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α-AMINOISOBUTYRIC ACID UPTAKE IN ISOLATED MOUSE FAT CELLS

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SUMMARY

1. The uptake of the amino acid analogue, α-aminoisobutyric acid into mouse isolated fat cells has been studied.

2. α-Aminoisobutyric acid was found to be transported into and accumulated within the cells by a process that was both temperature and energy-dependent.

3. α-Aminoisobutyric acid entry appears to be a complex phenomenon that may comprise at least two components, a saturable one, and one similar to passive diffusion. In addition, α-aminoisobutyric acid uptake was shown to be both sodium and potassium-dependent.

4. Adrenaline and ACTH markedly inhibited α-aminoisobutyric acid uptake. When added together with these lipolytic hormones, insulin restored α-aminoisobutyric acid uptake towards normal. Furthermore, under certain conditions, insulin alone stimulated α-aminoisobutyric acid uptake.

5. It is suggested that the intracellular concentration of free fatty acids, possibly that of ATP, may explain the action of these different hormones upon the uptake of α-aminoisobutyric acid by the fat cells, although an additional direct effect upon the transport mechanism *per se* cannot be excluded.

INTRODUCTION

Previous studies have shown that adipose tissue is able to utilize several amino acids as precursors in the biosynthesis of long chain fatty acids, and of proteins[1–10]. Furthermore, although little is known of the intimate mechanisms by which these processes are regulated, hormones such as insulin[1–3,5–11], and growth hormone[7,10,12] have been found to accelerate the metabolism of several amino acids *in vitro*, whereas others, such as adrenaline, ACTH[10,12], and glucocorticoids[10,12,13] were inhibitory.

More recently, experiments performed in intact adipose tissue with a non-metabolizable amino acid analogue, α-aminoisobutyric acid, indicated the existence of a transport mechanism for amino acids which rather closely resembled that found in diaphragm muscle. However, this α-aminoisobutyric acid transport differed from that of the diaphragm by being unresponsive to anabolic hormone stimulation[14].

We therefore decided to evaluate α-aminoisobutyric acid uptake in isolated fat cells, and to study some hormonal influences upon this process. In particular, because of their opposite effect on protein synthesis in these cells, the role of insulin and of

two lipolytic hormones (ACTH and adrenaline) on α-aminoisobutyric acid uptake has been evaluated.

A preliminary report of these results has been published[15].

MATERIALS AND METHODS

30-day-old male Swiss mice obtained from S. Ivanovas (Kisslegg im Allgäu, Germany) and fed *ad libitum* with Altromin 'R' laboratory chow (Kunath Company, Aarau, Switzerland) were used throughout these studies. They weighed about 18–25 g.

The isolated fat cells from the epididymal fat pads were prepared according to RODBELL[16], usually using the pooled fat pads from 50–100 mice. After being gassed with a CO_2–O_2 (5:95, by vol.) mixture they were incubated at 37.5° (pH 7.35) in 30-ml stoppered plastic vials placed in a metabolic shaker (Gallenkamp and Co., London). The final volume of the incubation medium in each vial was 1.5 ml (60–100 mg cells). Unless indicated otherwise, a Krebs–Ringer bicarbonate buffer containing 3.5 g/100 ml (w/v) dialyzed human serum albumin (Swiss Red Cross, Berne, Switzerland) was used. When the concentrations of sodium or potassium in the incubation medium were altered, sodium was replaced by choline, and potassium was replaced by sodium. The concentration of substrates, of hormonal or non-hormonal agents added to the incubation medium, as well as the incubation time, are specified with each experiment.

The measurement of intracellular water and of α-aminoisobutyric acid spaces was made according to the method described by CROFFORD *et al.*[17] for the estimation of intracellular glucose space in rat isolated fat cells. As this method proved insufficiently versatile for dynamic studies and could not be adapted to a washing procedure for rapid removal of labelled extracellular α-aminoisobutyric acid, the net uptake of α-aminoisobutyric acid by the fat cells was, in subsequent experiments, measured as follows: incubations were stopped by rapid chilling, and all further steps were performed at 0–2°. The cell suspensions were transferred from the incubation vials to washing tubes. These were long-stem, 10-ml plastic tubes at the bottom of which a small piece of plastic tubing (1 mm inner diameter) had been inserted through a bored hole which was resealed with glue (Araldit, Ciba, Basel). The tubing was then glued along the outside wall in order to permit rapid removal of the washing medium with a syringe, without undue stirring and loss of fat cells. The cells were washed twice with 5 ml of cold (2°) Krebs–Ringer bicarbonate buffer containing 1% human albumin. Addition of the washing buffer was followed by a quick (10 sec) centrifugation at 200 × *g* which made the cells float to the surface. Following the first centrifugation, the washing buffer was rapidly removed through the plastic tubing described above. Following the second centrifugation, the washing buffer was not removed and the floating cells were carefully collected from the surface using a 0.5-ml automatic pipette (Eppendorf, Hamburg, Germany). They were then poured onto a preweighed Millipore filter (25 mm diameter, 5.0 μ mean pore size, Millipore Filter, Bedford, Mass.) supported on a Millipore microanalysis filter holder. Vacuum filtration was performed using a water pump, and the cells remained unruptured on the filter, as evidenced by the existence of a measurable intracellular water space (Table I). The filter was then removed from the filter holder and dried. The drying process was completed in 30 min with the filters placed approx. 20 cm from a 150-W

incandescent lamp. When dry, the filters were reweighed, and the dry weight of the cells calculated. Each filter was then transferred to a liquid scintillation counting vial containing 20 ml of a scintillation solution[18] with 3 g/100 ml Cab-O-Sil (Packard Instrument). In this solution, the Millipore filter dissolved completely, and the precipitated albumin and cellular residue remained finely suspended. The presence of dried fat cells produced no quenching of ^{14}C radiation, provided the weight of fat did not exceed 150 mg.

At the end of the second wash, only about 8% of the initial radioactivity was still present in the total incubation medium. Furthermore, filtration of the washed adipose cells left only a minimal amount of labelled extracellular fluid on the Millipore filter. This contamination was accounted for in each experiment by running the following control: a series of cell suspensions was treated exactly as described above (washings; centrifugations; filtration) but they were kept at 0° and were not incubated. These control values, which were remarkably constant within any one experiment, were a measure of the radioactivity either trapped on the cells or present in the extracellular fluid remaining on the Millipore filter following filtration. They were subtracted from all subsequent values obtained with incubated adipose cells. The net α-aminoisobutyric acid uptake so obtained was expressed as μmoles per 10 g of dried fat cells. All samples were counted in an EX-2 model 314 Packard liquid scintillation spectrometer which exhibited an efficiency of 65% for ^{14}C and of 17% for ^{3}H under our conditions. Control experiments carried out with isolated fat cells established that α-amino[1-^{14}C]isobutyric acid was not metabolized, as no counts were recovered in either CO_2, total lipids or protein.

Collagenase was purchased from Worthington Biochemical Laboratories (Freehold, N.J., U.S.A.). α-Amino[1-^{14}C]isobutyric acid and [^{14}C]urea were secured from the Radiochemical Centre, Amersham, Buckinghamshire, England; [^{3}H]inulin from New England Nuclear, Boston, Mass., U.S.A. Unlabelled α-aminoisobutyric acid was supplied by Sigma Chemical, St. Louis, Mo., U.S.A. All organic and inorganic reagents were purchased from E. Merck (Darmstadt, Germany). Pork insulin (10 times recrystallized) was a gift of the Novo Laboratories, Copenhagen, Denmark. Samples of the third international standard for corticotrophin (ACTH 5 I.U. per vial) were supplied by the Department of Biological Standards, National Institute for Medical Research, London. Adrenaline was a gift of the Vifor Laboratories (Geneva, Switzerland). Ouabain (strophanthin G) was purchased from Fluka (Buchs, Switzerland), oligomycin from Sigma Chemical, St. Louis, Mo., U.S.A.

RESULTS

The existence of a measurable intracellular urea space in isolated mouse fat cells is shown in Table I. The value of about 9 μl per 100 mg dry weight of cells is somewhat greater than that reported for rat isolated adipose tissue[17], a fact that may be related to species and/or age differences, as very young mice were used in these studies. The intracellular α-aminoisobutyric acid space, estimated by subtracting [^{3}H]inulin space from α-amino[^{14}C]isobutyric acid space, was 17 times greater than the intracellular urea space, clearly indicating that the fat cells were able to concentrate the amino acid analogue. Table I further shows that this concentration process was potassium-dependent, an observation that will be further discussed below.

Because of the many technical difficulties involved in space measurements in isolated fat cells, subsequent experiments were then concerned only with net α-aminoisobutyric acid uptake (see MATERIAL AND METHODS). Since the α-aminoisobutyric acid space greatly exceeded the intracellular water space, it is likely that, under most conditions, net α-aminoisobutyric acid uptake may be considered as related to, if not synonymous with, α-aminoisobutyric acid accumulation within the adipocytes.

TABLE I

INTRACELLULAR UREA AND α-AMINOISOBUTYRIC ACID SPACES IN ISOLATED FAT CELLS

Incubations were carried out for 30 min in 0.5 ml Krebs–Ringer bicarbonate buffer containing 3.5 g/100 ml albumin and 10 mM sodium pyruvate. The concentrations of the markers were: inulin, 0.12 mg/ml; urea, 5 mM; α-aminoisobutyric acid, 0.5 mM. The radioactivities were: [^{3}H]inulin, 10 μC/ml; urea and α-amino[1-^{14}C]isobutyric acid, 0.15 μC/ml. Intracellular urea space computed as the urea space *minus* the inulin space. Results are expressed as μl/100 mg dry cells (mean ± S.E.).

K^+ added in incubation medium (mequiv/l)	*Number of experiments*	*[^{14}C]Urea–[^{3}H]inulin*	*α-Amino[1-^{14}C]isobutyric acid–[^{3}H]inulin*
6	17	9.64 ± 0.31	165.23 ± 7.18
0	6		47.17 ± 1.19

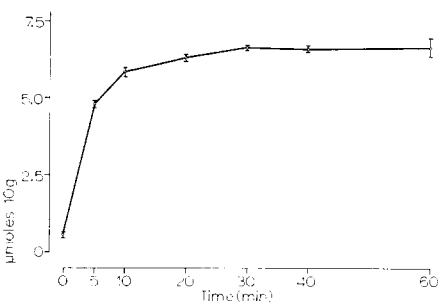

Fig. 1. Effect of duration of incubation on α-aminoisobutyric acid uptake by isolated fat cells. Incubations were carried out in 1.5 ml Krebs–Ringer bicarbonate buffer containing 3.5 g/100 ml albumin, 10 mM unlabelled sodium pyruvate and 2 mM α-amino[1-^{14}C]isobutyric acid. Each point is the mean of 4 values ± S.E.

The basic requirements of α-aminoisobutyric acid uptake in adipose cells are summarized in Figs. 1 and 2. As illustrated in Fig. 1, α-aminoisobutyric acid uptake increased rapidly with time, reaching a maximum in 20–40 min. This maximal level was then maintained up to 60 min. As shown in Fig. 2A, α-aminoisobutyric acid uptake was temperature-dependent: little uptake occurred at 8°, but it increased when the temperature was raised to 17° and 37°. The energy requirement was further illustrated by the finding that the addition of oligomycin (1 μg/ml) to the incubation medium markedly decreased the uptake of α-aminoisobutyric acid, indicating that the concentrative process was dependent upon a normally operating oxidative metabolism (Fig. 2B). However, when no substrate was added to the incubation medium, whether from the very beginning of the experiment, *i.e.* from the time of collagenase treatment of the adipose tissue onwards, or during the actual incubation of the

isolated fat cells only, the α-aminoisobutyric acid uptake was only slightly reduced (Figs. 2B and 2C). Accordingly, it would seem that, under the experimental conditions used, the cells contained stores of energy-yielding substrates (*e.g.* glycogen, fatty acids) in amounts adequate to insure near-optimal transport of the amino acid analogue towards the intracellular space and for at least 1 h.

The addition of one of several neutral amino acids, such as isoleucine, proline, leucine, glycine, phenylalanine, valine, serine, threonine, methionine or alanine, reduced the uptake of α-aminoisobutyric acid (Fig. 3). Alanine was the most potent inhibitor of α-aminoisobutyric acid uptake, followed by methionine, threonine and serine. These observations suggest the existence of competition between different amino acids for the same transport process, although other mechanism(s) may be involved.

As shown in Fig. 4, when the concentration of α-aminoisobutyric acid in the incubation medium was raised from 1 to 40 mM, the uptake of α-aminoisobutyric acid was progressively increased. Up to about 6 mM, the initial uptake increased hyperbolically with increasing extracellular levels of the amino acid analogue, but it did not reach a maximum. Instead, net uptake continued to increase in a linear fashion at α-aminoisobutyric acid concentrations greater than 6 mM. The curvilinear relation observed between extracellular α-aminoisobutyric acid concentrations and net α-aminoisobutyric acid uptake suggests that this uptake is a complex phenomenon that could involve at least two components, *e.g.* a transport mechanism that would exhibit saturation kinetics, and a linear process rather similar to passive diffusion[19].

As α-aminoisobutyric acid appeared to accumulate in fat cells by means of an energy-dependent process, and as transport of several substrates, amino acids in

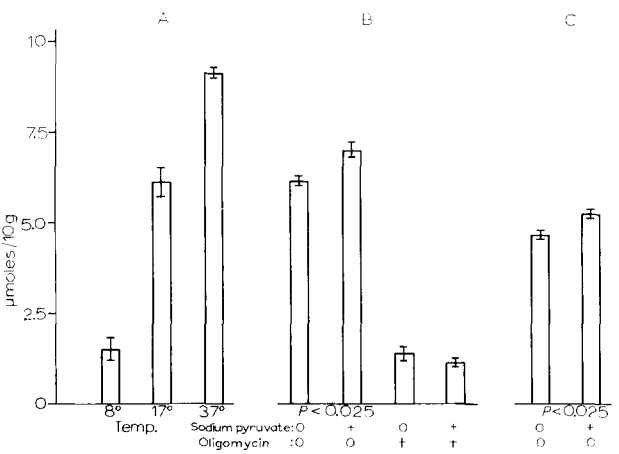

Fig. 2. Energy requirement for α-aminoisobutyric acid uptake in isolated fat cells. A. Krebs–Ringer bicarbonate buffer containing 3.5 g/100 ml albumin, 1 mM unlabelled sodium pyruvate, 2 mM α-amino[1-^{14}C]isobutyric acid. Incubation time 30 min. Each bar is the mean of 5 values ± S.E. B. Krebs–Ringer bicarbonate buffer containing 3.5 g/100 ml albumin, 10 mM unlabelled sodium pyruvate, 2 mM α-amino[1-^{14}C]isobutyric acid. Incubation with collagenase in the presence of sodium pyruvate, followed by incubation of cells with or without sodium pyruvate. Oligomycin, when present, 1 μg/ml. Incubation time 60 min. Each bar is the mean of 6 values ± S.E. C. Incubations carried out as described in B, except that the incubation with collagenase was in the absence of sodium pyruvate, and was followed by incubation with or without sodium pyruvate. Incubation time 60 min. Each bar is the mean of 6 values ± S.E.

particular, has been shown to depend at least partly upon the presence of sodium or potassium[20–28], it was of interest to test whether these cations would also influence α-aminoisobutyric acid uptake in our preparation.

The influence of sodium and potassium was therefore investigated by measuring net α-aminoisobutyric acid uptake as a function of increasing concentrations of either cation in the presence of the physiological concentration of the other. It was observed that maximal α-aminoisobutyric acid uptake occurred when sodium concentration was around 140–150 mM (Fig. 5). As shown in Fig. 6, potassium had a biphasic action, a maximal α-aminoisobutyric acid uptake being obtained between 6–9 mM. Below and above these concentrations, α-aminoisobutyric acid uptake was less, although

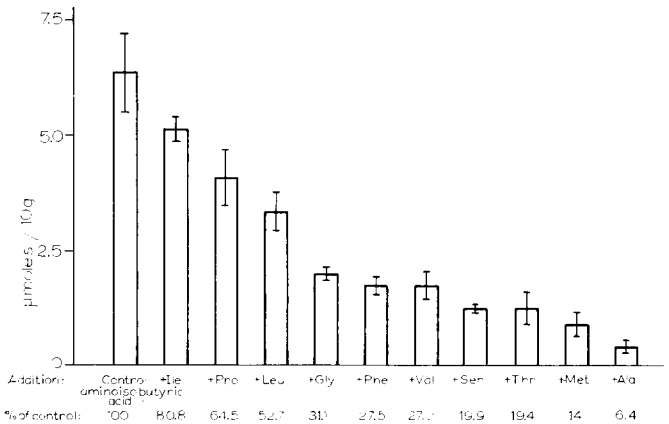

Fig. 3. Effect of neutral amino acids on α-aminoisobutyric acid uptake by isolated fat cells. Krebs–Ringer bicarbonate buffer containing 3.5 g/100 ml albumin, 10 mM sodium pyruvate, 1 mM α-amino[1-^{14}C]isobutyric acid with or without 20 mM unlabelled amino acid. Incubation time 10 min. Each bar is the mean of 4 values ± S.E.

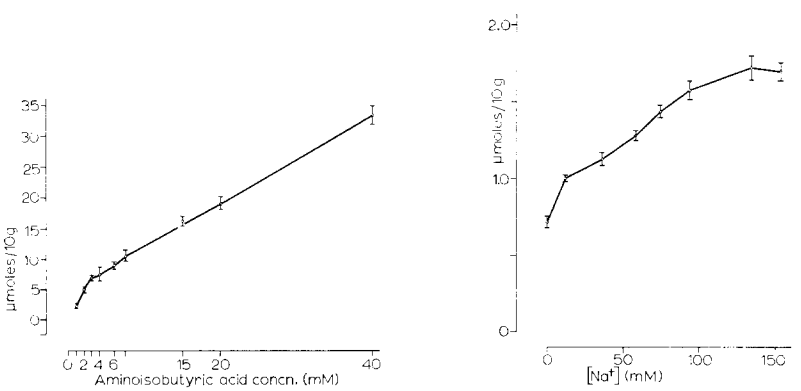

Fig. 4. Relationship between α-aminoisobutyric acid uptake by isolated fat cells and extracellular α-aminoisobutyric acid concentrations. Krebs–Ringer bicarbonate buffer containing 3.5 g/100 ml albumin and 10 mM sodium pyruvate. Incubation time 60 min. Sodium concn. reduced by ½ the amino acid concentrations. Each point mean of 6 values ± S.E.

Fig. 5. Effect of sodium on α-aminoisobutyric acid uptake by isolated fat cells. Krebs–Ringer bicarbonate buffer containing 3.5 g/100 ml albumin, 6 mM unlabelled potassium pyruvate and 0.25 mM α-amino[1-^{14}C]isobutyric acid. Cells preincubated first for 40 min at various sodium concns. without α-aminoisobutyric acid, then incubated for 60 min in the presence of α-aminoisobutyric acid. Sodium replaced by choline. Each point mean of 6 values ± S.E.

the lowest value was observed when potassium was completely absent, a finding that was in keeping with the reduced intracellular α-aminoisobutyric acid space previously observed by omitting potassium from the incubation medium (Table I). It should be mentioned that the inhibitory effect of sodium- or potassium-free media upon α-aminoisobutyric acid uptake by fat cells is of the same order of magnitude as that observed in muscle[20] and bone[23] cells. The relationship between sodium, potassium, and α-aminoisobutyric acid penetration into fat cells is further suggested by Table II. As can be seen, ouabain at concentrations ranging from 0.01 to 1 mM decreased the uptake of α-aminoisobutyric acid, an inhibitory effect that was clearly of lesser magnitude than that obtained by depleting the medium of potassium. One should add that the lowest concentrations of ouabain used were comparable to those described in studies related to the problem of electrolyte transport and of lipolytic activity in isolated fat cells[29,30].

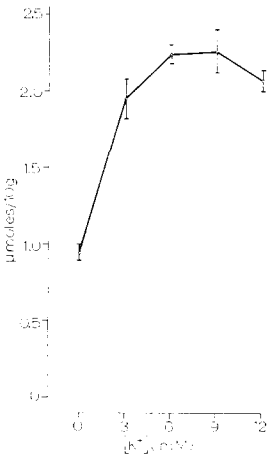

Fig. 6. Effect of potassium on α-aminoisobutyric acid uptake by isolated fat cells. Krebs–Ringer bicarbonate buffer containing 3.5 g/100 ml albumin, 10 mM unlabelled sodium pyruvate and 0.5 mM α-amino[1-^{14}C]isobutyric acid. Cells preincubated first for 40 min at various potassium concns. without α-aminoisobutyric acid, then incubated for 60 min in the presence of α-aminoisobutyric acid. Osmolarity kept constant by adjusting sodium concn. Each point mean of 6 values ± S.E.

TABLE II

EFFECT OF OUABAIN ON THE UPTAKE OF α-AMINOISOBUTYRIC ACID BY ISOLATED FAT CELLS

Incubations were carried out in 1.5 ml Krebs–Ringer bicarbonate buffer containing 3.5 g/100 ml albumin. No unlabelled energy-substrate added. Cells preincubated first for 60 min in the presence of ouabain, without α-aminoisobutyric acid; then incubated for 30 min in the presence of ouabain and 0.5 mM α-amino[1-^{14}C]isobutyric acid. Results are expressed as μmoles/10 g dried fat cells. Each figure is the mean of 6 values ± S.E. The significance of the difference from the controls (P) is <0.0025 in all instances.

Ouabain (μ*M*)	*Net α-amino[1-^{14}C]-isobutyric acid uptake*	*Inhibition* (%)
0	3.33 ± 0.02	
10	2.90 ± 0.06	13.0
100	2.30 ± 0.15	31.0
1000	2.23 ± 0.08	33.0

In the next series of experiments, the influence of insulin and of two lipolytic hormones (ACTH and adrenaline) upon α-aminoisobutyric acid uptake was studied. Insulin failed significantly to increase the uptake of α-aminoisobutyric acid when 10 mM unlabelled pyruvate or glucose was present in the incubation medium. However, when the glucose concentration was lowered to 0.5 mM, a small but highly significant effect of the hormone upon α-aminoisobutyric acid uptake was observed (Table III). This suggested that, when energy-yielding substrates were in excess, α-aminoisobutyric acid uptake had reached an optimum and could not be stimulated further by insulin. In contrast, at a low glucose concentration, the energy-yielding reactions necessary for α-aminoisobutyric acid uptake were possibly becoming limiting and were increased by the addition of the hormone. This interpretation was strengthened by the experiments shown in Table IV. As can be seen, α-aminoisobutyric acid

TABLE III

EFFECT OF INSULIN ON THE UPTAKE OF α-AMINOISOBUTYRIC ACID BY ISOLATED FAT CELLS

Incubations were carried out in 1.5 ml Krebs–Ringer bicarbonate buffer containing 3.5 g/100 ml albumin. Cells preincubated first for 60 min in the presence of insulin and unlabelled substrate as indicated; then incubated for 40 min in the presence of insulin, unlabelled substrate and 2 mM α-amino[1-^{14}C]isobutyric acid. Results are expressed as μmoles/10 g dried fat cells. Each figure is the mean of 6 values ± S.E.

Unlabelled substrate	*Net α-amino[1-^{14}C]isobutyric acid uptake*	
	Controls	*Insulin (1 munit/ml)*
0.5 mM glucose	4.05 ± 0.20	5.04** ± 0.11
1.0 mM glucose	4.31 ± 0.35	4.81* ± 0.26
10.0 mM glucose	4.69 ± 0.23	5.05* ± 0.14
10.0 mM sodium pyruvate	4.44 ± 0.12	4.38* ± 0.15

* Not significant.
** Difference from the controls $P < 0.0025$.

TABLE IV

EFFECT OF INSULIN AND OF OLIGOMYCIN, SINGLY OR COMBINED, ON THE UPTAKE OF α-AMINOISOBUTYRIC ACID BY ISOLATED FAT CELLS

Incubations were carried out in 1.5 ml Krebs–Ringer bicarbonate buffer containing 3.5 g/100 ml albumin. Cells preincubated first for 60 min in the presence of insulin and/or oligomycin, and unlabelled substrate as indicated; then incubated for 40 min with the same compounds and 2 mM α-amino[1-^{14}C]isobutyric acid. Results are expressed as μmoles/10 g dried fat cells. Each figure is the mean of 6 values ± S.E.

Unlabelled substrate	*Net α-amino[1-^{14}C]isobutyric acid uptake*		
	Controls	*Oligomycin (1 μg/ml)*	*Oligomycin (1 μg/ml) + insulin (1 munit/ml)*
10 mM glucose	4.13 ± 0.18	0.48 ± 0.07	2.78 ± 0.10
10 mM sodium pyruvate	4.07 ± 0.05	0.38 ± 0.05	0.40 ± 0.06

uptake was dramatically curtailed when oligomycin was added to the glucose-containing medium as well as to the pyruvate-containing medium. Moreover, the further addition of insulin to the medium containing glucose restored α-aminoisobutyric acid uptake towards normal, whereas no such effect was observed in the presence of pyruvate.

The effects of ACTH and adrenaline are summarized in Table V, which shows that both of these lipolytic hormones markedly reduced α-aminoisobutyric acid uptake when added *in vitro*. This was true whether glucose (10 mM), pyruvate (1 or 10 mM), or no substrate was added to the incubation medium. In all cases, the superimposed addition of insulin to the medium containing the lipolytic agents restored α-aminoisobutyric acid uptake towards normal. Moreover, when fat cells were incubated in the absence of added substrate, insulin alone had a clear-cut stimulatory effect upon the uptake of the amino acid analogue. As illustrated in Fig. 7, the uptake of α-aminoisobutyric acid was found to decrease progressively as the concentrations of adrenaline

TABLE V

EFFECT OF ACTH, ADRENALINE, AND INSULIN ON THE UPTAKE OF α-AMINOISOBUTYRIC ACID BY ISOLATED FAT CELLS

Incubations were carried out in 1.5 ml Krebs–Ringer bicarbonate buffer containing 3.5 g/100 ml albumin. Cells preincubated first for 60 min in the presence of hormones and unlabelled substrate as indicated; then incubated for 40 min with the same compounds and 2 mM α-amino[1-^{14}C]isobutyric acid; ACTH, 1 μg/ml; adrenaline, 1 μg/ml; insulin, 1 munit/ml. Results are expressed as μmoles/10 g fat cells. Each figure is the mean of 6 values ± S.E. All lipolytic effects significantly different from controls at $P < 0.005$. Insulin effect significantly different from controls at $P < 0.005$. All lipolytic effects significantly different from effects of lipolytic hormone + insulin at $P < 0.005$.

Hormone	*Unlabelled substrate*	*Net α-amino[1-^{14}C]-isobutyric acid uptake*
0	10 mM glucose	4.69 ± 0.23
ACTH		2.38 ± 0.09
ACTH + insulin		3.25 ± 0.12
Adrenaline		2.42 ± 0.04
Adrenaline + insulin		3.21 ± 0.11
0	10 mM sodium pyruvate	4.02 ± 0.05
ACTH		1.68 ± 0.09
ACTH + insulin		4.03 ± 0.23
Adrenaline		2.07 ± 0.26
Adrenaline + insulin		3.70 ± 0.13
0	1 mM sodium pyruvate	6.57 ± 0.13
ACTH		2.50 ± 0.06
ACTH + insulin		5.00 ± 0.25
0	1 mM sodium pyruvate	6.04 ± 0.06
Adrenaline		1.32 ± 0.02
Adrenaline + insulin		4.05 ± 0.12
0	No substrate	3.84 ± 0.07
Insulin		4.76 ± 0.12
ACTH		1.99 ± 0.10
ACTH + insulin		4.43 ± 0.15
Adrenaline		1.03 ± 0.21
Adrenaline + insulin		2.03 ± 0.08

were raised from 0.25 to 1 μg/ml. Finally, two xanthine derivatives, caffeine (10 mM) and theophilline (1 mM), were similar to the lipolytic hormones in their ability to reduce markedly the α-aminoisobutyric acid uptake by the isolated fat cells (Table VI).

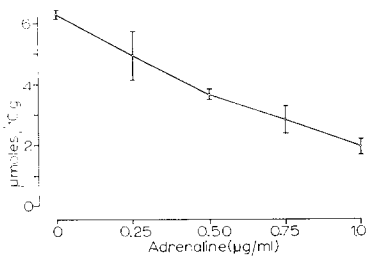

Fig. 7. Effect of adrenaline on the uptake of α-aminoisobutyric acid by isolated fat cells. Krebs–Ringer bicarbonate buffer containing 3.5 g/100 ml albumin, 1 mM unlabelled sodium pyruvate, 2 mM α-amino[1-^{14}C]isobutyric acid. Cells preincubated first for 60 min in the presence of adrenaline and unlabelled substrate, then incubated for 40 min with added α-aminoisobutyric acid. Each point mean of 6 values ± S.E.

TABLE VI

EFFECT OF XANTHINES ON THE UPTAKE OF α-AMINOISOBUTYRIC ACID BY ISOLATED FAT CELLS

Incubations were carried out in 1.5 ml Krebs–Ringer bicarbonate buffer containing 3.5 g/100 ml albumin. No substrate added. Cells preincubated first for 60 min in the presence of xanthines, without α-aminoisobutyric acid; then incubated for 30 min in the presence of xanthines and 2 mM α-amino[1-^{14}C]isobutyric acid. Caffeine, 10 mM; theophilline, 1 mM. Results are expressed as μmoles/10 g dried fat cells. Each figure is the mean of 6 values ± S.E.

Xanthines	*Net α-amino[1-^{14}C]-isobutyric acid uptake*
0	3.33 ± 0.02
Caffeine	0.24 ± 0.08
Theophilline	0.86 ± 0.16

DISCUSSION

These experiments demonstrate that isolated fat cells take up α-aminoisobutyric acid from the incubation medium, as previously reported for intact adipose tissue[14]. Since α-aminoisobutyric acid cannot be incorporated into proteins, fat or CO_2 (ref. 14, and our own controls), it is likely, although not yet proven, that it remains in free solution in the intracellular fluid. In addition, it appears that at low concentrations (0.5 mM) α-aminoisobutyric acid is not only taken up, but that it is accumulated in the adipose cells against a concentration gradient (Table I). This concentrative process is an energy-requiring one (Figs. 1 and 2), resembling that described for other tissues[32]. The entry of α-aminoisobutyric acid into fat cells appears to be a complex phenomenon that is consistent with the existence of at least two components: a saturable process, and a process similar to passive diffusion (Fig. 4). However, it must be stressed that the present data do not suffice to bring about a clear understanding of the processes involved, and that more kinetic studies are needed.

Of additional interest is the observation that the net movement of α-aminoisobutyric acid into the cells was dependent upon the presence of both sodium and potassium (Figs. 5 and 6, Tables I and II).

The net uptake of α-aminoisobutyric acid into isolated adipose cells appears to be responsive to various hormonal stimuli. Thus adrenaline and ACTH were markedly inhibitory whereas insulin was, under certain conditions, stimulatory by itself or could restore towards normal the decrease in α-aminoisobutyric acid uptake induced by the lipolytic hormones (Tables III–V). Whether these changes arose from modification of entry or of exodus cannot presently be resolved.

Although it is indeed quite speculative, we would like to suggest that the finding that energy was necessary for α-aminoisobutyric acid to accumulate in fat cells may give some clue as to the nature of the opposing effects of insulin and lipolytic agents. Thus, the most pronounced stimulatory effect of insulin was seen under conditions in which the energy supply was limited, *e.g.*, during the presumed inhibition of mitochondrial ATP production by oligomycin. Under these conditions, insulin could restore α-aminoisobutyric acid uptake towards normal when glucose, but not pyruvate, was the added energy-yielding substrate. This suggested that the hormone was exerting its effect by increasing the supply of intracellular glucose, and thus of energy, through metabolic pathways of glucose that were unaffected by oligomycin treatment (Table IV). Similarly, insulin clearly increased α-aminoisobutyric acid uptake when the availability of energy was apparently limited (low glucose concentration, no substrate added to the incubation medium), whereas it was ineffective in the presence of ample amounts of energy-yielding substrates (Tables III and V). Finally, a substantial stimulatory effect of insulin was observed in the presence of ACTH and adrenaline, both of which caused a large reduction of α-aminoisobutyric acid transport whether or not glucose or pyruvate was present in the medium (Table V).

The effects of insulin (even in the absence of substrate) and of lipolytic hormones upon α-aminoisobutyric acid uptake may be further related to the supply of energy in the following way: in the presence of lipolytic agents (ACTH, adrenaline, xanthine derivatives), the production of cyclic AMP is known to increase, thereby stimulating the lipolytic process and increasing the intracellular concentration of free fatty acids, as previously reported by many investigators (reviewed in ref. 33). The concentration of free fatty acids within the fat cells might then play a key role in controlling α-aminoisobutyric acid uptake: when reaching sufficiently high concentrations, free fatty acids could possibly have effects such as uncoupling, as previously reported in other tissues[34,35], and decrease the energy needed within the cells for optimal α-aminoisobutyric acid accumulation. Insulin would be expected to relieve the inhibitory effect of the lipolytic hormones by decreasing lipolysis and thereby lowering the intracellular concentration of free fatty acids, consequently increasing the energy available (presumably ATP) for α-aminoisobutyric acid uptake. This insulin effect could be related to either its stimulatory effect upon glucose entry[36], or to its inhibitory effect on hormone-stimulated lipase and adenyl cyclase activity[37,38]. Indeed, preliminary experiments from our laboratory do indicate that there exists an inverse relationship between lipolytic activity and net α-aminoisobutyric acid uptake. Thus, as adrenaline-induced lipolysis proceeded with time, net α-aminoisobutyric acid uptake progressively decreased. Similarly, as lipolysis was stimulated by increasing concentrations of adrenaline or caffeine, the inhibitory effect upon net α-aminoisobutyric acid uptake

became more marked. The superimposed addition of insulin to adrenaline-treated cells resulted in a decreased free fatty acids release while α-aminoisobutyric acid uptake was restored towards normal[39]. This possible role of free fatty acids in regulating α-aminoisobutyric acid uptake, a role previously suggested for the regulation of the pattern of glucose metabolism within these cells[29] is possibly strengthened by the finding that although adipose 'ghosts' (a preparation completely devoid of lipids)[40] were shown to take up α-aminoisobutyric acid in a way similar to the one described in these studies, no alteration of α-aminoisobutyric acid entry was observed in the presence of lipolytic hormones (T. CLAUSEN AND M. RODBELL, personal communication). Furthermore, it has been recently shown that insulin increased ATP concentrations, as well as incorporation of $^{32}P_i$ into ATP in isolated fat cells incubated in the absence of glucose. Lipolytic agents inhibited the incorporation of $^{32}P_i$ into ATP, while simultaneously decreasing ATP concentrations, effects that were reversed by the superimposed addition of insulin[41]. Clearly, actual measurements of intracellular ATP and free fatty acids levels in the different metabolic situations used in the present experiments are needed to substantiate the hypothesis of possible interrelations between free fatty acids, available energy, and net α-aminoisobutyric acid uptake.

Although much emphasis has been placed on energy availability in the interpretation of possible regulatory mechanisms of α-aminoisobutyric acid uptake by adipose cells, the stimulatory effect of insulin, or the inhibitory effect of the lipolytic hormones (particularly in the absence of added energy-yielding substrates) may represent 'membrane' effects, not directly related to either free fatty acid or ATP levels within the cells but to the transport systems *per se*. The very nature of the latter, as well as our understanding of their relationship to the ionic environment are, however, still incompletely understood in adipose tissue, despite its extensive exploration in a number of other types of cells[42–44].

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REFERENCES

1 M. G. HERRERA AND A. E. RENOLD, *Biochim. Biophys. Acta*, 44 (1960) 165.
2 M. E. KRAHL, *Biochim. Biophys. Acta*, 35 (1959) 556.
3 B. M. CARRUTHERS AND A. I. WINEGRAD, *Am. J. Physiol.*, 202 (1962) 605.
4 D. D. FELLER, in A. E. RENOLD AND G. F. CAHILL, JR., *Handbook of Physiology*, Section 5, American Physiological Society, Washington, 1965, p. 363.
5 J. CHRISTOPHE AND C. WODON, *Bull. Soc. Chim. Biol.*, 48 (1966) 1367.
6 J. CHRISTOPHE, C. WODON AND J. CAMUS, *Bull. Soc. Chim. Biol.*, 48 (1966) 1391.
7 H. M. GOODMAN, *Endocrinology*, 73 (1963) 421.
8 H. M. GOODMAN, *Am. J. Physiol.*, 206 (1964) 129.
9 B. JEANRENAUD AND M. TOUABI, *Proc. Intern. Conf. Use Radioactive Isotopes Pharmacol., Geneva, 1968*, in the press.
10 M. G. HERRERA AND A. E. RENOLD, in A. E. RENOLD AND G. F. CAHILL, JR., *Handbook of Physiology*, Section 5, American Physiological Society, Washington, 1965, p. 375.
11 L. V. MILLER AND P. M. BEIGELMAN, *Proc. Soc. Exptl. Biol. Med.*, 122 (1966) 73.
12 J. CHRISTOPHE AND C. WODON, *Rev. Ferment. Ind. Aliment.*, 18 (1964) 177.

13 G. R. PHILIPPS, M. G. HERRERA AND A. E. RENOLD, *Biochim. Biophys. Acta*, 106 (1965) 234.
14 H. M. GOODMAN, *Am. J. Physiol.*, 211 (1966) 815.
15 B. JEANRENAUD, M. TOUABI AND A. E. RENOLD, *Proc. 3rd Intern. Meeting Endocrinol., Marseille, 1968*, in the press.
16 M. RODBELL, *J. Biol. Chem.*, 239 (1964) 375.
17 O. B. CROFFORD, W. STAUFFACHER, B. JEANRENAUD AND A. E. RENOLD, *Helv. Physiol. Pharmacol. Acta*, 24 (1966) 45.
18 G. A. BRAY, *Anal. Biochem.*, 1 (1960) 279.
19 M. TOUABI AND B. JEANRENAUD, in preparation.
20 D. M. KIPNIS AND J. E. PARRISH, *Federation Proc.*, 24 (1965) 1051.
21 K. P. WHEELER AND H. N. CHRISTENSEN, *J. Biol. Chem.*, 242 (1967) 1450.
22 K. P. WHEELER AND H. N. CHRISTENSEN, *J. Biol. Chem.*, 242 (1967) 3782.
23 J. P. ROSENBUSCH, B. FLANAGAN AND G. N. NICHOLS, *Biochim. Biophys. Acta*, 135 (1967) 732.
24 T. R. RIGGS, L. M. WALKER AND H. N. CHRISTENSEN, *J. Biol. Chem.*, 233 (1958) 1479.
25 G. A. VIDAVER, *Biochemistry*, 3 (1964) 662.
26 G. A. VIDAVER, *Biochemistry*, 3 (1964) 795.
27 G. A. VIDAVER, *Biochemistry*, 3 (1964) 799.
28 G. A. VIDAVER, *Biochemistry*, 3 (1964) 803.
29 R. J. HO AND B. JEANRENAUD, *Biochim. Biophys. Acta*, 144 (1967) 61.
30 R. J. HO, B. JEANRENAUD, TH. POSTERNAK AND A. E. RENOLD, *Biochim. Biophys. Acta*, 144 (1967) 74.
31 D. M. KIPNIS AND M. W. NOALL, *Biochim. Biophys. Acta*, 28 (1958) 226.
32 H. N. CHRISTENSEN, *Biological Transport*, Benjamin, New York, 1962, p. 54.
33 B. JEANRENAUD, *Rev. Physiol. Biochem. Exptl. Pharmacol.*, 60 (1968) 57.
34 R. J. GUILLORY AND E. RACKER, *Biochim. Biophys. Acta*, 153 (1968) 490.
35 B. C. PRESSMAN AND H. A. LARDY, *Biochim. Biophys. Acta*, 21 (1956) 458.
36 O. B. CROFFORD AND A. E. RENOLD, *J. Biol. Chem.*, 240 (1965) 14.
37 R. L. JUNGAS AND E. G. BALL, *Proc. Natl. Acad. Sci. U.S.*, 56 (1966) 757.
38 R. W. BUTCHER, J. G. T. SNEYD, C. R. PARK AND E. W. SUTHERLAND, *J. Biol. Chem.*, 241 (1966) 1651.
39 J. D. VASSALLI AND B. JEANRENAUD, in preparation.
40 M. RODBELL, *J. Biol. Chem.*, 242 (1967) 5744.
41 D. HEPP, D. R. CHALLONER AND R. H. WILLIAMS, *J. Biol. Chem.*, 243 (1968) 4020.
42 H. N. CHRISTENSEN, H. AKEDO, D. L. OXENDER AND C. G. WINTER, in J. T. HOLDEN, *Amino Acid Pools*, Elsevier, Amsterdam, 1962, p. 527.
43 W. D. STEIN, *Theoret. Exptl. Biol.*, 6 (1967) 177.
44 H. N. CHRISTENSEN, *Perspectives Biol. Med.*, 10 (1967) 471.