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CHANGES IN AMINO ACID TRANSPORT IN THE RAT PANCREAS IN RESPONSE TO FASTING AND FEEDING

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SUMMARY

Transport of amino acids (in vitro) in the rat pancreas is affected by the nutritional state of the animal. A fast of 24 h (young animals) or 48 h (adult animals) reduces the rate of amino acid uptake in the isolated rat pancreas in vitro. In contrast, refeeding of animals after a fast shows an increase in transport in young as well as adult animals.

The effects of refeeding after a fast are mimicked to a significant extent by injection of mixtures of pancreozymin and carbamylcholine. Addition of these agents in vitro has no effect.

The incorporation of amino acids into the total proteins of the rat pancreas follows the pattern of amino acid uptake. Even at high external levels of glycine (5 mM), incorporation increases although the glycine level in the cell is in excess of 25 mM. Reduction of glycine uptake by ouabain by 75 % results in a substantial (44 %) diminution of amino acid incorporation into proteins. The data suggest that inhibition of amino acid incorporation under the various metabolic conditions examined is due largely to a decreased availability of amino acids.

INTRODUCTION

We have previously reported [1] that during development, amino acid transport in rat pancreas shows considerable change. Transport is high and Na^+-dependent several days before birth. Immediately after birth, and before suckling, transport activity rapidly falls to a minimum, and there is little Na^+ dependence. Within 12–24 h after birth, there is a rapid increase in transport.

Subsequent to our original report [1] of the drop in transport activity at birth, we made the observation that the early restoration of transport activity in the newborn was associated with food intake. Newborn animals not allowed to suckle did not show increased transport activity 24 h after parturition. This observation suggested to us

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that there might be a relationship between food intake and rate of amino acid transport in the pancreas. In addition, since the synthesis of the digestive enzymes by the pancreas may also be related to the dietary state (Snook [2]), we decided to examine whether a change in amino acid uptake will cause a parallel change in incorporation into proteins and whether the rate of amino acid incorporation into proteins affects amino acid transport.

MATERIALS AND METHODS

All experiments were carried out with Sprague-Dawley rats. The techniques used to prepare the pancreas as well as the details for measuring amino acid transport and extracellular space were described in an earlier communication (Cheneval and Johnstone [1]).

Incorporation of ^{14}C-labeled amino acids into proteins was measured in the following way. After incubation, the pancreas was homogenized in 1 ml of 5 % trichloroacetic acid. The homogenate was centrifuged for 5 min at $1000 \times g$. An aliquot of the supernatant was counted by liquid scintillation to estimate the amount of acid-soluble [^{14}C]glycine. The pellet was washed twice with 5 % trichloroacetic acid containing 2 mM non-radioactive glycine. After the final wash, the pellet was dissolved in 0.2 ml 2M NaOH and counted. The radioactivity in the pellet was taken to represent amino acid incorporation into protein.

Paper chromatographic analysis using isobutanol/acetic acid/water (5 : 4 : 1, by vol.) showed at least 95 % of the acid-soluble radioactivity migrated as a single component corresponding to authentic glycine. No other radioactive components were detected by this technique.

Fasting, feeding and pancreozymin injections. Newborn animals which were to be fasted were removed from the dam within the hour following their birth and before the first suckling. The animals were kept in one cage in the animal room at 20–22 °C without further precautions. We established that the newborn animals cannot withstand temperatures of 28–30 °C with high relative humidity. All the newborns survived a 24–30 h fast at 20–22 °C. The same procedure was used to fast older animals. Adult rats were fasted for 24–48 h in specially prepared cages.

To determine the effect of feeding after a fast, newborn animals and animals up to a week in age were replaced with the dam for 3 h. The dam had been kept with 4 or 5 of the original litter to maintain her milk supply. Fasting adult rats were also allowed to feed for a period of 3 h. After this period, the animals were decapitated and the pancreas excised immediately. Injections of pancreozymin (and the other agents) were made from a solution of isotonic NaCl either intraperitoneally (for adults) or subcutaneously in the dorsal region (for newborns) 0.5 h before decapitation.

Pancreozymin-cholecystokinin, secretin and carbamylcholine were obtained from Sigma Chem. Co., St. Louis, Mo. Insulin was obtained from Connaught Medical Research Laboratories (University of Toronto). [^{14}C]Glycine and 2-amino-[^{14}C]isobutyrate were obtained from New England Nuclear Corp., Boston, Mass.

RESULTS

Before examining the effects of potential regulators on amino acid transport and incorporation, we followed glycine uptake and incorporation as a function of age

TABLE I

UPTAKE AND INCORPORATION OF GLYCINE IN RAT PANCREAS

Transport and incorporation of amino acids were measured in normal Ringer medium containing phosphate buffer, pH 7.4, at 37 °C [1]. The duration of incubation was 15 and 90 min. [1-^{14}C]glycine was used at concentration of 0.25 mM, specific activity 0.4 Ci/mol. The values are expressed as mmol glycine per kg of pancreas (wet weight). The means values ± S.D. are given. The number of experimental observations is in parentheses.

Age	After 15 min of incubation		At steady state (90 min of incubation)	
	Uptake	Incorporation	Uptake	Incorporation
Fetus				
18 days	0.82	0.06	2.87	0.53
20 days	0.53	0.02	1.81 ± 0.16 (6)	0.46 ± 0.04 (6)
21–22 days	—	—	1.08 ± 0.04 (3)	0.17 ± 0.01 (3)
Newborn at birth	0.35 ± 0.02 (4)	0.01 ± 0.0 (4)	0.80 ± 0.05 (6)	0.07 ± 0.01 (6)
3 days after birth	0.58 ± 0.02 (6)	0.03 ± 0.01 (6)	1.78 ± 0.15 (4)	0.40 ± 0.05 (4)
Adult	0.91 ± 0.09 (6)	0.025 ± 0.0 (6)	1.66 ± 0.23 (12)	0.28 ± 0.07 (12)

of the animal. The results in Table I summarize our observations. Two time points were selected for examination, 15 min, when the rate of uptake was still close to the initial rate, and 90 min, when the uptake had reached steady state. The same pattern for uptake versus age was obtained at 15 and 90 min. It may be seen that even at the point of minimal uptake, the cellular concentration of acid-soluble [^{14}C]glycine was several-fold greater than the concentration in the medium. It is noteworthy that incorporation into proteins followed the same pattern as uptake, minimal uptake and incorporation occurring at birth. The observation that incorporation of ^{14}C into proteins followed the same pattern as uptake into the acid-soluble pool, suggested to us that incorporation might be closely tied to the available glycine concentration. In most organs, the requirements for amino acids for protein synthesis are met at very low levels of amino acid [3–6]. Thus, in systems capable of accumulating amino acids, the sites for amino acid incorporation are saturated at low extracellular amino acid levels and elevation of extracellular amino acid has little effect on incorporation into proteins [3–6]. In pancreas, Jamieson and Palade [7] showed, however, that incorporation into proteins increased when the leucine concentration was elevated from 0.4 to 4 mM.

The results in Fig. 1 show that in rat pancreas, as the external level of [^{14}C]-glycine was raised, the incorporation of ^{14}C into proteins was increased in an almost parallel manner. Since the endogenous glycine will dilute the [^{14}C]glycine, we estimated the size of the glycine pool in the pancreas from adult rats using a Technicon Amino Acid Analyser. This value was found to be 1.2–1.5 mmol/kg (wet weight of pancreas). Using this value, the specific activity of the cellular glycine concentration was computed from the ^{14}C-labelled and unlabelled material and incorporation (in μmol glycine) plotted as a function of extracellular glycine concentration (see upper curve, Fig. 1). (It was assumed that the endogenous glycine concentration did not change during the incubation period since experiments (unpublished) showed that the loss of [^{14}C]glycine from these cells is slow with a first-order rate constant of 0.025

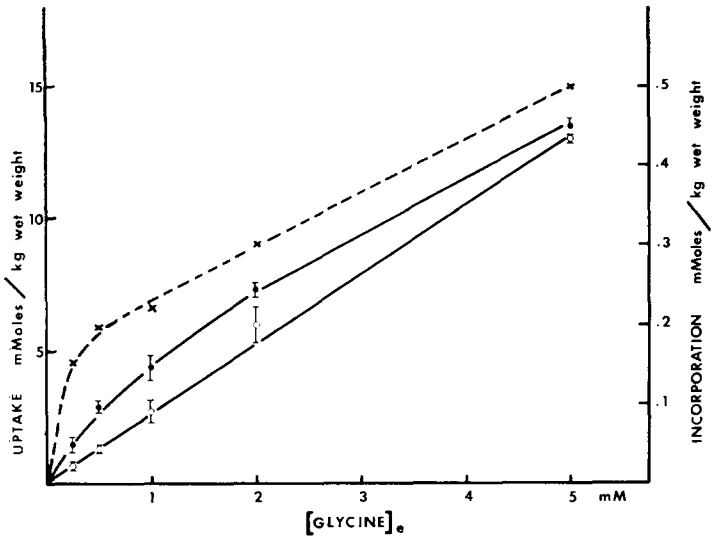

Fig. 1. Transport (○—○) and incorporation (●—●) of [1-^{14}C]glycine as a function of external concentration of glycine in the adult rat pancreas after 10 min incubation. Incorporation (×---×) computed after correction for intracellular dilution with non-radioactive glycine (see text).

min^{-1}.) Despite the correction for dilution, it is evident that incorporation increased as the medium glycine concentration increased to 5 mM and until the cellular glycine concentration was at least 25 mM, suggesting that even a these levels, the protein-synthesizing machinery was not saturated.

We have previously shown that the transport of three amino acids (2-amino-isobutyric acid, cycloleucine and glycine) varied considerably as a function of age of the animal [1]. This variation apparently occurred with incorporation as well (Table I). The marked dependence of incorporation on amino acid level may be the cause for this relationship.

Relation between nutritional state and transport activity

The possible causes for the minimal levels of uptake and incorporation at birth followed by restoration of activity within 24 h of birth were examined. The results in Table II show that fasted 1-day-old animals had the same or less transport activity as newborn animals in contrast to their fed litter mates. Therefore, we examined the effects of fasting and refeeding on transport and incorporation of amino acids at different ages.

The data in Table II show the effects of fasting on 2-aminoisobutyrate transport in animals of different ages. Two aspects are significant: (1) In the adult, fasting for 24 h did not alter the transport activity. In young animals, there was a pronounced effect of fasting on amino acid transport, which was most marked in the newborn animal (2). Refeeding has an effect opposite to fasting. At all ages, refeeding for at least 3 h after a fast increased transport activity. If the animals were killed 1–2 h after a meal, the response to "refeeding" was variable and generally much smaller. Therefore all refeedings were carried out for 3 h. In the young animal transport and

TABLE II

THE RESPONSE OF α-AMINOISOBUTYRATE UPTAKE TO FASTING AND FEEDING

Transport of 2-aminoisoburytic acid. Conditions of incubation as given in Table I. The uptake was measured after 90 min of incubation. The figures in square brackets are calculated using the values for the newborn as the control (100 %) value. The duration of the fast was 24 h.

Age	Normally fed (control)	Fasted 24 h (percent of control)	Fasted 24 h and refed for 3 h (percent of control)
1 day after birth	100 %	35.2 ± 5.1 (13)	61.1 ± 4.6 (6)
	[Newborn at birth = 100 %]	[88.5 ± 12.8]	[154.0 ± 11.5]
2 days after birth	100 %	73.5 ± 8.6 (12)	130.0 ± 1.7 (7)
7 days after birth	100 %	79.0 ± 1.1 (6)	130.8 ± 7.3 (4)
Adult	100 %	100.3 ± 9.5 (8)	163.2 ± 21.2 (11)

incorporation of glycine into proteins was altered similarly by fasting and refeeding. In the adult animal the response to changes in the nutritional state was generally less than in younger animals.

Since these responses were seemingly mediated by availability of food, we considered the possibilities that hormonal or nerve stimulation of the pancreas by stimuli from the intestine might be responsible for these changes. We found that addition of pancreozymin-cholecystokinin, secretin, acetylcholine or carbamylcholine to the incubation flask was without effect on uptake or incorporation of amino acids. The lack of effect of pancreozymin and carbamylcholine on incorporation of ^{14}C-labelled amino acids in pancreas in vitro has been reported [8, 9]. However, an injection of a mixture of pancreozymin and 10^{-5} M carbamylcholine stimulated transport and incorporation of [1-^{14}C]glycine in a manner similar to refeeding (Table III). Separately, these agents were much less effective. The increased activity was evident about 30 min after the injection and was most apparent in the young animals. In contrast with 3-day-old animals, no response to pancreozymin and carbamylcholine was evident in the newborn unless insulin and glucose were also injected. A similar requirement for insulin and glucose to evoke a pancreozymin response was already shown by Albritton et al. [10]. Insulin and glucose evoked no response if given independently. In adult animals, pancreozymin-carbamylcholine treatment after a prolonged (48 h) fast stimulated incorporation significantly. It is apparent that in the adult the effect of refeeding was less marked on glycine uptake than on 2-aminoisobutyrate uptake.

In the present study, the data suggest that the agents which alter incorporation of amino acids into protein do so indirectly by altering the transport of free amino acids. As shown in Fig. 1, incorporation closely followed glycine concentration. Moreover, when 75 % of the transport activity was reduced by ouabain, incorporation was reduced by 44 %. In contrast, inhibition of incorporation by cycloheximide by 90 % had no effect on uptake of glycine (results not shown). These data support the proposal that protein synthesis may become dependent on the rate of amino acid transport under conditions of limited availability of amino acids (Riggs and Walker [23]).

TABLE III

GLYCINE UPTAKE AND INCORPORATION: RESPONSE TO FASTING, FEEDING AND PANCREOZYMIN TREATMENT

Conditions of incubation given in Table I. A pancreozymin/carbamylcholine mixture dissolved in isotonic NaCl was used for injection. 5 Crick units/100 g body weight and 10^{-5} μmol carbamylcholine were injected. The figures in square brackets are calculated using the values for the newborn as the control (100 %) value.

Age	Normally fed (control)		Fasted 24 h (percent of control)		Fasted 24 h and refed for 3 h (percent of control)		Fasted 24 h and injected with pancreozymin (percent of control)	
	Uptake	Incorporation	Uptake	Incorporation	Uptake	Incorporation	Uptake	Incorporation
1 day after birth	100 %	100 %	61.0 ± 3.9 (4)	14.0 ± 2.3	70.4 ± 6.2 (3)	35.8 ± 2.1	61.0 ± 4.7 (7)	14.4 ± 1.2
	[Newborn at birth = 100 %]		[97.5 ± 6.2]	[85.8 ± 1.4]	[112.5 ± 10.0]	[220.0 ± 12.9]	80.5 ± 4.7 (6)*	37.2 ± 4.7
							[129.0 ± 7.5]*	[228.5 ± 28.6]
3 days after birth	100 %	100 %	67.0 ± 18.0 (11)	75.0 ± 22.5	130.0 ± 8.4 (6)	135.0 ± 32.5	102.0 ± 11.2 (5)	135.0 ± 20.0
Adult	100 %	100 %	79.5 ± 8.4 (10)**	103.5 ± 10.7	105.0 ± 12.6 (7)**	128.6 ± 32.0	109.5 ± 8.4 (6)**	175.0 ± 10.7

* In this case, pancreozymin/carbamylcholine solution also contained 10 munits of insulin and 0.5 μmol glucose.

** The duration of the fast was 24 h for animals up to 3-days-old and 48 h for older animals.

DISCUSSION

The data presented show that to an appreciable extent, transport of amino acids by the pancreas depends on the nutritional state of the animal. The response is far from simple. Fasting per se for 24 h does not affect transport of amino acids in the adult animal whereas a 48-h fast does cause a decrease in uptake. In contrast, in newborn animals or very young animals fasting for 24 h decreases the ability of the pancreas to transport amino acids. At all ages tested, refeeding after a fast, increases the ability of the pancreas to transport amino acids. To a considerable extent, the effect of refeeding can be duplicated by in vivo injections of mixtures of carbamylcholine and pancreozymin, suggesting that these agents may be involved in regulating amino acid transport in the pancreas in response to the dietary state.

The data also suggest that the level of amino acid incorporation into the proteins of the pancreas may be regulated by the availability of amino acids. Incorporation is closely tied to the free amino acid level and changes in uptake are reflected in incorporation. Elevation of transport, either by increasing the amino acid level or addition of secretagogues, stimulates incorporation, while inhibition of transport by ouabain decreases incorporation. Inhibition of protein synthesis on the other hand has no effect on the level of uptake. The observations suggest that the availability of free amino acids, even at high concentration, may play a major role in determining the rate of protein synthesis in the pancreas. In this regard the pancreas is distinctive since in many organs, the optimal amino acid requirements for protein synthesis are met at low extracellular levels of amino acids [3–6, 11]. Since the pancreas is amongst the most active of mammalian organs in accumulation of a variety of amino acids (Begin and Scholefield [12]), this means that very much higher levels of amino acids are required to saturate the protein-synthesizing mechanism in the pancreas than in other organs. Considering the known capacity of the pancreas to synthesize protein, it is perhaps significant that optimal rates are not attained unless amino acids are freely available at high concentration.

The most dramatic response to the nutritional state occurs in newborn animals which are removed from the dams immediately after birth. Both transport and incorporation fall to low levels below the prenatal ones. In a newborn animal, after a 24-h fast, the levels of uptake and incorporation are elevated within 3 h post suckling but do not reach the value of the normally fed controls.

There is little agreement in the literature on the effect of secretagogues on de novo synthesis of pancreatic proteins. Thus, Webster and his colleagues [13–17], as well as Reggio et al. [18], Farber and Sidransky [19], Rothman and Wells [20] contend that injection of pancreozymin or acetylcholine substitutes stimulated the synthesis of hydrolytic proteins. Other investigators (Jamieson and Palade [7], Kramer and Poort [21], Hokin and Hokin [8], and Dickman et al. [22]) were unable to show increased synthesis in response to secretagogues. Part of the difference may be the fact that administration of the stimulating agent in vivo is associated with an increase in synthesis, whereas in vitro addition is generally without effect. However, it is not clear that this is the only reason for the differences in observation.

Similarly, there is little agreement on the role of fasting and refeeding on protein synthesis in the pancreas. Jamieson and Palade [7] did not observe any increase in enzyme synthesis in response to pancreozymin or carbamylcholine (added

in vitro) with pancreases from fasted animals. However, they did not compare synthesis in fasted and non-fasted animals. Webster [14] observed a decreased incorporation of amino acids into pancreatic proteins in fasted animals which could be elevated on administration of pancreozymin in vivo.

The present results are consistent with the observations that fasting and refeeding as well as injection of pancreozymin and carbamylcholine into fasted animals increase amino acid incorporation (protein synthesis). Addition of pancreozymin to the incubation flask had no effect. The present data also suggest that these changes are brought about largely by alterations in the free amino acid level, particularly in young animals.

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