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THE RELATIONSHIP BETWEEN THE TRANSPORT OF GLUCOSE AND CATIONS ACROSS CELL MEMBRANES IN ISOLATED TISSUES

VIII. THE EFFECT OF MEMBRANE STABILIZERS ON THE TRANSPORT OF K^+, Na^+ AND GLUCOSE IN MUSCLE, ADIPOCYTES AND ERYTHROCYTES

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SUMMARY

1. The effects of various categories of membrane stabilizers on Na^+–K^+ fluxes and the transport and metabolism of glucose have been characterized in *in vitro* experiments with soleus muscles, whole epididymal fat pads, isolated fat cells and erythrocytes prepared from fed rats.

2. Within a certain concentration range, tetracaine, lidocaine, chlorpromazine, imipramine and thiomebumal were all found to induce a prompt and marked decrease in the release of ^{42}K from preloaded soleus muscles. Higher concentrations induced K^+ loss and lysis of the cells.

3. When present at concentrations where the ^{42}K release was inhibited the abovementioned compounds suppressed or abolished the stimulating effect of insulin, hyperosmolarity and trypsin on 3-*O*-methylglucose transport and glucose uptake in soleus muscles and epididymal fat pads. In these tissues, the basal rates of glucose uptake or 3-*O*-methylglucose transport were not significantly diminished. In erythrocytes, both influx and efflux of 3-*O*-methylglucose was inhibited by relatively high concentrations of tetracaine.

4. The conversion of D-[$^{14}C_6$]glucose into glycogen (in muscle) or CO_2 and triglycerides (in isolated fat cells) was considerably inhibited by tetracaine and lidocaine, both in the absence and in the presence of insulin.

5. The effect of the membrane stabilizers tested on sugar transport does not appear to be the result of altered Na^+–K^+ distribution across the plasma membrane, and the inhibitory effects on K^+ efflux and sugar transport are not strictly correlated. The inhibitory effects on these transport processes rather seem to be two different manifestations (with different thresholds) of an increased overall structural stability of the plasma membrane.

6. It is concluded that membrane stabilizers (or the phenomenon of membrane stabilization) are of importance not only for the exchange of water and electrolytes, but that the transport and metabolism of glucose in the major targets for insulin

action may be quite markedly suppressed by several of the drugs belonging to this wide category of compounds.

INTRODUCTION

Recently, Hales[1] and Hales and Perry[2] demonstrated that local anesthetics inhibit the insulin-stimulated metabolism of glucose in isolated fat cells. These drugs diminish the excitability of muscle and nerve by reducing the permeability of the plasma membrane to cations[3-6], and their effect on glucose metabolism suggested that cations might be involved in sugar transport or the activation of the sugar transport system. The commonly used local anesthetics belong to a large category of chemically diverse compounds which share the property of reducing the permeability to cations and water in a variety of tissues[7-13].

Some of these membrane stabilizers (phenothiazines[14-16], diphenylhydantoin[17,18] and barbiturates[19,20]) have been found to decrease glucose tolerance or to produce hyperglycemia. The phenothiazine derivatives have already a long time ago been shown to decrease glucose uptake in rat hemidiaphragm[21], and recently it was reported that the antiepileptic diphenylhydantoin inhibits the uptake of 3-*O*-methylglucose in the isolated intact rat diaphragm muscle[22].

This suggested that the phenomenon of membrane stabilization is not only of importance for the exchange of water and electrolytes, but that carrier-mediated transport of sugars might be influenced also, perhaps as a secondary phenomenon. As a part of the study of relationships between the transport of cations and glucose, and in order to determine whether membrane stabilization is of more general significance for the transport and metabolism of glucose, the effect of various types of membrane stabilizers was tested using three different cell types. This report presents experiments with tetracaine, lidocaine, chlorpromazine, imipramine and thiomebumal, which were selected because they have been widely used in experimental and clinical work, and at the same time represent different categories of membrane stabilizers.

In muscle and adipocytes, these compounds were found to cause little if any suppression of basal 3-*O*-methylglucose transport. However, in erythrocytes, and in soleus muscle and epididymal fat pads stimulated by insulin, hyperosmolarity or trypsin, they were all found to cause a prompt and marked suppression of 3-*O*-methylglucose transport. The collective evidence indicates that the function of the glucose transport system and in particular its activation is impaired by membrane stabilizers, and that this effect is not directly related to changes in the distribution or fluxes of Na^+ and K^+ across the plasma membrane.

METHODS

Experiments with soleus muscles

All soleus muscles were prepared from fed Wistar rats in the weight range 60–70 g. The procedures for the isolation of intact muscles, the measurement of uptake and release of 3-*O*-methylglucose, inulin space, K^+ content, glucose uptake and the incorporation of D-[$^{14}C_6$]glucose into glycogen have been described in detail in earlier reports in this series[23-25]. The efflux of K^+ was assessed by loading

the muscles for 60 min in Krebs–Ringer bicarbonate buffer[26] containing 3 μCi/ml of $^{42}K^+$ and the same concentration of K^+ as the standard buffer, *i.e.* 5.93 mM. The washout of ^{42}K activity was followed using the same technique as for the measurements of 3-*O*-[^{14}C]methylglucose release, with the sole difference that the tubes used for incubation during the washout periods were directly counted in a Packard Auto-gamma spectrometer. The ^{42}K activity of the muscles was determined under the same counting conditions, and after correction for the decay of ^{42}K, the rate coefficient of $^{42}K^+$ release was calculated as earlier described[27].

The initial rate of Na^+ influx was assessed by measuring the amount of $^{22}Na^+$ taken up during a 10-min incubation at 30 °C. In order to obtain a more precise determination of a unidirectional flux, the incubation took place in the presence of ouabain ($1 \cdot 10^{-3}$ M) and was followed by a wash at 0 °C, during which a major part of the extracellular $^{22}Na^+$ was removed without significant losses of the $^{22}Na^+$ taken up in the intracellular space. Separate control experiments (Kohn, P. G. and Clausen, T., unpublished) showed that under steady-state conditions (constant Na^+ content in the muscles) the rate of Na^+ efflux was almost identical with the rate of Na^+ influx determined by the above mentioned method. The ^{22}Na activity of the muscles was determined in a Packard Auto-gamma spectrometer and expressed as μmoles of Na^+ taken up/g wet weight per min.

Experiments with isolated fat cells and epididymal fat pads

Isolated fat cells were prepared by collagenase treatment of epididymal fat pads from fed Wistar rats weighing 100–120 g[28]. After washing (5 times) in Krebs–Ringer bicarbonate buffer containing 1% dialyzed bovine serum albumin and 2.5 mM D-glucose, the cells were distributed with an automatic polyethylene pipette (Eppendorf) into plastic counting vials containing 2 ml of the same buffer with 0.1 μCi/ml of D-[$^{14}C_6$]glucose and the additions indicated in the legend. After 60 min of incubation at 37 °C in a Gallenkamp metabolic agitator moving 80 cycles/min, the amount of ^{14}C activity incorporated into CO_2 and triglycerides was determined as described elsewhere[29,30].

The method used for the measurement of 3-*O*-methylglucose efflux from whole epididymal fat pads has been described in an earlier report[27].

Experiments with erythrocytes

The rats used for the preparation of soleus muscles were killed by decapitation, and the blood from the neck vessels was directly collected and immediately mixed with heparin–saline (20 I.U./ml). The blood was mixed with Krebs–Ringer bicarbonate buffer and the erythrocytes separated by centrifugation at $2000 \times g$ for 10 min. After aspiration of the supernatant and the "buffy coat", the erythrocytes were washed twice in a large (10-fold) volume of Krebs–Ringer bicarbonate buffer, and then incubated at 30 °C in a volume of 2–5 ml containing 3-*O*-[^{14}C]methylglucose (final hematocrit 5–10%).

Aliquots of 300 μl were withdrawn from the suspension and centrifuged for 45 s at $10000 \times g$ in a Beckman microcentrifuge (catalogue No. 314300). The tip of the polyethylene centrifuge tube containing the erythrocytes was cut off and after suspension in 5% trichloroacetic acid and centrifugation, the ^{14}C activity of the clear colorless supernatant was determined in a liquid scintillation spectrometer.

After correction for wet weight and trapped extracellular fluid, the amount of 3-*O*-[^{14}C]methylglucose taken up or retained in the erythrocytes was calculated and expressed as μmoles/g of cells or as per cent of the initial content. The validity of this method was checked in recovery experiments with simultaneous measurements of the changes in the ^{14}C activity of the extracellular phase.

Chemicals, isotopes and hormones

All chemicals were of analytical grade. 3-*O*-Methylglucose was obtained from Calbiochem (Los Angeles), pancreatic trypsin (EC 3.4.4.4) from Nutritional Biochemicals Corp. (Cleveland), and the reagents for the enzymatic determination of glucose from Kabi (Uppsala). 3-*O*-[^{14}C]Methylglucose (spec. act. 50 Ci/mole), [hydroxymethyl-^{14}C]inulin (spec. act. 9 Ci/mole), D-[$^{14}C_6$]glucose (spec. act. 335 Ci/mole), and ^{22}Na (spec. act. 3 Ci/mmole) were products of the Radiochemical Centre, Amersham, England. ^{42}K (spec. act. 100 Ci/mole) was purchased from the Danish Atomic Energy Commission, Isotope Laboratory, Risø. Mono-component pork insulin lot No. MC-S-970 (25 I.U./mg, purified by chromatography) was a gift from the Novo Research Laboratories (Copenhagen).

RESULTS

Electrolyte distribution

Although the membrane stabilizers used in the present study have been shown to alter the permeability to cations in a variety of tissues[7–12], there is no information available about their effects on the isolated rat soleus muscle. Therefore, the changes in Na^+–K^+ transport and K^+ content induced by tetracaine, lidocaine, chlorpromazine, imipramine and thiomebumal were characterized in preliminary experiments. From Figs 1 and 2 it can be seen that within a certain concentration range, all of the compounds tested produced a rather prompt suppression of the washout of $^{42}K^+$ from preloaded muscles. In some cases (in particular at the higher concentrations of the drugs) this decrease was followed by a rise towards (or above) the control level. This is illustrated in detail for tetracaine, which was the most frequently used drug in the present study (Fig. 2). It can be seen that the release of $^{42}K^+$ is inhibited by concentrations down to 0.1 mM. 0.5 mM was found to produce the same decrease as 1.0 mM, and may therefore represent the level of maximal response. With 4 mM of the local anesthetic, the inhibitory effect was only seen within the first 10 min after its addition, whereas in all of the following efflux tubes there was a rapid loss of ^{42}K activity from the muscles.

From Fig. 3 it appears that upon 60 min of exposure to tetracaine, lidocaine or chlorpromazine the K^+ content may be slightly elevated at the lower concentrations of the drugs. Higher levels produce a loss of K^+, which may be even more pronounced than that induced by ouabain ($1 \cdot 10^{-3}$ M) within the same time interval (K^+ content: 55.0 ± 1.9 μmoles/g wet weight)[23].

The data in Table I provide further evidence for an inhibitory effect of membrane stabilizers on cation transport. It appears that the uptake of $^{22}Na^+$ is suppressed by tetracaine in a concentration range extending considerably below that which was found to inhibit $^{42}K^+$ efflux. At the highest concentrations (2–3 mM), the values for $^{22}Na^+$ uptake tend to rise towards the control level. This may be

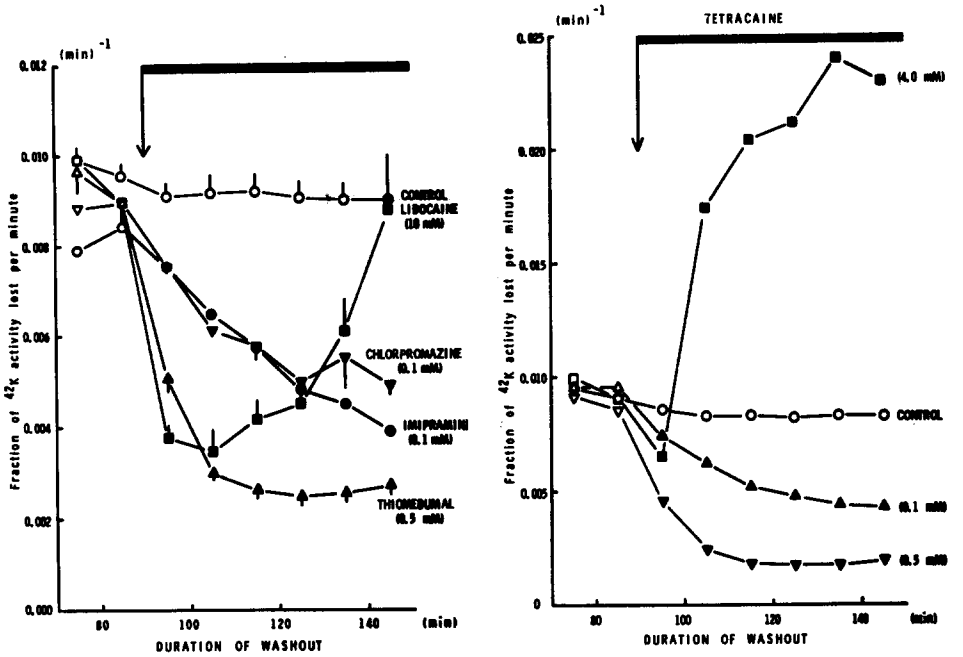

Fig. 1. Effect of lidocaine, thiomebumal, chlorpromazine and imipramine on the rate coefficient of $^{42}K^+$ release from rat soleus muscle. The muscles were loaded in Krebs-Ringer bicarbonate buffer containing 1 mM pyruvate and $^{42}K^+$ (3 μCi/ml) for 60 min at 30 °C. They were then washed out into a series of tubes containing unlabelled buffer with pyruvate (1 mM). The fraction of ^{42}K activity lost per min is shown as a function of the washout time. ○—○, controls; ■—■, lidocaine (10 mM); ▲—▲, thiomebumal (0.5 mM); ▼—▼, chlorpromazine (0.1 mM); ●—●, imipramine (0.1 mM). Each point represents the mean of 3–6 observations and vertical bars indicate S.E. where this exceeds the size of the symbols.

Fig. 2. Effect of tetracaine on the rate coefficient of $^{42}K^+$ release from rat soleus muscle. Details as for Fig. 1. ○—○, controls; ▲—▲, tetracaine (0.1 mM); ▼—▼, tetracaine (0.5 mM); ■—■, tetracaine (4.0 mM). Each point represents the mean of 2–6 observations.

the result of a nonspecific increase in the overall permeability of the plasma membrane. It should be noted that in the presence of tetracaine, the muscles developed contractures. At the high concentrations (2–4 mM), these were apparent within the first 10 min of exposure, whereas with 0.5 mM, contractures were only occasionally seen after a period of 60–90 min.

Similar effects were produced by lidocaine (15–20 mM), chlorpromazine (0.25–1.0 mM) and imipramine (0.5–1.0 mM). In the frog sartorius muscle, lidocaine (3–7 mM) has been shown to produce a gradual increase in resting tension, which was associated with a rise in $^{45}Ca^{2+}$ efflux[31], and it seems likely that in rat soleus muscle also, the membrane stabilizers may gain access to the cytoplasm and interfere with the accumulation of Ca^{2+} in the sarcoplasmic reticulum.

The transport and metabolism of glucose in muscle

The following graphs illustrate the effects of the membrane stabilizors on the transport and metabolism of glucose in muscle, adipocytes and erythrocytes. From

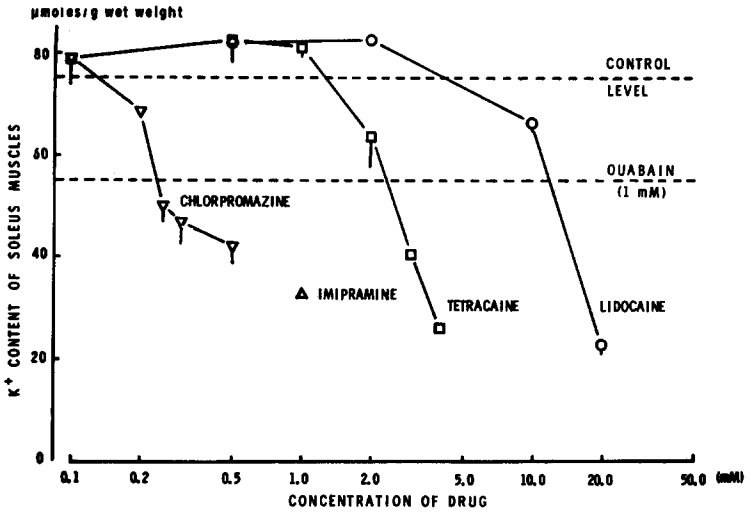

Fig. 3. Effect of chlorpromazine, imipramine, tetracaine, lidocaine and ouabain on the K^+ content of rat soleus muscle. Details as for Fig. 1. 90 min after the onset of washout, various concentrations of the drugs indicated were added to the efflux medium, and following further 60 min of washout the K^+ content of the muscles was determined by flame photometry. Each point represents the mean of 3–12 observations and vertical bars indicate S.E.

TABLE I

THE EFFECT OF TETRACAINE ON $^{22}Na^+$ UPTAKE IN SOLEUS MUSCLE

The muscles were incubated at 30 °C for 15 min in 2 ml Krebs–Ringer bicarbonate buffer containing 1 mM pyruvate, 1 mM ouabain without or with tetracaine at the concentrations indicated. $^{22}Na^+$ was then added to the incubation medium and after 10 min of exposure to the label, the muscles were quickly blotted on wet filter paper mounted on an ice-block and transferred to ice-cold buffer without label. After three washes of 10-min duration in ice-cold buffer, the amount of $^{22}Na^+$ activity retained was determined and expressed as μmoles of Na^+ on the basis of the specific activity of extracellular $^{22}Na^+$ during the 10-min exposure to the label.

Incubation medium Krebs–Ringer bicarbonate buffer with ouabain (1 mM)	*$^{22}Na^+$ uptake (μmoles/g per min) (mean ± S.E.)*	*No. of experiments*	*Significance P of difference between control and experimental*
Control	0.762 ± 0.036	(8)	—
Tetracaine (0.005 mM)	0.628 ± 0.023	(3)	> 0.05
Tetracaine (0.01 mM)	0.574 ± 0.017	(5)	< 0.01
Tetracaine (0.05 mM)	0.493 ± 0.010	(6)	< 0.01
Tetracaine (0.1 mM)	0.469 ± 0.027	(6)	< 0.01
Tetracaine (0.5 mM)	0.452 ± 0.035	(3)	< 0.01
Tetracaine (1 mM)	0.446 ± 0.023	(3)	< 0.01
Tetracaine (2 mM)	0.569 ± 0.030	(3)	< 0.02
Tetracaine (3 mM)	0.556 ± 0.041	(3)	< 0.02

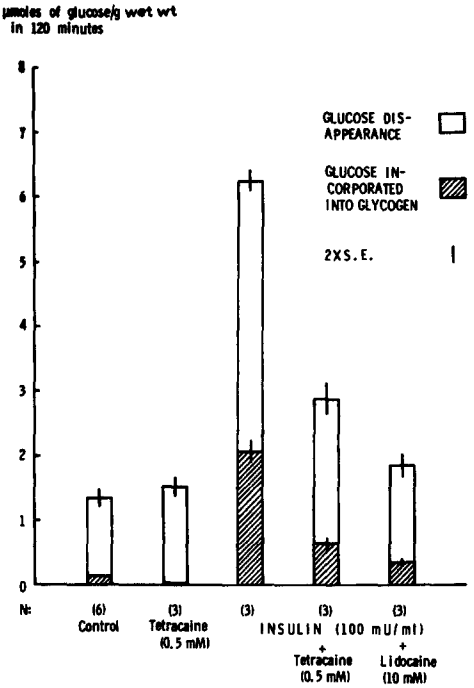

Fig. 4. Effect of tetracaine, lidocaine and insulin on glucose uptake and its incorporation into glycogen by rat soleus muscle. Soleus muscles were incubated for 120 min at 30 °C in 1 ml of Krebs–Ringer bicarbonate buffer containing 1 mM pyruvate and 1 mM D-[$^{14}C_6$]glucose (0.2 μCi/ml) without or with the additions indicated. Uptake of glucose is expressed as μmoles/g wet wt of tissue disappearing from the incubation medium, and the fraction hereof incorporated into glycogen is indicated by the hatched parts of the columns. The column heights represent the values from (N) observations and 2×S.E. is denoted by the vertical bars.

TABLE II

EFFECT OF TETRACAINE ON THE INCORPORATION OF ^{14}C ACTIVITY INTO GLYCOGEN *IN VIVO*

24-h fasted Wistar rats (90–110 g) were given an intraperitoneal injection of 1 ml 154 mM NaCl without (controls) or with tetracaine (4 mM). 10 min later, 1 ml of a solution containing 5.6 mM D-[$^{14}C_6$]glucose (0.5 μCi/ml) and 1 munit/ml of insulin was injected intraperitoneally. 60 min after the second injection, the animals were killed and the diaphragms excised and processed for the determination of ^{14}C activity in total glycogen[25]. The results are given as cpm per g wet weight of diaphragm muscle with the number of observations in parentheses.

	^{14}C activity in glycogen (cpm/g wet weight)		*Significance P of difference*
Controls	36516±3785	(6)	<0.005
Tetracaine-treated	20766±2438	(6)	

Fig. 4 it appears that whereas tetracaine (0.5 mM) produced no significant change in the basal rate of glucose uptake in rat soleus muscle, the stimulating effect of a supramaximal dose of insulin (100 munits/ml) was considerably suppressed. Lidocaine was found to cause a slightly more marked suppression when added at a 20-fold higher concentration. Both tetracaine and lidocaine produced a substantial decrease in the incorporation of D-[$^{14}C_6$]glucose into glycogen, both in the absence and in the presence of insulin.

The data shown in Table II indicate that also under *in vivo* conditions tetracaine suppresses the incorporation of ^{14}C activity from D-[$^{14}C_6$]glucose into the glycogen of diaphragm muscle. In order to characterize the effects of membrane stabilizers

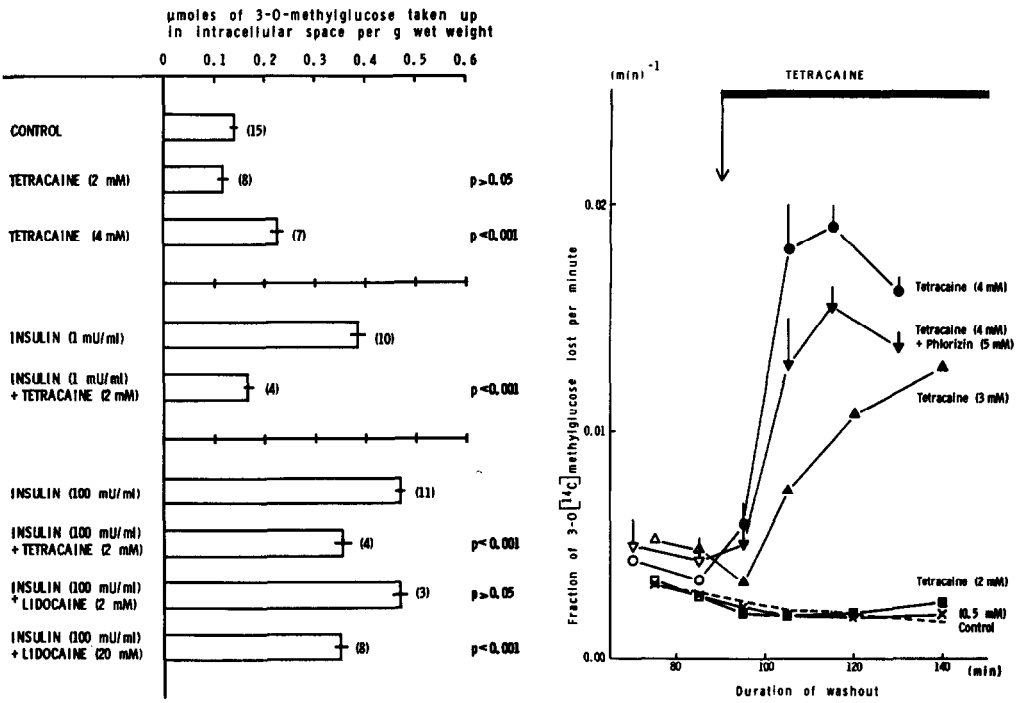

Fig. 5. Effects of tetracaine, lidocaine and insulin on the uptake of 3-*O*-methylglucose by rat soleus muscle. Soleus muscles were incubated for 60 min at 30 °C in 2 ml of Krebs–Ringer bicarbonate buffer containing 1 mM pyruvate, 1 mM 3-*O*-[^{14}C]methylglucose without or with the additions indicated. The columns indicate the uptake of 3-*O*-methylglucose into the space not available to inulin. The number of observations is given in parentheses and 2×S.E. is denoted by the horizontal bars. The significance of the difference between the uptake obtained in the absence and in the presence of local anesthetics has been assessed by calculating *P* values for the experiments without or with insulin (1 or 100 munits/ml), respectively.

Fig. 6. Effects of tetracaine and phlorizin on the rate coefficient for 3-*O*-methylglucose release from soleus muscle. The muscles were loaded in Krebs–Ringer bicarbonate buffer containing 1 mM pyruvate and 1 mM 3-*O*-[^{14}C]methylglucose (1 μCi/ml) for 60 min at 30 °C. They were then washed out into a series of tubes containing buffer with pyruvate (1 mM), but no 3-*O*-methylglucose. The fraction of ^{14}C activity lost per min is shown as a function of time. Each point represents the mean of 3–11 observations, and vertical bars indicate S.E. where this exceeds the size of the symbols. — — —, controls; ×—×, tetracaine (0.5 mM); ■—■, tetracaine (2 mM); ▲—▲, tetracaine (3 mM); ●—●, tetracaine (4 mM); ▼—▼, tetracaine (4 mM) and phlorizin (5 mM).

on the processes of glucose transport, the next series of experiments were performed using the non-metabolized sugar 3-*O*-methylglucose, which in earlier studies with soleus muscles had been found to be transported by a process closely similar to that mediating the uptake of glucose[23].

From Fig. 5 it appears that whereas 2 mM of tetracaine produces a barely significant inhibition of the basal uptake of 3-*O*-methylglucose, the same concentration of the drug abolishes the stimulating effect of a submaximal concentration of insulin (1 munit/ml) and suppresses the rise produced by supramaximal levels of the hormone (100 munits/ml). Again, lidocaine is a considerably less potent inhibitor of insulin-stimulated sugar transport. Other experiments (data not presented) showed that also at a considerably higher concentration of 3-*O*-methylglucose (20 mM), tetracaine (2 mM) almost abolished the effect of insulin (1 munit/ml) without producing any significant change in the basal uptake of 3-*O*-methylglucose.

On the other hand, 4 mM of tetracaine was found to stimulate the uptake of 3-*O*-methylglucose. This, together with the complex response of $^{42}K^+$ efflux illustrated above, made it desirable to evaluate the effect of membrane stabilizers by following the time-course of 3-*O*-methylglucose release, which has earlier been shown to yield a very sensitive parameter for sugar permeability[23].

Fig. 6 shows the effect of tetracaine (0.5–4 mM) on the washout of ^{14}C-labelled 3-*O*-methylglucose from soleus muscles which had been preloaded with the sugar for 60 min. At a concentration (0.5 mM), where the local anesthetic had been shown to produce a clear suppression of $^{42}K^+$ washout, the release of 3-*O*-methylglucose is not significantly altered. At the higher concentrations, however, (3–4 mM), where a marked rise in $^{42}K^+$ release (and K^+ loss) is seen, there is a considerable increase in the rate coefficient of 3-*O*-methylglucose washout. This increase is only slightly diminished by phlorizin (5 mM), which was otherwise found to abolish the effect of a variety of contitions known to stimulate the efflux of 3-*O*-methylglucose[23]. A high concentration of lidocaine (20 mM) was found to produce a similar (phlorizin-resistant) and marked rise in the release of 3-*O*-methylglucose.

This indicates that the stimulating effect of high concentrations of local anesthetics on the release (and uptake, Fig. 5) of 3-*O*-methylglucose does for a major part not reflect a rise in carrier-mediated transport, but should rather be considered as the result of a nonspecific loss of plasma membrane integrity, a phenomenon which may also account for the considerable loss of K^+ described above (Fig. 3). This is not so surprising in view of the experience that high (so-called toxic) concentrations of local anesthetics produce lysis of erythrocytes and irreversible upheaval of action potential propagation in nerves[32,33].

Whereas tetracaine and lidocaine caused little or no inhibition neither of basal glucose uptake, nor of basal influx and efflux of 3-*O*-methylglucose, they were consistently found to suppress the stimulating effect of insulin (and other factors) on all of these processes. Fig. 7 shows that the marked stimulation produced by a supramaximal dose of the hormone can be almost entirely abolished by tetracaine (2.0 mM). As in the uptake experiments (Fig. 5), it can be seen that tetracaine – as compared to lidocaine – is a considerably more potent inhibitor of insulin-stimulated 3-*O*-methylglucose release. Thus 0.5 mM tetracaine causes the same decrease (about 50%) as 10 mM lidocaine. The relative anesthetic potency of these two drugs has been shown to differ by a factor of 10 (ref. 34). It should be noted that although

0.1 mM tetracaine produced a clear suppression of $^{42}K^+$ release, the stimulating effect of insulin (1 munit/ml) was not significantly altered by this concentration of the drug.

In order to determine whether the inhibitory effect of local anesthetics on sugar transport were related to a rise in the K^+ content of the tissue, the effect of tetracaine was tested in the presence of ouabain. The decrease in insulin-stimulated (100 munits/ml) 3-*O*-methylglucose efflux induced by tetracaine was not significantly altered by ouabain (1 mM). Since the final K^+ content of the muscles was 33.5 ± 1.4 μmoles/g wet wt, it seems unlikely that the inhibition was the outcome of a rise in the intracellular K^+ concentration over and above that found in untreated muscles. Furthermore, these experiments exclude the possibility that the effect of tetracaine is related to changes in the rate of active Na^+–K^+ transport.

Since several reports indicate that Ca^{2+} is of significance for the effect of local anesthetics on excitability and Na^+ transport[35], the extracellular Ca^{2+} concentration was varied in a series of experiments. This caused no significant change in the response to tetracaine, and even in a Ca^{2+}-free milieu containing 0.5 mM EGTA, 1 mM of the local anesthetic suppressed the stimulating effect of insulin (100 munits/ml) on 3-*O*-methylglucose release to almost exactly the same level as in buffers containing 1.27 or 2.54 mM Ca^{2+}.

The specificity of the effect was evaluated using other stimuli for the efflux of 3-*O*-methylglucose.

From Figs 8 and 9 it can be seen that the increase in 3-*O*-methylglucose release produced by hyperosmolarity (mannitol, 200 mM) or trypsin (1 mg/ml), is also suppressed by tetracaine (2.0 mM). It should be noted that the inhibitory effect of

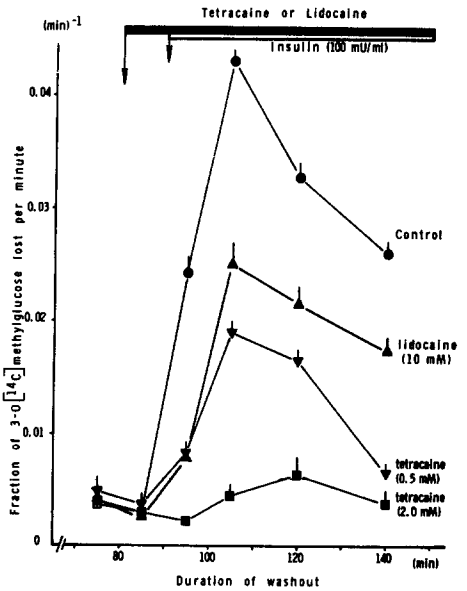

Fig. 7. Effects of tetracaine, lidocaine and insulin on the rate coefficient for 3-*O*-methylglucose release from soleus muscle. Details as for Fig. 6. ●—●, insulin (100 munits/ml) alone; ▼—▼, tetracaine (0.5 mM) and insulin (100 munits/ml); ■—■, tetracaine (2.0 mM) and insulin (100 munits/ml); ▲—▲, lidocaine (10 mM) and insulin (100 munits/ml). Each point represents the mean of 3–6 observations.

the membrane stabilizers on insulin-stimulated 3-*O*-methylglucose release has a very short time lag. This is illustrated in Fig. 10, from which it appears that within 10 min after the onset of exposure to tetracaine (2.0 mM) or thiomebumal (0.5 mM), the stimulating effect of insulin (1 munit/ml) was clearly suppressed. The other membrane stabilizers (chlorpromazine and imipramine) which had been shown to inhibit the efflux of $^{42}K^+$ were also found to suppress the stimulating effect of insulin

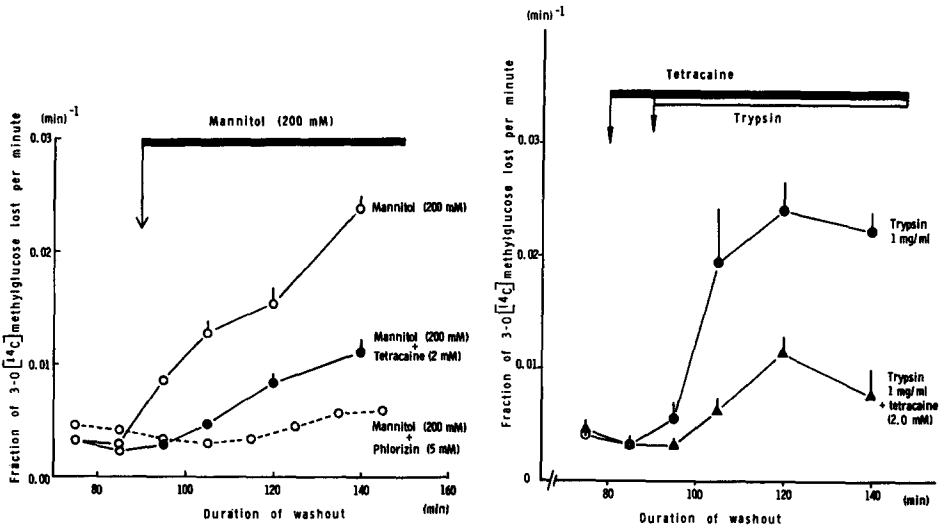

Fig. 8. Effects of hyperosmolarity, phlorizin and tetracaine on the rate coefficient for 3-*O*-methylglucose release from soleus muscle. Details as for Fig. 6. ○—○, mannitol (200 mM) alone; ○----○, mannitol (200 mM) and phlorizin (5 mM); ●—●, mannitol (200 mM) and tetracaine (2 mM). Each point represents the mean of 3–6 observations.

Fig. 9. Effects of trypsin and tetracaine on the rate coefficient for 3-*O*-methylglucose release from soleus muscle. Details as for Fig. 6. ●—●, trypsin (1 mg/ml) alone; ▼—▼, trypsin (1 mg/ml) and tetracaine (2 mM). Each point represents the mean of 3–6 observations.

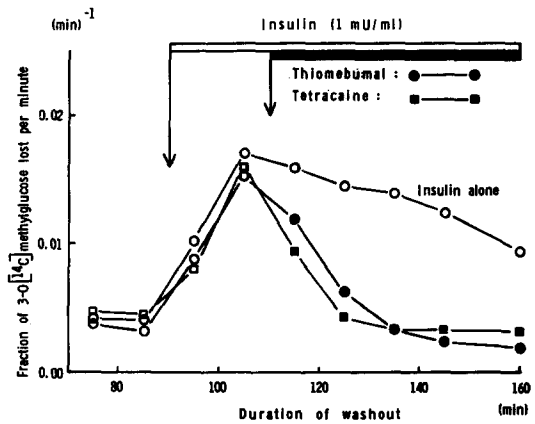

Fig. 10. Effects of tetracaine and thiomebumal on insulin-stimulated release of 3-*O*-methylglucose from soleus muscle. Details as for Fig. 6. ○—○, insulin (1 munit/ml) alone; ●—●, thiomebumal (0.5 mM) and insulin (1 munit/ml); ■—■, tetracaine (2 mM) and insulin (1 munit/ml). Each point represents the mean of 2–3 observations.

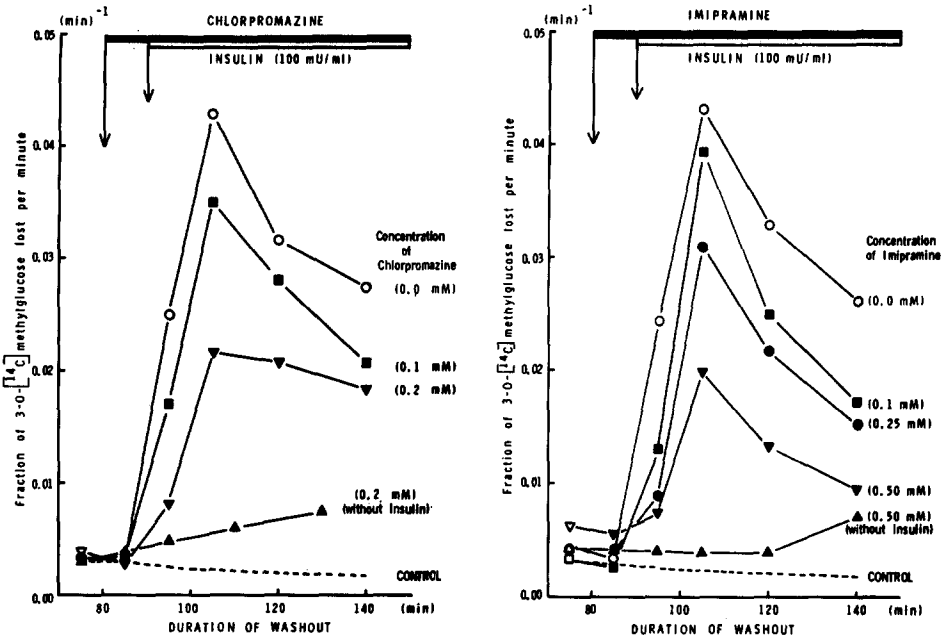

Fig. 11. Effects of chlorpromazine and insulin on the rate coefficient for 3-*O*-methylglucose release from soleus muscle. Details as for Fig. 6. ○—○, insulin (100 munits/ml) alone; ■—■, chlorpromazine (0.1 mM) and insulin (100 munits/ml); ▼—▼, chlorpromazine (0.2 mM) and insulin (100 munits/ml); ▲—▲, chlorpromazine (0.2 mM) alone; ----, controls. Each curve represents the mean of 3 observations.

Fig. 12. Effects of imipramine and insulin on the rate coefficient for 3-*O*-methylglucose release from soleus muscle. Details as for Fig. 6. ○—○, insulin (100 munits/ml) alone; ■—■, imipramine (0.1 mM) and insulin (100 munits/ml); ●—●, imipramine (0.25 mM) and insulin (100 munits/ml); ▼—▼, imipramine (0.5 mM) and insulin (100 munits/ml); ▲—▲, imipramine (0.5 mM) alone; ----, controls. Each curve represents the mean of 3 observations.

on 3-*O*-methylglucose release in a dose-dependent fashion (Figs 11 and 12). Like the local anesthetics, the higher concentrations of chlorpromazine (0.2–0.5 mM) and imipramine (0.5–1.0 mM) were found to produce a progressive stimulation of the basal rate of 3-*O*-methylglucose release. Again, this effect might be the outcome of impaired integrity of the plasma membrane, a conclusion also supported by the fact that at these concentration levels the drugs caused a considerable loss of K^+ (Fig. 3).

As noted for the local anesthetics, the inhibitory effects of chlorpromazine and thiomebumal, were not specifically related to the action of insulin, since both compounds brought about a significant decrease in the hyperosmolarity-stimulated 3-*O*-methylglucose release.

Adipocytes

In isolated fat cells, tetracaine and lidocaine were found to suppress not only the effect of insulin or hyperosmolarity on glucose metabolism, but also the basal conversion of glucose into CO_2 and triglycerides (Fig. 13). It should be noted that when present at high concentrations, both tetracaine (4 mM) and lidocaine (10 mM)

blocked the metabolism of glucose completely. This together with the fact that relatively low concentrations of tetracaine (1.0 mM) and lidocaine (2.0 mM) produced a marked inhibition of basal glucose metabolism also, suggests that adipocytes are more susceptible and respond more promptly to the action of local anesthetics. However, the higher temperature (37 °C) might have contributed to the accentuated response.

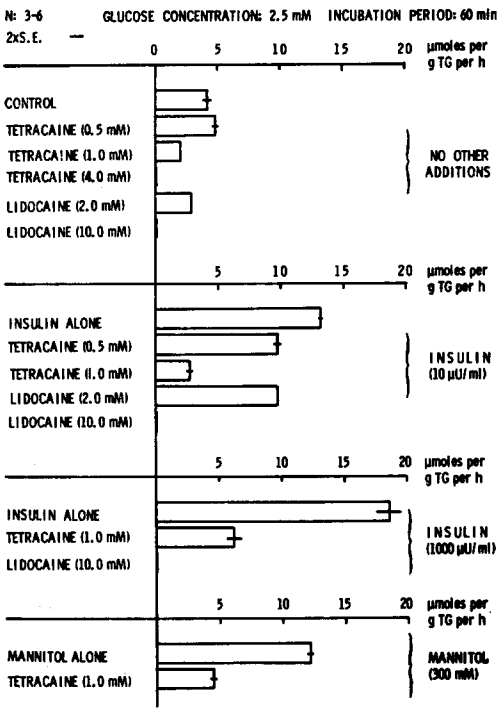

Fig. 13. Effects of tetracaine, lidocaine, insulin and mannitol on glucose metabolism in isolated fat cells. Isolated fat cells were prepared from epididymal fat pads and suspended in 2 ml Krebs–Ringer bicarbonate buffer (10 mg cells per ml) containing bovine serum albumin (1%) and 2.5 mM D-[$^{14}C_6$]glucose (0.2 μCi/ml) without or with the additions indicated. After incubation for 60 min at 37 °C, the metabolism was arrested by the addition of 200 μl 5 M H_2SO_4 and the amount of ^{14}C activity converted into CO_2 and triglycerides determined. The columns indicate the amount of D-[$^{14}C_6$]glucose converted into CO_2 and triglycerides (μmoles/g triglycerides (TG) per h). Each value represents the mean of 3–6 observations and 2 × S.E. is denoted by the horizontal bars.

Since these experiments yielded little direct information about the changes in glucose transport, another series of experiments were performed in order to assess the effect of tetracaine and lidocaine on the permeability to 3-*O*-methylglucose in adipocytes. As can be seen from Figs 14 and 15, neither of the two compounds produced any inhibition of the basal rate of 3-*O*-methylglucose release from whole epididymal fat pads, but both suppressed the stimulating effect of insulin on this process. Again, tetracaine seems to be considerably more potent than lidocaine. At variance with the results obtained by measuring glucose metabolism in the isolated fat cells, lidocaine alone caused a delayed rise in sugar transport. Also in the presence

of insulin, lidocaine and tetracaine induced this late increase, which was associated with a loss of K^+ of 62 and 53%, respectively ($P < 0.001$). Thus it seems likely that the pronounced inhibition of basal and insulin-stimulated glucose metabolism seen in the isolated fat cells exposed to these compounds may be the result of partial or total loss of cell integrity and ensuing impairment of glucose metabolism.

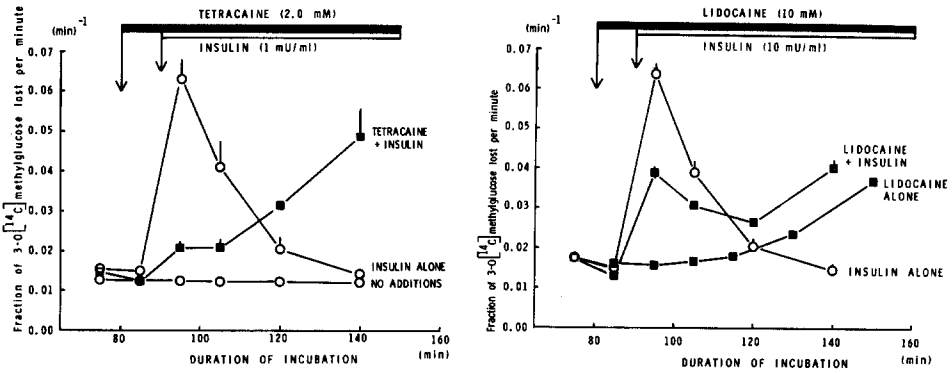

Fig. 14. Effects of tetracaine and insulin on the rate coefficient for 3-*O*-methylglucose release from epididymal adipose tissue. Whole epididymal fat pads were loaded in Krebs–Ringer bicarbonate buffer containing bovine serum albumin (1%) and 1 mM 3-*O*-[^{14}C]methylglucose (1 μCi/ml) for 60 min at 37 °C. They were then washed out into a series of polyethylene vials containing buffer with 1% albumin and no 3-*O*-methylglucose. The fraction of ^{14}C activity lost per min is shown as a function of time. Each point represents the mean of 3–6 observations, and vertical bars indicate S.E., where this exceeds the size of the symbols. ○—○, controls; ○—○, insulin (1 munit/ml) alone; ■—■, insulin (1 munit/ml) and tetracaine (2.0 mM).

Fig. 15. Effects of lidocaine and insulin on the rate coefficient for 3-*O*-methylglucose release from epididymal adipose tissue. Details as for Fig. 14. ○—○, insulin (1 munit/ml) alone; ■—■, lidocaine (10 mM) alone; ■—■, insulin (1 munit/ml) and lidocaine (10 mM). Each point represents the mean of 3–6 observations, and vertical bars indicate S.E., where this exceeds the size of the symbols.

Erythrocytes

From the data presented above it would appear that membrane stabilizers have little if any effect on the basal transport of 3-*O*-methylglucose (and glucose) and that in the presence of various stimuli, the rate of sugar penetration is not suppressed to levels lower than the basal. In order to determine whether the inhibitory effect of these compounds were restricted to the regulated component of sugar transport in insulin-sensitive tissues only, some measurements of 3-*O*-methylglucose transport in erythrocytes from the same animal were included in the present study.

From Figs 16 and 17 it can be seen that 3-*O*-methylglucose is rapidly taken up or released by rat erythrocytes, and that phlorizin (1 mM) suppresses the efflux. Under basal conditions, measurements of the initial rate of uptake indicated that the transport of 3-*O*-methylglucose shows saturation kinetics with an apparent K_m of 5.68 mM and a V of 11.8 μmoles/g per h. It seems likely, therefore, that as earlier shown for soleus muscles, this sugar is transported by a carrier-mediated process in the erythrocytes also.

It appears (Figs 16 and 17) that both the uptake and the efflux of 3-*O*-methylglucose is inhibited by a relatively high concentration of tetracaine (2–4 mM; only

the results obtained with 4 mM are shown). Several experiments showed that lower concentrations of the local anesthetic (0.1–1.0 mM) produced no significant change in 3-*O*-methylglucose transport. At variance with the muscles and adipocytes, the erythrocytes showed no lytic phenomena when exposed to 4 mM tetracaine. Whereas lysis of the cells might lead to erronous overestimation of the inhibitory effect of tetracaine on the uptake of 3-*O*-methylglucose, the effect on efflux would rather be underestimated. The relatively close agreement between the two estimates argues that tetracaine does in fact inhibit the transport of 3-*O*-methylglucose in red cells.

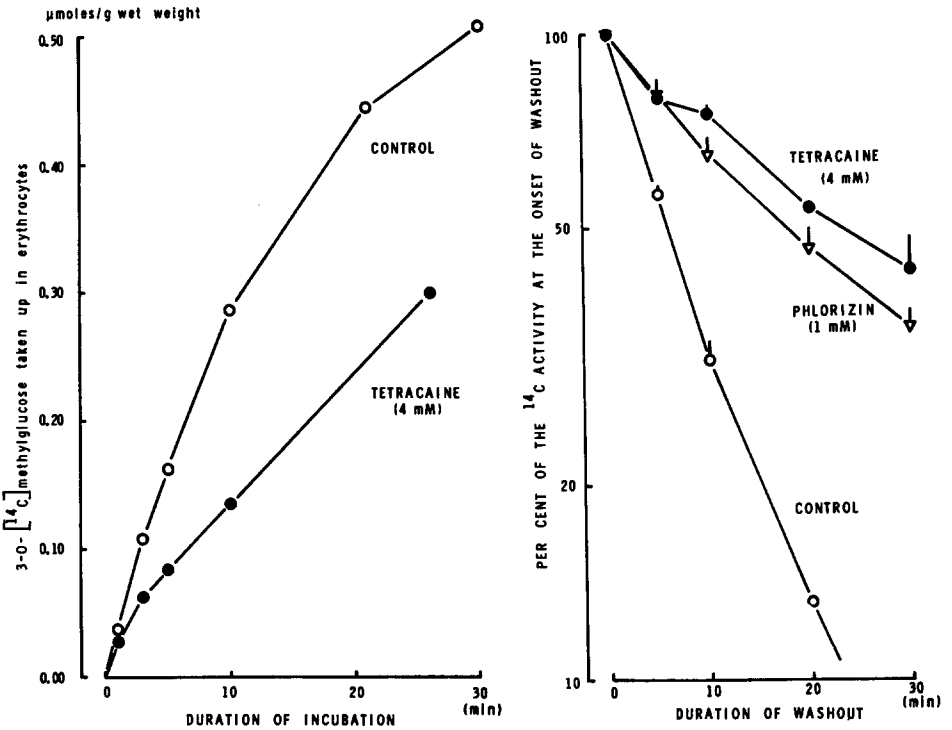

Fig. 16. Effects of tetracaine on the uptake of 3-*O*-methylglucose in rat erythrocytes. Washed fresh erythrocytes were incubated for the indicated intervals of time in polyethylene vials containing 2 ml of Krebs–Ringer bicarbonate buffer with 1 mM 3-*O*-[^{14}C]methylglucose (0.1 μCi/ml) without or with tetracaine (4 mM). The amount of 3-*O*-methylglucose taken up is expressed as μmoles per g of erythrocytes (after correction for "trapped" extracellular phase). Each point represents the mean of duplicate determinations.

Fig. 17. Effect of tetracaine and phlorizin on the release of 3-*O*-methylglucose from rat erythrocytes. Washed fresh erythrocytes were incubated for 60 min in 2 ml Krebs–Ringer bicarbonate buffer containing 1 mM 3-*O*-[^{14}C]methylglucose (1 μCi/ml). After two washes in a 20-fold volume of ice-cold buffer the packed erythrocytes were distributed in polyethylene vials containing 5 ml buffer without 3-*O*-methylglucose and without or with the additions indicated. They were then incubated at 30 °C, and samples withdrawn at the intervals indicated. The ^{14}C activity of the erythrocytes was determined and expressed as per cent of the level at the onset of washout at 30 °C. Each point represents the mean of 3 determinations and S.E. is denoted by the vertical bars.

DISCUSSION

For a general appraisal of the effects of membrane stabilizers it is important that the inhibitory effect of these compounds on the permeability to ions and water is often exerted within a certain (and sometimes rather narrow) concentration range. Larger doses will frequently produce an immediate or delayed rise in the overall permeability of the plasma membrane, and eventually a lysis with severe impairment of basal functions. The present data indicate that mammalian skeletal muscle (and possibly adipocytes also) share this characteristic response pattern with a number of other tissues previously studied. Therefore, a satisfactory evaluation of the effects of membrane stabilizers on the transport and metabolism of glucose requires the determination of both dose–response relationships and the time-course of action. For each of the compounds tested in this study, it has been possible to define conditions where the permeability to K^+, Na^+ and glucose is diminished, and in the following, some emphasis will be laid on discussing the significance and possible interrelations of these phenomena of membrane stabilization.

Ion transport

Although it is evident from several electrophysiological studies that membrane stabilizers decrease the permeability to Na^+ in nerve and muscle[5,7,9], their effects on cation transport have rarely been assessed in direct measurements of ion fluxes. Shanes[3] found that cocaine inhibits the permeability to K^+ in frog sartorius muscle, and later it was demonstrated that this local anesthetic decelerates the release of $^{42}K^+$ from desheathed sciatic nerves of the toad[36]. Others have shown that cocaine inhibits the permeability to K^+ in rabbit atria[37], and a variety of local anesthetics were found to inhibit the transport of Na^+ and K^+ in erythrocytes[38]. More recently, chlorpromazine and propranolol were found to suppress the efflux of K^+ from perfused rat hearts[12]. Furthermore, it was demonstrated that chlorpromazine inhibits the influx of Na^+ in muscle[6]. The present data indicate that in mammalian skeletal muscle, several categories of membrane stabilizers inhibit the efflux of K^+ and influx of Na^+.

In agreement with earlier reports[36], the effect on $^{42}K^+$ release was found to be prompt in onset, and in some instances, the rate coefficient was maintained at a rather low level for more than 1 h. This might lead to a rise in the K^+ content, but as can be seen from Fig. 3, the measurements performed at the end of the efflux experiments only showed a slight increase at the lowest concentration of the drugs. In most of the experiments, the K^+ content was either unchanged or lowered. This may in part be the outcome of a secondary rise in K^+ efflux, but it seems likely that the influx of K^+ was inhibited also[37].

The transport and metabolism of glucose

The major conclusion to be drawn from the present study is that membrane stabilizers inhibit the transport of glucose. The observation that tetracaine produces a decrease in the basal rate of 3-*O*-methylglucose transport in erythrocytes indicates that this effect is not restricted to insulin-sensitive cells, and that it may be the outcome of impaired function of the glucose transport system.

In muscle, the inhibitory effect is only seen when the glucose transport system

is activated by certain stimuli, and the experiments with whole epididymal fat pads indicate that the same may be the case for fat cells.

In all three cell types studied, the inhibitory effect of the membrane stabilizers on sugar transport had a very short time-lag, indicating a direct action on the permeability properties of the plasma membrane. An alternative mode of action is suggested by the fact that with certain concentrations of the membrane stabilizers, the K^+ content of the soleus muscles is slightly elevated (Fig. 3).

However, after 20 min of exposure to 2 mM tetracaine, the K^+ content of the muscles was not significantly changed, although the insulin-stimulated efflux of 3-*O*-methylglucose was almost completely suppressed. Furthermore, the experiments with lidocaine (10–20 mM) and chlorpromazine (0.2–0.5 mM) show that even when the final K^+ content is considerably lowered, the insulin-stimulated 3-*O*-methylglucose transport is clearly decreased throughout the 60 min of exposure to these drugs. This, together with the fact that tetracaine (1 mM) produced the same suppression of the insulin-stimulated 3-*O*-methylglucose efflux in normal and K^+ depleted cells, argues that the effect of membrane stabilization on sugar transport is not secondary to a rise in the intracellular K^+ level over and above that prevailing under normal conditions.

Furthermore, the previous papers in this series[23,25,39] have demonstrated that in muscle even considerable losses of K^+ induced by ouabain or incubation in the absence of K^+ produces little or no change in the basal and insulin-stimulated transport of glucose and 3-*O*-methylglucose.

The observation that the inhibitory effect of membrane stabilizers on the efflux of K^+ and 3-*O*-methylglucose shows a closely similar time-course might indicate a rather direct relationship between the processes of sugar and ion transport. However, a comparison of the dose–response relationship for the effect of tetracaine on 3-*O*-methylglucose efflux and $^{42}K^+$ efflux (or $^{22}Na^+$ influx) shows that the ion transport is more susceptible to the local anesthetics than sugar transport. Thus, whereas 0.1 mM of tetracaine caused no significant inhibition of insulin-stimulated 3-*O*-methylglucose efflux in soleus muscles, this concentration of the drug decreased $^{42}K^+$ efflux by 48% and $^{22}Na^+$ influx by 41%.

On the other hand, it is possible that the effect of membrane stabilizers on excitability, ion transport and sugar transport are different manifestations of the same basic structural modification in the plasma membrane.

It is generally assumed that the transfer of sugars across the plasma membrane requires a certain mobility of membrane components. Thus it has been proposed that carriers might mediate the transport of glucose by a translocation of sites capable of stereospecific binding of the sugar molecule. It seems likely that the mobility of such carriers depends on the rigidity of the overall membrane structure. Since the composition of membrane lipids is essential for the permeability to ions and water[40,41], and since membrane stabilizers have been shown to inhibit the passage of ions across artificial membranes composed of lipids only[35,42], it is reasonable to assume that the phenomenon of membrane stabilization is the outcome of decreased mobility of lipid components in the natural plasma membrane also. This might present a steric hindrance to the translocation of various carriers situated in the membrane. Since the structures involved in the transfer of ions and sugars are not necessarily the same, an inhibition of ion transport may be detectable before

(*i.e.* at lower concentrations of the membrane stabilizers) the overall mobility of the lipid components of the plasma membrane has reached a point where the structures mediating the transfer of sugars are no longer capable of functioning at a normal rate.

In particular, under conditions where the translocation of sugar carrier sites is accelerated, steric hindrances due to increased rigidity of the lipid components of the plasma membrane can be imagined to represent a limitation for the total capacity for the transfer of sugar molecules. This may explain how membrane stabilizers can prevent activation of the glucose transport system without causing any clearly detectable reduction in basal permeability.

The inhibitory effect of local anesthetics on the insulin-stimulated conversion of glucose into glycogen can partly be accounted for as secondary to a decrease in glucose uptake. However, the fact that the fraction of glucose incorporated into glycogen was significantly diminished by tetracaine under basal conditions also indicates that the membrane stabilization leads to an inhibition of glucose metabolism which may be separated from the reduced sugar permeability. The considerable inhibition of the conversion of glucose into triglycerides and CO_2 produced by the local anesthetics in fat cells would also indicate that the metabolism of glucose is rather susceptible to membrane stabilizers. Earlier studies have demonstrated that membrane stabilizers (local anesthetics, thiomebumal) inhibit metabolism and energy production[44,45]. In contrast to other metabolic inhibitors (cyanide, ethacrynic acid and 2,4-dinitrophenol), which have earlier been shown to accelerate sugar transport in muscle and adipose tissue[23,27], the membrane stabilizers apparently cause no stimulation of the phlorizin-sensitive transport of 3-*O*-methylglucose. Therefore, it is as yet difficult to exclude the possibility that the inhibitory effect of membrane stabilizers is related to impairment of glucose metabolism. The rapid onset of the inhibition argues against a primary action on intracellular processes, but an unambiguous identification of the primary site of action will require studies of the effects on sugar transport in purified membranes or artificial membranes.

The present report seems to allow the conclusion that the phenomenon of membrane stabilization includes a reduced permeability to one of the most important substrates for cellular metabolism. It was recently shown that chlorpromazine inhibits the uptake of glucose in erythrocytes[45], and that local anesthetics suppress the influx and the efflux of α-aminoisobutyric acid in rat soleus muscle[46]. This indicates that membrane stabilizers can reduce the exchange of a variety of charged and uncharged organic compounds across cellular membranes.

The inhibitory effects of membrane stabilizers on the transport and metabolism of glucose in the major targets for insulin action may in some instances explain the hyperglycemic or diabetogenic effects of certain of these drugs[14–20]. At the same time the observations may elucidate some of the mechanisms of insulin resistance or insulin action.

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