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MULTIPLE EFFECTS OF SULPHYDRYL REAGENTS ON SUGAR TRANSPORT BY RAT SOLEUS MUSCLE

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Iodoacetate, over the range 0.2–2 mM, stimulated the uptake of D-xylose by rat soleus muscle and inhibited anaerobic lactate production by soleus muscle. Stimulation of sugar transport is considered to be due to the resultant fall in ATP. *p*-Chloromercuribenzene sulphonate (0.5–2 mM) stimulated xylose uptake to a lesser extent than iodoacetate and induced a proportionately smaller fall in ATP, consistent with the inhibitory effect of *p*-chloromercuribenzene sulphonate on lactate production. Under certain conditions, *p*-chloromercuribenzene sulphonate stimulated sugar transport without affecting the ATP level. This suggests that whereas *p*-chloromercuribenzene sulphonate can be expected to stimulate sugar transport through the lowering of muscle ATP, it may also act through some other mechanism. No stimulatory effect on xylose uptake was observed when muscles were exposed to *N*-ethylmaleimide (0.02–2 mM) either for brief (1 min) or more prolonged (30 min) periods. Because *N*-ethylmaleimide induced a marked fall in muscle ATP, it is surprising that *N*-ethylmaleimide did not stimulate sugar transport; in most experiments this inhibitor actually inhibited sugar transport. *N*-Ethylmaleimide inhibited the stimulation of sugar transport by 2,4-dinitrophenol and anoxia; this inhibitory effect appears to explain why *N*-ethylmaleimide itself did not stimulate sugar transport. *p*-Chloromercuribenzene sulphonate also inhibited 2,4-dinitrophenol-stimulated xylose uptake by a mechanism which seems similar to that of *N*-ethylmaleimide; this could explain in part the modest stimulatory effect of this inhibitor on muscle sugar transport.

Introduction

A number of laboratories have reported that sulphydryl reagents stimulate basal sugar transport in diaphragm [1,2], skeletal [3–5] and smooth [6] muscles. The mechanism whereby sulphydryl reagents exert their stimulatory effect is still unknown. As sugar transport is stimulated by metabolic inhibitors which lower muscle ATP [7,8], this could provide an explanation for some of the reported effects of sulphydryl blockade. Unlike muscle, sugar transport by the adipocyte is not activated by lowering the ATP content [9]. In adipose tissue, low concentrations of sulphydryl reagents stimulate the uptake of glucose [10–13] and 3-*O*-methylglucose [14]. Hence, the possibility that sulphydryl reagents may stimulate sugar transport in muscle through some mechanism other than the lowering of ATP levels cannot be excluded.

The studies presented in this paper were undertaken to clarify the nature of the stimulatory effects(s) of sulphydryl blockade on muscle sugar transport. As reported below, marked differences were observed between the effects of the inhibitors studied. Thus, iodoacetate stimulates sugar trans-

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port consistent with its ability to lower muscle ATP. *N*-Ethylmaleimide also lowers muscle ATP, but, paradoxically, inhibits sugar transport. *p*-Chloromercuribenzene sulphonate stimulates sugar transport; but to a lesser extent than iodoacetate; this inhibitor also promotes a modest fall in muscle ATP.

Methods

Soleus muscles weighing approx. 30 mg were obtained from Sprague Dawley rats (70–90 g) fed ad libitum. Muscles were incubated under an atmosphere of O_2/CO_2 (95:5, v/v); anaerobic incubations were under N_2/CO_2 (95:5, v/v). The basic ('bicarbonate') medium contained NaCl (118 mM), KCl (4.8 mM), $CaCl_2$ (2.6 mM), $MgSO_4$ (1.2 mM), KH_2PO_4 (1.2 mM) and $NaHCO_3$ (25 mM); prior to use the medium was gassed with the appropriate gas mixture. Additions to, or variations from, this basic medium are detailed in the text.

Xylose uptake was determined using the method of Korbl et al. [8]. In this procedure the muscles are first preincubated under the test conditions; the uptake of D-[U-^{14}C]xylose (final concn. 10 mM, spec. act. 0.03 μCi/μmol) is then measured over a 5-min period at 37°C, using D-[1-^{3}H]sorbitol (10 mM, spec. act. 0.1 μCi/μmol) as the extracellular marker.

For the determination of ATP, muscles were homogenized in 5% trichloroacetic acid and the extract thus obtained was assayed using luciferase in an LKB-Wallac bioluminometer, Model 1250. Lactate was measured by the method of Barker and Summerson [15].

Materials

D-[U-^{14}C]xylose was from Amersham International, Amersham. D-[1-^{3}H]sorbitol was from New England Nuclear Corp., Boston. Luciferase was from LKB-Wallac, Turku, Finland. Iodoacetate was obtained from Hopkin and Williams Ltd., Essex, 2,4-dinitrophenol was from British Drug Houses Ltd., Poole; *p*-chloromercuribenzene sulphonate was from Sigma Chemical Co., St. Louis, and *N*-ethylmaleimide from Calbiochem-Behring (Aust.) Pty. Ltd., Sydney.

Statistics

To minimise the effect of biological variation between individual animals, wherever possible the experiments were designed using paired controls. One muscle from each pair was incubated under test conditions, while the second served as the control. The results of these experiments were analyzed for statistical significance using Student's *t*-test as applied to paired samples. Where it was not possible to use paired controls, muscles taken from litter mates were distributed randomly among the experimental groups and the results subjected to statistical analysis using the standard Student's *t*-test.

Results

Effects of iodoacetate

Xylose uptake was progressively stimulated when soleus muscles were exposed for 30 min to concentrations of iodoacetate over the range 0.2–2.0 mM (Fig. 1). The stimulatory effect of 2 mM iodoacetate was evident 5 min after exposure to the inhibitor, and reached its maximum value by 30 min (Fig. 2). This effect did not persist, but

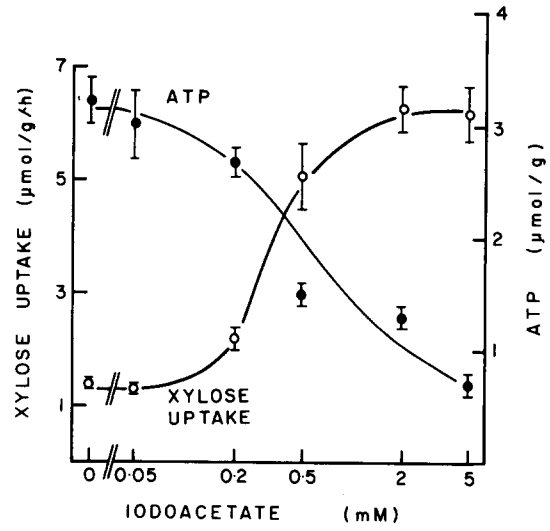

Fig. 1. Effect of iodoacetate on xylose uptake and ATP levels. Soleus muscle pairs were preincubated for 30 min at 37°C. At this point, iodoacetate (0.5–5 mM) was added to one muscle from each pair and the incubation continued for a further 30 min at 37°C. Values are mean ± S.E. of five determinations.

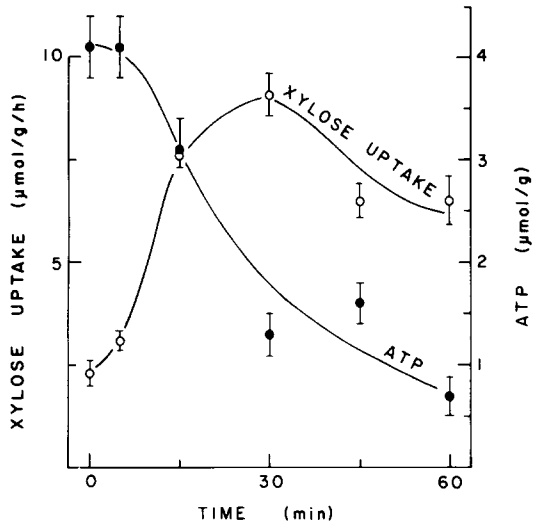

Fig. 2. Effect of 2 mM iodoacetate on xylose uptake and ATP levels. Soleus muscle pairs were preincubated for 30 min at 37°C. At this point, iodoacetate (2 mM) was added to one muscle from each pair and the incubation continued for up to 60 min as shown. Values are mean±S.E. of five determinations.

declined slowly over the next 30 min. The activation of sugar transport induced by iodoacetate was comparable in magnitude to the stimulatory effects of other insulin-like agents which we have studied in soleus muscle (e.g., 2,4-dinitrophenol [8], EDTA [16] and hyperosmolarity [17]) but was less than the effect of insulin [8].

The ability of agents such as anoxia and uncouplers of oxidative-phosphorylation to activate muscle sugar transport has been explained in terms of their lowering the ATP level [8]. From Figs. 1 and 2 it is apparent that the stimulatory effect of iodoacetate on sugar transport was associated with a fall in ATP. We have also observed that many of the agents which activate sugar transport in soleus muscle, irrespective of their mode of action, require ATP to promote all or part of their stimulatory action [8,17,18]. From Fig. 2 it is clear that the decline in the stimulatory effect of iodoacetate occurs over a period when ATP has fallen considerably. When muscle ATP was completely depleted by prolonged incubation (90 min) under anaerobic conditions, the ability of iodoacetate (2 mM) to activate sugar transport was severely curtailed (anaerobic control, 4.2 ± 0.2 μmol/g per h vs. anaerobic + iodoacetate, 4.9 ± 0.2 μmol/g per h; $P < 0.05$, $n = 7$). These observations suggest that, like the other agents studied, there is also an ATP-dependent step involved in at least part of the action of iodoacetate.

Because this inhibitor has classically been used to inhibit muscle glycolysis, we examined the effect of iodoacetate on glycolysis in anaerobic soleus muscle. Lactate production was completely blocked within 10–15 min by exposure to 2 mM iodoacetate (Fig. 3). Lower concentrations of iodoacetate inhibited lactate production to a lesser extent; the effect of 0.5 mM iodoacetate was apparent at 15 min and that of 0.3 mM iodoacetate at 30 min.

Effect of N-ethylmaleimide

The stimulatory effects of *N*-ethylmaleimide on muscle sugar transport previously reported were observed following brief exposure of the muscle to the inhibitor [2,19]. We have examined the effects of *N*-ethylmaleimide (0.02–2 mM) on xylose uptake following both brief (1 min) and more prolonged (30 min) exposure*. No stimulatory effect of *N*-ethylmaleimide was observed in any of these experiments (Fig. 4). In short-term experiments there was no effect of *N*-ethylmaleimide up to 0.1 mM; at a concentration of 1 mM (which is similar to that used by previous workers [2,19]), *N*-ethylmaleimide actually inhibited xylose uptake. Xylose uptake was also inhibited when muscles were exposed to *N*-ethylmaleimide (0.05–2 mM) for 30 min. Brief exposure to *N*-ethylmaleimide did not affect muscle ATP; however, ATP levels were depressed when muscles were incubated for 30 min in the presence of *N*-ethylmaleimide concentrations in excess of 0.05 mM (Fig. 4).

Because the lowering of muscle ATP levels is usually associated with increased sugar transport [7,8], the failure of *N*-ethylmaleimide to stimulate sugar transport shown in Fig. 4 is somewhat surprising. Accordingly, we asked whether *N*-ethylmaleimide might block the mechanism whereby a fall in the ATP level leads to the activation of the sugar transport system. To test this possibility,

* To limit the duration of exposure to *N*-ethylmaleimide to these times, an equimolar amount of mercaptoethanol was added at the appropriate time and allowed to react for a further 5 min. Control experiments showed that sugar transport was unaffected by such a mixture of *N*-ethylmaleimide and mercaptoethanol.

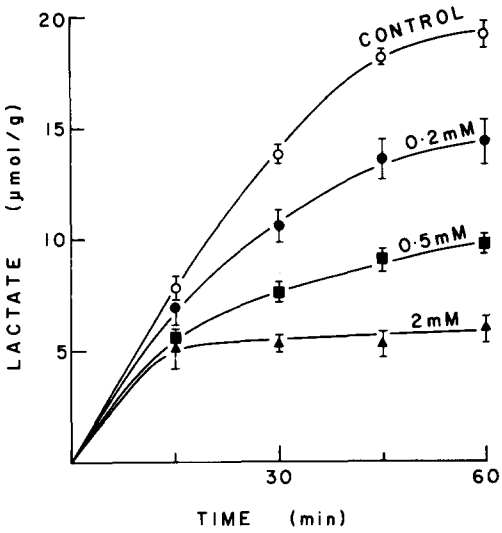

Fig. 3. Effect of iodoacetate on lactate production by anaerobic soleus muscle. Soleus muscles were incubated under anaerobic conditions for up to 60 min at 37°C in an original volume of 1 ml bicarbonate medium containing 0–2 mM iodoacetate. At the times shown, 200 μl of the incubation medium was withdrawn and its lactate concentration determined. Values are mean ± S.E. of five determinations.

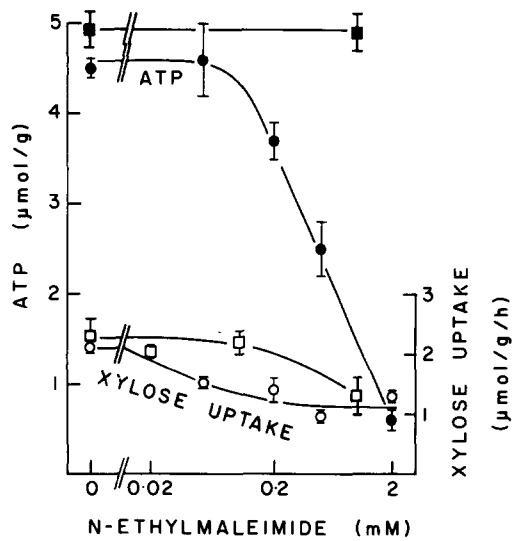

Fig. 4. Effect of *N*-ethylmaleimide on xylose uptake and ATP levels. Soleus. muscle pairs were preincubated for 30 min at 37°C. At this point, *N*-ethylmaleimide (0.05–2 mM) was added to one muscle from each pair and the incubation continued for either 1 min (□, ■) or 30 min (○, ●). *N*-Ethylmaleimide was neutralized by the addition of an equimolar amount of mercaptoethanol; after a further 5 min the muscles were either transferred to fresh media containing the radioactive test sugars for the determination of xylose uptake, or subjected to assay for ATP. Values are mean ± S.E. of six determinations.

we examined the effect of *N*-ethylmaleimide under conditions where sugar transport was stimulated by 2,4-dinitrophenol or by anoxia. Exposure to *N*-ethylmaleimide (0.2 mM) for 30 min inhibited 2,4-dinitrophenol- and anoxia-stimulated sugar transport (Table I). Brief exposure (1 min) to *N*-ethylmaleimide also inhibited 2,4-dinitrophenol-stimulated xylose uptake (Fig. 5). This effect became apparent at a concentration of 1 mM *N*-ethylmaleimide, which is similar to the concentration which inhibits basal sugar transport after brief exposure to the inhibitor (Fig. 4). However, from the data presented in Fig. 5, it is clear that the effect of *N*-ethylmaleimide on 2,4-dinitrophenol-stimulated sugar transport is not merely a reflection of a lowered basal transport rate, but is due to the inhibition of the action of 2,4-dinitrophenol itself. We have monitored ATP levels in muscles incubated under the same conditions as described in Table I; *N*-ethylmaleimide (0.2 mM) did not itself affect the ATP level, nor did it prevent

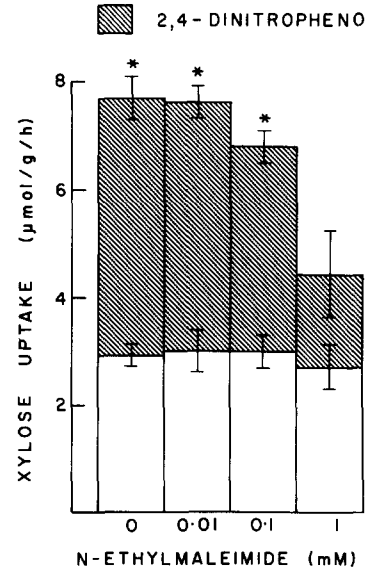

Fig. 5. Inhibition of 2,4-dinitrophenol-stimulated xylose uptake by brief exposure to *N*-ethylmaleimide. Soleus muscles were preincubated for 30 min at 37°C in bicarbonate medium, then exposed for 1 min to *N*-ethylmaleimide. The *N*-ethylmaleimide was neutralized by the addition of an equimolar amount of mercaptoethanol and incubation for a further 5 min. The muscles were transferred to fresh bicarbonate medium ± 2,4-dinitrophenol (0.5 mM) and incubated for 10 min. Values are mean ± S.E. of five determinations. * 2,4-Dinitrophenol vs. control, $P < 0.001$.

TABLE I

EFFECT OF *N*-ETHYLMALEIMIDE ON THE STIMULATION OF XYLOSE UPTAKE BY 2,4-DINITROPHENOL AND ANOXIA

Soleus muscles were incubated for 60 min at 37°C under 95% O_2/5% CO_2 or 95% N_2/5% CO_2. Where indicated, the following additions were made: *N*-ethylmaleimide (0.2 mM, 30 min before the end of the incubation period); 2,4-dinitrophenol (0.5 mM, 10 min before the end of the incubation period). Values are mean±S.E. of six determinations. n.s., not significant.

Incubation details		Xylose uptake (μmol/g per h)	
Atmos	Additions	Control	*N*-ethylmaleimide
O_2/CO_2	–	1.8±0.3	2.0±0.3
	2.4-dinitrophenol	7.0±0.6	3.4±0.6
		$P<0.005$	n.s.
O_2/CO_2	–	2.0±0.2	1.8±0.2
N_2/CO_2	–	4.6±0.4	3.3±0.3
		$P<0.005$	$P<0.005$

2,4-dinitrophenol or anoxia from lowering muscle ATP (data not presented).

Effect of p-chloromercuribenzene sulphonate

Xylose uptake was stimulated when muscles were exposed for 30 min to concentrations of *p*-chloromercuribenzene sulphonate in excess of 0.5 mM (Fig. 6). The effect of this inhibitor, however, was considerably lower than that of iodoacetate. Stimulation was evident after exposure to *p*-chloromercuribenzene sulphonate (2 mM) for 15 min and was maximal by 30 min (Fig. 7); this effect was maintained over the next 30 min. Of the three inhibitors tested, *p*-chloromercuribenzene sulphonate had the least effect on ATP levels; exposure to *p*-chloromercuribenzene sulphonate (2 mM) for 60 min lowered the ATP content by only 25% (Fig. 7). This is reflected in the effect of this inhibitor on muscle glycolysis (Fig. 8); *p*-chloromercuribenzene sulphonate (2 mM) inhibited the production of lactate by anaerobic muscle by 20–38%. Here, again, the effect of *p*-chloromercuribenzene sulphonate differed from that of iodoacetate. Thus, although the inhibitory effect of 2 mM *p*-chloromercuribenzene sulphonate on lactate production was comparable with that of 0.2 mM iodoacetate, the effect of *p*-chloromercuribenzene

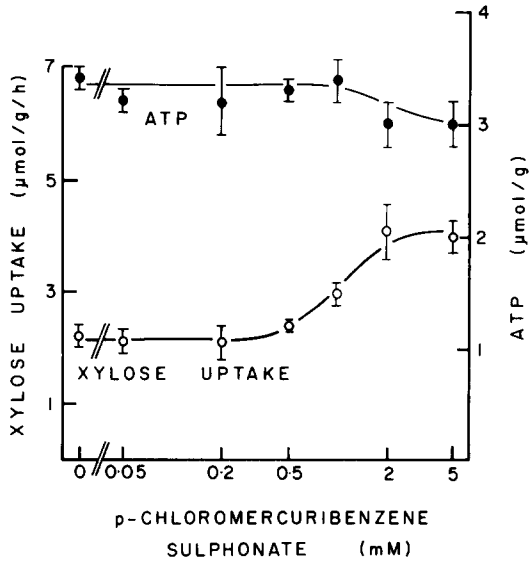

Fig. 6. Effect of *p*-chloromercuribenzene sulphonate on xylose uptake and ATP levels. Soleus muscle pairs were preincubated for 30 min at 37°C. At this point, *p*-chloromercuribenzene sulphonate (0.05–5 mM) was added to one muscle from each pair and the incubation continued for a further 30 min at 37°C. Values are mean±S.E. of five determinations.

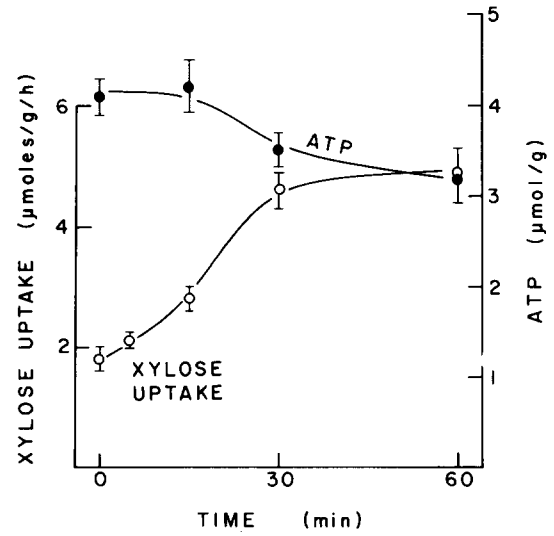

Fig. 7. Effect of 2 mM *p*-chloromercuribenzene sulphonate on xylose uptake and ATP levels. Soleus muscle pairs were preincubated for 30 min at 37°C. At this point, *p*-chloromercuribenzene sulphonate (2 mM) was added to one muscle from each pair and the incubation continued for up to 60 min as shown. Values are mean±S.E. of five determinations.

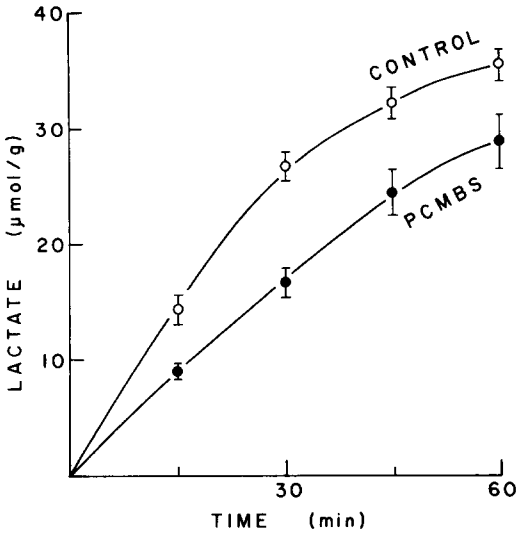

Fig. 8. Effect of 2 mM *p*-chloromercuribenzene sulphonate on lactate production by anaerobic soleus muscle. Soleus muscles were incubated under anaerobic conditions±2 mM *p*-chloromercuribenzene sulphonate (PCMBS). Other details same as legend to Fig. 3. Values are mean±S.E. of five determinations.

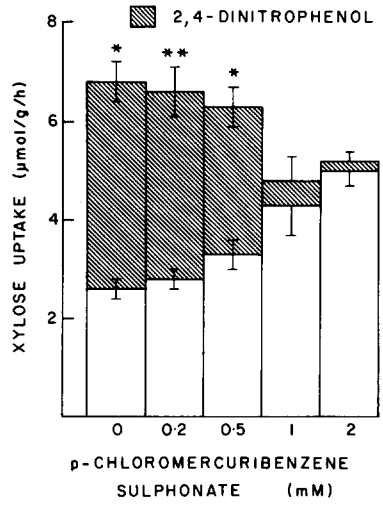

Fig. 9. Inhibition of 2,4-dinitrophenol-stimulated xylose uptake by *p*-chloromercuribenzene sulphonate. Soleus muscle pairs were incubated for 30 min at 37°C in the presence of *p*-chloromercuribenzene sulphonate as shown; 10 min before the end of this period, 2,4-dinitrophenol (0.5 mM) was added to one muscle from each pair. Values are mean±S.E. of six determinations. 2,4-Dinitrophenol vs. control, * $P<0.001$, ** $P<0.005$.

sulphonate is rapid in onset and remains essentially constant over the period of the experiment, whereas the effect of iodoacetate appears more slowly and tends to increase with time (cf. Figs. 3 and 8).

The rather limited stimulatory effect of *p*-chloromercuribenzene sulphonate on sugar transport shown in Figs. 6 and 7 suggested that this inhibitor, like *N*-ethylmaleimide, may also antagonize the stimulatory effect of those agents which act through the lowering of muscle ATP levels. This was found to be so; *p*-chloromercuribenzene sulphonate inhibited the stimulation of xylose uptake by 2,4-dinitrophenol (Fig. 9). This experiment was complicated by the fact that *p*-chloromercuribenzene sulphonate itself stimulates sugar transport and thus raises the baseline. Nevertheless, from the data shown it is clear that the inhibitory effect of *p*-chloromercuribenzene sulphonate on 2,4-dinitrophenolstimulated xylose uptake is not due simply to the increase in the baseline value up to the level in 2,4-dinitrophenol-treated muscles.

Discussion

Stimulation of sugar transport by sulphydryl reagents

The three sulphydryl reagents used for these studies (iodoacetate, *N*-ethylmaleimide and *p*-chloromercuribenzene sulphonate) were selected because all have been reported to stimulate the uptake of non-metabolizable glucose analogues in a variety of muscle types [1–6]. In contrast to these observations, studies concerned with the action of sulphydryl reagents on the uptake of glucose, as distinct from its analogues, have variously reported either no change [20–22], stimulation [19], or inhibition [23]. As glucose uptake will reflect both the transport and ongoing metabolism of the sugar, and in view of the potential for these inhibitors to affect glucose metabolism, it is, perhaps, not surprising that differences of this kind would be observed.

Using fat pad and isolated fat cells, it has been shown that the effects of sulphydryl reagents on glucose utilization are concentration-dependent; low concentrations (up to approx. 100 μM) stimulate, and high concentrations (above approx. 100

μM) inhibit [10,12,13,24,25]. The stimulatory effect seen with low concentrations of sulphydryl reagents suggests that this could be a membrane-localized effect [12]. Due to the tendency to equate glucose utilization with transport in adipose tissue studies, there are relatively few reports concerned with the action of sulphydryl reagents on the transport of glucose analogues in adipose tissue. Whereas these analogue studies have all used inhibitor concentations in the millimolar range, which would have inhibited glucose utilization, no inhibitory effects on glucose transport were observed [12,26,27]; in one study *N*-ethylmaleimide was observed to stimulate the uptake of 3-*O*-methylglucose by adipocytes [14]. Conversely, Kikuchi and Larner [28] have reported that *N*-ethylmaleimide (0.1–0.5 mM) inhibits basal 2-deoxyglucose uptake by adipocytes. Because 2-deoxyglucose uptake reflects both transport and phosphorylation, one cannot be certain that this effect was on sugar transport alone.

It would be naive to expect that all sulphydryl reagents would share a common action on muscle sugar transport. Because sugar transport in muscle is activated by agents which lower ATP [7,8], this provides one mechanism for the action of sulphydryl reagents. Sugar transport in the adipocyte is not activated by agents which lower ATP [9]; hence the effect of sulphydryl reagents in this tissue suggests that there is at least a second possible mechanism which may also apply to muscle. If one considers the various sulphydryl reagents themselves, then differences in their ability to enter the cell and to react with different sulphydryl groups in a variety of proteins must influence their effect on sugar transport. Of the three inhibitors tested, *N*-ethylmaleimide enters the cell the most rapidly [29], iodoacetate enters more slowly [29,30], and *p*-chloromercuribenzene sulphonate enters poorly if at all [31]. Furthermore, it cannot be assumed that the reactivity of these reagents is always restricted to sulphydryl groups. Thus, iodoacetate will also react with amino groups, though more slowly than with sulphydryl groups [29,30], and *N*-ethylmaleimide will also react with amino- and imidazole groups [29,30]; *p*-chloromercuribenzene sulphonate is the most specifically directed towards sulphydryl groups [31].

This potential for diversity is well illustrated by the results presented in this paper. Thus, iodoacetate stimulated sugar transport to the same extent as other insulinomimetric agents which we have studied [8,16,17]; *p*-chloromercuribenzene sulphonate stimulated sugar transport to a lesser extent than iodoacetate and *N*-ethylmaleimide inhibited rather than stimulated sugar transport.

The stimulatory effect of iodoacetate is accompanied by a fall in muscle ATP (Figs. 1 and 2). Kono and Colowick [1] noted that the fall in ATP (43%) induced by iodoacetate in rat diaphragm was less than that induced by 2,4-dinitrophenol, and concluded that the stimulatory effect of iodoacetate on sugar transport was not mediated through the lowering of muscle ATP levels. This assumes that there is a simple relationship between the muscle ATP level and the stimulation of sugar transport; this is not so [4,8]. We have been unable to demonstrate the stimulatory effect of iodoacetate in the absence of any effect on ATP. We cannot exclude completely the possibility that iodoacetate may also act through some ATP-independent mechanism, masked by its effect on ATP levels. Nevertheless, from the experiments presented in this paper and those reported previously by Korbl et al. [8], we believe that the stimulatory effect of iodoacetate is most reasonably explained in terms of its effect on ATP level.

p-Chloromercuribenzene sulphonate induces a small, but consistent, stimulation of sugar transport in soleus muscle, accompanied by a modest fall in muscle ATP. These effects of *p*-chloromercuribenzene sulphonate are similar to the effects of *p*-hydroxymercuribenzoate in rat diaphragm reported by Kono and Colowick [1]. The effect of *p*-chloromercuribenzene sulphonate on ATP levels is consistent with its inhibitory effect on glycolysis. All this suggests a priori that the stimulatory effect of *p*-chloromercuribenzene sulphonate on sugar transport, like that of iodoacetate, could be due simply to its effect on ATP levels. However, from the data shown in Fig. 4 and 5, it can be seen that the stimulatory effect of *p*-chloromercuribenzene sulphonate can be observed under certain conditions where there is no effect on muscle ATP; specifically, exposure to 1 mM *p*-chloromercuribenzene sulphonate for 30 min (Fig. 6), and 2 mM *p*-chloromercuribenzene

sulphonate for 15 min (Fig. 7). This raises the possibility that *p*-chloromercuribenzene sulphonate may also stimulate sugar transport by some mechanism other than the lowering of ATP. The fact that *p*-chloromercuribenzene sulphonate does lower muscle ATP tends to mask the presence of such an alternative mechanism. These experiments suggest, rather than confirm, that such an alternative, perhaps similar to the system in the adipocyte, may also be present in muscle.

N-Ethylmaleimide did not stimulate sugar transport in soleus muscle, even when muscles were exposed briefly to low concentrations of the inhibitor (Fig. 4). This is in accord with Bihler's observation that *N*-ethylmaleimide does not stimulate basal sugar transport in diaphragm [32]. Conversely, Carlin and Hechter [2] reported that brief exposure to *N*-ethylmaleimide stimulates sugar transport in rat diaphragm. Because they measured xylose uptake over a 90-min period, one must ask whether the stimulatory effect of *N*-ethylmaleimide which they observed was due in fact to leakage of the sugar into the treated muscle.

Inhibitory effects of sulphydryl reagents

Paradoxically, although *N*-ethylmaleimide lowers muscle ATP, it does not stimulate sugar transport; this suggests that *N*-ethylmaleimide must somehow be able to block the mechanism whereby a fall in ATP activates sugar transport. This is confirmed with our observation that *N*-ethylmaleimide inhibits the stimulatory effects of 2,4-dinitrophenol and anoxia (Table I). The concentration of *N*-ethylmaleimide used in these experiments (0.2 mM) was chosen because it was found to have little or no effect on muscle ATP levels. *N*-ethylmaleimide does not prevent 2,4-dinitrophenol or anoxia from lowering muscle ATP; hence its action must be to inhibit the subsequent activation mechanism. In this way it could be expected that *N*-ethylmaleimide would block the stimulation of sugar transport which otherwise should have resulted from its own effect on muscle ATP levels.

It has long been recognized that *N*-ethylmaleimide inhibits the stimulatory effect of insulin on sugar transport in muscle [21,32–35] and adipocytes [12,14,26], and the effects of oxidants in adipocytes [26,27]. The inhibitory effect of *N*-ethylmaleimide on the stimulation of sugar transport by 2,4-dinitrophenol and anoxia, reported above, and the observation that *N*-ethylmaleimide also blocks ouabain-stimulated sugar transport [32], suggest that *N*-ethylmaleimide may inhibit a broader range of insulinomimetic agents than hitherto suspected.

The inhibitory effect of *p*-chloromercuribenzene sulphonate on 2,4-dinitrophenol-stimulated xylose uptake appears to be similar to that of *N*-ethylmaleimide. Unlike *N*-ethylmaleimide, *p*-chloromercuribenzene sulphonate does stimulate sugar transport, albeit to a lesser extent than the other agents which we have studied [8,16,17]. One must now consider whether the inhibitory effect of *p*-chloromercuribenzene sulphonate on the activation of sugar transport, demonstrated in Fig. 9, could have served to limit the magnitude of the stimulatory effect of *p*-chloromercuribenzene sulphonate itself.

The initial aim of these studies was to define in soleus muscle the stimulatory effect of sulphydryl reagents on sugar transport which has been described elsewhere. Whether there is such an effect, specific to these agents and distinct from their inhibitory effect on energy metabolism, remains in doubt. In that these agents do affect muscle ATP levels, this itself will tend to activate sugar transport and thus mask any intrinsic effect of the sulphydryl reagents themselves. Furthermore, because of the ability of *N*-ethylmaleimide and *p*-chloromercuribenzene sulphonate to block the activation of sugar transport, this too will tend to obscure any insulin-like action these agents might otherwise possess. It is clear, from the diversity of their effects, that iodoacetate, *p*-chloromercuribenzene sulphonate and *N*-ethylmaleimide must affect a variety of different sulphydryl groups. If we are to establish that there is a mechanism whereby sulphydryl reagents can activate muscle sugar transport, independent of lowering ATP, it will be necessary to find some other sulphydryl reagents with specificities essentially limited to this function, so that they neither affect ATP nor exhibit the same inhibitory effects as *N*-ethylmaleimide or *p*-chloromercuribenzene sulphonate.

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