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EFFECTS OF DIPHENYLHYDANTOIN ON THE TRANSPORT OF Na^+ AND K^+ AND THE REGULATION OF SUGAR TRANSPORT IN MUSCLE *IN VITRO**

I. BIHLER AND P. C. SAWH

Department of Pharmacology and Therapeutics, Faculty of Medicine, University of Manitoba, Winnipeg 3, Manitoba (Canada)

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SUMMARY

1. The distribution of the nonmetabolized glucose analog 3-*O*-methyl-D-[^{14}C]-glucose and the intracellular concentration of Na^+ and K^+ were measured in "intact" rat hemidiaphragms, *in vitro*.

2. 5,5-Diphenylhydantoin (DPH) at concentrations of 0.1 and 0.5 mM produced an increase in internal K^+ and a decrease in internal Na^+, consistent with stimulation of the Na^+ pump. Higher concentrations, 1.0 and 5.0 mM had the opposite effect. Both the stimulatory and the inhibitory effects were potentiated by increased external K^+ (16 mM).

3. Sugar transport was inhibited by DPH whenever internal K^+ was increased and Na^+ decreased and, conversely, sugar transport was stimulated following the opposite ionic changes. The changes in sugar transport were highly and significantly correlated to changes in internal Na^+ and K^+. These effects were apparent on efflux, as well as on influx of sugar. Inhibition was present with and without insulin but was greater in the presence of the hormone. The inhibitory effect of DPH was abolished by 10^{-5} M ouabain, which blocks the Na^+ pump but not by 10^{-9} M ouabain.

4. The ionic changes are evidence for a stimulatory effect of DPH on ion transport which changes to inhibition at higher drug concentrations. The effects on sugar transport provide additional evidence for inhibition of sugar transport by low internal Na^+ (or high K^+) and support the postulated regulatory effect of internal Na^+ and/or K^+ on the sugar transport mechanism in skeletal muscle.

INTRODUCTION

Recent work in this laboratory[1-3] and elsewhere[4-7] has shown that insulin-sensitive transport and metabolism of sugar in muscle and adipose tissue is stimulated by factors which inhibit the Na^+ pump. We have found[3] that the increase in both influx and efflux of sugar is correlated with changes in the intracellular levels of Na^+ or K^+, or both, consequent to the inhibition of the Na^+ pump and have, therefore,

Abbreviation: DPH, 5,5-diphenylhydantoin.

* These results were presented[1] in part to the 24th International Congress of Physiological Sciences, Washington, D.C., 1968.

proposed that the Na^+ pump, through its effects on internal ion levels exerts a regulatory effect on the sugar transport mechanism. According to this hypothesis, stimulation of the Na^+ pump leading to an increase in internal K^+ and decrease in internal Na^+ should inhibit sugar transport. In recent experiments we have demonstrated such an effect[8], using incubation in a high-K^+ medium, a procedure known to stimulate the Na^+ pump in muscle[9,10]. To provide further evidence on this point we report here the effects on sugar transport of 5,5-diphenylhydantoin (DPH). This drug alters the intracellular ion distribution[11], apparently through stimulation of the Na^+ pump[12], without causing membrane depolarization and without concomitant changes in the ionic composition or the osmolarity of the incubation medium. It should, therefore, provide additional evidence on the relation between sugar transport and the intracellular levels of Na^+ and K^+. At the same time, the present study provides new data on the effect of diphenylhydantoin on ionic levels and the function of the Na^+ pump in intact muscle tissue.

METHODS

Sugar transport was studied as described[2,3] previously by following the distribution of the nonmetabolized glucose analog, 3-*O*-methyl-D-[^{14}C]glucose in the "intact" rat hemidiaphragm[13], incubated in vitro in Krebs–Henseleit bicarbonate buffer. DPH was dissolved in 50% ethanol and a small volume of this solution was added to the incubation medium; an identical amount of ethanol was added to the paired control hemidiaphragms. The radioactivity of tissue extracts and media was determined by liquid scintillation spectrometry and the levels of Na^+ and K^+ were measured by atomic absorption spectrophotometry. Where applicable, the data were corrected for extracellular space taken to equal the mannitol space determined in the same tissue sample. Sugar influx was expressed as percentage penetration, (sugar in intracellular water/sugar in medium) $\times$ 100, reached at the end of an incubation period with sugar. In the efflux measurements sugar-loaded tissues were incubated for 10 min in a sugar-free medium and the amount of sugar lost was expressed as percentage of the sugar content in the paired control hemidiaphragms which were loaded with sugar but not washed out. As discussed earlier[2,3,8], these expressions for penetration and efflux are of a semiquantitative nature only. Statistical evaluation of results was done by Student's t test.

RESULTS

The effect of DPH on the intracellular levels of K^+ and Na^+ is shown in Fig. 1. It shows the mean changes in intracellular concentrations of Na^+ and K^+ in tissues treated with the drug for 50 min when compared to ion concentrations in control tissues incubated without the drug. At DPH concentrations of 0.1 and 0.5 mM the levels of K^+ were significantly increased ($P < 0.02$ and $P < 0.001$, respectively) and those of Na^+ significantly decreased ($P < 0.05$ and $P < 0.001$, respectively). The effect of 0.5 mM DPH amounted to a 30% decrease in internal Na^+. The changes in K^+ were always opposite and roughly equivalent to those in Na^+. The direction of these changes was consistent with stimulation of the Na^+ pump. With higher concentrations of DPH, 1.0 and 5.0 mM, the effect was reversed, and a highly significant increase in

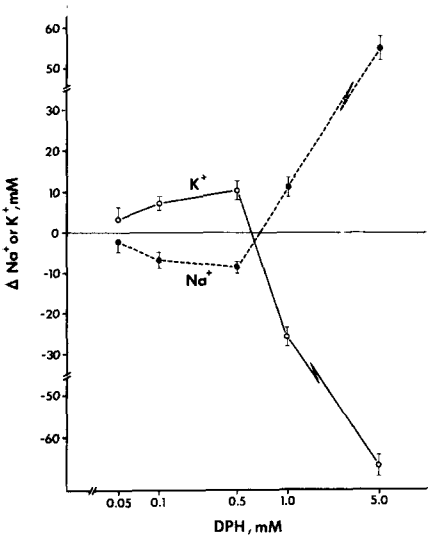

Fig. 1. The effect of diphenylhydantoin on intracellular levels of Na^+ and K^+. Incubation for 50 min in the presence of insulin, 0.25 munit/ml, and the concentrations of DPH shown on the abscissa. The ordinate refers to differences between drug treated and control hemidiaphragms. Data are means ± S.E. of 6 to 20 determinations. For statistical comparisons, see text.

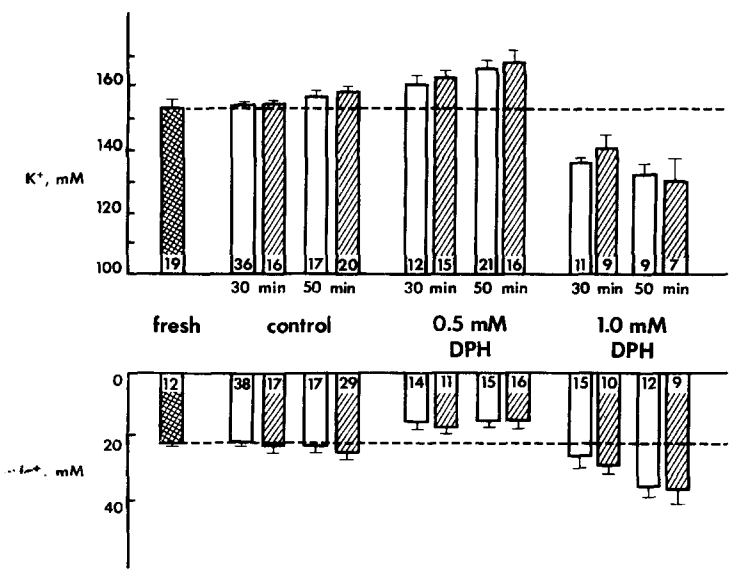

Fig. 2. The time course of changes in intracellular Na^+ and K^+ levels. The hatched bars refer to the presence of 0.25 munit/ml of insulin. Concentrations of DPH and duration of incubation are indicated under the bars. The height of the bars indicates means ± S.E. with the No. of experiments given at the base of each bar. The dotted line is to facilitate comparison with initial ion levels.

internal Na^+ and decrease in internal K^+ was observed. Thus, the effect of DPH on ion levels is clearly biphasic. Insulin, 0.25 munit/ml was present in all the experiments shown in Fig. 1 but identical results were obtained in the absence of the hormone (see below).

Fig. 2 illustrates the time course of changes in internal Na^+ and K^+ under various conditions. In the absence of DPH ion levels remain essentially stable. With 0.5 mM DPH an increase in internal K^+ and a corresponding decrease in internal Na^+ is evident at 30 min ($P < 0.005$, for both Na^+ and K^+, with and without insulin) and becomes somewhat greater at 50 min. The effect of 5 mM DPH to depress ion gradients was significant at 30 min and much greater at 50 min. Insulin did not influence these changes.

TABLE I

EFFECT OF DIPHENYLHYDANTOIN ON THE PENETRATION OF 3-METHYLGLUCOSE

Following preincubation for 20 min, the tissues were incubated for 30 min with 5 mM 3-methylglucose. One hemidiaphragm of each pair was exposed to the drug during preincubation and incubation. The figures are means of differences ± S.E. between paired hemidiaphragms with the number of pairs given in brackets.

Diphenylhydantoin (mM)	*Δ % Penetration*	
Insulin:	*None*	*0.25 munit/ml*
0.005	+ 0.9 ± 1.3 (9)	
0.02	+ 1.3 ± 1.2 (8)	− 1.7 ± 1.1 (11)
0.05	− 0.4 ± 0.8 (10)	− 0.4 ± 0.8 (10)
0.1	− 1.2 ± 1.3 (15)	− 7.1 ± 0.9 (22)*
0.5	− 5.7 ± 0.6 (9)*	−12.6 ± 0.8 (31)*
1.0	+ 6.1 ± 0.4 (4)*	+ 9.0 ± 1.9 (6)**
5.0	+14.4 ± 1.6 (5)**	+24.5 ± 1.5 (6)*

* $P < 0.0005$ (paired comparison).
** $P < 0.005$ (paired comparison).

Table I shows the effect of graded concentrations of DPH on sugar penetration. With 0.5 mM DPH sugar transport was strongly inhibited in the absence and even more in the presence of a submaximal dose of insulin, 0.25 munit/ml. With 0.1 mM DPH the inhibitory effect was significant only in the presence of insulin. The greater inhibitory effect of DPH in the presence of insulin is in keeping with a similar difference in the effect of high external K^+ (ref. 8,21). With higher concentrations of DPH, 1.0 and 5.0 mM, sugar transport was significantly increased with and without added insulin. The biphasic nature of these effects shows a clear relation to the changes in internal ion levels (Fig. 1), with inhibition of sugar transport when the normal transmembrane gradients of Na^+ and K^+ were increased, and stimulation when the ionic gradients were decreased. These results agree with earlier observations that sugar transport was inhibited when the level of internal Na^+ was decreased and that of K^+ increased in a high-K^+ medium[8]. A correlation analysis of the pooled data is shown below (Figs. 3A and 3B).

The effect of high concentrations of DPH is not due to a nonspecific increase in membrane permeability: penetration of the "non-transported" sugar, L-glucose re-

mained low and unchanged by 5 mM DPH (Δ % penetration = 0.4 $\pm$ 0.25 (5), $P >$ 0.1). In addition, the distribution of mannitol was not altered by 1.0 or 5.0 mM DPH.

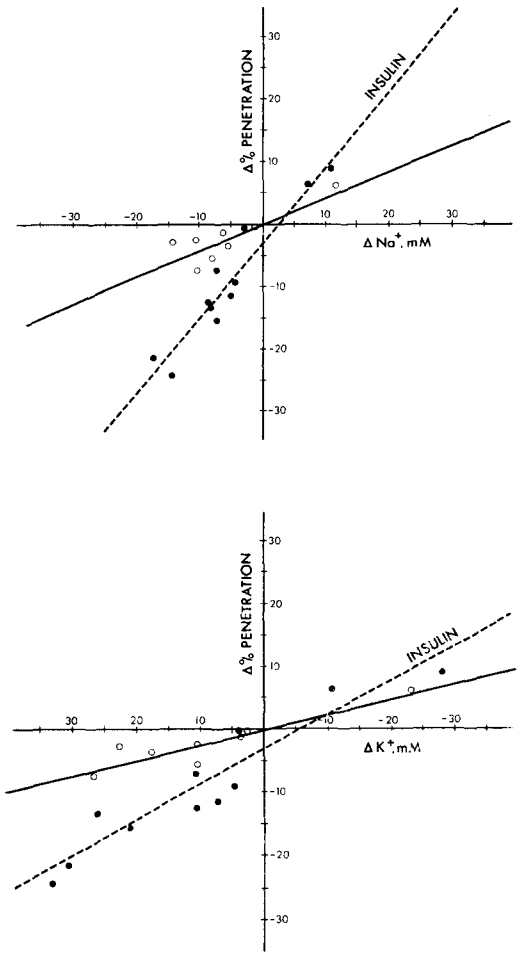

Fig. 3. Correlation between changes in sugar penetration and changes in internal Na^+ (A) and internal K^+ (B). Each point is the mean of differences in paired experiments (6 to 31 pairs). Data include 8 points from an earlier study on the effect of high external K^+ (BIHLER AND SAWH[8]). See text for correlation analysis. Note: To emphasize the similarity in correlation of sugar transport to Na^+ and K^+, the abscissa for K^+ (Fig. 2B) is reversed, with negative values to the right of the zero point.

It has been shown before that influx and efflux of sugar in muscle are affected in the same manner by various regulatory factors[3,7,8]. Table II indicates that this applies to DPH as well. A low concentration of the drug caused a decrease in insulin-stimulated efflux of sugar into sugar-free medium and a high concentration of DPH caused an increase in efflux. The changes in ion content under these conditions are also shown in Table II. They are consistent with those in Fig. 1 and are statistically significant although to total time of exposure to DPH (15 min) was shorter. Table II also indicates that the rate of efflux depends on the duration of exposure to insulin,

TABLE II

EFFECT OF DIPHENYLHYDANTOIN ON THE EFFLUX OF 3-METHYLGLUCOSE

Tissues were loaded for 30 min in 10 mM 3-methylglucose and washed out in sugar-free medium for 10 min. DPH was present during the last 5 min of loading and during the 10 min of washout. Insulin (0.25 munit/ml) added as indicated. The data give the amount of sugar lost from the tissue (mean ± S.E.) in percent of the amount loaded in the paired control. The data for ions refer to differences between control and drug treated tissue at the end of the washout period. The number of pairs for each figure is given in brackets.

	Sugar lost during 10-min washout (%)	*Δ intracellular ions (mM)*	
		Na^+	K^+
Insulin*	61.7 ± 1.8 (8) ***		
Insulin* + 0.5 mM DPH	53.2 ± 1.6 (4) $P < 0.01$	− 5.6 ± 1.7 (5) $P < 0.025$	+ 5.4 ± 1.3 (5) $P < 0.01$
Insulin**	52.5 ± 2.1 (4) ***		
Insulin** + 5.0 mM DPH	63.4 ± 2.2 (4) $P < 0.05$	+15.6 ± 4.4 (5) $P < 0.0125$	−18.2 ± 3.7 (5) $P < 0.005$

* Insulin present during loading and washout.
** Insulin present during washout only.
*** Difference between lines 1 and 3: $P < 0.02$.

efflux from tissue exposed to insulin during the loading and washout periods being significantly greater ($P < 0.02$) than from tissue exposed to the hormone only during washout. The gradual onset of the stimulatory effect in muscle is well known, and the time course of its effect on sugar efflux has recently been described[7].

To explore the striking reversal of effects of DPH when its concentration was doubled from 0.5 to 1.0 mM, we measured these effects in the presence of 16 mM K^+. Such a concentration of external K^+ increases intracellular K^+, decreases intracellular Na^+ and inhibits sugar transport to about the same extent as 0.5 mM DPH[8]. Table III shows the results of these experiments together with appropriate controls, illustrating the effects of DPH or of high K^+ alone. The data indicate that a concentration of DPH (0.05 mM) which is too low to be effective either when alone (Expt. B) or in the presence of insulin (Expt. D), increased ionic gradients and inhibited sugar transport in the presence of 16 mM K^+ (Expts. C and F), over and above the effect of 16 mM K^+ alone (Expts. A and E). This effect was seen both in the presence (Expt. F) and absence (Expt. C) of insulin. On the other hand, the effect of 0.5 mM DPH, a concentration causing maximal inhibition of sugar transport and increase in ionic gradients (Expt. G), was reversed to stimulation of sugar transport and decrease in ionic gradients in the presence of 16 mM K^+ (Expt. H), effects seen in a normal medium only with higher concentrations of DPH (Fig. 1 and Table I). Thus, increased external K^+ appears to potentiate the effects of DPH, eliciting effects which one would expect from a higher concentration of DPH if used alone.

The effect of low concentrations of DPH to increase internal K^+ and decrease internal Na^+ may be due to stimulation of the Na^+ pump. The data in Table IV indicate that 0.5 mM DPH, a concentration maximally effective in inhibiting sugar transport (Table I), had no effect in the presence of 10^{-5} M ouabain, a concentration known to strongly inhibit the Na^+ pump. In contrast, DPH had the expected inhibitory effect when added to 10^{-9} M ouabain, a concentration which does not inhibit

TABLE III

EFFECTS OF DIPHENYLHYDANTOIN IN THE PRESENCE OF INCREASED EXTERNAL K^+

Incubation with 5 mM 3-methylglucose as in Table I. 0.25 munit/ml insulin added as indicated. 10 mM KCl added to normal medium (6 mM K^+) to give total concentration of 16 mM K^+, where indicated. The figures are means of differences ± S.E. in paired experiments, with the number of pairs given in brackets. Expts. A and E from BIHLER AND SAWH[8]. Effects in Expts. B and D are not significant, all others are (paired comparison).

Expt.	*Medium*	*Δ intracellular ions (mM)*		*Δ% Penetration of sugar*
		Na^+	*K^+*	
A	Normal (6 mM K^+) 16 mM K^+	− 5.6 ± 1.3 (13)*	+17.8 ± 2.9 (13)*	− 3.7 ± 0.6 (11)*
B	Normal 0.05 mM DPH	− 1.2 ± 3.5 (6)	+ 2.6 ± 4.4 (6)	− 0.4 ± 0.8 (10)
C	16 mM K^+ 16 mM K^+ + 0.05 mM DPH	− 4.7 ± 1.2 (6)**	+ 9.1 ± 2.1 (6)**	− 3.5 ± 1.2 (4)***
D	Insulin Insulin + 0.05 mM DPH	− 2.1 ± 2.8 (6)	+ 3.3 ± 3.2 (6)	− 0.4 ± 0.8 (10)
E	Insulin (6 mM K^+) Insulin, 16 mM K^+	− 7.4 ± 1.5 (20)*	+21.1 ± 3.5 (20)*	−15.7 ± 2.0 (11)*
F	Insulin, 16 mM K^+ Insulin, 16 mM K^+ + 0.05 mM DPH	− 7.2 ± 1.5 (6)**	+12.2 ± 2.1 (6)**	− 8.6 ± 0.6 (6)*
G	Insulin Insulin + 0.5 mM DPH	− 8.9 ± 1.5 (16)**	+10.7 ± 2.3 (16)**	−12.6 ± 0.8 (31)*
H	Insulin + 0.5 mM DPH Insulin, 16 mM K^+ + 0.5 mM DPH	+16.1 ± 1.8 (15)*	−21.3 ± 3.2 (15)*	+ 5.1 ± 0.9 (15)*

* $P < 0.0005$.
** $P < 0.005$.
*** $P < 0.02$.

TABLE IV

COMBINED EFFECTS OF DIPHENYLHYDANTOIN AND OUABAIN ON SUGAR PENETRATION

Incubation for 30 min with 5 mM 3-methylglucose. 0.25 munit/ml insulin was added to all tissues and 0.5 mM DPH was added to one of each pair of hemidiaphragms. The figures are means of differences ± S.E. in paired experiments with the number of pairs given in brackets.

Medium	*Δ % penetration of sugar*	
Ouabain 10^{-5} M Ouabain 10^{-5} M + DPH	+0.7 ± 0.4 (6)	(not significant)
Ouabain 10^{-9} M Ouabain 10^{-9} M + DPH	−4.3 ± 0.4 (4)	$P < 0.025$

the Na^+ pump and, in fact, stimulates it in this preparation[1]. Thus, the effect of DPH is abolished when the Na^+ pump is blocked, and it may be concluded that for DPH to be effective the Na^+ pump must be intact and susceptible to stimulation.

The mean values of changes in sugar penetration together with the corresponding changes in internal levels of Na^+ and K^+ are plotted in Figs. 3A and B. The pooled data include results with four concentrations of DPH, with three different concentrations of elevated external K^+ (data reported previously[8]) and with the two agents combined. Correlation analysis showed that the coefficient of correlation between Δ% sugar penetration and Δ mM internal Na^+ is $r = 0.952$ ($P < 0.001$) and $r = 0.844$ ($P < 0.01$), with and without 0.25 munit/ml insulin, respectively. For the correlation between Δ% sugar penetration and Δ mM internal K^+, $r = -0.952$ ($P < 0.001$) and $= -0.915$ ($P < 0.005$), with and without insulin, respectively. Thus, the correlations are both strong and statistically highly significant. For reasons of scale Fig. 3 does not include the data with 5.0 mM DPH. However, with this concentration also, sugar transport and changes in internal Na^+ and K^+ were reasonably well correlated in the absence of insulin. In its presence, however, sugar transport was lower than expected from the plot. Such deviations are to be expected at high transport rates because, firstly, percentage penetration appreciably underestimates influx under these conditions, and secondly, because increases in transport are limited by the maximal transport capacity and the stimulatory effects of insulin and any other factor cannot be additive beyond this limit.

DISCUSSION

We have shown earlier[2,3] that the well known stimulation of sugar transport in muscle by factors inhibiting the Na^+ pump is linked to changes in intracellular concentrations of Na^+ or K^+, or both, and we have suggested that the Na^+ pump, through its effect on internal levels of Na^+ and K^+, may exert a regulatory effect on sugar transport. Recently, we have also demonstrated the converse effect[8], *i.e.* inhibition of sugar transport through incubation in high-K^+ media, a treatment known to activate the Na^+ pump in muscle[9,10]. However, an increase in external K^+ inevitably leads to changes either in osmolarity of the medium (addition of KCl to the basal medium) or in the concentration of external Na^+ (isosmotic substitution of K^+ for Na^+). It has been shown that hyperosmolarity[14] and a decrease in external Na^+ (ref. 15) may

increase sugar transport, thereby counteracting the effect of high external K^+. Furthermore, the effects of hyperosmolarity and low external Na^+ on the Na^+ pump have not been well studied. Nor has it been excluded that high external K^+ might influence sugar transport directly, by a mechanism not involving the Na^+ pump. It seemed desirable, therefore, to study the interaction between sugar transport and internal levels of Na^+ and K^+ by means not involving these complications. The anticonvulsant drug diphenylhydantoin (DPH) is well suited for this purpose. As first shown by WOODBURY[11], the administration of DPH to rats *in vivo* increases the tissue concentrations of K^+ and decreases those of Na^+ in several organs of the body. FESTOFF AND APPEL[12] showed that DPH activated the (Na^+,K^+)-ATPase of rat brain synaptosomes if the concentrations of Na^+ and K^+ were within certain narrow limits. Synaptosomal uptake of K^+ was also stimulated by DPH under these conditions[16]. Other evidence consistent with stimulation of Na^+ and K^+ transport by DPH includes enhancement of renal Na^+ reabsorption in the dog[17], inhibition of insulin secretion *in vitro* with a concomitant decrease in the internal Na^+ of pancreatic islets[18], and antagonism of the K^+-depleting effect of digitalis in the normal dog heart *in vivo*[19]. The results presented here also demonstrate an effect of DPH on intracellular levels of Na^+ and K^+. Collectively, these data represent direct evidence for an effect of DPH on Na^+ and K^+ transport and are consistent with an action of the drug on the Na^+ pump mechanism. Thus, it seems reasonable to conclude that the changes in internal ion concentrations and in the activity of the (Na^+,K^+)-ATPase are both the expressions of a direct effect of DPH on the Na^+ pump mechanism. However, alternate mechanisms of action such as an effect of DPH on passive ion fluxes or an indirect effect on the Na^+ pump are not strictly excluded by the present evidence. In any case, whatever its mechanism of action, it is well established that DPH affects the internal levels of Na^+ and K^+, and this appears to be the effect utilized for modulation of sugar transport in muscle.

Effects on internal Na^+ and K^+

The results in Figs. 1 and 2 and Table III show that moderate concentrations of DPH enhance the normal gradients of Na^+ and K^+ across the cell membrane while higher concentrations have the opposite effect. As shown in Fig. 2, the effect of moderate concentrations of DPH is not merely a slowing of the spontaneous passive fluxes of Na^+ and K^+ but an actual increase in Na^+ and K^+ gradients when compared to ion levels at the start of incubation. The effect of DPH is clearly biphasic, that of lower concentrations being consistent with stimulation of the Na^+ pump and that of higher concentrations with inhibition. The reversal in effect is striking, since mere doubling of the DPH concentration from 0.5 to 1.0 mM is sufficient to convert maximal stimulation to strong inhibition. Another and perhaps related effect is illustrated in Table III, showing that high (16 mM) external K^+ shifts to a lower concentration both the threshold of the stimulatory effect of DPH and the point of reversal from stimulation to inhibition. The observed shift of the stimulatory part of the DPH dose–response curve to lower drug concentrations might be explained by synergism with the stimulatory effect of high external K^+ itself. This, however, cannot be the explanation for the shift of the inhibitory part of the dose–response curve. A more likely explanation for the biphasic nature and apparent ion dependence of the effects of DPH on Na^+ transport would be that changes in the ionic environment induced by high external

K^+ (or possibly DPH itself), *e.g.* a decrease in internal Na^+ or in ion binding to some cellular sites, could modify the interaction betweeen the drug and its receptors. In this context it is perhaps significant that another group of drugs specifically affecting the Na^+ pump, namely the cardiac glycosides, also have a biphasic effect which may be modified by K^+.

If one accepts that the effect of DPH on sugar transport is linked to its effect on ion transport (see below), Table IV provides additional indirect evidence. The data indicate that inhibition of sugar transport and, by inference, stimulation of the Na^+ pump by DPH are abolished when the pump is blocked by a high concentration of ouabain (10^{-5} M). This suggests that a functioning Na^+ pump is required for the effect of DPH, a relationship difficult to reconcile with an effect of DPH on passive ion fluxes. In contrast, the effect of DPH is not altered by a very low concentration of ouabain (10^{-9} M); it appears, in fact, to be additive to the Na^+ pump stimulating (and sugar transport inhibiting) effect of such low concentrations of cardiac glycosides[1] (I. BIHLER AND P. C. SAWH, unpublished data).

These effects of DPH on ion transport in intact muscle incubated *in vitro* may be considered a logical link between the effects of DPH after systemic administration *in vivo* and the effects on enzyme systems in broken cell preparations. Our data agree with the results obtained with brain synaptosomes[12] indicating that the effect of DPH may depend in extent and direction on the concentrations of Na^+ and K^+. An earlier study on brain microsomal (Na^+,K^+)-ATPase[20] revealed only inhibition by DPH but was done in an ionic environment which would preclude any stimulatory effect[12].

Effects on sugar transport

Sugar transport and the intracellular concentrations of Na^+ and K^+ in muscle show a consistent parallelism: a decrease in internal K^+ and increase in internal Na^+ is accompanied by stimulation of the specific sugar transport mechanism[1-3,5-7], whereas the opposite change, an increase in K^+ and decrease in Na^+ is accompanied by inhibition of sugar transport[1,8]. The present study provides further evidence in support of this relationship between sugar transport and internal Na^+ and/or K^+ and for the suggestion[2,8] that the changes in sugar transport may be causally related to those in ion levels. Unlike the effect of high external K^+, the effect of DPH is not complicated by changes in osmolarity or in the ionic composition of the medium. Another advantage of DPH is that, depending on its concentration and the ionic environment, the same drug may either increase or decrease the internal levels of Na^+ and K^+. Table I shows that the changes in sugar transport induced by DPH are qualitatively fully consistent with its effect on internal ionic levels: when the ionic gradients were increased by low concentrations of DPH sugar transport was inhibited and, conversely, when the ionic gradients were decreased by high levels of DPH sugar transport was increased. Thus, whether high external K^+ (ref. 8) or DPH are used, sugar transport is inhibited when internal Na^+ is decreased and internal K^+ is increased. Also, either a decrease or an increase in sugar transport may be induced by the same agent, DPH, depending on its effect on ion transport. The inhibition of sugar transport by DPH was greater and became apparent at a lower concentration of DPH if insulin was present. It appears that, as noted previously[8,21], the inhibitory response is greater if the sugar transport system is already stimulated, *e.g.* by insulin.

As with other modulators of sugar transport, the inhibitory and stimulatory effects of DPH were also seen when sugar efflux was measured (Table II). The effects on sugar transport shown in Table IV are also consistent with the effects of DPH and of ouabain on internal ion levels and their proposed relation to sugar transport. The inhibition of Na^+ transport by 10^{-5} M ouabain is well known; details on the effect of 10^{-9} M ouabain will be published separately.

The above conclusions based on the direction of changes in sugar transport and internal ion levels are further strengthened by a quantitative analysis of the pooled data from this and a previous study[8] on the effect of high external K^+ (Figs. 3A and 3B). The strong and highly significant correlation between internal concentrations of Na^+ and K^+ and sugar transport thus represents the counterpart to the similar correlation observed in tissues exposed to K^+-free medium[3]. At the same time, the occurrence of this correlation in a great variety of experimental conditions argues strongly in favour of a causal relationship.

This is not to say, of course, that variations in internal Na^+ and/or K^+ are the only or primary factors modulating sugar transport in muscle. We have recently[8] discussed the possibility that the modulating effects of variations in internal Na^+ or K^+, of muscular contraction and even of insulin[22] may involve a specific membrane-bound pool of Ca^{2+} as the ultimate and common mediator of modulating influences.

It cannot be excluded that DPH may have a direct effect on this hypothetical pool and preliminary data suggest that some drugs affecting membrane Ca^{2+}, the so-called membrane stabilizers may affect sugar transport in this manner. However, the present evidence, in conjunction with earlier observations indicates that such an explanation need not be invoked in the case of DPH and strongly supports an effect of DPH *via* internal Na^+ or K^+ levels. It thus provides additional support for the hypothesis[2,8] that the internal levels of Na^+ or K^+ or both, may modulate the activity of the sugar transport mechanism in insulin-sensitive tissues. It is still not possible to decide, however, which of the two ions is responsible for the effect, nor do the present data provide direct evidence on the nature of the link between changes in internal Na^+ or K^+ and Ca^{2+} fluxes.

Independently of any effects on sugar transport, this work provides a clearcut demonstration of the biphasic effect of DPH on ion levels in isolated muscle, consistent with a dual action on the Na^+ pump. This action of DPH may be relevant to the mechanism of its therapeutic effects as an anticonvulsant and cardiac antiarrhythmic drug.

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REFERENCES

1 I. BIHLER, *Proc. Int. Union Physiol. Sci.*, 7 (1968) 45.
2 I. BIHLER, *Biochim. Biophys. Acta*, 163 (1968) 401.
3 I. BIHLER AND P. C. SAWH, *Biochim. Biophys. Acta*, 225 (1971) 56.

4 T. CLAUSEN, *Biochim. Biophys. Acta*, 120 (1966) 361.
5 R. J. HO AND B. JEANRENAUD, *Biochim. Biophys. Acta*, 144 (1967) 61.
6 J. LETARTE AND A. E. RENOLD, *Biochim. Biophys. Acta*, 183 (1969) 350.
7 T. CLAUSEN, *Biochim. Biophys. Acta*, 183 (1969) 625.
8 I. BIHLER AND P. C. SAWH, *Biochim. Biophys. Acta*, 241 (1971) 302.
9 P. HOROWICZ AND C. J. GERBER, *J. Gen. Physiol.*, 48 (1965) 489.
10 D. M. KIPNIS AND J. E. PARRISH, *Fed. Proc.*, 24 (1965) 1051.
11 D. M. WOODBURY, *J. Pharmacol. Exp. Ther.*, 115 (1955) 74.
12 B. W. FESTOFF AND S. H. APPEL, *J. Clin. Invest.*, 47 (1968) 2752.
13 T. KONO AND S. P. COLOWICK, *Arch. Biochem. Biophys.*, 93 (1961) 514.
14 T. CLAUSEN, J. GLIEMAN, J. VINTEN AND P. G. KOHN, *Biochim. Biophys. Acta*, 211 (1970) 233.
15 D. ILSE AND S. ONG, *Biochim. Biophys. Acta*, 211 (1970) 602.
16 A. V. ESCUETA AND S. H. APPEL, *J. Clin. Invest.*, 49 (1970) 27a.
17 A. KOCH, R. HIGGINS, M. SANDE, J. TIERNEY AND R. TULIN, *Physiologist*, 5 (1962) 168.
18 S. KIZER, M. VARGA-GORDON, K. BRENDEL AND R. BRESSLER, *J. Clin. Invest.*, 49 (1970) 52a.
19 B. J. SCHERLAG, R. H. HELFANT, M. A. RICCIUTI AND A. N. DAMATO, *Am. J. Physiol.*, 215 (1968) 1288.
20 M. D. RAWSON AND J. H. PINCUS, *Biochem. Pharmacol.*, 17 (1968) 573.
21 T. CLAUSEN AND P. G. KOHN, *J. Physiol.*, 210 (1970) 138P.
22 I. BIHLER AND P. C. SAWH, *Proc. Can. Fed. Biol. Soc.*, 13 (1970) 75.