increased the hepatic uptake of AIB by a mechanism which resembled system A. This glucagon-sensitive system could be monitored by the use of the model amino acid MeAIB. In contrast, the insulin-stimulated system exhibited little or no affinity for MeAIB and will be referred to as system B. On the basis of other reports that the hepatic transport of AIB is almost entirely Na^+ dependent and the present finding that the uptake of 2-aminobicyclo [2,2,1] heptane-2-carboxylic acid (BCH) was not stimulated by either hormone, we conclude that system B is Na^+ dependent. Furthermore, insulin added to the perfusate of livers from glucagon-pretreated donors suppressed the increase in AIB or MeAIB uptake. Depending upon the specificities of systems A and B, both of which are unknown for liver tissue, the insulin/glucagon ratio may alter the composition of the intracellular pool of amino acids.

Key words: insulin, glucagon, transport, amino acids, diabetes

Amino acid transport in liver cells, controlled by various hormones, may be an important factor in regulating hepatic metabolism by altering the composition of the amino acid pools. Thus, it is known that substrate supply can regulate gluconeogenesis (1). Using α-aminoisobutyric acid (AIB) as a monitor of transport, numerous investigations have shown that its accumulation in the liver is increased in response to such hormones as glucocorticoids (2), growth hormone (3), insulin (4, 5), and glucagon (6, 7). Furthermore, the hormonal stimulation of hepatic amino acid uptake has been demonstrated both in vivo (8, 9) and in vitro (5, 10). Of the hormones used, insulin and glucagon have been the most rigorously investigated, perhaps because of their importance in the regulation of carbohydrate metabolism.

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Both insulin (4, 5, 10) and glucagon (6, 7) stimulate the transport of AIB yet they are recognized as metabolically antagonistic hormones. The purpose of the present study was to distinguish between the stimulatory effects of insulin and glucagon using model amino acids and to establish whether or not their antagonistic effects also could be described in terms of hepatic amino acid transport.

MATERIALS AND METHODS

Determination of Amino Acid Uptake in Vivo

One hour prior to sacrifice, 1.0 μCi (10 μCi per ml 0.9% saline) of radioactively labeled amino acid was injected via the tail vein. Injections were spaced 5 min apart to allow time for removal of the liver exactly 60 min later. The animals were sacrificed by decapitation; the livers were removed, rinsed in 0.9% saline, and weighed. The entire liver was immersed in 15.0 ml of 0.9% saline. After homogenization in a Sorvall Omnimixer, a 0.5 ml aliquot of the homogenate was mixed with 10.0 ml of Bray's fluid and the radioactivity was determined in a scintillation spectrometer. The dose of radioactivity injected was determined daily by counting 0.5 ml of a 1:1000 dilution of the labeled amino acid. Results are reported as percent of the injected radioactivity recovered in the liver, i.e., (cpm per liver/total cpm injected) × 100.

Isolated Liver Perfusion

Liver perfusion was performed in a manner similar to that of Miller (11), with a few modifications. Human red cells were isolated from whole blood by centrifugation at 4°C. The cells were then washed 3 times by centrifugation in chilled Ringer solution. The packed cells, 30 ml, were stored in an Erlenmeyer flask containing 1 ml of a solution of 38 mg of glucose, 300 units of penicillin G, and 3.0 g of streptomycin sulfate dissolved in Ringer solution. The flasks were covered with parafilm, stored between 2°C and 4°C, and the cell suspensions were used during the next 72 h. The cells were diluted on the day of perfusion with 70 ml of Krebs-Ringer bicarbonate buffer, pH 7.4, containing 2.1 g of fraction V BSA, 1,000 units of heparin, and 250 mg of glucose. The portal vein and the thoracic vena cava cannulas were made from Intramedic polyethylene tubing. The flow rate of the perfusate averaged 1–3 ml/g liver/min. A preliminary perfusion of 30 min was performed before the labeled amino acid and hormones (where indicated), were added to the perfusate. The first sample was collected following a 3-min mixing time and was recorded as the zero time point for the subsequent calculations.

Samples of perfusate, 1.0 ml, were removed at 0, 10, 20, 30, 45, 60, 90, and 120 min; each was deproteinized by the addition of 4.0 ml of absolute ethanol. After centrifugation, a 1.0 ml aliquot of supernatant was added to 10.0 ml of Bray's scintillation fluid and the mixture was counted in a scintillation spectrometer (Packard). The percent of the amino acid accumulated by the liver was calculated from the decrease in radioactivity in the perfusate; i.e., [(cpm/total perfusate at t_x)/(cpm/total perfusate at t_o)] × 100 gives the percent uptake per total liver. The total perfusate volume used in the calculation was adjusted at each time to account for the removal of the 1.0 ml sample. The percent uptake per liver was then multiplied by 10.0/liver weight so that the data were normalized and reported as the percent uptake per 10 g of liver tissue. Samples (1 g) of liver, taken at the termination of randomly selected experiments, were homogenized, and the radioactivity was determined. In all cases, 95–100% of the radioactivity removed from the perfusate was recovered in the liver; less than 1.0% was found in the bile.

The final amino acid concentration in the perfusate was 1.2 mM, representing approximately 2×10^6 cpm. When glucagon-pretreatment was used, 10.0 μg of the hormone per 100 g body weight were injected subcutaneously 1 h prior to the removal of the liver. Hormones given in vitro were added at the same time as the labeled amino acid and in the concentration indicated in the text. "Glucagon-free" insulin and "insulin-free" glucagon were the generous gifts of Dr. Mary Root of Eli Lilly. The cAMP content of the perfused livers was determined by the use of a radioimmunoassay kit purchased from New England Nuclear Corporation, Boston, Massachusetts. Instructions for tissue preparation and the assay protocol were supplied with the kit.

Experimental Diabetes

Experimental diabetes was induced by an intravenous injection of alloxan monohydrate (50 mg per 100 g body weight) in 0.9% saline. The animals, which had been starved 48 h before the injection, were refed immediately after treatment and given a 1% glucose solution instead of water for the first 24 h. For those rats receiving insulin, 4 units of protamine zinc insulin (Lilly) were given subcutaneously each day. Control rats received an equal volume of 0.9% saline. Blood glucose levels were estimated by the method of Feteris (12). Rats were considered diabetic when the plasma glucose content was greater than 300 mg per 100 ml of plasma 48 h after the treatment with alloxan. Plasma was obtained by centrifugation of blood samples in the presence of 0.25 ml of anticoagulant solution containing potassium oxalate (4.0 g/100 ml) and sodium fluoride (4.0 g/100 ml) in glass distilled water. To 0.1 ml of plasma, 5.0 ml of o-toluidine reagent were added and the mixture was heated for 10 min in a boiling water bath. A 0.1 ml aliquot of a standard containing 100 mg of glucose per 100 ml was included during each set of incubations. The cuvettes were then removed and allowed to cool to room temperature by placing them in tap water for 3 min. The absorbance at 635 nm was determined for each tube during the following 30 min. All samples were run in duplicate while the standards were in triplicate. The data were expressed as mg glucose per 100 ml of plasma.

Reagents

The radioactive amino acids, 1-^{14}C-BCH, 1-^{14}C-AIB, 1-^{14}C-cycloleucine, and 1-^{14}C-MeAIB were purchased from New England Nuclear Corporation, Boston, Massachusetts. The unlabeled BCH was the kind donation of Dr. Halvor N. Christensen, The University of Michigan. The o-toluidine reagent, heparin, Fraction V BSA, alloxan monohydrate, and unlabeled amino acids were obtained from Sigma Chemical Company. Fisher Scientific Company was the source for the components of the liquid scintillation fluid. All other reagents and chemicals were of the highest quality obtainable.

RESULTS

Hepatic Uptake of AIB Stimulated by Glucagon and Insulin

Figure 1 shows the stimulation of AIB accumulation in livers perfused with a single, 1.0 μg dose of glucagon. The livers treated with glucagon accumulated 46.7 ± 6.1% of the added AIB per 10 g of tissue, compared with a control of 20.4 ± 0.8%. Although the AIB levels in the control livers approached a steady state after 60 min, the stimulated uptake was nearly linear during this time and continued to increase even after 120 min. The elevated uptake could be observed as early as 10 min following the addition of glucagon.

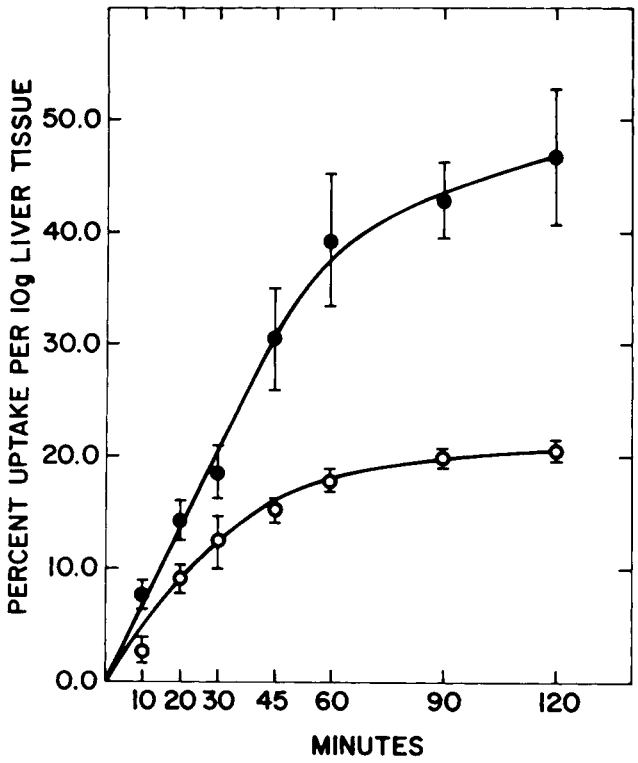

Fig. 1. Accumulation of AIB in isolated, perfused livers. Livers were taken from normal rats and perfused with (●—●) or without (○— ○) 1.0 μg of glucagon added in vitro. The hormone and the labeled amino acid were added 3 min prior to time zero. The results are the average ± SD of the data obtained from 3–6 livers.

As seen in Fig. 2, the addition of 1.0 μg of insulin to the perfusate increased the final accumulation of AIB to 29.7 ± 0.4% while 5 μg increased it to 46.9 ± 1.8%. These stimulations of AIB uptake by the perfused liver following the in vitro administration of glucagon and insulin are in accord with the earlier work of Chambers et al. (2).

Interrelation of Glucagon- and Insulin-Regulated Transport

The stimulation of AIB uptake in livers exposed individually to glucagon and insulin was confirmed in the preceding section. However, these 2 hormones also have antagonistic actions which may be manifested as opposing effects on amino acid transport. Glucagon exerts its effect on AIB uptake via cAMP production (6, 13) whereas insulin is known to exert its antagonistic influence on metabolism either by diminishing the cAMP levels (14) or by counteracting the actions of the nucleotide (15). Stimulation by both hormones could be explained by the existence of separate hormone-sensitive systems. The antagonistic effect of insulin may be explained by an inhibition of the glucagon-sensitive system. If this is indeed the case, it is conceivable that a dose of insulin might be found which could suppress the glucagon-stimulated AIB uptake without simultaneously stimulating the insulin-responsive system. Figure 3 shows typical results for the addition of insulin in 2 doses (1 or 5 μg). It is apparent that the livers obtained from donor rats, pretreated with 10 μg of glucagon/100 g body weight, exhibited an accumulation after 3 h of 41.4 ± 3.4%

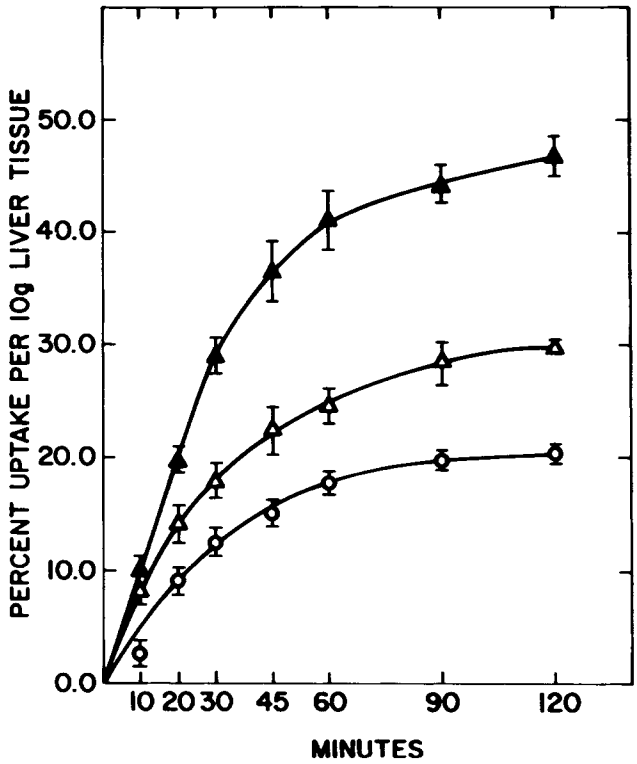

Fig. 2. Uptake of AIB by normal livers perfused with insulin. Controls are the open circles (o—o); livers treated with 1.0 or 5.0 μg of insulin are designated by open (△—△) and closed (▲—▲) triangles, respectively. Data are the average ± SD of 3–6 determinations.

compared with the control of 20.4 ± 0.8%. When livers from glucagon-pretreated rats were subsequently perfused with 1 μg of insulin, the AIB accumulation was reduced to 32.0 ± 0.9% after 2 h. Doses of 2 and 3 μg also suppressed the effect of glucagon but to lesser degrees (data not shown). A 5 μg dose of insulin, however, enhanced the uptake to 50.7 ± 4.2% of the AIB. Thus it appears that doses of 3 μg or less of insulin suppressed the glucagon effect more than they stimulated the simultaneous uptake. The 5 μg dose, however, yielded an overriding stimulation compared with its concomitant inhibition of the glucagon effect. These data suggest the existence of 2 hormonally responsive processes. Both glucagon and insulin stimulate AIB uptake when used individually. On the other hand when used together insulin has the capability of suppressing the effect of glucagon.

Antagonistic Effect of Insulin on the Glucagon-Stimulated Amino Acid Transport

In the preceding sections, the existence of 2 separate hormonally sensitive processes was suggested as well as an inhibitory effect of insulin on the glucagon-stimulated process. The distinction of these 2 phenomena was complicated by their simultaneous occurrence. Glucagon-stimulated uptake is thought to have the specificity of the A- or alanine-preferring transport as described for Ehrlich ascites cells (7, 16). Since Christensen et al. (17) have shown that monomethylation of the α-amino nitrogen of amino acids restricts their transport to the A system, it seemed likely that N-methyl AIB (MeAIB)

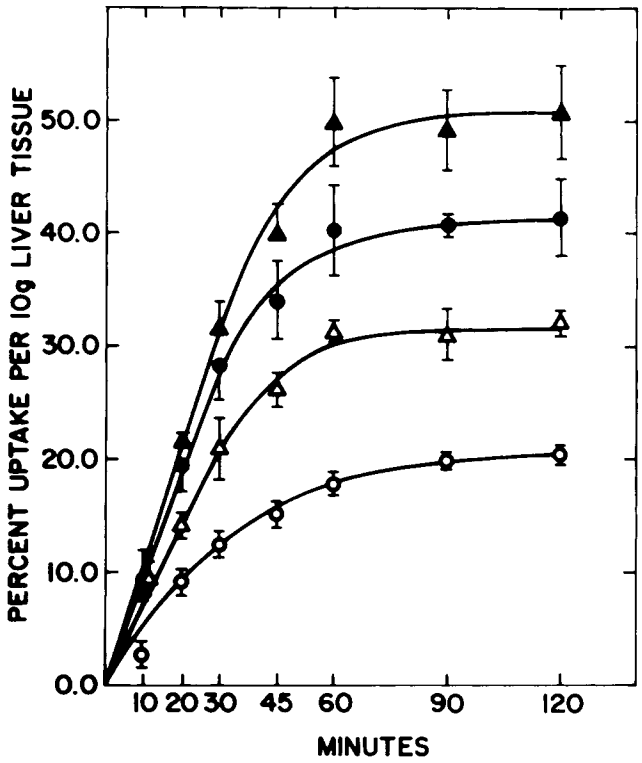

Fig. 3. Accumulation of AIB in perfused livers from rats pretreated with 10 μg of glucagon per 100 g body weight plus insulin added in vitro. Livers from pretreated rats (●—●) were exposed to 1.0 μg (△—△) or 5.0 μg (▲—▲) of insulin. Control livers are shown by the open circles (○—○). Insulin was added at the same time as the labeled amino acid. Data are the average ± SD of 3–6 perfusions.

would selectively respond to glucagon but not to insulin. This amino acid might be used to distinguish the inhibitory effect of insulin from its stimulatory action.

Pretreatment in vivo with glucagon markedly stimulated the hepatic transport of MeAIB. Figure 4 shows that the maximal uptake by livers from the treated animals reached 45.9 ± 2.8% after 60 min while the controls were 24.6 ± 0.9% of the MeAIB. As anticipated, perfusion of normal livers with 5 μg of insulin had no effect on the uptake of MeAIB (Fig. 5). Thus the accumulation of MeAIB is stimulated by glucagon but not by insulin.

Since MeAIB responded well to the stimulatory effect of glucagon but did not respond to insulin, it appears that the latter hormone does not influence the A transport system. It is conceivable, however, that the insulin might exert its antagonistic effect by suppressing the glucagon-stimulated uptake of MeAIB. Both 1 and 5 μg of insulin added to the perfusate of a liver from a glucagon-pretreated rat suppressed the uptake of MeAIB (Fig. 4). These data substantiate the existence of an antagonistic effect of insulin on the glucagon-stimulated uptake of amino acids by the liver.

Absence of Na^+-Independent L Transport in Hormone-Stimulated Amino Acid Transport

Although the stimulated transport of AIB induced by glucagon is known to require Na^{π} (7), the Na^+ dependence of the newly recognized component of AIB uptake needed

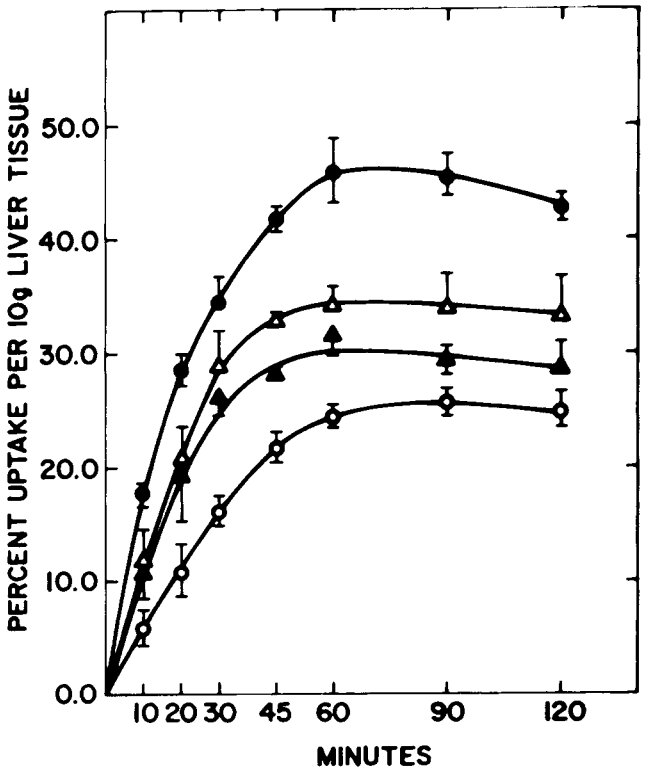

Fig. 4. Uptake of MeAIB in livers from glucagon-pretreated rats (10 μg per 100 g body weight) (•—•) and the effect of adding 1.0 μg (△—△) or 5.0 μg (▲—▲) insulin to the perfusate. Control livers are shown by the open circles (○—○). Data are the average ± SD of 3–6 determinations.

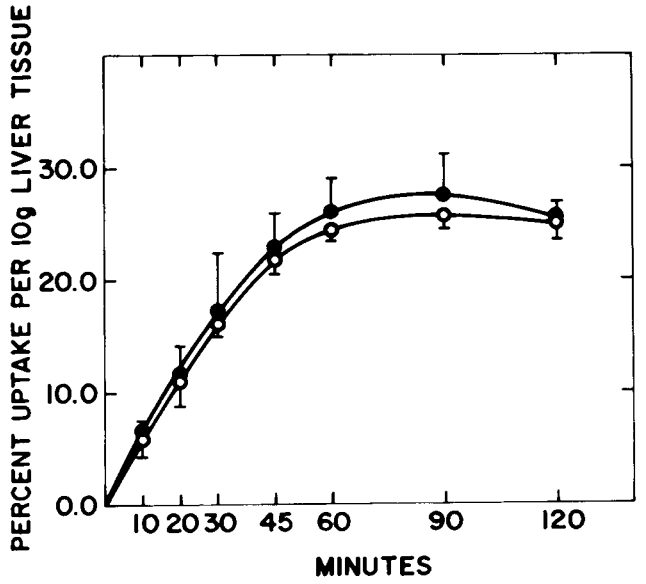

Fig. 5. Uptake of MeAIB in normal and insulin-treated livers. Effect of perfusion with (•—•) or without (○—○) the addition of 5.0 μg of insulin is shown. Data are the average ± SD of 3–6 perfusions.

TABLE I. Effect of Insulin and Glucagon on BCH Uptake in Isolated Perfused Liver*

Hormone added	Time of perfusion (min)						
	10	20	30	45	60	90	120
Control	5.4	9.2	11.7	11.7	12.0	14.0	13.5
5.0 μg insulin[a]	4.5	6.9	10.7	13.4	14.4	13.5	13.5
Glucagon[b] 10 μg/100 g body weight	4.0	7.9	9.5	12.0	13.6	12.9	13.8

*Data are the averages of 2 perfusions for each condition and are expressed as the percent of the added BCH accumulated per 10 g liver tissue.
[a]5.0 μg of insulin were added to the perfusate at the same time as the labelled amino acid.
[b]Glucagon was injected subcutaneously 1 hr prior to liver isolation.

TABLE II. Effect of Insulin and Glucagon on Hepatic cAMP Levels in Isolated Perfused Liver*

Hormone added	cAMP content after 120 min
Control	0.92 ± 0.20
1 μg glucagon	1.40 ± 0.20[b]
1 μg insulin	0.72 ± 0.02[c]
5 μg insulin	0.90 ± 0.08[c]
Glucagon pretreatment[a]	2.17 ± 0.30[d]
Glucagon pretreatment[a] plus 1 μg insulin	1.50 ± 0.30[e]
Glucagon pretreatment[a] plus 5 μg insulin	1.23 ± 0.20[f]

*Livers were perfused with glucagon or insulin (see Materials and Methods) for 120 min, then immediately submersed in liquid nitrogen and stored frozen until assayed. Data are expressed as nmoles cAMP per g liver and are the average of 3–6 livers.
[a]Glucagon (10 μg/100 g body weight) was injected subcutaneously 1 h prior to liver isolation. Insulin, when given, was added to the perfusate of the livers taken from glucagon-pretreated donors.
[b]$p < 0.02$ when compared to the control.
[c]not statistically different when compared to the control.
[d]$p < 0.001$ when compared to the control.
[e]$p < 0.05$ when compared to glucagon pretreatment.
[f]$p < 0.01$ when compared to glucagon pretreatment.

to be determined. Evidence for Na^+ dependence was obtained indirectly by determining the absence of any stimulation on the uptake of 2-aminobicyclo[2,2,1] heptane-2-carboxylic acid (BCH), a model amino acid carried only by the Na^+-independent L (leucine-preferring) system (18). When ^{14}C-BCH was introduced into the perfusate, control livers accumulated approximately 14% of the added amino acid per gram of liver tissue (Table I); neither the addition of 5 μg of insulin nor pretreatment of the donor rat with glucagon had any effect on the uptake of BCH. Thus both the insulin- and the glucagon-stimulated transport of the liver are believed to occur by Na^+-dependent systems.

Effect of Glucagon and Insulin on Hepatic cAMP Levels

It is believed that the effect of glucagon on amino acid uptake is mediated by cAMP. Insulin can oppose the action of glucagon either by suppressing cAMP levels (14) or by counteracting the metabolic effects of this nucleotide (15). To demonstrate that the antagonism of glucagon and insulin on the uptake of AIB was accompanied by alterations of cAMP levels, measurements were made of this nucleotide in all the foregoing experi-

ments following their termination. As seen from Table II, glucagon administered either in vitro or in vivo, increased the hepatic cAMP. Glucagon perfused in vitro increased the cAMP from a normal of 0.92 to 1.40 nmoles/g liver whereas pretreatment of the donor rat yielded a level of 2.17 nmoles/g. Tews et al. (6) observed a maximal stimulation of AIB transport when the cAMP levels were approximately 3 nmoles/g liver. Insulin, on the other hand, did not affect the normal content of cAMP as already observed by Park et al. (14).

Consistent with the amino acid transport data, 1 or 5 μg of insulin depressed the cAMP levels to 1.50 ± 0.30 and 1.23 ± 0.20 nmoles per g liver, respectively, when introduced into the perfusate of livers from glucagon-pretreated donor rats. Therefore, it may be concluded that insulin lowered the glucagon-stimulated levels of cAMP. It may also be concluded that glucagon and insulin each stimulated AIB uptake by a different mechanism, the former via a cAMP-mediated process as suggested by Tews et al. (19) and the latter via a system not involving this cyclic nucleotide. Although insulin decreased the glucagon-stimulated transport and also partially lowered the level of cAMP, it may not be assumed that these processes are necessarily related.

Table II shows that a cAMP level of 1.40 nmoles/g liver was associated with a stimulated AIB uptake (Fig. 1) whereas 1 μg of insulin, which reduced the cAMP from 2.17 to 1.50 nmoles/g, suppressed the accumulation of AIB (Fig. 4). The reason for the divergent effects on AIB uptake for the same apparent cAMP concentration is presently unknown.

AIB Transport Studied in the Diabetic Rat

Although it is known that experimental alloxan diabetes is accompanied by a hyperglucagonemia (20), no effort has been made to demonstrate that this phenomenon results in a stimulation of hepatic amino acid transport. Based on the preceding studies it would be expected that AIB accumulation should be elevated in the diabetic state and that this increased uptake could be counteracted by insulin. Rats were injected with alloxan monohydrate and the uptake of AIB measured at the times indicated for a total of 14 days (Fig. 6). Before treatment with alloxan, approximately 6% of the injected AIB was recovered in the liver. In constrast, 48 h after injecting the alloxan, the accumulation was 17.4 ± 0.7%. On the 8th day the animals were divided into 2 groups; 1 received 4 units of protamine zinc insulin daily while the controls received saline. Within 24 h, treatment with insulin reversed the diabetes-induced stimulation of AIB transport (Fig. 6). Those rats receiving insulin accumulated 5% of the injected amino acid per liver, while the AIB uptake in the animals given saline remained elevated at 20%. To support the hypothesis that the insulin had reversed an action of glucagon upon the liver, the plasma glucose levels were determined in the same animals. As expected, plasma glucose levels exceeded 300 mg per 100 ml 48 h after the administration of alloxan and continued to increase for the duration of the experiment (Data not shown). Treatment of these rats with daily injections of insulin decreased the plasma glucose to control values resulting in a general profile which was similar to that of the AIB transport. This reversal of the elevated glucose levels may be caused by a decrease in the circulating glucagon (21, 22).

Hepatic Accumulation of Other Model Amino Acids in Diabetic Rats

As already described, MeAIB serves as a monitor for the stimulation of the glucagon-sensitive system A but not of the insulin-sensitive transport. To prove that the stimulation of AIB uptake in diabetes is the result of the hyperglucagonemia, MeAIB uptake was monitored in livers from control and diabetic rats. Figure 7 shows that diabetes resulted

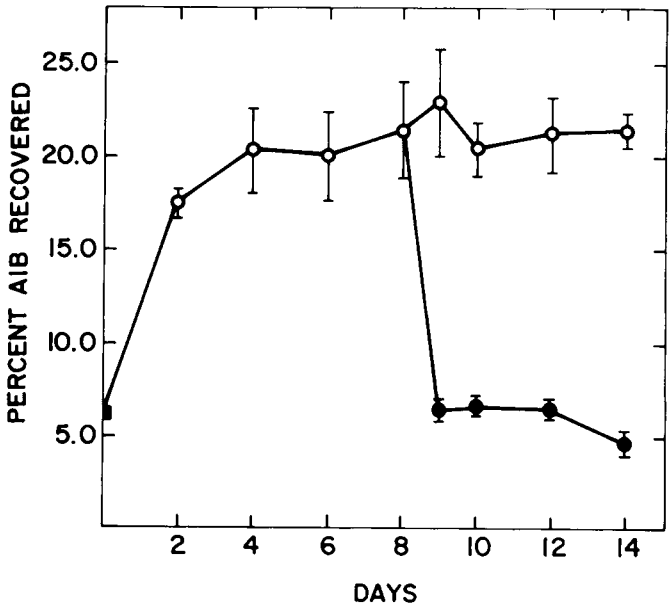

Fig. 6. Percent of the total AIB injected recovered in the livers of alloxan diabetic rats. On day 8, one half of the remaining animals received insulin (●—●) as described in the section on Materials and Methods; controls (○—○) were given an equal volume of saline. Each point is the average ± SD of 4 rats.

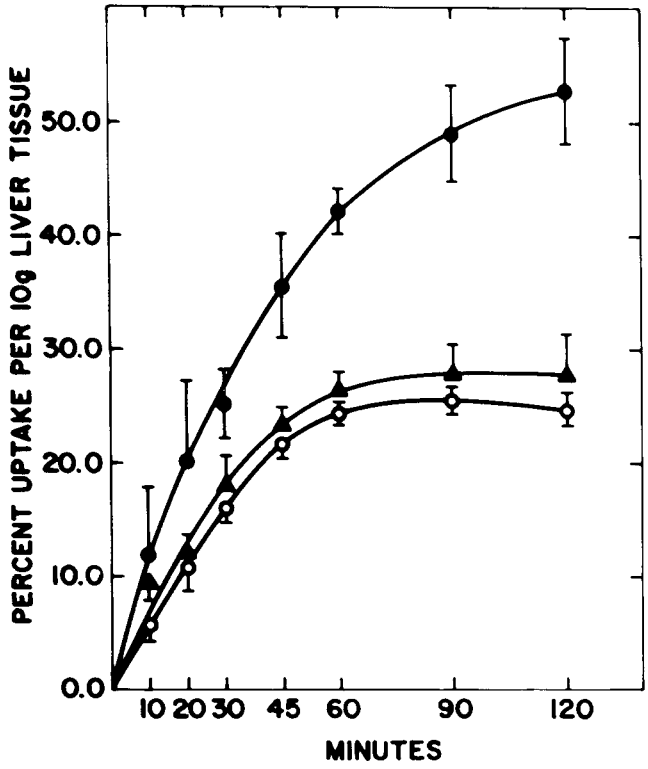

Fig. 7. Uptake of MeAIB in livers from diabetic rats perfused with (▲—▲) or without (●—●) 5.0 μg of insulin. Control livers are shown as the open circles (○—○). Insulin was added in vitro at the same time as the labeled amino acid. Each curve is the average ± SD of 3–6 livers.

TABLE III. Effect of Insulin on Hepatic cAMP Levels in Isolated, Perfused Livers From Alloxan-Diabetic Rats*

Treatment	cAMP content after 120 min
Control	0.92 ± 0.20
Diabetic	2.69 ± 0.20[b]
Diabetic plus 5.0 μg insulin[a]	1.10 ± 0.08[c]

*Diabetes was induced by injecting alloxan as described in Materials and Methods. The same livers as those used for Fig. 7 were employed for these determinations. After perfusion for 120 min, the livers were submersed in liquid nitrogen and stored frozen until assayed. The data are the average ± SD of 3–6 livers and are expressed as nmoles cAMP per g liver tissue.

[a]Insulin was added to the perfusate at the beginning of the experimental period as described in Materials and Methods. [b]$p < 0.001$ when compared to the control. [c]$p < 0.001$ when compared to the diabetic.

TABLE IV. In Vivo Uptake of BCH and Cycloleucine in Livers of Alloxan-Diabetic Rats*

	BCH	Cycloleucine
Control	2.08 ± 0.14 (5)	2.19 ± 0.15 (4)
Diabetic	2.53 ± 0.18 (3)[a]	6.38 ± 0.32 (3)[b]

*Determination of uptake is described in Materials and Methods. Data are reported as percent of injected amino acid recovered in the liver after 60 min. The number of rats is shown in parenthesis.

[a]$p < 0.02$ when compared to the control

[b]$p < 0.001$ when compared to the control

in a twofold stimulation in accumulation of MeAIB, following 2 h of perfusion. When 5 μg of insulin were introduced into the perfusate, the stimulated accumulation of MeAIB was abolished (Fig. 7). These results confirm those previously described for the glucagon-pretreated rats.

Because of the known hyperglucagonemia, hepatic cAMP levels are also elevated in alloxan diabetes (23). Treatment with insulin decreases the cAMP content and reverses the glucagon-induced changes in carbohydrate metabolism (14). In accord with these observations the data of Table III show the increased levels of cAMP in perfused livers from alloxan-diabetic rats. Perfusion of these livers with 5 μg of insulin reduced the cAMP content to near control values. Thus the interrelation of glucagon and insulin as studied in alloxan-diabetes support the evidence provided in the preceding section using only exogenous hormones.

The limitation of uptake to Na^+ dependent systems was also tested by determining the uptake of BCH and cycloleucine (Table IV). The uptake of BCH which monitors the Na^+-independent L system (18) was not affected to the same degree as cycloleucine, a model amino acid transported by both Na^+-dependent A and the Na^+-independent L systems (16).

DISCUSSION

Although both insulin (4, 5, 10) and glucagon (6, 7) stimulate the uptake of AIB by the liver, the present report demonstrates that they do not enhance a common transport mechanism. Glucagon stimulated system A, mediated by cAMP (6, 7, 13), as suggested by

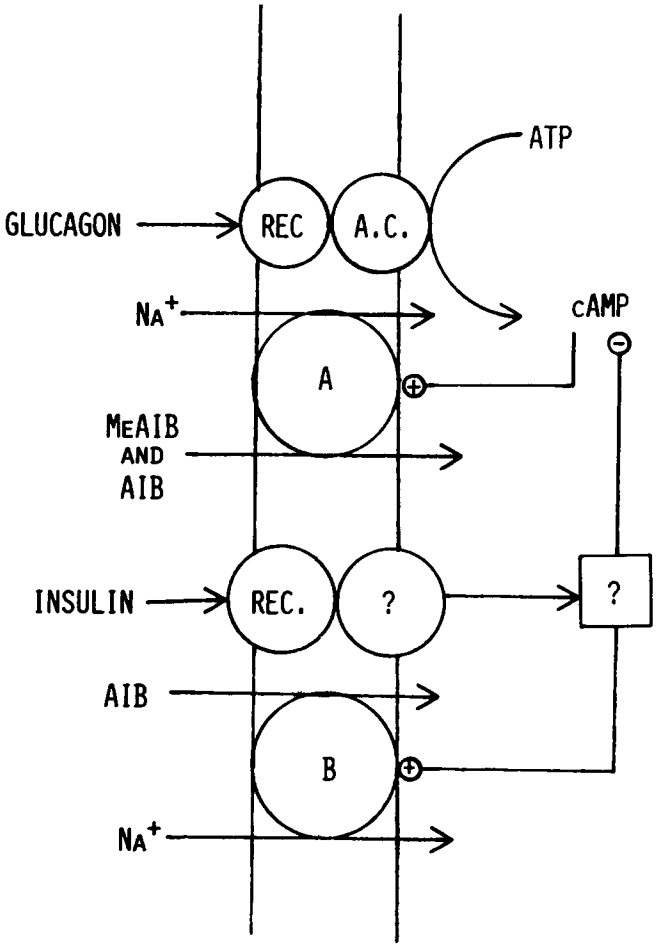

Fig. 8. A schematic diagram showing the relationship between the pancreatic hormones insulin and glucagon and Na^+-dependent amino acid transport at the liver plasma membrane.

the response of MeAIB to the hormone whereas insulin did not affect the methylated model amino acid. For the time being, we suggest that the insulin-sensitive transport simply be called system B. Thus, the hepatic uptake of AIB can be assumed to be the result of a combination of systems A and B. Previous investigations have suggested the existence of 2 systems for AIB uptake in the liver (24, 25). Furthermore, Flory and Neuhaus (24) have shown that the transport of AIB is almost entirely Na^+ dependent, thus system B is probably also Na^+ dependent. In support of this concept is the fact that neither insulin nor glucagon affected the hepatic accumulation of BCH, a model amino acid carried entirely by the Na^+-independent L-process (18).

According to LeCam and Freychet (7, 26) the systems for neutral amino acid transport, present in the Ehrlich ascites tumor cell, are also found in the liver. These authors showed that AIB uptake was only partially inhibited by MeAIB (7). The uninhibited component was attributed to system ASC (26), despite the fact that Christensen has shown that ASC exhibits no affinity for AIB (27). In any event it appears that the liver possesses 2 AIB transporting systems, 1 inhibited by MeAIB, the other not. It is con-

ceivable that the latter may be the same as the insulin-sensitive process described in the present report. For the time being the provisional designation of system B is preferred to distinguish the insulin-stimulated process from the glucagon-sensitive system A.

Exposure of rats to whole-body irradiation (γ rays) has been used to study the role of amino acid transport in controlling hepatic metabolism (28, 29). The radiation-induced uptake of amino acids by the liver occurs via system A and results from an excessive release of glucagon from the pancreas (29, 30). A stimulation of amino acid uptake caused by exposure to γ rays was accompanied by both gluconeogenic and glycogenic states (29). Since the effect of glucagon on hepatic transport appears to involve the A system, which transports many of the gluconeogenic amino acids, it appears likely that the specificity of the A system is an important factor in regulating the gluconeogenic state of the liver. In contrast, the insulin-sensitive system B may present the liver with an entirely different spectrum of amino acids. What effect this may have on hepatic metabolism must await a determination of the specificity of system B.

It is apparent that insulin has the ability to regulate hepatic, neutral amino acid transport in 3 distinct ways. First, insulin can act on the pancreas to decrease the circulating levels of glucagon and, therefore, can suppress the stimulation of system A by glucagon. Secondly, the data reported here show that insulin can modulate the effect of glucagon on system A at the liver cell. Finally, insulin can cause the elevation of an additional Na^+-dependent system, namely, system B. Unger has proposed that the insulin/glucagon molar ratio is more important in homeostasis than is the absolute concentration of either hormone (20). It is possible that the I/G ratio is reflected as an A/B transport ratio at the liver membrane (Fig. 8) and thereby controls the composition of the intracellular amino acid pool.

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