Effects of insulin and guanosine 5'-[γ -thio]triphosphate on fatty acid synthesis and lipolysis within electroporabilized fat-cells

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1. Exposure to electric fields ('electroporation') has been used to investigate the mechanism of the action of insulin on the regulation of fatty acid synthesis and lipolysis in isolated rat fat-cells. 2. Exposure of the cells to electric fields (six pulses at 2 or 3 kV/cm) permitted the uptake of a number of low- M_r molecules normally excluded from cells, including sucrose (M_r 342), EDTA (M_r 394) and propidium iodide (M_r 668). At least 90 % of the cells were found to be permeable to these species. 3. Insulin stimulated the synthesis of fatty acids in electroporated (2 kV/cm) cells to a similar extent (0.68 ± 0.19 to 6.7 ± 0.7 μ g-atoms of glucose carbon/h per g, or 3.3 ± 0.6 to 11.2 ± 1.6 μ g-atoms of H_2O hydrogen/h per g) to that observed in non-electroporated cells. Guanosine 5'-[γ -thio]triphosphate (GTP[S]; 0.5 mM) also stimulated fatty acid synthesis (2-fold) and flux of glucose carbon into triacylglycerol glycerol (3-fold) in these cells, but had no effect on these parameters in non-electroporated cells. 4. Lipolysis in the electroporated cells was stimulated by isoprenaline and also by GTP[S], but only the effects of isoprenaline were inhibited by insulin. 5. Exposure to a higher field strength (3 kV/cm) gave results qualitatively similar to those described above, although the effects of insulin and isoprenaline were diminished. 6. These studies provide evidence against a role for changes in Ca^{2+} , and probably also Na^+ , K^+ or Mg^{2+} , in insulin action on fat-cells, but may support a role for GTP-binding proteins.

INTRODUCTION

Permeabilization of cells provides an attractive means of studying the mechanism of insulin action, because it allows the possibility of manipulating the concentration of small molecules within the cytosol.

Of the methods which may be used to render the plasma membrane of mammalian cells permeable [1,2], treatment with detergents such as digitonin [3] and saponin [4] have been previously employed in studies of insulin action with fat-cells. A potential problem with this approach is the excessive leakage of large molecules, including proteins, through the pores induced by detergents (see [3]). This risks the loss of important protein-protein interactions, such as those which may be involved in the activation of protein kinases by insulin [5–7]. Although *Staphylococcus aureus* α -toxin [8,9], which forms smaller pores (approx. 2 nm), might offer one solution to this problem, in our hands this agent causes extensive lysis of fat-cells (G. A. Rutter, unpublished work).

We have therefore turned to the use of 'electroporation' to investigate insulin signalling in rat epididymal fat-cells by transiently exposing the cells to high electric fields. In other cells, this treatment is known to cause the formation of rather small (1–3 nm) pores in the plasma membrane [2,10] and has been valuable in elucidating the signalling roles of Ca^{2+} and other second messengers [11–14].

While this work was in progress, Shibata *et al.* [15] reported the successful application of electroporation to the study of the regulation of phosphodiesterase activity and glucose transport in fat-cells. Here, we extend these findings to show further that under appropriate conditions electroporated fat-cells retain metabolic competence over a time scale in which low- M_r species can be introduced into the cells. This has allowed us to investigate the effects of metal ions and guanosine 5'-[γ -thio]triphosphate (GTP[S]) on the control by insulin and isoprenaline of fatty acid synthesis and triacylglycerol (TG) breakdown.

EXPERIMENTAL

Materials

D-[U- ^{14}C]Glucose (230 Ci/mol), D-[U- ^{14}C]fructose (230 Ci/mol), D-[U- ^{14}C]glucose 6-phosphate (150 Ci/mol), [U- ^{14}C]sucrose (approx. 500 Ci/mol) and ethylenediaminetetra[2- ^{14}C]acetic acid ([^{14}C]EDTA; 40 Ci/mol) were obtained from Amersham International, Amersham, Bucks., U.K. Digitonin (AnalaR) was from BDH, Poole, Dorset, U.K. Protein kinase A inhibitor peptide and GTP[S] were obtained from Sigma, Poole, Dorset, U.K. Sources of other reagents were as given previously [16].

Methods

Isolation of fat-cells and exposure to electric fields. Cells were isolated from epididymal fat-pads of 160–190 g male Wistar rats as described previously [16], and resuspended in buffer A, comprising 140 mM-NaCl, 4.7 mM-KCl, 1.25 mM- $CaCl_2$, 1.25 mM- $MgSO_4$, 2.4 mM- NaH_2PO_4 , 10 mM-Hepes, 15 mM- $NaHCO_3$, 1 mM-glucose and 1 % (w/v) dialysed BSA, pH 7.4, gassed thoroughly with O_2/CO_2 (19:1), at 37 °C. Before exposure to electric fields, cells were washed once in 10 vol. of 'intracellular buffer' (buffer B), comprising 125 mM-KCl, 4 mM-NaCl, 1 mM-EGTA, 1 mM- $MgCl_2$, 2.4 mM- KH_2PO_4 , 12.5 mM-Hepes, 5 mM-2-oxoglutarate, 1 mM-malonate, 1 mM-glucose, 5 mM- $NaHCO_3$ and 1 % (w/v) dialysed BSA, pH 7.2, and gassed with O_2/CO_2 as for buffer A. Cells were resuspended in this buffer at about 0.2 g dry wt./ml.

Exposure of the cells to electric fields ('electroporation') was performed in a volume of 1 ml in disposable plastic cuvettes from Bio-Rad, with six pulses from the Gene Pulsar (Bio-Rad) at a capacitance of 25 μ F (giving a pulse duration of approx. 40 μ s; [2]). The cuvette was inverted between exposures to ensure complete mixing of the cell suspension, and the polarity was reversed for the final three pulses. Discharges of 0.8 kV or 1.2 kV (to give field strengths of 2.0 or 3.0 kV/cm, respectively, in

Abbreviations used: GTP[S], guanosine 5'-[γ -thio]triphosphate; TG, triacylglycerol; G-protein, guanine nucleotide regulatory protein.

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the 0.4 cm cuvettes) were used routinely. Up to 6 ml of cells was electroporated for an individual experiment (total time < 10 min), and incubations were always begun 15–25 min after electroporation.

Assay of fatty acid synthesis and flux of glucose carbon into triacylglycerol glycerol. Incubations were performed in a total volume of 1 ml at 37 °C and were initiated by adding 0.5 ml of cell suspension to an equal volume of either buffer A or buffer B (electroporated cells) plus 0.1–0.5 μ Ci of D-[U- 14 C]-glucose, 1–2 mCi of $^3\text{H}_2\text{O}$, and insulin and other additions as required. Incubations were continued for 30 min, and tubes were inverted at 5–10 min intervals.

Radioactivity associated with TG fatty acids and TG glycerol was determined as described elsewhere [17]. Fatty acids were finally redissolved in toluene-based scintillant containing 0.4 % (w/v) 2,5-diphenyloxazole and 0.03 g of 1,4-bis-(5-phenyloxazol-2-yl)benzene; radioactivity associated with TG glycerol was measured in a xylene-based scintillant (Unisolve E; Koch-Light, Haverhill, Suffolk, U.K.).

Lipolysis. This was measured in cells incubated as described above, but in the absence of radioisotopes. Glycerol released was measured [18] either after acidification of the whole incubation with 1 % (v/v) HClO_4 (Table 2), or in the acidified infranatant (Table 3).

Measurement of sucrose and EDTA uptake, and accessible spaces. Incubations were performed in a volume of 1 ml, essentially as described above, but in the presence of 1 mM-sucrose or EDTA, and 1 μ Ci of the ^{14}C -labelled compound/ml. For measurement of the H_2O -accessible space, 10 μ Ci of $^3\text{H}_2\text{O}$ /ml was also added. Samples (0.2 ml) were removed at the given intervals into polypropylene centrifuge tubes (4 mm $\times$ 45 mm), overlaid with 0.1 ml of dinonyl phthalate, and the cells were rapidly separated from the medium by centrifugation at 10000 g (5 s). Radioactivity associated with cells and medium was determined by scintillation counting in 10 ml of xylene-based scintillant [19].

Measurement of propidium iodide uptake. Fluorescence (500 nm excitation, 600 nm emission) of cell suspensions (2 ml) containing 10 μM -propidium iodide was measured in the thermostatically regulated cell holder of a Perkin-Elmer LPS-5 spectrofluorimeter. The suspensions were continuously stirred and maintained at 37 °C.

Other methods. ATP was measured in neutralized HClO_4 extracts of cells suspensions by luciferin-luciferase assay [20].

RESULTS AND DISCUSSION

Effects on fat-cell permeability of exposure to electric fields

Repeated exposure (six pulses) to an electric field of 2 or 3 kV/cm enabled fat-cells to take up low- M_r species, including sucrose (M_r 342) and EDTA (M_r 394 in the Mg^{2+} -bound form) in a time- and field-strength-dependent manner. Data from the experiment illustrated in Fig. 1 were fitted by non-linear regression (Fig P Software; Biosoft, Cambridge, U.K.) to a first-order expression, $y = A(1 - e^{-kt}) + B$, where k is the first-order rate constant, y is the sucrose or EDTA content, A is the increase in this parameter between $t = 0$ and $t = \infty$, and B is the content at $t = 0$. Calculated half-times (mean $\pm$ S.E.M. for 8 observations) for the uptake of sucrose and EDTA were 2.6 ± 1.0 and 7.5 ± 1.1 min respectively, after exposure at 2 kV/cm, and 0.5 ± 0.1 and 0.72 ± 0.06 min after exposure at 3 kV/cm. By contrast, half-times for uptake in untreated cells were in excess of 2 h.

Measurements of the difference between sucrose- and H_2O -inaccessible spaces (Table 1) after treatment with electric dis-

Fig. 1. Effect of exposure to electric fields on the uptake of (a) 1 mM-sucrose and (b) 1 mM-EDTA into fat-cells

Cells were incubated in medium B, and then given no further treatment (●), or exposed (six times) to fields of 2 kV/cm (■) or 3 kV/cm (▲), before measuring rates of uptake (see the Experimental section). Data were fitted as described in the text. These experiments were carried out on separate preparations of fat-cells, and this may explain the apparent difference in the final cell contents of sucrose and EDTA. In other experiments the final content of sucrose was similar to that of EDTA (e.g. see Table 1).

charge, or after complete permeabilization with 0.1 mg of digitonin/ml, indicated that at least 90 % of the cells were rendered leaky to sucrose after the electrical treatment. Similar results were also obtained with EDTA (not shown). This assumes that the residual sucrose-inaccessible/ H_2O -accessible space after the detergent treatment represents intracellular compartments

Table 1. Effects of exposure to electric fields on [^{14}C]sucrose and $^3\text{H}_2\text{O}$ spaces

The treatment of cells and incubation with radioisotopes is described in the Experimental section. Data are the means of observations on duplicate incubations which agreed to within 10 %.

		Accessible space ($\mu\text{l/g}$ dry mass)				
	Medium ...	A	B	B	B	B + digitonin (0.1 mg/ml)
Field strength (kV/cm ...		0	0	2.0	3.0	0
Incubation time	Radioisotope					
30 s	^3H	78.2	93.3	132	131	110
	^{14}C	35.1	24.7	53.0	63.9	81.5
	$^3\text{H}/^{14}\text{C}$	43.1	68.6	79.5	67.5	28.5
45 min	^3H	69.1	90.8	117	120	97.3
	^{14}C	28.7	25.8	97.7	101	83.7
	$^3\text{H}/^{14}\text{C}$	40.1	55.0	18.8	19.0	13.6

such as mitochondria and microsomes. The increased H_2O -accessible space in the electrically treated cells compared with the untreated cells presumably results from the osmotic potential created by non-diffusible large- M_r species within the treated cells (see below; [10]). Electrically treated cells (2 or 3 kV/cm) were also found to be fully leaky to the fluorescent DNA-binding stain propidium iodide (M_r 668; [4]). Again, no uptake was detectable into untreated cells.

The above experiments thus demonstrate that the electroporated cells were permeable to species of up to about M_r 1000. However, we were entirely unable to detect any increase in the leakage out of the cells of large molecules, including proteins such as lactate dehydrogenase, even at field strengths up to 5 kV/cm. This contrasts with the effects of detergents such as digitonin [3] or α -toxin from *Staphylococcus aureus* (G. A. Rutter, unpublished work), which can cause extensive damage to fat-cells and the leakage of enzymes.

Effects of electric discharge on the control by insulin of fatty acid synthesis

Six exposures to the 2 kV/cm electric field had only a modest effect on insulin-stimulated fatty acid synthesis by fat-cells, measured as flux of either glucose carbon or H_2O hydrogen [21] into saponifiable fatty acids (Table 2). However, some diminution of insulin-stimulated fatty acid synthesis was evident after treatment at 3 kV/cm. Flux of glucose carbon into TG glycerol was barely affected by exposure to either electric-field strength. In each case, closely similar results (not shown) were obtained whether incubations were performed for 15 or 30 min, consistent with near-linear rates of flux over the 30 min period.

Insulin-stimulated fatty acid synthesis within the electrically treated cells was entirely dependent on the presence of HCO_3^- (results not shown). Further, this process was also dependent on the presence of a substrate for mitochondrial oxidation, such as 2-oxoglutarate (plus malonate, added to allow uptake of 2-oxoglutarate by mitochondria). For example, electroporated cells in K^+ -based medium (buffer B) supplemented with insulin gave rates of conversion of glucose carbon into fatty acids in the absence of 2-oxoglutarate and malonate of 16% (2 kV/cm) and

12% (3 kV/cm) of those obtained in the presence of the substrates. This requirement resembles that for pyruvate (or other oxidizable substrate) for the activation of phosphodiesterase activity in electroporated fat-cells [15]. Our choice of 2-oxoglutarate plus malonate was intended to minimize isotope dilution when monitoring the flux of glucose carbon into fatty acids. However, after electroporation, rates of fatty acid synthesis in the presence of insulin measured by monitoring the flux of glucose carbon were somewhat lower than those measured by monitoring the incorporation of H_2O hydrogen (Table 2). Hence it appears that some isotope dilution on measuring glucose carbon flux into fatty acids may occur in the electroporated cells.

The electric-discharge treatment also facilitated the incorporation of carbon atoms derived from exogenously added glucose 6-phosphate into fatty acids. Rates (as ng-atoms/h per g; means of two observations) at 1 mM-glucose 6-phosphate and in the presence of 83 nM-insulin were 1.0, 5.5 and 17.5 after no further treatment or after six pulses at 2 or 3 kV/cm respectively. Assuming that 1 mol of glucose 6-phosphate can yield a maximum of 4 g-atoms of fatty acid carbon, then these rates represent an uptake of glucose 6-phosphate into the cell comparable with that of sucrose (about 0.5 and 3.0 nmol/h per g after treatment at 2 and 3 kV/cm respectively, calculated from the data of Fig. 1 and Table 1.)

Effects of electric discharge on the hormonal regulation of TG breakdown

The lipolytic response to the β -adrenergic agonist isoprenaline was largely preserved in the electrically treated cells (Table 2). Again, no stimulation of lipolysis was observed in the absence of 2-oxoglutarate plus malonate. The response to isoprenaline was not appreciably affected (results not shown) in either intact or electrically treated cells by the peptide inhibitor or protein kinase A (M_r 2200; [22]). This may give a minimum estimate of the molecular mass of species able to permeate the cell membrane after the electric-discharge treatment.

Strikingly, whereas insulin weakly potentiated the lipolytic action of 10 μ M-isoprenaline when cells were incubated in Na^+ -based medium, with intact cells incubated in K^+ -based medium

Table 2. Effects of exposure to electric fields on fat-cell metabolism and ATP content

Details of incubations and assays are given in the Experimental section. Data are means ($\pm$ S.E.M.) of three to six observations on three separate preparations†, or means of two incubations on a single preparation which agreed to within 10%. Abbreviation: n.d., not determined. Statistical significance was determined by using Student's *t* test: ** $P < 0.01$, *** $P < 0.001$, for the effect of insulin.

Parameter	Medium... Field strength (kV/cm)... Additions or omissions	A 0	B 0	B 2.0	B 3.0
Flux of glucose carbon into fatty acids (μ g-atoms/h per g)†	None	0.66 $\pm$ 0.23	0.29 $\pm$ 0.15	0.68 $\pm$ 0.19	1.3 $\pm$ 0.2
	83 nM-insulin	11.2 $\pm$ 1.3***	8.6 $\pm$ 0.5***	6.7 $\pm$ 0.7***	3.4 $\pm$ 0.5***
Flux of H_2O hydrogen into fatty acids (μ g-atoms/h per g)†	None	2.1 $\pm$ 0.5	1.6 $\pm$ 0.5	3.3 $\pm$ 0.5	2.6 $\pm$ 0.8
	83 nM-insulin	12.9 $\pm$ 1.0***	9.1 $\pm$ 0.9***	11.2 $\pm$ 1.6***	6.6 $\pm$ 0.3**
Flux of glucose carbon into TG glycerol (μ g-atoms/h per g)	None	0.53	0.64	1.7	1.7
	83 nM-insulin	2.3	2.4	3.8	2.9
Glycerol release (μ mol/h per g)	None	0.64	0.44	0.69	1.1
	10 μ M-isoprenaline	18.4	11.4	5.5	4.4
	10 μ M-isoprenaline plus 83 nM-insulin	25.2	7.2	3.6	3.8
ATP content (nmol/g)	None	n.d.	222	127	56.6
	2-Oxoglutarate and malonate omitted	237	200	52.3	18.0

Table 3. Effect of GTP[S] on fatty acid synthesis, flux of glucose into TG glycerol and lipolysis in untreated and electrically treated cells

Incubations were performed as described in the Experimental section. Data are means ($\pm$ S.E.M.) of four incubations on two separate preparations†, or are means of duplicate incubations on one preparation; duplicates agreed within 10%. The concentrations of additions were 83 nM-insulin and 0.5 mM-GTP[S]. Cells were preincubated with GTP[S] for 15 min before assay. Statistical significance was determined by unpaired Student's *t* test: **P* < 0.05, ****P* < 0.001, for the effect of GTP[S]. Abbreviation: n.d., not determined.

Parameter	Field strength (kV/cm)...	Medium...	A	B	B†
		Additions	0	0	2.0
Flux of glucose carbon into fatty acids (μ g-atoms/h per g)	None		2.0	0.3	1.0 $\pm$ 0.04
	Insulin		7.1	8.7	6.47 $\pm$ 1.3
	GTP[S]		2.7	0.5	2.38 $\pm$ 0.19***
	GTP[S] plus insulin		7.5	5.7	4.66 $\pm$ 0.29
Flux of H ₂ O hydrogen into fatty acids (μ -atoms/h per g)	None		4.5	1.3	2.69 $\pm$ 0.09
	Insulin		7.5	10.2	7.56 $\pm$ 1.4
	GTP[S]		4.6	1.4	3.36 $\pm$ 0.2*
	GTP[S] plus insulin		8.3	6.8	5.29 $\pm$ 0.3
Flux of glucose carbon into TG glycerol (μ g-atoms/h per g)	None		0.9	0.26	0.91 $\pm$ 0.12
	Insulin		7.5	10.2	1.81 $\pm$ 0.15
	GTP[S]		1.2	0.21	2.91 $\pm$ 0.15***
	GTP[S] plus insulin		1.6	0.87	3.07 $\pm$ 0.14***
Glycerol release (μ mol/h per g)	None		< 0.1	n.d.	< 0.1
	Insulin		< 0.1	n.d.	< 0.1
	GTP[S]		0.69	n.d.	1.8
	GTP[S] plus insulin		0.50	n.d.	2.0

insulin substantially reversed the effects of this agent. Furthermore, electrical treatment did not block this effect of insulin. This is consistent with the activation of an insulin-sensitive phosphodiesterase in the electroporated cells, as reported recently by Shibata *et al.* [15], and observed previously in digitonin-permeabilized cells [3].

Effect of GTP[S] on fatty acid synthesis and lipolysis

GTP[S] (0.5 mM) stimulated TG breakdown and glycerol release in cells exposed to a field of 2 kV/cm (Table 3). A much weaker stimulation was observed in intact cells. These observations are consistent with enhanced entry of this non-hydrolysable analogue into the treated cells, followed by G-protein-mediated activation of adenylate cyclase and an increase in intracellular cyclic AMP [23,24]. This then leads not only to the activation of lipolysis, but also to enhanced re-esterification of fatty acids, and hence the observed increase in flux of glucose carbon into TG glycerol (Table 3). Similar observations have been made in isolated intact fat-cells in response to cyclic AMP mobilization by adrenaline [25].

In cells treated at 2 kV/cm, and in the absence of insulin, GTP[S] also stimulated flux of glucose carbon and H₂O hydrogen into fatty acids by approx. 2.0-fold and 1.5-fold respectively (Table 3). These parameters were barely affected in untreated cells, incubated in either Na⁺- or K⁺-based media. It seems unlikely that this stimulation results from cyclic AMP mobilization, however, given that increases in cyclic AMP in these cells are associated with the inhibition of acetyl-CoA carboxylase and fatty acid synthesis [25–28]. Consistent with this, in the presence of insulin, GTP[S] had an inhibitory effect on the fluxes of carbon and hydrogen atoms into fatty acids in the electroporated cells.

Conclusion

Electroporation provides a novel system for exploring the roles of small molecules in the control of fat-cell metabolism by insulin and other hormones [15]. After exposure to an appropriate field strength (e.g. six pulses of 2 kV/cm), metabolism of glucose continues in the electroporated cells at rates comparable

with those observed in untreated cells, under conditions where low-*M_r* species (< 1000) can be introduced into the cytosol. The rate of loss of intracellular coenzymes and intermediate metabolites is apparently quite small, as linear rates of metabolism were evident for up to 30 min. Presumably this is in part due to the limited number and size of the 'pores' generated by the electroporation and in part the result of extensive binding of these small molecules to enzymes and other macromolecules within the cell. It should be noted that in electroporated cells rates of fatty acid synthesis from glucose 6-phosphate were only about 0.1% of those from glucose (in the presence of insulin), which indicates that in these cells most of the glucose still enters via the glucose transporter and not through the pores. This is consistent with the observations by Shibata *et al.* [15], who observed that rates of glucose transport in electroporated cells were very low in the absence of insulin, but were comparable with those for intact cells in the presence of the hormone.

At a higher field strength (3 kV/cm), however, although the uptake of small molecules is accelerated (Fig. 1), this is countered by an inhibition of metabolism, which is probably the result of increased rates of loss of essential cofactors and intermediates.

There is one very striking difference between fat-cells before and after electroporation, and that is the need for the addition of 2-oxoglutarate (or other substrate for mitochondrial oxidation; [15]) for the lipogenic response to insulin, or the lipolytic response to isoprenaline. Addition of such a substrate appears to be necessary to ensure adequate levels of intracellular ATP (Table 1), and perhaps also the production of reducing equivalents [NAD(P)H]. It is not obvious, however, why pyruvate produced from added glucose is no longer sufficient for mitochondrial ATP [or NAD(P)H] synthesis. Possible explanations are that (i) extramitochondrial ATP hydrolysis is enhanced in the electroporated cells, perhaps owing to activation of ATP-driven membrane ion pumps, or (ii) mitochondrial citrate cycle and electron-transport activity are suppressed by the absence of Ca²⁺, a known regulator of these processes (see below and [29]).

We observed that sucrose and EDTA were equilibrated into the cells after about 15–20 min (2 kV/cm-treated cells) or about

5 min (3 kV/cm-treated cells). Since all assays were performed 15–25 min after electroporation, it is likely that EGTA (1.0 mM) was fully equilibrated into the cells, so that the cytosolic Ca^{2+} concentration was clamped in the nanomolar range. However, rates of fatty acid synthesis, at least in 2 kV/cm-treated cells, were not significantly different from those in non-electroporated cells. This would seem to make unlikely any role for changes in Ca^{2+} in the stimulation by insulin of fatty acid synthesis. This conclusion, however, is apparently in conflict with that of Pershadsingh *et al.* [30], who have argued for a role for Ca^{2+} in insulin action on fat-cells. Thus, those authors observed that loading the cells with the Ca^{2+} chelator quin-2 inhibited insulin-stimulated glucose uptake by approx. 50%. However, basal rates of glucose uptake were also lowered to a similar extent by this treatment, so that the fold stimulation elicited by insulin was barely affected. Hence it would appear difficult to argue that this result suggests an involvement for Ca^{2+} in signalling by insulin. Furthermore, recent direct measurement [31,32] of intracellular Ca^{2+} concentration within fat-cells using the Ca^{2+} indicator fura-2 have indicated that no change in this parameter occurs in response to insulin.

Our experiments with electroporated cells may also argue against a role for other metal ions, such as Na^+ , K^+ or Mg^{2+} , in insulin action, since it seems unlikely that the concentrations of these could be changed by exposure of the electroporated cells to insulin.

Finally, the observed stimulation of fatty acid synthesis by GTP[S] in cells exposed to a 2 kV/cm field would seem to be consistent with the recent finding [9] that non-hydrolysable GTP analogues promote the recruitment to the plasma membrane of the insulin-sensitive glucose carrier (Glut4; [33]) in α -toxin-permeabilized cells. Whether this analogue also enhances fatty acid synthesis by stimulating other insulin-sensitive systems (e.g. acetyl-CoA carboxylase and pyruvate dehydrogenase; [7]) within the permeabilized cells appears worthy of further investigation.

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