J. Pharm. Pharmacol. 1979, 31: 536–541 Received November 13, 1978

Effect of ethanol on adrenaline-stimulated glucose uptake in rat white adipose tissue

KOICHI ITAYA

Department of Physiological Chemistry, Faculty of Pharmaceutical Sciences, Hokkaido University, Sapporo 060, Japan

The effect of ethanol on adrenaline-stimulated glucose uptake by rat white adipose tissue has been examined in vitro. Ethanol (3%) inhibited the stimulatory effect of adrenaline on glucose uptake whereas it failed to inhibit the effect of adrenaline on free fatty acid production. Addition of calcium (12·5mM) to the incubation medium restored adrenaline's effect on glucose uptake. Addition of propranolol also restored the effect of adrenaline inhibited by ethanol. Ethanol did not inhibit insulin-stimulated glucose uptake. These results suggest that ethanol modifies the coupling of the adrenoceptor to the glucose transport system in adipose tissue that is stimulated by adrenaline.

Adrenaline stimulates lipolysis and glucose uptake by free fat cells and adipose tissue. The view has been expressed that the effect of adrenaline in stimulating glucose utilization might be secondary to the accumulation of free fatty acids (FFA) resulting from stimulated lipolysis (Cahill et al 1960; Blecher 1967). However, many reports show that stimulation of glucose oxidation by adrenaline occurs in the absence of stimulated lipolysis. The β-adrenoceptor blocking agent, propanolol, inhibits adrenaline-stimulated lipolysis at concentrations which do not inhibit adrenaline-stimulated glucose uptake in fat cells (Blecher et al 1969). On the contrary an α-adrenoceptor blocking agent, phenoxybenzamine, diminishes the stimulatory effect of adrenaline on glucose uptake in fat cells (Luzio et al 1974) and rat diaphragm (Saitoh et al 1974). I have previously reported the presence of α-receptors with inhibitory effects on lipolysis in rat brown adipose tissue (Itaya 1978). I have now examined the experimental condition which can specifically reverse the action of adrenaline on glucose uptake by rat white adipose tissue.

MATERIALS AND METHODS

Male rats of the Wistar-derived strain with free access to food were decapitated on reaching 200 to 250g. Epididymal and mesenteric fat pads were removed and divided into pieces of tissue weighing 50 to 100mg. Usually two or three pairs of tissue pieces were taken from each rat, and six to twelve rats were used in each experiment. One piece from each of a pair of tissues was placed in 1 ml of medium (Krebs-Ringer bicarbonate buffer with 2% bovine serum albumin, pH 7·3 with some glucose, usually 100 mg%) containing ethanol or one of the other agents being tested, while another pair of tissues from the same rat served as control. All flasks were equilibrated with 6% CO_2 in oxygen and incubated at 37 °C for 1 or 3 h.

The concentrations of glucose in the medium were determined (Hugget & Nixon 1957) after deproteinization with $Ba(OH)_2$ and $ZnSO_4$. Lactate content in the incubation medium was determined by the method of Barker & Summerson (1941). The concentration of FFA in the medium after incubation was determined as described previously (Itaya 1977; Itaya & Ui 1965).

In some experiments, some pieces of fresh epididymal and mesenteric adipose tissue were incubated with 1 mg of trypsin in 1 ml of the Krebs-Ringer buffer for 1 h and then washed twice with 1 ml of the buffer containing soybean trypsin inhibitor (0·1 mg ml^{-1}). The partially digested tissues were used to test the effect of ethanol and insulin on the treated tissue. At the same time another piece of adipose tissue from the same rat was preincubated in buffer without trypsin as control.

An experiment to find the effect of adrenaline or insulin on the rate of glucose oxidation by adipose tissue was done by incubating the tissue in 1 ml of the buffer containing D-glucose-U-^{14}C (0·2 μCi) for 3 h and measuring the $^{14}CO_2$ formed using the method of Bray & Goodman (1968).

RESULTS

Effect of ethanol on the stimulatory effect of adrenaline on glucose uptake

As shown in Table 1, the addition of ethanol (3%) to the incubation medium inhibited the effect of adrenaline on glucose uptake both by epididymal

Table 1. Effect of ethanol on glucose uptake and FFA release stimulated by adrenaline. Epididymal or mesenteric adipose tissues were incubated in the presence or absence of ethanol in 1 ml of Krebs-Ringer bicarbonate buffer pH 7·3 containing 2% bovine serum albumin at 37 °C for 3 h. Adrenaline, 5 μg ml^{-1}. Each value for 'Net effect' represents the means ± s.e.m. The numbers of experiments are given in parentheses. NS, not significant.

Tissue	Ethanol (%)	Control glucose uptake	Adren. (mg g^{-1}/3h)	Net effect	P
Epididymal	0	2·3	8·1	+ 5·8 ± 0·84 (6)	< 0·005
	3	1·8	2·0	+ 0·2 ± 0·14 (6)	NS
Mesentric	0	6·6	10·7	+ 4·1 ± 0·67 (6)	< 0·005
	3	5·8	5·8	— 1·0 ± 0·37 (6)	NS
		FFA release (μequiv g^{-1} 3h^{-1})			
Epididymal	0	3·3	13·5	+ 10·2 ± 0·80 (6)	< 0·001
	3	3·2	15·3	+ 12·1 ± 1·19 (6)	< 0·001
Mesenteric	0	0·3	10·4	+ 10·1 ± 0·93 (6)	< 0·001
	3	0·3	14·2	+ 13·9 ± 1·02 (6)	< 0·001

and by mesenteric adipose tissue, while the stimulatory effect of adrenaline on FFA release was not inhibited, rather lipolysis was enhanced a little. FFA release from control tissue was not influenced by ethanol, but in its presence basal uptake of glucose by control tissue was inhibited.

As shown in Figs 1 and 2, the adipose tissues incubated with ethanol showed saturation kinetics of the Michaelis-Menten type in glucose uptake and lactate production, suggesting that the basal glucose transport system of these tissues is active even in the presence of ethanol, and that the inhibitory effect of ethanol on the adrenaline-stimulated glucose uptake is not the result of damage to the plasma membrane of adipose tissue cells.

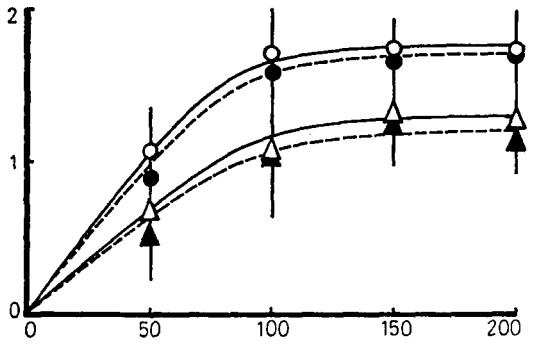

FIG. 1. Effect of concentration of glucose in the medium on the glucose uptake and the lactate production of epididymal adipose tissue in the presence and absence of ethanol (ordinate: mg g^{-1} 3 h^{-1}). Each of four pieces of epididymal adipose tissues from a rat was placed in the flask containing 50, 100, 150, or 200 mg% of glucose (abscissa), resprectively, and incubated for 3 h at 37 °C. Each bar represents the means ± s.e.m. of six observations. ○——○, glucose uptake in the absence of ethanol; ●- - -●, glucose uptake in the presence of ethanol; △——△, lactate production in the absence of ethanol; ▲- - -▲, lactate production in the presence of ethanol.

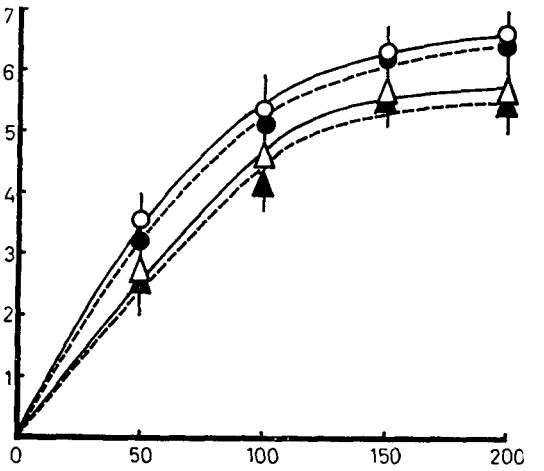

FIG. 2. Effect of concentration of glucose (abscissa: mg%) in the medium on the glucose uptake and lactate production (ordinate: mg g^{-1} 3 h^{-1}) or mesenteric adipose tissue in the presence or absence of ethanol. The conditions of incubation and the symbols were the same as Fig. 1.

Subsequent data relate only to epididymal adipose tissue since there were no qualitative differences between epididymal and mesenteric adipose tissue (Table 1, Figs 1 and 2).

Differential effect of ethanol on adrenaline- and insulin-stimulated glucose uptake

There is the possibility that ethanol directly inhibits hormone-enhanced glucose transport mechanism. If this were so, insulin-stimulated glucose uptake should be inhibited by ethanol. But it was hardly affected by ethanol (Figs 3 and 4), whereas adrenaline's effect on glucose uptake was reduced by the ethanol. Increasing the concentration of ethanol caused only a slight further decrease in basal

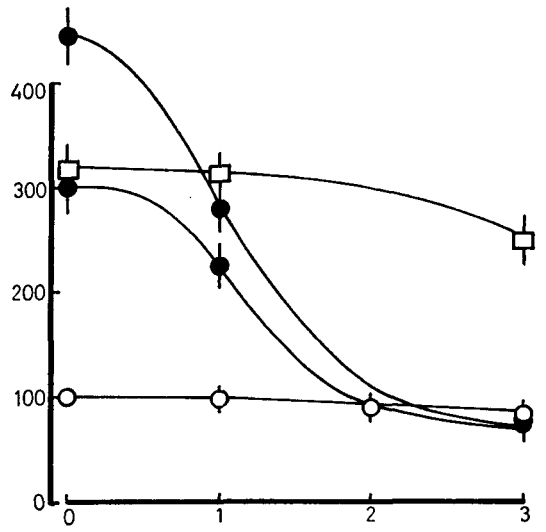

FIG. 3. Effect of concentration of ethanol (abscissa: %) on glucose uptake (ordinate: % of initial uptake) stimulated by adrenaline or insulin. Four pieces of epididymal adipose tissue from a rat were incubated in the presence of 0, 1, 2, or 3% of ethanol, respectively. The glucose uptake obtained in the absence of ethanol are expressed as 100%. For the test of hormone action, each of the four pieces of adipose tissue from a rat was placed in the flask containing medium, without ethanol and hormone, without ethanol and with hormone, with hormone and 1% of ethanol, or with hormone and 3% of ethanol, respectively. Each bar represents the mean ± s.e.m. of six observations. ○——○, control; ●——●, adrenaline 5μg ml^{-1} (two different experiments); □——□, insulin 0·02 unit ml^{-1}.

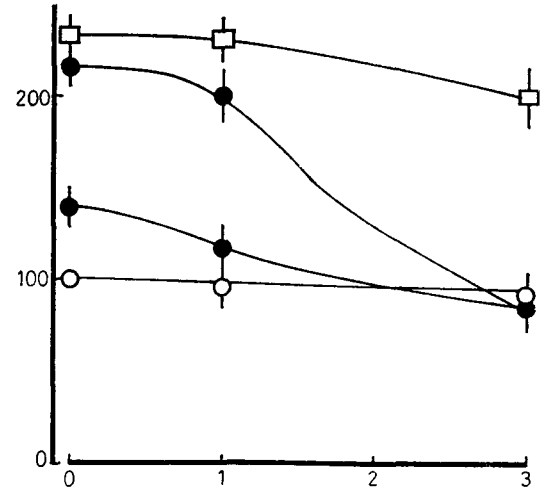

FIG. 4. Effect of concentration of ethanol (abscissa: %) on $^{14}CO_2$ production (ordinate: % of initial production) from glucose-U-^{14}C stimulated by adrenaline or insulin. For conditions of incubation, see the legend for Fig. 3. Glucose-U-^{14}C was added into the medium as 0·2 μCi ml^{-1}.

glucose uptake (Fig. 3). In the experiments with glucose-U-^{14}C ethanol depressed the production of $^{14}CO_2$ (Fig. 4). The effects of ethanol on the 'dose-response curves' of adrenaline and insulin are shown in Figs 5 and 6.

Adrenaline stimulates the lipolysis of adipose tissue, whereas insulin does not. Since the reduction of adrenaline's effect on glucose uptake in the presence of ethanol may be due to accummulation of FFA in adipose tissue and medium, the effect of insulin on glucose uptake was studied in the

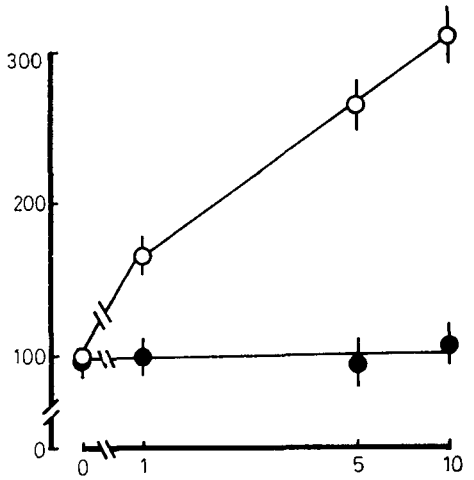

FIG. 5. Effect of ethanol on 'dose response curve' of adrenaline. Glucose uptake by control epididymal fat tissue is expressed as 100%. Each bar represents mean ± s.e.m. of six observations. ○——○, without ethanol; ●——●, with 3% of ethanol. Ordinate: % of initial glucose uptake. Abscissa: adrenaline concentration (μg ml^{-1}).

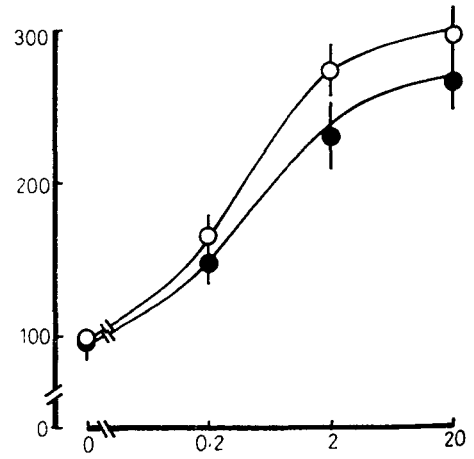

FIG. 6. Effect of ethanol on 'dose response curve' of insulin. Experiments conditions as Fig. 5, except for insulin. Ordinate: % of initial glucose uptake. Abscissa: insulin concentration (mU ml^{-1}).

presence of adrenaline. The results are in Table 2. FFA concentration in the medium was about 10 times control values. Insulin stimulated glucose uptake by adipose tissue even in the presence of high amounts of FFA, suggesting that the ethanol specifically inhibits the adrenaline-stimulated glucose uptake.

Table 2. Effect of adrenaline on insulin-stimulated glucose uptake in the presence of ethanol. Epididymal adipose tissue was incubated in the presence of 3% of ethanol. For other conditions, see legend for Table 1. Adrenaline, 5μg ml^{-1}. Insulin, 0·02 unit ml^{-1}.

Adrenaline	Control Glucose uptake	Insulin (mg g^{-1}/3h)	Net effect	P
−	1·5	4·8	+ 3·3 ± 0·20 (6)	0·001
+	1·4	4·6	+ 3·2 ± 0·20 (6)	0·001
	FFA release (μequiv g^{-1} 3h^{-1})			
−	1·6	0·7	− 0·9 ± 0·72 (6)	NS
+	14·9	12·7	− 2·2 ± 0·83 (6)	0·05

Since Luzio et al (1974) reported that phenoxybenzamine inhibited the stimulation of glucose uptake by adrenaline but not that by insulin and ACTH, the effect of ethanol on ACTH-stimulated glucose uptake was examined and found to have an inhibitory effect (Fig. 7).

Kono (1969) showed that the effect of insulin on glucose uptake diminished in the fat cells pretreated with trypsin. As shown in Table 3, neither insulin nor adrenaline stimulated glucose uptake in trypsin-treated adipose tissue, but adrenaline stimulated FFA release. Thus the glucose uptake mechanism stimulated by adrenaline also seems to be sensitive to trypsin digestion.

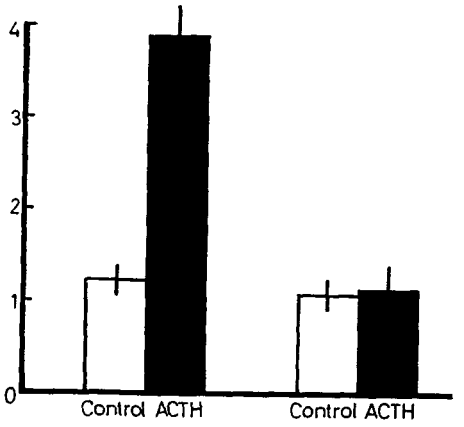

FIG. 7. Effect of ethanol on glucose uptake stimulated by ACTH (0·2 unit ml^{-1}). Each bar represents the mean ± s.e.m. of six observations on epididymal fat pads. Ordinate: glucose uptake (mg g^{-1} 3 h^{-1}).

Table 3. Disappearance of stimulatory effect of insulin and adrenaline on glucose uptake in trypsinized epididymal adipose tissue. Trypsin treatment: tissues were incubated with trypsin (1 mg ml^{-1}), then washed with the solution containing soybean trypsin inhibitor. For other conditions, see legend for Table 1 and 2.

Trypsin treatment	Glucose uptake (mg g^{-1}/3h) Control	Adrenaline	Net effect	P
−	1·6	4·0	+ 2·4 ± 0·40 (6)	< 0·01
+	1·3	1·4	+ 0·1 ± 0·32 (6)	NS
	Control	Insulin	Net effect	
−	1·4	4·3	+ 2·9 ± 0·41 (6)	< 0·001
+	1·4	1·6	+ 0·2 ± 0·31 (6)	NS
	FFA release (μeq g^{-1} 3h^{-1})			
	Control	Adrenaline	Net effect	
−	0·8	16·2	+ 15·4 ± 1·01 (6)	< 0·001
+	0·9	14·9	+ 14·0 ± 0·98 (6)	< 0·001

Conditions for the restoration of adrenaline's stimulation on glucose uptake

Experiments were designed to find conditions under which the stimulatory effect of adrenaline on glucose uptake reappeared.

Changing the incubation medium. Adipose tissues were first incubated for 2 h in medium containing 3% of ethanol but no glucose. Then they were transferred into fresh medium containing glucose but no ethanol, and incubated for 1 or 3 h. Adrenaline did not affect glucose uptake by the adipose tissue during the 1 h second incubation (Table 4), but it stimulated glucose uptake during the 3 h-second incubation.

Increased concentration of calcium. The concentration of calcium in Krebs-Ringer bicarbonate buffer solution is usually about 2·5 mM. When this is increased to 12·5 mM, although calcium phosphate precipitated a little, adrenaline stimulated the glucose uptake by adipose tissue even in the presence of 3% of ethanol to 50% of the control value (without ethanol) (Table 5).

Addition of β-blocker. Adrenaline stimulates glucose uptake even in the presence of ethanol when propranolol is added to the incubation medium. At 5μg ml^{-1}, propranolol reversed the inhibitory effect of ethanol on adrenaline-stimulated glucose

Table 4. Restoration of adrenaline-effect on glucose uptake by 3 h-incubation in the absence of ethanol. Epididymal adipose tissues preincubated with or without 3% ethanol for 2 h were transferred into fresh madium containing glucose and no ethanol. Other conditions as Table 1 and 2.

Preincubation (3% ethanol)	Incubation (h)	Glucose uptake (mg g^{-1}/3h) Control	Adren.	Net effect	P
−	1	1·3	2·4	+ 1·1 ± 0·38 (6)	< 0·05
+	1	1·5	1·4	− 0·1 ± 0·35 (6)	NS
+	3	2·7	4·3	+ 1·6 ± 0·36 (6)	< 0·01

Table 5. Effect of Ca ions on the inhibitory action of ethanol. Epididymal adipose tissues were incubated in the presence or absence of ethanol. Experimental conditions as in Table 1, except for the Ca^{2+} concentrations.

Ethanol (%)	Ca ions (mM)	Glucose uptake (mg g^{-1}/3h) Control	Adren.	Net effect	P
0	2·5	1·9	4·1	+ 2·2 ± 0·34 (6)	< 0·01
3	2·5	1·7	1·8	+ 0·1 ± 0·28 (6)	NS
3	12·5	1·6	2·5	+ 0·9 ± 0·32 (6)	< 0·05

uptake (Table 6). The effect was dose-dependent (Fig. 8), but above 30μg ml^{-1} propranolol did not stimulate the glucose uptake by adipose tissue incubated with ethanol.

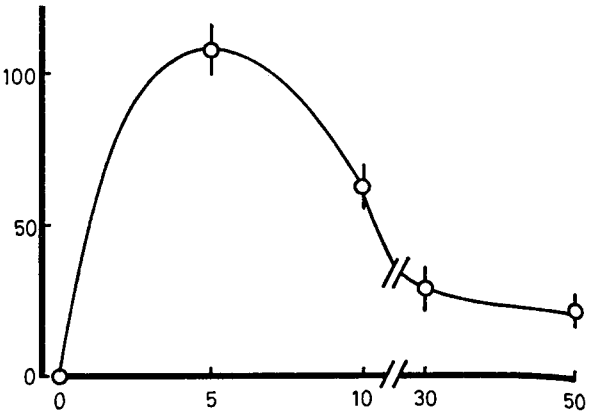

FIG. 8. Dose-dependent effect of propranolol (abscissa: μg ml^{-1}) on the inhibitory action of ethanol. Epididymal adipose tissue was incubated in the presence of ethanol. Concentration of propranolol; 0, 5, 10, 30, and 50μg ml^{-1}. Ordinate: recovery of adrenaline effect (%).

DISCUSSION

The present experiments show that the stimulatory effect of adrenaline on the uptake and oxidation of glucose in rat adipose tissue is diminished by the addition of ethanol to the incubation medium. Under the same conditions adrenaline still increased the release of FFA from the tissue. This suggests that the stimulation of uptake and oxidation of glucose by adrenaline is independent of its lipolytic effect in rat white adipose tissues.

Luzio et al (1974) demonstrated that the α-adrenoceptor blocking agent, phenoxybenzamine, inhibited the adrenaline-stimulated glucose uptake at a concentration that did not inhibit lipolysis and cyclic AMP formation in rat isolated fat cells. Saitoh et al (1974) also demonstrated that adrenaline stimulated glucose uptake and the accumulation of 3-*O*-methylglucose in the rat isolated diaphragm, this effect being blocked by an α-adrenoceptor blocking agent and potentiated by a β-adrenoceptor blocking agent added to the incubation medium. These results suggest that adrenaline-stimulated glucose uptake is mediated by α-receptors. Ethanol may inhibit stimulation of the α-receptor by adrenaline. However, this is unlikely because ethanol also inhibits the glucose uptake stimulated by ACTH, the action of which is independent of α-receptors.

Table 6. Effect of propranolol on the inhibitory action of ethanol. Epididymal adipose tissue was incubated. Other conditions as Table 1, except for the addition of 5μg ml^{-1} of propranolol.

Ethanol (%)	β-blocker (μg ml^{-1})	Glucose uptake (mg g^{-1}/3h) Control	Adren.	Net effect	P
0	0	2·2	5·1	+ 2·9 ± 0·29 (6)	0·001
3	0	2·0	2·5	+ 0·5 ± 0·31 (6)	NS
3	5	2·0	5·2	+ 3·2 ± 0·35 (6)	0·001

Lacko et al (1974) showed alcohols to inhibit the exchange transport of glucose in human erythrocytes, the degree of inhibition depending mainly upon the concentration of the alcohol in the membrane, and that the glucose transport system could bind two alcohol molecules. If this mechanism is active in adipose tissue, the glucose uptake stimulated by insulin should be also inhibited by ethanol, but it was not (Figs 3 and 4, Table 2).

Seeman et al (1971) found that a low concentration of ethanol (about the same as that I have used, 600mM) reduced the passive influx of Na^+, and a high concentration increased the passive Na^+ influx in frog sartorius muscle. This suggests that ethanol has some effects on the Na^+ pump and (Na^+-K^+)-ATPase. Hesketh et al (1976) showed that benzyl alcohol could fluidize the ring of lipids immediately surrounding penetrant proteins such as (Ca^{2+}-Mg^{2+})-ATPase and that this decrease in rigidity leads to activation of the enzyme, presumably by affecting the conformational flexibility of the protein. Dipple & Houslay (1978) also reported that benzyl alcohol progressively activated glucagon-stimulated adenylate cyclase from rat liver plasma membranes by modulating the fluidity of its lipid environment. These three results suggest that the effects of ethanol reported herein are due to its effect on membrane fluidity. Ethanol at the concentration used may dissociate the coupling of adrenoceptors to the glucose uptake system by modifying the rigidity of the membrane lipids.

This is likely because the addition of propranolol to the incubation medium restored the stimulatory

effect of adrenaline on glucose uptake by adipose tissue even in the presence of ethanol (Fig. 8 and Table 6). Lee (1977) has shown that propranolol has a membrane-fluidizing property. This seems to be active in the presence of ethanol. Ca ions also seem to stabilize the plasma membrane because the addition of Ca ions restored the action of adrenaline on glucose uptake of adipose tissue in the presence of ethanol (Table 5). Ca ions may interact with phospholipids in lipid bilayers in plasma membranes.

REFERENCES

Barker, S. B., Summerson, W. H. (1941) J. Biol. Chem. 138: 535–554

Blecher, M. (1967) Biochem. Biophys. Res. Commun. 27: 560–567

Blecher, M., Merlino, N. S., Ro'Ane, J. T., Flynn, P. D. (1969) J. Biol. Chem. 244: 3423–3429

Bray, G. A., Goodman, H. M. (1968) J. Lipid Res. 9: 714–719

Cahill, Jr., G. F., Leboeuf, B., Flinn, R. B. (1960) J. Biol. Chem. 235: 1246–1250

Dipple, I., Houslay, M. D. (1978) Biochem J. 174: 179–190

Hesketh, T. R., Smith, G. A., Houslay, M. D., McGill, K. A., Birdsall, N. J. M., Metcalfe, J. C., Warren, G. B. (1976) Biochemistry 15: 4145–4151

Hugget, A. G., Nixon, D. A. (1957) Biochem. J. 66: 12P

Itaya, K. (1977) J. Lipid Res. 18: 663–665

Itaya, K. (1978) J. Pharm. Pharmacol. 30: 632–637

Itaya, K., Ui, M. (1965) J. Lipid Res. 6: 16–20

Kono, T. (1969) J. Biol. Chem. 244: 1772–1778

Lacko, L., Wittke, B., Geck, P. (1974) J. Cell Physiol. 83: 267–274

Lee, A. G. (1977) Mol. Pharmacol. 13: 474–487

Luzio, J. P., Jones, R. C., Siddle, K., Hales, C. N. (1974) Biochim. Biophys. Acta 362: 29–36

Saitoh, Y., Itaya, K., Ui, M. (1974), Ibid. 343: 492–499

Seeman, P., Kwant, W. O., Goldberg, M., Chan-Wong, M. (1971) Ibid. 241: 349–355