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THE STIMULATING EFFECT OF 3′,5′-(CYCLIC)ADENOSINE MONOPHOSPHATE AND LIPOLYTIC HORMONES ON 3-*O*-METHYLGLUCOSE TRANSPORT AND $^{45}Ca^{2+}$ RELEASE IN ADIPOCYTES AND SKELETAL MUSCLE OF THE RAT

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(1) In order to assess the possible role of 3′,5′-(cyclic)adenosine monophosphate (cAMP) in the control of glucose transport, the effect of the nucleotide or agents known to increase its intracellular concentration on sugar transport or $^{45}Ca^{2+}$ washout were characterized in epididymal fat pads, free fat cells and soleus muscles of the rat. (2) When added to the incubation medium, cAMP (0.1–2.0 mM) stimulated 3-*O*-[^{14}C]methylglucose washout from fat pads. This effect was abolished by cytochalasin B, and additive to that induced by submaximal (10–25 μU/ml), but not by supramaximal (10 mU/ml) concentrations of insulin. (3) cAMP (2 mM) stimulated the conversion of [U-^{14}C]glucose into CO_2 and triacylglycerols. This effect was additive to that of insulin (100 μU/ml). (4) ACTH, glucagon, adrenaline, noradrenaline and salbutamol, which are all known to increase the cAMP content of adipose tissue, stimulated the washout of 3-*O*-[^{14}C]methylglucose and $^{45}Ca^{2+}$ from preloaded fat pads. The fractional losses of the two isotopes were significantly correlated ($P < 0.001$, $r = 0.73$). (5) In free fat cells, adrenaline (10^{-6} M) and salbutamol (10^{-5} M) stimulated the uptake of 3-*O*-[^{14}C]methylglucose, and salbutamol (10^{-5} M) did not interfere with the stimulating effect of insulin (25 μU/ml) on sugar uptake. (6) In rat soleus muscles, adrenaline and salbutamol produced a dose-dependent stimulation of the washout of 3-*O*-[^{14}C]methylglucose and $^{45}Ca^{2+}$. The effect of adrenaline on sugar efflux was abolished by propranolol. (7) It is concluded that the activation of the glucose transport system by insulin is unlikely to be mediated by a drop in the cellular concentration of cAMP. An increase in cAMP brought about by β-adrenoceptor agonists or lipolytic hormones may induce a mobilization of calcium ions from cellular pools into the cytoplasm, which in turn leads to the activation of the glucose transport system demonstrated in the present as well as in several earlier studies.

Introduction

It has been proposed that 3′,5′-(cyclic)adenosine monophosphate (cAMP) is important in the control of glucose transport in insulin-sensitive tissues. A decrease in the cellular cAMP content induced by insulin, ouabain, H_2O_2, nicotinic acid and adenosine has been shown to be associated with increased glucose utilization in adipocytes [1–3]. Recently, it was shown that the effects of insulin on 3-*O*-methylglucose transport and cAMP content in fat cells had the same time-lag [4]. Furthermore, when cAMP content was increased by ACTH, adrenaline, glucagon, isoprenaline or theophylline, glucose utilization was inhibited [5,6].

However, these studies provide little information about the effects of cAMP per se on the glucose transport system and leave room for the possibility that the cellular messenger eliciting activation of the glucose transport system may at the same time induce a decrease in the cAMP content.

In addition, there is substantial evidence that hormones, which can be assumed to increase the cAMP level in adipose tissue, free fat cells and skeletal muscle, stimulate glucose uptake, the conversion of glucose into triacylglycerols and CO_2 as well as the transport of non-metabolized sugars in adipocytes and skeletal muscle [7–15]. Recently, it was shown that isoprenaline markedly increases 3-*O*-methylglucose uptake into free fat cells. The fact that this effect was entirely abolished by propranolol indicates that it is mediated by β-adrenoceptors and is associated with a rise in the cellular cAMP content [16].

We have reported earlier that lipolytic hormones induce a stimulation of 3-*O*-methylglucose efflux from whole epididymal fat pads [14]. As this effect was later found to be associated with an acceleration of the washout of $^{45}Ca^{2+}$ [17], it seemed possible that cAMP could induce a stimulation of the glucose transport system via a mobilization of calcium ions from intracellular pools [18].

The present study explores this possibility in measurements of the washout of 3-*O*-[^{14}C]methylglucose and $^{45}Ca^{2+}$ from epididymal fat pads and soleus muscles of the rat. The experiments demonstrate that lipolytic hormones and a selective β_2-adrenoceptor agonist increase both parameters. Furthermore, β_2-adrenoceptor stimulation induces an increase in 3-*O*-[^{14}C]methylglucose washout, which is additive to that exerted by insulin. It is concluded that the activation of the glucose transport system induced by insulin or other agents is unlikely to be mediated by a drop in the cytoplasmic concentration of cAMP. Part of the present results have previously been reported in a preliminary form [19].

Methods

Experiments with whole epididymal fat pads

Washout experiments were performed essentially as described in earlier reports [14,17]. Whole epididymal fat pads were obtained from fed Wistar rats weighing 100–120 g and following a wash, loaded for 60 min in polyethylene counting vials containing 3 ml of buffer with ^{45}Ca (2 μCi/ml) or 3-*O*-[^{14}C]methylglucose (1 mM and 2 μCi/ml). Thereafter, the fat pads were washed out in a series of counting vials containing 2 ml of unlabelled buffer. At the end of washout, the radioactivity remaining in the tissue was determined, and by counting the radioactivity released into the washout vials, the fractional loss could be calculated as described earlier [14]. The conversion of glucose into triacylglycerols and CO_2 was measured by incubating whole fat pads for 30 min in Krebs-Ringer bicarbonate buffer containing 1% albumin, 5 mM D-glucose and 0.2 μCi/ml D-[U-^{14}C]glucose. The incubation was terminated by adding 0.2 ml 5 M H_2SO_4 to the incubation medium, the $^{14}CO_2$ evolved collected as described by Gliemann [20] and triacylglycerols extracted and counted according to the method of Dole [21].

Experiments with free fat cells

Free fat cells were prepared from epididymal fat pads obtained from fed Wistar rats weighing 120 or 180 g [22]. The uptake of 3-*O*-[^{14}C]methylglucose was measured as described by Whitesell and Gliemann [23]. The fat cells were preincubated for 5 or 15 min without or with the agents tested, and then incubated for 20 s in buffer containing 3-*O*-[^{14}C]methylglucose (1 mM and 5 μCi/ml). The sugar uptake was expressed as nmol/ml fat cells per 20 s. The equilibrium level was determined in each experiment using an incubation time of 40 min.

Experiments with soleus muscles

The procedures for the preparation and incubation of soleus muscles have been described in detail elsewhere [24]. Muscles weighing 25–35 mg were obtained from fed Wistar rats in the weight range 60–70 g and incubated in Krebs-Ringer bicarbonate buffer. In order to ensure adequate oxygenation, all experiments were performed at 30°C, and the muscles kept agitated by continuous aeration with a mixture of 95% O_2 and 5% CO_2.

Chemicals, isotopes and hormones

All chemicals were of analytical grade. Bovine serum albumin (Armour Co, Eastbourne, U.K.) was used after repeated dialysis against distilled water for 24 h at 4°C, followed by neutralization with NaOH. 3-*O*-Methyl-D-glucose was purchased from the Sigma Co, St. Louis, U.S.A., cytochalasin B from Aldrich-Europe, Beerse, Belgium, and cAMP from the Boehringer Co, Mannheim, F.R.G.

ACTH (Synachten®) was obtained from Ciba, Basel, Switzerland and highly purified glucagon from the Novo Industries, Copenhagen, Denmark. Salbutamol and insulin were gifts from the Glaxo Co, Ware, U.K. and Nordic Insulin, Gentofte, Denmark, respectively. $^{45}Ca^{2+}$ (1 Ci/mmol) was obtained from the Danish Atomic Energy Commission Isotope Laboratory, Risø, Denmark, and 3-*O*-[^{14}C]methylglucose (59 mCi/mmol) from Amersham International, Amersham, U.K. Experimental details are given in the legends to figures and tables.

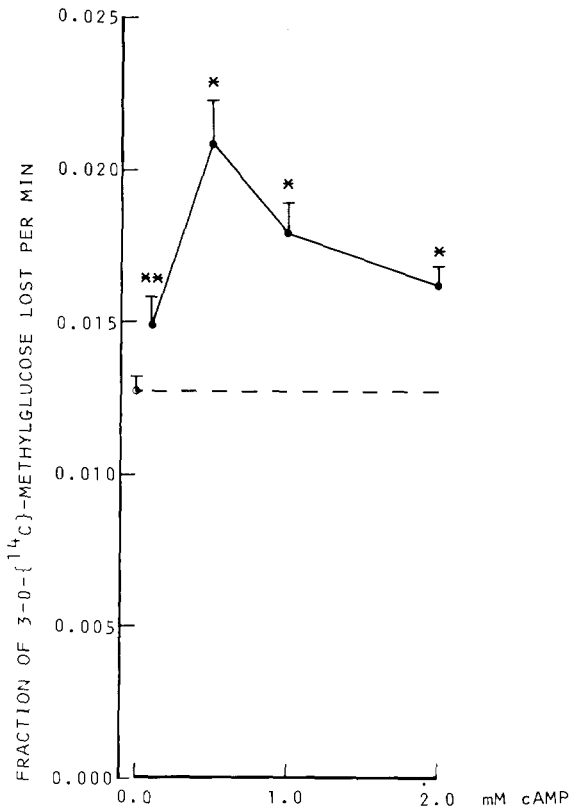

Fig. 1. Effect of varying concentrations of cAMP on the fractional loss of 3-*O*-[^{14}C]methylglucose from whole epididymal fat pads. The tissues were loaded by incubation for 60 min at 37°C in Krebs-Ringer bicarbonate buffer containing 1% albumin, 3-*O*-[^{14}C]methylglucose (2 μCi/ml and 1 mM) and 1.27 mM Ca^{2+}. They were then transferred through a series of tubes containing 2 ml unlabelled buffer without or with the additions indicated. At the end of washout, the ^{14}C activity retained in the tissue and released into each washout tube were determined. From this, the fractional loss of 3-*O*-[^{14}C]methylglucose was calculated [14]. Each point represents the mean of 4–12 measurements performed within the first 10 min after the addition of cAMP. The *P* values indicate the significance of the difference between the control level and the experimental points: *, $P < 0.001$, **, $P < 0.05$. Bars – S.E. — — —, control level.

Results

Experiments with whole epididymal fat pads

Over the concentration range 0.1–2.0 mM, cAMP induced a highly significant stimulation of 3-*O*-[^{14}C]methylglucose efflux from preloaded fat pads (Fig. 1). Maximum increase (63%) was obtained at 0.5 mM, and at 5 mM the response was not significantly different from that seen at 2 mM. The effect was blocked by cytochalasin B (5 μg/ml), indicating that it is not the outcome of nonspecific leakage of the plasma membrane. As can be seen from Fig. 2, the effect of cAMP is relatively rapid in onset and additive to that pro-

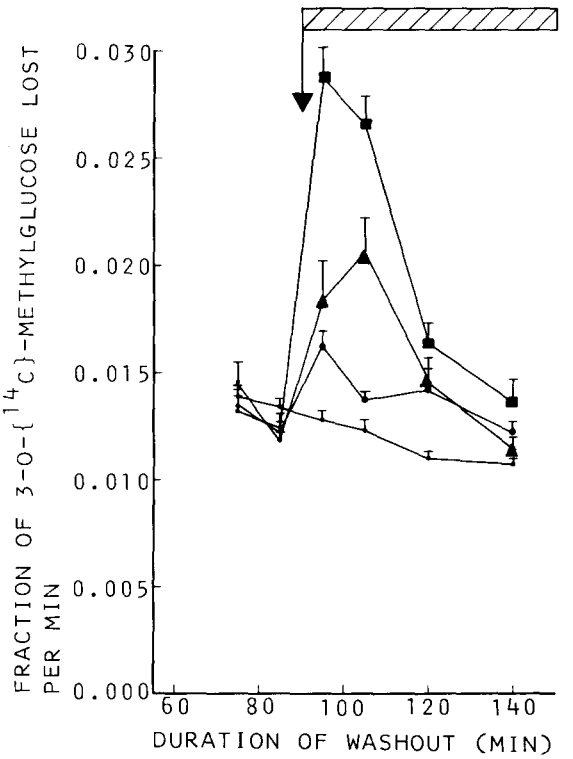

Fig. 2. Effect of 2 mM cAMP and 25 μU/ml insulin on the efflux of 3-*O*-[^{14}C]methylglucose from whole epididymal fat pads. Experimental conditions as described in the legend to Fig. 1. The compounds listed are added to the washout medium corresponding to the hatched bar. Each point represents the mean of 5–12 observations, with bars denoting S.E. •, control; ▲, insulin; ●, cAMP; ■, insulin + cAMP.

TABLE I

EFFECT OF INSULIN ± cAMP ON THE WASHOUT OF 3-*O*-[^{14}C]METHYLGLUCOSE FROM WHOLE EPIDIDYMAL FAT PADS

The fractional loss of ^{14}C-labelled 3-*O*-methylglucose was measured as described in the legend to Fig. 1. The values measured within the first 10 min after the addition of insulin are shown ± S.E. with the number of observations in parentheses.

Addition	Fraction of 3-*O*-[^{14}C]methylglucose lost per min	
Insulin (10 μU/ml)	0.0143 ± 0.0012 (7)	$P < 0.001$
Insulin (10 μU/ml) + cAMP (2 mM)	0.0270 ± 0.0022 (7)	
Insulin (25 μU/ml)	0.0184 ± 0.0018(10)	$P < 0.005$
Insulin (25 μU/ml) + cAMP (2 mM)	0.0285 ± 0.0011 (6)	
Insulin (10 mU/ml)	0.0691 ± 0.0058 (3)	$P > 0.8$
Insulin (10 mU/ml) + cAMP (2 mM)	0.0692 ± 0.0028 (3)	

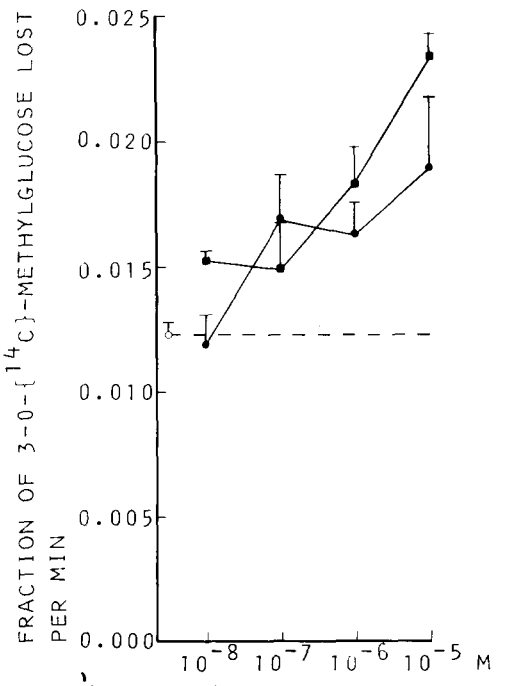

Fig. 3. Effect of varying concentrations of adrenaline and salbutamol on the fractional loss of 3-*O*-[^{14}C]methylglucose from whole epididymal fat pads. Experimental conditions as described in the legend to Fig. 1. Each point represents the mean of 3–11 observations performed in the interval from 10 to 20 min after the addition of the agent tested. Bars denote S.E. — — —, control level; ●, salbutamol; ■, adrenaline.

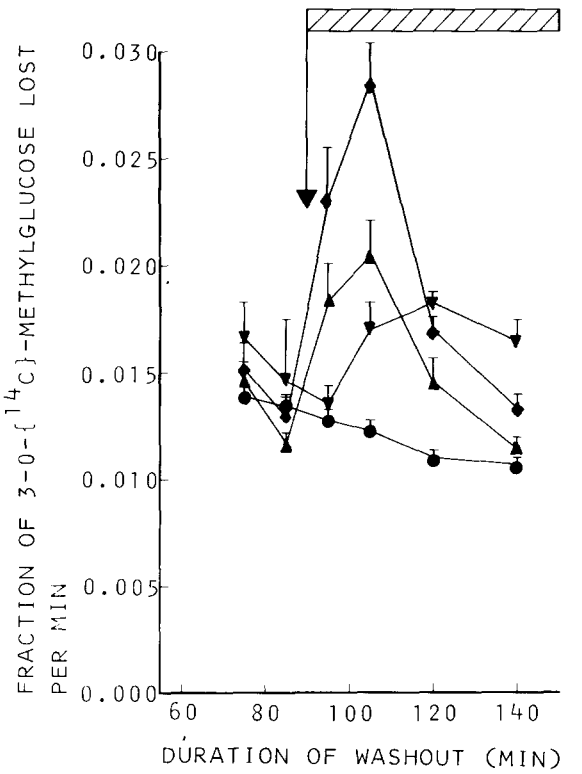

Fig. 4. Effect of $1 \cdot 10^{-5}$ M salbutamol and 25 μU/ml insulin on the efflux of 3-*O*-[^{14}C]methylglucose from whole epididymal fat pads. Experimental conditions as described in the legend to Fig. 1. The compounds listed are added to the washout medium corresponding to the hatched bar. Each point represents the mean of 5–11 observations with bars denoting S.E. ●, control; ▲, insulin alone; ◆, insulin + salbutamol; ▼, salbutamol alone.

duced by a submaximal concentration of insulin (25 μU/ml). Also, when added together with 10 μU/ml of insulin, cAMP (2.0 mM) gave a clear-cut further increase in the fractional loss of 3-*O*-[^{14}C]methylglucose. However, in the presence of a supramaximal concentration of the hormone (10 mU/ml), no effect of cAMP could be detected (Table I).

cAMP also stimulated the conversion of [U-^{14}C]glucose into CO_2 and triacylglycerols. 2 mM increased glucose metabolism from 2.7 ± 0.2 to 4.1 ± 0.5 μmol/g wet wt. per h ($n = 10$–11, $P < 0.02$). The addition of 100 μU/ml insulin gave a further increase to 6.8 ± 1.0 μmol/g per h ($n = 12$, $P < 0.05$).

Since only modest amounts of cAMP may gain access to the cytoplasm, the following experiments

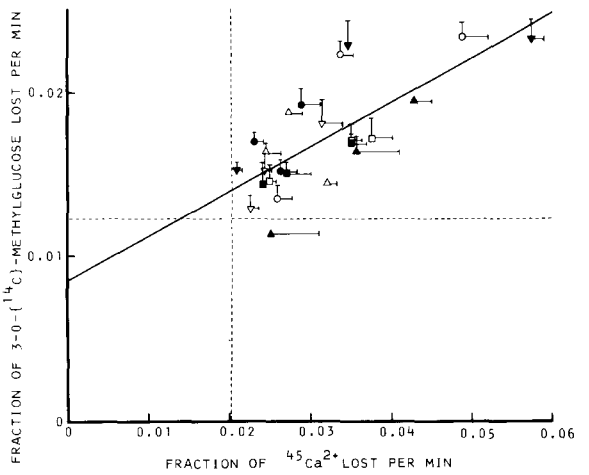

Fig. 5. Effects of $3 \cdot 10^{-7}$ M ACTH (△), $3 \cdot 10^{-7}$ M glucagon (▽), adrenaline at $1 \cdot 10^{-6}$ M (●), $1 \cdot 10^{-5}$ M (○) and $1 \cdot 10^{-4}$ M (▼), $1 \cdot 10^{-5}$ M noradrenaline (▲), and salbutamol at $1 \cdot 10^{-5}$ M (□) and $1 \cdot 10^{-4}$ M (■) on the fractional loss of $^{45}Ca^{2+}$ and 3-*O*-[^{14}C]methylglucose from whole epididymal fat pads. Experimental conditions as described in the legends to Figs. 1 and 2. Following 60 min of loading in buffer containing 2 μCi/ml $^{45}Ca^{2+}$ or 3-*O*-[^{14}C]methylglucose, the washout of the two isotopes was performed as described for the sugar washout experiments. For each agent tested, the fractional losses of ^{45}Ca and 3-*O*-[^{14}C]methylglucose were both measured in each of the three washout periods following its addition (see Fig. 2). The three results thus obtained with each concentration of the different agents are presented by the same symbol, and using the method of least squares, a regression line was calculated ($P < 0.001$, $r = 0.73$). Each of the 24 points represent the mean of 3–9 observations with bars denoting S.E. The dashed lines indicate the fractional losses measured in the absence of any stimulus (10–11 observations) (control levels).

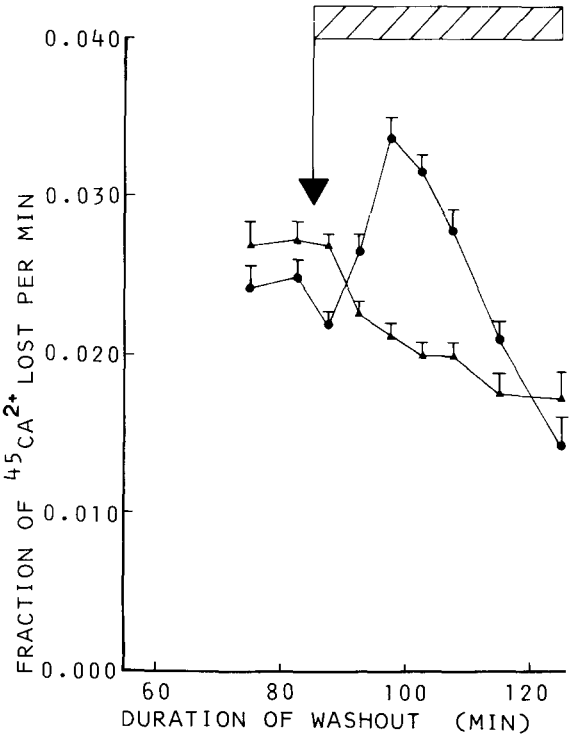

Fig. 6. Effect of salbutamol on the fractional loss of $^{45}Ca^{2+}$ from whole epididymal fat pads. Experimental conditions as described in the legend to Fig. 5. The fractional loss of $^{45}Ca^{2+}$ was measured in 5 min periods. Each point represents the mean of 3–5 observations with bars denoting S.E. ▲, control; ●, with salbutamol.

were performed using agents known to increase the cAMP content of fat pads.

As can be seen from Fig. 3, both adrenaline and salbutamol induced a dose-dependent stimulation of 3-*O*-[^{14}C]methylglucose efflux. This effect developed with a time-lag, reaching its maximum in the second 10 min period of exposure. The effect of salbutamol was additive to that of a submaximal concentration of insulin (25 μU/ml) and somewhat later in onset (Fig. 4).

Stimulation of the adenylate cyclase has been found to be associated with a mobilization of calcium ions from cellular pools which can be detected as a rise in the fractional loss of $^{45}Ca^{2+}$ from preloaded tissues or cells [18,25,26]. In fat pads and fat cells, lipolytic agents have been demonstrated to stimulate the washout of $^{45}Ca^{2+}$ [17,27], and it was of interest, therefore, to assess the possible relationships between this effect and the stimulation of sugar transport. ACTH, glucagon, salbutamol, adrenaline and noradrenaline were all found to induce a statistically significant rise in the fractional loss of $^{45}Ca^{2+}$ as well as that of 3-*O*-[^{14}C]methylglucose. Measurements performed in the first three time intervals after the addition of the lipolytic agents under otherwise identical conditions (Fig. 5) showed that the fractional losses of the two isotopes were significantly correlated ($P < 0.001$, $r = 0.73$). The effect of adrenaline was more pronounced and earlier in onset than that of salbutamol. This together with the observation that the α-adrenoceptor blocking agent thymoxamine ($1 \cdot 10^{-5}$ M), when added together with $1 \cdot 10^{-5}$ M adrenaline, reduced the fractional loss of $^{45}Ca^{2+}$ from 0.049 ± 0.003 to

0.028 ± 0.001 ($P < 0.005$) within the first 10 min of exposure, indicates that a substantial part of the adrenaline-induced stimulation of $^{45}Ca^{2+}$ release is mediated via α-adrenoceptors. On the other hand, it should be noted that the β_2-adrenoceptor specific agonist salbutamol (at $1 \cdot 10^{-5}$ M) increased the fractional loss of $^{45}Ca^{2+}$ from 0.020 ± 0.001 to 0.038 ± 0.003 (ten vs. seven observations, $P < 0.001$). The time-course of this effect is shown in Fig. 6, from which it can be seen that the maximum rise is reached between 10 and 15 min after the onset of exposure. With cAMP alone (0.1–2.0 mM), it was not possible to detect any significant increase in the washout of $^{45}Ca^{2+}$ from fat pads.

Experiments with free fat cells

In order to determine the effects of β-adrenoceptor stimulation on sugar influx, the uptake of 3-*O*-[^{14}C]methylglucose was measured in free fat cells. Since the experiments with whole epididymal fat pads were performed using animals weighing 100–120 g, these measurements were done with fat cells isolated from animals weighing 120 as well as 180 g. Following 40 min of equilibration with 3-*O*-[^{14}C]methylglucose, the water space available to the sugar was 2.4 ± 0.1 and 1.3 ± 0.1% for the 120 and 180 g rats, respectively (mean of fifteen and nine observations with five and three cell preparations from the two groups of animals).

Within the first 20 s of exposure to 3-*O*-[^{14}C]methylglucose, the untreated cells obtained from these two groups of animals showed an uptake corresponding to, respectively, 9 and 16% of these equilibrium levels. $1 \cdot 10^{-6}$ M adrenaline and $1 \cdot 10^{-5}$ M salbutamol both stimulated the uptake of 3-*O*-[^{14}C]methylglucose in the cells obtained from either group of animals (Table II). The relative increase in sugar uptake was somewhat larger than that achieved in the washout experiments. In the fat cells obtained from the 180 g rats, statistically significant effects of adrenaline were only seen following 15 min of preexposure. A submaximal concentration of insulin (25 μU/ml) induced a clear-cut stimulation of sugar uptake, and the increase was not significantly affected by the presence of $1 \cdot 10^{-5}$ M salbutamol. In the presence of 25 μU/ml insulin, salbutamol appeared to induce a further stimulation, but this was not statistically significant.

Experiments with soleus muscles

Both adrenaline and salbutamol produced a dose-dependent stimulation of 3-*O*-[^{14}C]methyl-

TABLE II

EFFECT OF ADRENALINE, SALBUTAMOL AND INSULIN WITHOUT OR WITH SALBUTAMOL ON THE UPTAKE OF 3-*O*-[^{14}C]-METHYLGLUCOSE IN ISOLATED FREE FAT CELLS

Fat cells were isolated from epididymal fat pads obtained from rats weighing 120 or 170–180 g [22] and preincubated for either 5 or 15 min without or with the agents listed. They were then incubated for 20 s in buffer containing 1 mM 3-*O*-[^{14}C]methylglucose (5 μCi/ml) [23]. The amount of sugar taken up within this interval of time is given as the mean ± S.E., and the number of incubation tubes is indicated in the parentheses. The significance of the difference between the controls and the experimental values is indicated by *P*.

Experimental conditions		3-*O*-[^{14}C]Methylglucose uptake (nmol per ml fat cells in 20 s)				
Rat wt. (g)	Preincubation (min)	Controls	Adrenaline ($1 \cdot 10^{-6}$ M)	Salbutamol ($1 \cdot 10^{-5}$ M)	Insulin (25 μU/ml)	Insulin (25 μU/ml) + salbutamol ($1 \cdot 10^{-5}$ M)
120	5	$2.2 \pm 0.2(15)$	$4.5 \pm 0.6(12)$ $P < 0.001$	$3.0 \pm 0.4(11)$ $P < 0.05$	$7.4 \pm 1.3(9)$ $P < 0.001$	$8.5 \pm 1.3(9)$ $P < 0.001$
180	5	$2.1 \pm 0.3(16)$	$3.0 \pm 0.5(8)$ $P < 0.10$	$2.4 \pm 0.4(8)$ $P > 0.10$	$5.6 \pm 0.6(8)$ $P < 0.001$	$6.4 \pm 0.6(8)$ $P < 0.001$
180	15	$2.1 \pm 0.3(16)$	$5.4 \pm 1.0(8)$ $P < 0.001$	$4.3 \pm 0.8(8)$ $P < 0.005$	$7.0 \pm 0.9(8)$ $P < 0.001$	$8.0 \pm 0.8(8)$ $P < 0.001$

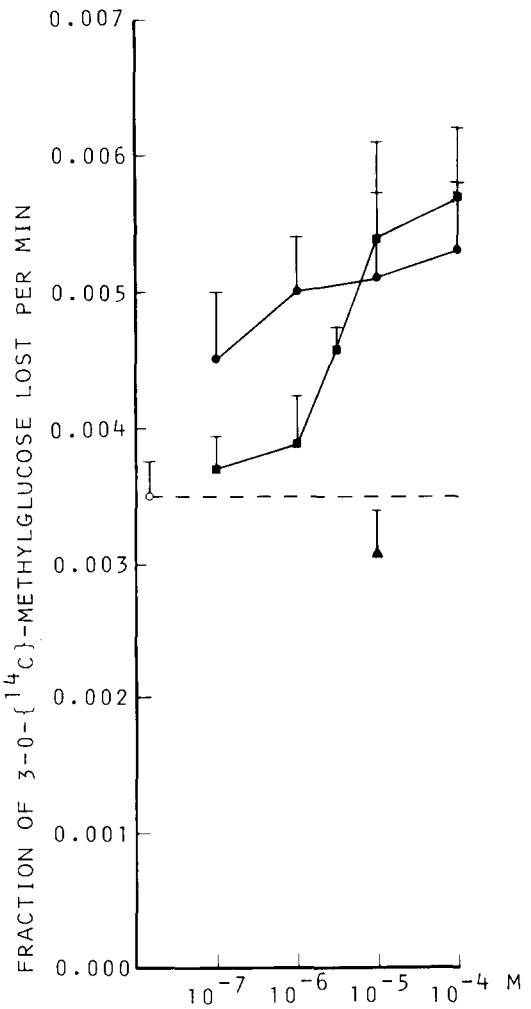

Fig. 7. Effect of varying concentrations of adrenaline and salbutamol on the fractional loss of 3-*O*-[^{14}C]methylglucose from rat soleus muscles. Muscles were loaded by incubation for 60 min in Krebs-Ringer bicarbonate buffer containing 1 mM 3-*O*-[^{14}C]methylglucose (2 μCi/ml). They were then transferred through a series of tubes containing 2 ml of unlabelled buffer without or with the additions indicated. At the end of washout, the ^{14}C activity retained in the tissue and that released into each washout tube were determined. From this, the fractional loss of 3-*O*-[^{14}C]methylglucose was calculated. Each point represents the mean of measurements performed within the first 10 min after the addition of the agent tested. Bars denote S.E. Each point represents the mean of 3–12 observations. At one concentration of $1 \cdot 10^{-5}$ M adrenaline, the effect of $2 \cdot 10^{-5}$ M propranolol was tested by adding the drug to the efflux medium from the onset of washout. ■, adrenaline; ●, salbutamol; ▲, adrenaline + propranolol; O----, control.

TABLE III

EFFECT OF ADRENALINE AND SALBUTAMOL ON THE FRACTIONAL LOSS OF $^{45}Ca^{2+}$ FROM RAT SOLEUS MUSCLE

Soleus muscles were loaded for 60 min in Krebs-Ringer bicarbonate buffer containing $^{45}Ca^{2+}$ (2 μCi/ml). They were then transferred through a series of tubes containing 2 ml unlabelled buffer without or with the additions indicated. At the end of washout, the $^{45}Ca^{2+}$ activity retained in the tissue and the amount released into each washout tube were determined. The fractional loss of $^{45}Ca^{2+}$ activity was calculated as described elsewhere [14]. The values measured within the first 10 min after addition of the agents tested are shown ± S.E. with the number of observations in parentheses.

Addition	Fraction of $^{45}Ca^{2+}$ lost per min	*P*
Control	0.0049 ± 0.0001(4)	
Adrenaline ($1 \cdot 10^{-5}$ M)	0.0060 ± 0.0002(4)	< 0.005
Salbutamol ($1 \cdot 10^{-5}$ M)	0.0044 ± 0.0001(4)	< 0.02
Ouabain ($1 \cdot 10^{-3}$ M)	0.0061 ± 0.0003(8)	
Ouabain ($1 \cdot 10^{-3}$ M) + adrenaline ($1 \cdot 10^{-5}$ M)	0.0074 ± 0.0005(8)	< 0.05
Ouabain ($1 \cdot 10^{-3}$ M) + salbutamol ($1 \cdot 10^{-5}$ M)	0.0087 ± 0.0004(8)	< 0.001

glucose efflux (Fig. 7). As in the experiments with fat pads, the increase in the fractional loss reached its maximum between 10 and 20 min after the onset of exposure. None of these effects was very pronounced, reaching statistical significance only at $1 \cdot 10^{-6}$ M for salbutamol ($P < 0.05$) and at $6 \cdot 10^{-6}$ M for adrenaline ($P < 0.01$). $2 \cdot 10^{-5}$ M propranolol blocked the effect of adrenaline (Fig. 7).

Also in soleus muscles, adrenaline induced a rise in the fractional loss of $^{45}Ca^{2+}$ (Table III). Salbutamol, however, produced a modest decrease. Since stimulation of the β_2-adrenoceptors leads to hyperpolarization and a marked decrease in intracellular Na^+ [28], the efflux of calcium ions may be impeded. In order to avoid the stimulating effect of salbutamol and adrenaline on the active Na^+,K^+ transport, the experiments were repeated in the presence of ouabain. Under these conditions, where it is known that neither compound can induce hyperpolarization or changes in in-

tracellular Na^+, there is a significant rise in the fractional loss of $^{45}Ca^{2+}$ (Table III).

Discussion

The major purpose of the present study has been to analyze the possible direct or indirect effects of cAMP on glucose transport. The direct addition of cAMP to epididymal fat pads was found to stimulate glucose utilization and the washout of 3-*O*-[^{14}C]methylglucose. More significantly, lipolytic agents as well as the selective β_2-adrenoceptor agonist salbutamol, all of which have been shown to increase the cellular cAMP contents [29,30], were found to stimulate 3-*O*-methylglucose transport both in fat pads, free fat cells and soleus muscles. Most of these observations confirm previous studies, but add the further information that the effects of cAMP, adrenaline and salbutamol are additive to that of submaximal concentrations of insulin. This seems incompatible with the idea that the insulin-induced activation of the glucose transport system can be elicited by a decrease in the cytoplasmic concentration of cAMP. From analogous experiments demonstrating additive effects of insulin and adrenaline on active electrogenic Na^+,K^+ transport in rat soleus muscle, it was concluded that the insulin-induced activation of the Na^+,K^+-pump is unlikely to be mediated by a decrease in cellular cAMP [31].

The mechanisms by which cAMP may induce stimulation of sugar transport were explored in measurements of $^{45}Ca^{2+}$ washout. The fractional loss of $^{45}Ca^{2+}$ from a preloaded tissue is to a large extent determined by the cytoplasmic concentration of free calcium ions available for transport [32,33], and this in turn depends on the rate by which $^{45}Ca^{2+}$ is released from the major cellular pools in the mitochondria and the endoplasmic reticulum. In keeping with this, we have shown that H_2O_2, *p*-chloromercuriphenylsulfonic acid and 2,4-dinitrophenol, which are known to induce a loss of calcium from isolated mitochondria, also bring about a pronounced and early rise in the fractional loss of $^{45}Ca^{2+}$ from preloaded fat pads and muscles [34]. Although the present experiments cannot exclude that part of the stimulating effect of lipolytic agents on $^{45}Ca^{2+}$ washout is the result of an increased rate of calcium transport across the plasma membrane, it seems reasonable to assume that it reflects a mobilization of Ca^{2+} from cellular pools. Thus, pretreatment of fat cells with adrenaline has been shown to decrease the calcium contents of the mitochondria [35], and in isolated liver cells, glucagon was found to decrease the exchangeable fraction of calcium in the mitochondria [26].

Several studies have demonstrated that a rise in the cytoplasmic Ca^{2+} level, whether induced by agents releasing calcium from cell organelles or by stimulating the influx of extracellular calcium, is associated with increased permeability for sugars (for reviews, see Ref. 36–38). Furthermore, linear correlations were found between the rise in the fractional losses of $^{45}Ca^{2+}$ and 3-*O*-[^{14}C]methylglucose induced by a variety of glucose transport stimuli [34,39], indicating that calcium ions liberated into the cytoplasm induce a dose-dependent activation of the glucose transport system. The present study has established a similar significant correlation for the action of lipolytic agents in fat pads (Fig. 5). In view of this evidence, the stimulating effect of these compounds on 3-*O*-methylglucose transport may be understood as the result of α- or β-adrenoceptor-mediated increases in the cytoplasmic Ca^{2+} level.

β-Adrenoceptor stimulation leads to an increase in the cellular cAMP content, and cAMP as well as dibutyryl cAMP were found to stimulate the washout of $^{45}Ca^{2+}$ from preloaded livers and liver cells [18,25,26]. However, in the present study, the addition of cAMP produced no significant rise in the fractional loss of $^{45}Ca^{2+}$ from fat pads, possibly because the plasma membrane is less permeable to the nucleotide. Thus, at variance with the correlation described above, exogenous cAMP seems to activate the glucose transport system without causing any detectable change in $^{45}Ca^{2+}$ efflux.

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