Effect of K^+, Valinomycin, Tetraphenylborate and Ouabain on Lipolysis by White Fat Cells[1]

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SUMMARY

The omission of K^+ from the phosphate buffer used for isolation and incubation of white fat cells potentiated the lipolytic action of theophylline but inhibited that of catecholamines. Omission of K^+ reduced the lipolytic action of growth hormone and glucocorticoid in the absence of glucose from the medium. Valinomycin, a macrocyclic antibiotic which increases the K^+ permeability of natural and artificial lipid membranes, inhibited the lipolytic action of theophylline if K^+ was present in the medium. In the absence of K^+ valinomycin had the opposite effect. Valinomycin did not mimic the stimulatory effect of insulin on glucose metabolism in buffer plus K^+ where it acted as an antilipolytic agent. Ouabain stimulated glucose metabolism and inhibited the lipolytic action of norepinephrine only if K^+ was present in the buffer. However, ouabain potentiated the lipolytic action of theophylline. These results indicate that the activation of lipolysis in fat cells does not have an absolute dependency upon K^+ but can be indirectly influenced by alterations in medium K^+ or by drugs which influence K^+ influx and efflux across cell membranes.

INTRODUCTION

Mosinger and Kujalova (1) postulated that the presence of K^+ in the medium was required for the hormonal activation of lipolysis in white adipose tissue. Recently it was reported that omission of K^+ or addition of ouabain inhibited the lipolytic action of catecholamines (2, 3) and either had no effect (3) or increased (2) the lipolytic action of theophylline.

The present studies were designed to investigate the possibility that the actions of growth hormone plus glucocorticoid, norepinephrine, and theophylline on free white fat cells might have an absolute dependency upon the presence of K^+ in the buffer. The effects on fat cell lipolysis of valinomycin, an antibiotic which increases the K^+ permeability of membranes (4–6), tetraphenylborate, a K^+ complexing agent (7), and of ouabain, a glycoside which inhibits a K^+-dependent ATPase (8), were also investigated. The principal conclusion from these studies is that the presence of K^+ in the medium can influence but is not required for the activation of lipolysis in white fat cells.

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MATERIALS AND METHODS

White fat cells were isolated from the parametrial adipose tissue of Sprague-Dawley female rats starved for 18 hr prior to sacrifice. The rats (130–150 g) were maintained on laboratory chow for 7–14 days after arrival from the Charles River Labs. Cells were isolated by a modification of the procedure of Rodbell (9) after digestion of adipose tissue for about 1 hr with crude bacterial collagenase (Worthington) at a concentration of 0.5 mg/ml in Krebs-Ringer phosphate buffer which contained albumin, 4%; NaCl, 128 mM; $CaCl_2$, 1.4 mM; $MgCl_2$, 1.4 mM; Na_2HPO_4, 10 mM

(pH adjusted to 7.4 with HCl); and KCl, 5.2 mM except in those experiments in which the cells were to be incubated in K^+-free buffer. The buffer was made up fresh daily and the pH adjusted to 7.4 after addition of bovine fraction V albumin powder obtained from the Pentex Company (P-55) or the Armour Pharmaceutical Company (No. 27,008 referred to as A-27). In all experiments in which cells were incubated in K^+-free buffer they were isolated and washed in buffer without K^+. Under these conditions K^+ was released to the medium during digestion of adipose tissue with collagenase in K^+-free buffer. The final concentration of K^+ was around 2 mM if 1 g of tissue was incubated in 3–4 ml of collagenase solution. There was no further release of appreciable amounts of K^+ during the incubation period since the K^+ content of the medium in which the cells had been incubated for 4 hr was 0.1 mM or less. The K^+-free albumin buffer used in each experiment was also checked for K^+ content and contained less than 0.1 mM. All K^+ determinations were done by internal standard flame photometry using an Instrumentation Labs No. 143 flame photometer.

The fat cells were incubated for 4 hr in 17×100 mm plastic culture tubes. Initial control values were obtained on cells incubated for 5 min. The values for each experiment were based on the average of duplicate tubes using pooled fat cells obtained from the adipose tissue of 3 or more rats. All experiments were replicated at least 4 times with different groups of animals. The results were evaluated by paired comparisons using the Student t test.

Glycerol was determined by a fluorimetric modification of the enzymatic procedure of Vaughan (10) on a 50-μl aliquot of the medium. DPNH formation was measured with a filter fluorimeter (American Instrument Company, Silver Spring, Maryland). The wavelength of the incident light was 340 mμ and the secondary filter transmitted light above 410 mμ. Free fatty acids were determined on the remainder of the incubation medium and cells by a modification of the procedure of Dole and Meinertz (11) in which hexane was substituted for heptane. In some experiments 0.1–0.3 μC of uniformly or randomly labeled D-glucose-^{14}C (New England Nuclear or Schwartz BioResearch) were added to each tube. The incorporation of labeled glucose into carbon dioxide, total lipid, and fatty acid was determined by the procedure of Rodbell (9). The amount of cells added to each tube was calculated from the total fatty acid content assuming 3 mmoles of fatty acid per gram of cells (9).

Bovine growth hormone (NIH-GH-B6) was a gift of the Endocrinology Study Section of the National Institutes of Health, and dexamethasone (9α-fluoro-11β,17α,21-trihydroxy-16α-methyl-1,4-pregnadiene-3,20-dione) was a gift of Merck. The crystalline insulin was a gift of the Eli Lilly Company, and valinomycin of Dr. J. C. MacDonald of the National Research Council of Canada (Saskatoon, Saskatchewan). The valinomycin (2.4 mg) was dissolved in 0.5 ml of absolute ethanol and then diluted to a volume of 10 ml with 4% albumin buffer. In all experiments with valinomycin, the same volume (25 μl) of 5% ethanol was added to control flasks. Sodium tetraphenylborate (K&K Labs.), L-norepinephrine bitartrate (CalBiochem), ouabain octahydrate (Sigma), and theophylline (Mallinckrodt) were used as received from the suppliers except that theophylline was recrystallized from ethanol. Stock solutions of all drugs and hormones were stored at —15°.

RESULTS

The lipolytic action of theophylline was considerably greater in K^+-free buffer than in regular buffer containing 5 mM K^+ (Figs. 1 and 2). The cells in the experiments shown in Fig. 1 were incubated in P-55 albumin and there was almost no lipolytic response to 0.2 mM theophylline; those in Fig. 2, which were incubated in A-27 albumin, showed a marked acceleration of lipolysis with 0.2 mM theophylline. The P-55 albumin apparently contains a considerable amount of an inhibitor of lipolysis whose effects can be largely overcome by

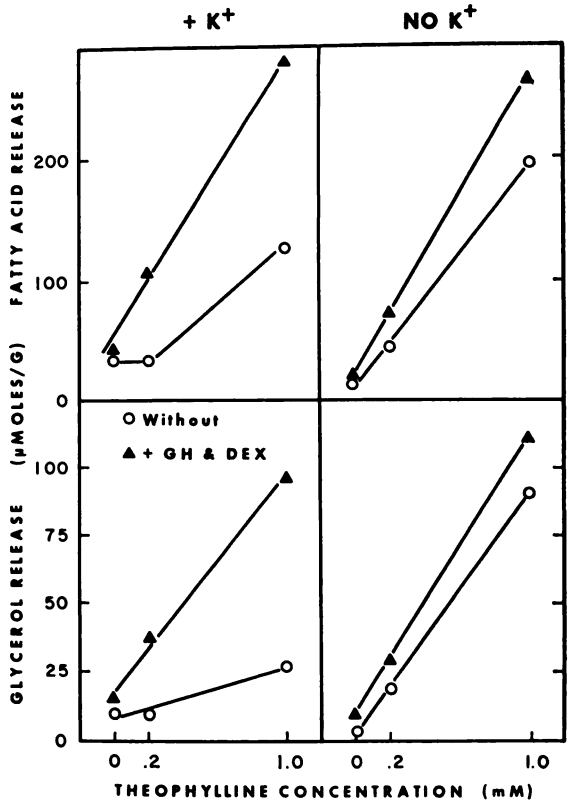

FIG. 1. *Effect of omission of medium K^+ on the lipolytic action of theophylline and growth hormone plus glucocorticoid*

Free white fat cells (8 mg of cells per flask) were incubated for 4 hr in 1.5 ml of P-55 albumin without added glucose. The values are the means of 7 paired replications; the filled triangles represent those in the presence of growth hormone (GH), 0.75 μg/ml, and of dexamethasone (DEX), 0.015 μg/ml; open circles represent those in the absence of these hormones.

increasing the concentration of the lipolytic agent. Previous studies indicated that in cells incubated with P-55 albumin there was no lipolytic action of growth hormone and glucocorticoid unless a lipolytic agent such as theophylline was present (12). Similar results were seen in the studies shown in Fig. 1. The increase in lipolysis due to theophylline alone was considerably greater in K^+-free buffer, but that due to growth hormone plus dexamethasone in the presence of theophylline was about the same in the presence as in the absence of K^+ (Fig. 1).

The lipolytic action of 0.05 μg/ml of norepinephrine was reduced by about one-half in K^+-free buffer as compared to buffer with K^+ (Fig. 2). Under identical conditions the lipolytic action of theophylline was 60% greater in K^+-free buffer (Fig. 2).

The addition of valinomycin inhibited the lipolytic action of theophylline on fat cells incubated in buffer containing K^+ (Table 1 and Fig. 3). The addition of 100 μU/ml of insulin produced an inhibition of the lipolytic action of theophylline comparable to that due to valinomycin, but insulin increased the metabolism of glucose while valinomycin almost completely blocked glucose metabolism (Table 1).

The antilipolytic action of valinomycin was dependent upon the presence of K^+ in the medium and valinomycin actually potentiated the lipolytic action of theophylline in the absence of K^+ (Fig. 3). Valinomycin also inhibited the lipolytic action

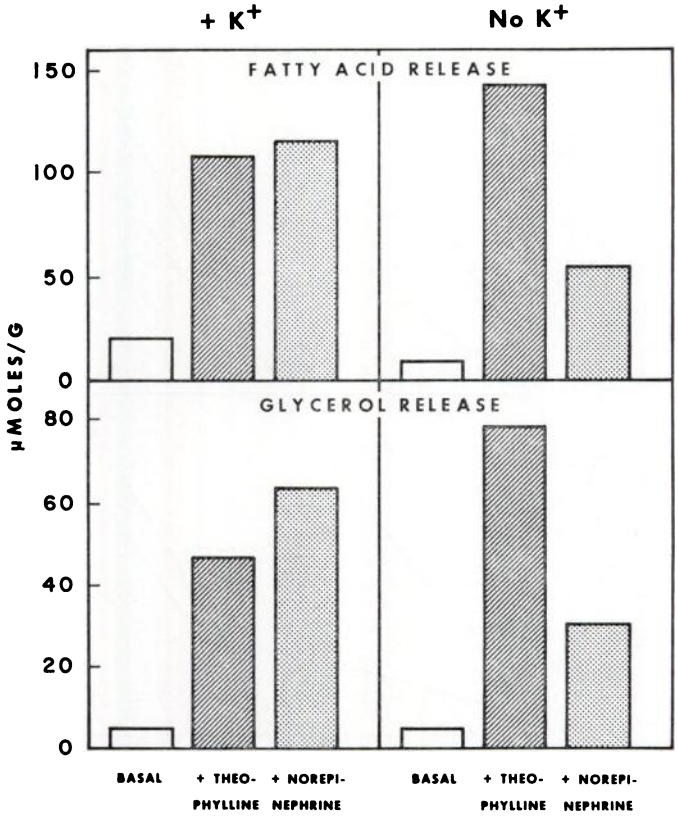

FIG. 2. *Differential effect of omission of K^+ on lipolytic action of theophylline versus norepinephrine*

Free white fat cells (16.5 mg in presence of K^+ and 17 mg in the absence of K^+) were incubated for 4 hr in 1.5 ml of 4% A-27 albumin containing glucose (0.3 mM). The values are the means of 4 paired experiments; those in the presence of 0.2 mM theophylline are represented by striped bars, and those in the presence of 0.05 μg/ml of L-norepinephrine are represented by stippled bars. The increase in both fatty acid and glycerol release due to theophylline was significantly greater ($P < 0.05$ by paired comparisons) in the absence of K^+ from the medium while those due to norepinephrine were significantly lower in the absence of K^+.

TABLE 1

Comparison of antilipolytic action of insulin with that of valinomycin

Free white fat cells (0.6 mg) were incubated for 4 hr in 1.5 ml of 4% P-55 albumin containing glucose uniformly labeled with ^{14}C (0.3 mM). The values, in micromoles per gram, are the means of 6 paired experiments, and the differences due to insulin (100 μU/ml) and valinomycin (4 μg/ml) are the means ± standard errors of the paired differences. Theophylline (1 mM) was present in all tubes, as was K^+. Glucose conversion to fatty acid was less than 0.01 μmole/g even in the presence of insulin.

Parameter	Basal	Difference due to insulin, 100 μU/ml	Difference due to valinomycin, 4 μg/ml
Glycerol release	125	−35 ± 9	−33 ± 8
Fatty acid release	235	−62 ± 13	−85 ± 11
Glucose to carbon dioxide	0.10	+0.29 ± 0.10	−0.10 ± 0.20
Glucose to glyceride-glycerol	0.12	+2.22 ± 0.60	−0.12 ± 0.01

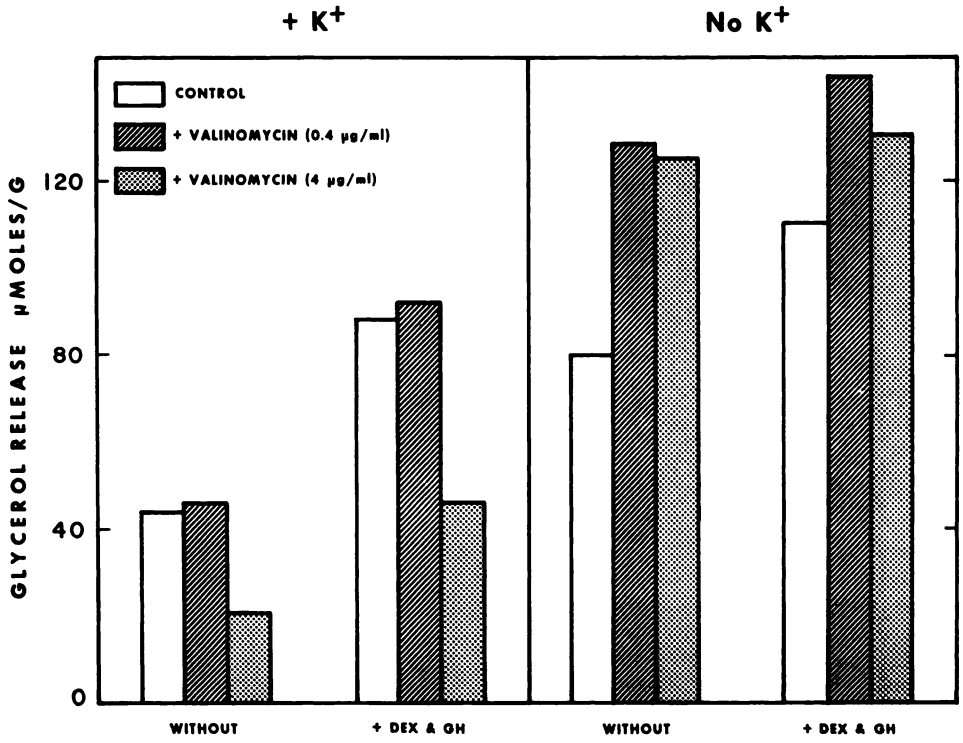

FIG. 3. *Potassium requirement for antilipolytic action of valinomycin*

Free fat cells (8 mg per tube) were incubated for 4 hr in 1.5 ml of P-55 albumin containing glucose (0.3 mM). The values are the means of 4 paired experiments, and 1 mM theophylline was present in all tubes. The basal value for glycerol release was 7 in the presence of K^+ and 3 in the absence of K^+. DEX represents dexamethasone at a concentration of 0.015 µg/ml; GH represents growth hormone at a concentration of 0.75 µg/ml.

of growth hormone and dexamethasone in the presence but not in the absence of K^+ from the medium (Fig. 3).

Ouabain has been reported to inhibit the lipolytic action of catecholamines (2, 3) but not that of theophylline (2). The results in Fig. 4 indicated that 10^{-5} M ouabain actually enhanced the lipolytic action of theophylline in both the presence and absence of K^+ from the medium ($P < 0.05$ by paired comparisons). However, ouabain did not inhibit the further increase in lipolysis due to growth hormone and dexamethasone (Fig. 4).

Ouabain at a concentration of 5×10^{-5} M stimulated glucose metabolism in the presence of K^+ in the medium and inhibited the lipolytic action of norepinephrine (Table 2). These effects of ouabain were not seen in the absence of K^+ from the medium.

Tetraphenylborate was added in the experiments shown in Table 3 to see whether removal of any traces of K^+ present in or released to the medium by fat cells would affect the lipolytic action of growth hormone plus glucocorticoid or of theophylline. Tetraphenylborate (0.3 mM) did not significantly affect the stimulation of glycerol release due to theophylline or growth hormone plus dexamethasone in either the presence or absence of K^+ from the medium. Tetraphenylborate did inhibit the stimulation by these substances of fatty acid release, but only when K^+ was present in the medium.

Omission of K^+ reduced the lipolytic action of growth hormone plus glucocorticoid to a greater extent in the absence than in the presence of glucose in the medium (Fig. 1 and Table 3). In cells incubated with

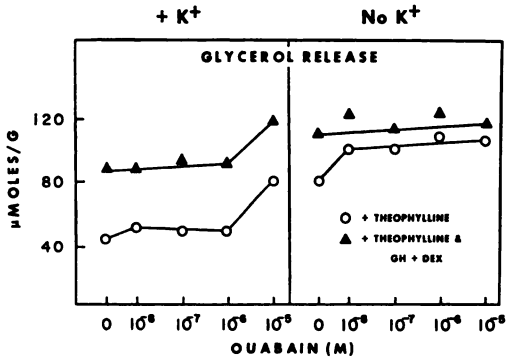

FIG. 4. *Effect of ouabain on lipolytic action of heophylline*

Free white fat cells (8 mg per tube) were incubated for 4 hr in 1.5 ml of 4% P-55 albumin containing glucose (2.8 mM). The values are the means of 4 paired experiments, and 1 mM theophylline was present in all tubes. The open circles represent values with theophylline (1 mM) alone; and filled triangles those with growth hormone, 0.75 µg/ml, and dexamethasone, 0.015 µg/ml, in addition to theophylline. The basal value for glycerol release in the absence of theophylline was 7 in the presence of K^+ and 3 in the absence of K^+.

glucose there was a lipolytic action of growth hormone and glucocorticoid in the absence of K^+ (Table 3).

DISCUSSION

The omission of K^+ from the buffer used for isolation and incubation of white fat cells reduced the lipolytic action of low concentrations of catecholamines (Fig. 2). Previously Ho *et al.* (3) reported that omission of K^+ inhibited the lipolytic action of low concentrations of ACTH or catecholamines but that these effects could be overcome by increasing the concentration of the hormones. The results of Ho *et al.* (3) and those reported here suggest that the lipolytic action of ACTH and catecholamines is influenced by K^+ but does not have an absolute dependence on K^+ as suggested by Mosinger and Kujalova (1).

The potentiation of the lipolytic action of theophylline by omission of K^+ is interesting since theophylline is thought to activate lipolysis by decreasing the breakdown of 3′,5′-adenosine cyclophosphate (3′,5′-AMP). Ho *et al.* (3) reported that prior incubation of adipose tissue in K^+-free buffer or in the presence of ouabain resulted in an inhibition of the adenyl cyclase activity of homogenates of adipose tissue. However, this cannot account for the potentiation of the lipolytic action of theophylline on fat cells by ouabain or omission of K^+. The adenyl cyclase activity responsible for the basal formation of 3′,5′-AMP must either not be influenced by K^+ lack or there are other effects of K^+ lack which more than offset any inhibition of

TABLE 2

Ouabain action on fat cell metabolism in presence and absence of K^+

Free white fat cells (16.5 mg in the presence of K^+ and 17 mg in the absence of K^+) were incubated for 4 hr in 1.5 ml of 4% A-27 albumin containing glucose uniformly labeled with ^{14}C (0.3 mM). The basal values, in micromoles/per gram, are the means of 4 experiments, and the differences due to ouabain (0.05 mM) are the means ± standard errors of the paired differences. Glucose conversion to fatty acid was less than 0.01 µmole/g even in the presence of insulin.

	+K+		−K+	
Norepinephrine (µg/ml)	Basal	Difference due to ouabain	Basal	Difference due to ouabain
		Glucose to glyceride-glycerol		
0	3.0	+2.2 ± 0.5	5.6	+0.5 ± 0.8
0.05	2.2	+2.0 ± 0.7	6.1	0 ± 0.3
		Glucose to carbon dioxide		
0	0.32	+0.18 ± 0.1	0.8	+0.06 ± 0.15
0.05	0.33	+0.11 ± 0.04	0.9	0 ± 0.03
		Glycerol release		
0	6	+2.5 ± 1	3	0 ± 0.5
0.05	67	−17 ± 4	31	−4 ± 2

TABLE 3

Tetraphenylborate and fat cell lipolysis

Free white fat cells (8 mg/tube in series A with glucose 0.3 mM and 9.5 mg/tube in series B without glucose) were incubated for 4 hr in 1.5 ml of 4% P-55 albumin. The basal values in series A are the means of 4 experiments while those in series B are the means of 6 experiments. The differences due to tetraphenylborate (TPB) at a concentration of 0.3 mM are the means ± standard error of the paired differences. GH + DEX represent growth hormone at a concentration of 0.7 μg/ml, and dexamethasone at a concentration of 0.015 μg/ml. Theophylline was present in all tubes at a concentration of 1 mM.

Additions besides theophylline	Series A with glucose			Series B without glucose	
	K^+ (mM)	Basal	Difference due to TPB, 0.3 mM	Basal	Difference due to TPB, 0.3 mM
			Fatty acid release (μmoles/g)		
0	6	112	−30 ± 15	99	−46 ± 20
GH + DEX	6	174	−53 ± 20	178	−51 ± 16
0	0	120	+11 ± 14	154	−15 ± 16
GH + DEX	0	186	+11 ± 12	191	−34 ± 23
			Glycerol release (μmoles/g)		
0	6	44	0 ± 6	35	0 ± 11
GH + DEX	6	88	+2 ± 7	60	+12 ± 9
0	0	80	+13 ± 15	89	+2 ± 13
GH + DEX	0	110	+10 ± 6	95	−4 ± 2

adenyl cyclase. Since the lipolytic action of cyclic 3′,5′-AMP (13) or of dibutyryl 3′,5′-AMP (14 and unpublished observations) is enhanced by omission of K^+ from phosphate buffer, it is possible that K^+ lack either inhibits the breakdown at 3′,5′-AMP or enhances the activation of triglyceride lipase by the nucleotide.

Valinomycin accelerates the energy-linked K^+ uptake by rat liver mitochondria (15) and causes marked increases in the permeability of natural (4, 5) and experimental bimolecular lipid membranes (6) to K^+ but not to Na^+. Valinomycin could act as a pore or carrier to transport K^+ through lipid membranes and would therefore decrease the intracellular K^+ content of cells incubated in K^+-free buffer. This could account for the stimulation of theophylline action in K^+-free buffer by valinomycin since it appears that the lower the intracellular K^+ content the more lipolysis is accelerated by theophylline. In the presence of 5 mM K^+ in the buffer, valinomycin may stimulate an energy-dependent influx of K^+ into fat cells in addition to increasing the passive K^+ efflux. The utilization of energy for active K^+ uptake might reduce the energy available for activation of lipolysis and account for the inhibitory effect of valinomycin on theophylline-induced lipolysis. The activation of lipolysis by theophylline requires energy derived from oxidative phosphorylation in both brown (16) and white fat cells (17).

Growth hormone plus glucocorticoid was able to accelerate lipolysis in K^+-free buffer containing glucose even in the presence of 0.3 mM tetraphenylborate. The addition of this amount of tetraphenylborate should have been sufficient to complex any traces of K^+ released to the medium, and suggests that the action of these hormones is not dependent upon extracellular K^+ if glucose is also present.

Tetraphenylborate has other effects on cellular metabolism which may be unrelated to its ability to complex K^+ since at higher concentrations and in the absence of albumin it uncoupled energy transfer reactions in rat liver mitochondria (18). However, 0.3 mM tetraphenylborate, the concentration used in this paper, had no effect on basal glucose metabolism or the action of insulin on fat cells incubated in K^+-free buffer (19). Possibly the formation of potassium tetraphenylborate was responsible for the reduction of fatty acid

but not of glycerol release seen only in cells incubated in buffer containing K^+ (Table 3).

Ouabain did not inhibit the potentiation of theophylline-induced lipolysis by growth hormone and glucocorticoid, which is rather different from the inhibitory effect of ouabain on the lipolytic action of catecholamines. The mechanism by which these hormones influence lipolysis differs since the lipolytic action of growth hormone and glucocorticoid involves a lag period of at least 1 hour which is blocked by inhibitors of RNA and protein synthesis such as ultraviolet light (20), X-radiation (20), puromycin (21), cycloheximide (22), and actinomycin-D (21, 22). The protein made under the influence of these hormones appears to affect the formation of 3′,5′-AMP (12). In contrast, the lipolytic action of cateholamines is not affected by any of the above-mentioned agents and is rapid in onset (20–22). Catecholamines are thought to activate adenyl cyclase with a resultant increase in intracellular accumulation of 3′,5′-AMP which accelerates lipolysis (23).

The inhibition of catecholamine-induced lipolysis and stimulation of glucose metabolism by ouabain in buffer containing K^+ confirms the work of Ho *et al.* (3, 24) and extends their findings by demonstrating that these actions of ouabain are not seen in the absence of K^+ (Table 2). Kypson *et al.* (14) reported that ouabain (10^{-4} M) inhibited the lipolytic action of epinephrine but not of norepinephrine. Their data indicated that ouabain inhibited the action of both catecholamines but only the inhibition of epinephrine action was statistically significant (14). The present results and those of Ho *et al.* (3, 24) do not agree with those of Kujalova and Mosinger (25), who reported that ouabain blocked glucose uptake by adipose tissue.

The effects of ouabain were similar to those of the K^+-free buffer in that both inhibited the lipolytic action of norepinephrine and potentiated that of theophylline. Since ouabain blocks the activity of the transport system responsible for the uptake of K^+ into cells (8), it is not surprising that its effects in buffer containing K^+ were similar to those produced by omission of K^+ from the buffer.

Despite the fact that ouabain and insulin both stimulate glucose metabolism, there is little similarity in their mechanisms of action. Insulin is able to stimulate glucose metabolism in the absence of K^+ (19) while ouabain has no effect on glucose metabolism in K^+-free buffer (Table 2). But of even more importance is the finding that insulin inhibited (Table 1), while ouabain potentiated, the lipolytic action of theophylline (Fig. 4).

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