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Effects of Na^+ on Sugar and Amino Acid Transport in Striated Muscle *

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Evidence has been presented recently which suggests that the sodium ion is essential for the active transport [1] of a variety of organic solutes. This requirement was first demonstrated by Riklis and Quastel (1) who showed that Na^+ was needed for the active transport of sugars by guinea pig intestine. Subsequently, Csaky and Zollicoffer (2, 3), using rat and toad intestinal preparations, reported that Na^+ was required for the active transport of the monosaccharides glucose and 3-O-methylglucose, the amino acids L-tyrosine and DL-phenylalanine, and the pyrimidine, uracil. Crane, Miller, and Bihler (4, 5) also observed an obligatory Na^+ requirement for active sugar transport by intestinal epithelium, and more recently, Kleinzeller and Kotyk (6) reported a similar requirement for the active transport of galactose by renal tubular epithelium.

The purpose of this study was to examine the effect of Na^+ on a nonactive mediated transport system as well as on an active transport process. Striated muscle was selected for this purpose, since in this tissue, in contrast to intestine and kidney, glucose is transported by a nonactive mediated system (7). Amino acids, however, are transported by both active and nonactive mediated systems and a process characterized by the kinetics of passive diffusion (8, 9). Furthermore, both sugar and amino acid-mediated transport in this tissue are responsive to the hormone insulin.

The results of this study indicate that in striated muscle *1*) the nonactive mediated transport of sugars is not Na^+ dependent; *2*) the active transport of the amino acid α-aminoisobutyric acid (AIB) is Na^+ dependent; *3*) transport systems requiring Na^+ are inhibited by the glycoside, strophanthin K; and *4*) the insulin response of both sugar and amino acid transport can be differentially affected by either the absence of Na^+ or addition of strophanthin.

Methods

Animals. Male rats of the Sprague-Dawley strain,[2] weighing 120 to 150 g, were used in all experiments. Female frogs (*Rana pipiens*) were obtained commercially.[3]

Muscle preparations. Three *in vitro* muscle preparations were used: *1*) the cut rat hemidiaphragm described by Gemmill (10), *2*) the intact rat diaphragm described by Kipnis and Cori (11), and *3*) the frog sartorius muscle. After excision, both diaphragm preparations were rinsed briefly in buffer and blotted before incubation. The frog sartorius muscles were carefully dissected and severed at their tendinous insertions and placed in Ringer's solution at 4° C for 16 to 18 hours before experimental use.

Experimental procedures. The muscle preparations were incubated in sufficiently large volumes of buffer (30 ml for intact diaphragms; 5 ml for cut diaphragms and frog sartorius muscles) to avoid significant changes in the extracellular concentration of the penetrating solute during the experiment. Although a variety of buffers was used, isosmolarity was maintained at 300 mOsm per L for the rat diaphragm and 240 mOsm per L for the frog sartorius muscle. Choline chloride was substituted isosmotically for Na^+ when indicated and the medium buffered with Tris, 5×10^{-4} M. Control experiments indicated that this concentration of Tris did not affect the transport of the various solutes studied, the total water content of the tissue, or its extracellular space. When-

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[1] Active transport, in contradistinction to nonactive transport, in this paper, refers to movement or accumulation of solute against a concentration gradient.

[2] Holtzman Rat Co., Madison, Wis.

[3] Schettle Frog Farm, Stillwater, Minn.

ever used, crystalline zinc insulin [4] was present at a concentration of 0.4 U per ml.

Sugar transport was studied with *a*) 2-deoxyglucose (2DG), a sugar transported by the glucose transport system and phosphorylated by hexokinase to form 2-deoxyglucose-6-phosphate (2DG-6-P), which is not further metabolized (7), and *b*) galactose, a sugar transported by a mechanism independent of that involved in glucose penetration (12, 13). Upon completion of the incubation period in experiments involving 2DG, the hemidiaphragms were quickly excised, blotted, frozen in an ether-CO_2 slurry, and weighed. The intracellular content of free 2DG and 2DG-6-P was determined as described previously (7) except that the colorimetric procedure of Waravdekar and Saslaw for deoxysugars was used (14). In all of the experiments reported in this study, free 2DG was not demonstrated intracellularly, and hence the rate of accumulation of 2DG-6-P was a measure of the rate of 2DG transport.

Galactose transport was studied with galactose-1-C^{14}. After incubation, the tissue was homogenized in 10 vol of 0.008 N acetic acid. This procedure extracted > 98% of the tissue radioactivity. Samples of the acetic acid extract were plated on stainless steel planchets and assayed in a windowless gas flow counter; all values were corrected to infinite thinness. In contrast to the results obtained at extracellular galactose concentrations of 10^{-2} to 10^{-3} M, where greater than 95% of the tissue radioactivity was in the form of free galactose, the predominant form of tissue radioactivity at extracellular concentration of 10^{-5} to 10^{-6} M was galactose-1-phosphate. Free galactose and galactose-1-phosphate were identified by paper and column chromatography with authentic standards and acid hydrolysis. The characteristics and kinetics of galactose transport and galactokinase activity of striated muscle constitute the topic of a separate report (13).

Amino acid transport was studied with the nonmetabolizable amino acid AIB-1-C^{14}. AIB was extracted by homogenizing the tissues in 0.008 N acetic acid as previously described (8). Samples of these tissue extracts were plated and assayed as described for galactose-1-C^{14}.

[4] Eli Lilly and Co., Indianapolis, Ind.

TABLE I

*2-Deoxyglucose (2DG) penetration into the intact rat diaphragm in the presence and absence of Na^+**

Medium	Na^+	Intracellular 2DG-6-P†	
		Control	Insulin
	mEq/L	*μmoles/ml*	*μmoles/ml*
Tris-choline chloride‡	0	3.3 ± 1.3	14.5 ± 0.9
Krebs-phosphate	137	3.9 ± 0.7	14.5 ± 1.2
Krebs-phosphate-Tris‡	137	3.2 ± 0.4	

* Intact diaphragms were incubated in 0.01 M 2DG at 37° C for 30 minutes. Each value represents the mean ± SD of 4 to 12 experiments.
† 2DG-6-P = 2-deoxyglucose-6-phosphate.
‡ Tris, 5×10^{-4} M.

The tissue content of Na^+ was measured in two ways: *1*) Diaphragms were digested in concentrated nitric acid for 1 hour at 50° C, and Na^+ was determined in these extracts by flame photometry with appropriate internal standards, and *2*) Na^{22} was added to incubation medium, and after equilibration with the tissue Na^+ (less than 15 minutes incubation at 37° C is required for isotopic equilibration), Na^{22} content was measured with a windowless scintillation counter. Concomitant measurements of Na^+ content by these two methods agree within 5%.

ATP was measured by a modification of the luciferin-luciferase assay method of Strehler and Totter (15). After incubation, diaphragms were rapidly frozen in liquid nitrogen, pulverized, and extracted with 10 vol of cold water that was then heated at 100° C for 10 minutes. Samples of this extract were added to the enzyme assay system, and light production was measured in a Farrand photofluorimeter.

The extracellular space was measured by sucrose-C^{14} distribution. Tissue water was determined by drying to constant weight.

Calculations. Intracellular concentration was calculated by the following formula:

$$C_i = \frac{C_t - \left(C_m \times \frac{S_s}{S_w}\right)}{1 - \frac{S_s}{S_w}},$$

TABLE II

Galactose penetration into the intact rat diaphragm and frog sartorius muscle in the presence and absence of Na^+

Tissue	Medium	Na^+	Intracellular galactose*	
			Control	Insulin
		mEq/L	*mμmoles/ml*	*mμmoles/ml*
Intact rat diaphragm	Krebs-phosphate	137	0.75 ± 0.13	3.67 ± 0.59
Intact rat diaphragm	Tris-choline	0	0.95 ± 0.27	3.39 ± 0.2
Frog sartorius muscle	Frog-Ringers	114	19.24 ± 6.8	45.4 ± 4.7
Frog sartorius muscle	Tris-choline	0	23.5 ± 6.9	50.0 ± 1.98

* Intact diaphragms were incubated in 1.5×10^{-6} M galactose-1-C^{14} for 60 minutes at 37° C. Frog sartorius muscles were incubated in 1.5×10^{-5} M galactose-1-C^{14} for 180 minutes at 20 to 22° C. Each value represents the mean ± SD of 4 to 12 experiments.

TABLE III

Effect of Na^+ on the temperature coefficient (Q_{10}) of 2DG and galactose penetration in the presence and absence of insulin

Sugar	Na^+	Intracellular sugar				Q_{10}	
		Control		Insulin			
		27°	37°	27°	37°	Control	Insulin
	mEq/L	*μmoles/ml*					
2-Deoxyglucose†	137*	1.9	3.9	8.0	15.5	2.0	1.9
	0	1.4 ± 0.2	3.6 ± 0.6	8.6 ± 0.5	15.6 ± 0.5	2.5	1.7
		mμmoles/ml					
D-Galactose†	137	3.7 ± 0.8	3.6 ± 0.6	7.3 ± 0.4	11.9 ± 0.6	1.0	2.3
	0	4.4 ± 0.4	4.6 ± 0.5	7.9 ± 0.2	12.8 ± 0.3	1.0	2.3

* Values taken from Kipnis and Cori (7).
† Intact diaphragms incubated in 0.01 M 2-deoxyglucose for 30 minutes and in 6×10^{-6} M galactose for 45 minutes. Values represent mean ± SE of the mean of 3 to 6 experiments.

where C_i = concentration per milliliter intracellular water; C_t = concentration per milliliter tissue water; C_m = concentration per milliliter incubation medium; S_s = sucrose-C^{14} distribution in milliliters per gram; S_w = total tissue water in milliliters per gram.

Chemicals. Galactose-1-C^{14} (SA, 0.5 to 6.5 mc per mmole) and sucrose-C^{14} (SA, 0.5 to 5.7 mc per mmole),[5] AIB-1-C^{14} (SA, 17 mc per mmole),[6] and 2-deoxyglucose[7] were obtained commercially.

Results

Effect of sodium on 2DG and galactose transport. Complete isosmotic replacement of Na^+ in the incubation medium did not alter the basal penetration (i.e., without added insulin) of 2DG (Table I). Furthermore, insulin-stimulated transport in the absence or presence of Na^+. Tris, 5×10^{-4} M, did not in itself affect sugar transport. Galactose penetration and its stimulation by insulin in both rat diaphragm and frog sartorius muscle are, likewise, not Na^+ dependent (Table II).

The temperature coefficients (Q_{10}) of basal and insulin-stimulated penetration of both 2DG and galactose are not affected by Na^+ (Table III). Whereas the basal penetration of 2DG is characterized by a $Q_{10} \sim 2.0$, the Q_{10} of basal galactose transport, even at concentrations as low as 6×10^{-6} M, is 1.0. The Q_{10} for the transport of both sugars in the presence of insulin, however, is 1.7 to 2.3.

[5] California Corp. for Biochemical Research, Los Angeles, Calif.

[6] Isotopes Specialties Corp., Burbank, Calif.

[7] Mann Research Laboratories, New York, N. Y.

Effect of sodium on AIB transport. In contrast to 2DG and galactose penetration, Na^+ exerted a profound influence on AIB transport (Table IV). In the intact diaphragm preparation, the absence of extracellular Na^+ caused a marked reduction in the basal penetration rate of AIB and complete loss of insulin responsiveness.

Intracellular/extracellular concentration gradients greater than 1, the criteria used in this study for assessing the presence of active transport, are not observed in the intact rat diaphragm after 60 to 120 minutes of incubation unless insulin is added. In order to examine the effect of Na^+ on active AIB transport in the absence of added insulin, the cut rat diaphragm was used, since in this preparation concentration gradients greater than 1 are readily developed in the absence of the hormone (16). In the absence of Na^+, the intracellular/extracellular concentration ratio never

TABLE IV

Effect of Na^+ on α-aminoisobutyric acid (AIB) penetration into the rat diaphragm

Medium	Rat diaphragm preparation	Na^+	$\frac{\text{Intracellular}}{\text{Extracellular}}$ AIB ratio*	
			Control	Insulin
		mEq/L		
Krebs-phosphate	Intact	137	0.83±0.05	1.73±0.05
Tris-choline	Intact	0	0.52±0.02	0.59±0.07
Krebs-phosphate	Cut	137	1.83±0.58	3.32±0.69
Tris-choline	Cut	0	1.07±0.03	1.10±0.08

* Intact rat diaphragms were incubated in 4.8×10^{-6} M AIB-1-C^{14} for 60 minutes at 37° C; cut diaphragms were incubated in 1×10^{-6} M AIB-1-C^{14} for 60 minutes at 37° C. All values represent the mean ± SD of 4 to 12 experiments.

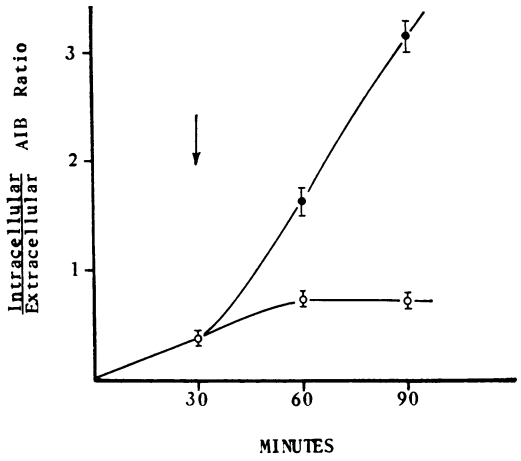

FIG. 1. RECOVERY OF ACTIVE α-AMINOISOBUTYRIC ACID (AIB) TRANSPORT. Intact diaphragms were incubated in the presence of insulin (0.4 U per ml) for 30 minutes in Tris-choline buffer. Diaphragms (●——●) were then removed and incubated in Krebs-phosphate buffer for an additional 30 to 60 minutes and compared to control tissues (○——○) remaining in Tris-choline buffer. AIB concentration, 1×10^{-5} M. Each point represents the mean ± SE of the mean of at least three experiments.

exceeded 1 with or without insulin, suggesting that equilibrium with the medium was reached by nonactive mediated transport, diffusion, or both, and that active transport was inhibited (Table IV). The addition of Na^+ to the incubation medium rapidly reverses the inhibition of active transport and restores insulin responsiveness (Figure 1).

The Q_{10} of AIB penetration in the absence of Na^+ is 1.9, with or without insulin (Table V), suggesting that under these conditions AIB penetration still represents mediated transport. AIB penetration was examined in the presence and absence of Na^+ at low AIB concentrations (10^{-6} M), where active mediated transport is predominant, and at high AIB concentrations (10^{-2} M), where active transport is saturated and nonactive penetration is predominant (Table VI). In order to observe the effects of Na^+ on the rate of AIB penetration at high concentrations, 1×10^{-2} M, incubations were performed for 20 minutes so that C_i/C_o values would be less than 1, the diffusion equilibrium. In contrast to the results obtained at low concentrations, AIB penetration at high concentrations is neither insulin responsive nor sodium dependent.

The active transport of AIB progressively decreased as the Na^+ content of the incubation medium was isosmotically replaced with choline chloride (Figure 2). To ascertain whether the effect of Na^+ on AIB accumulation is mediated primarily by alterations in influx or by changes in efflux, initial rates of AIB influx and efflux in the presence and absence of Na^+ were determined. Influx was measured during short periods of incubation (i.e., 10 to 30 minutes) when the intracellular level of AIB was low and efflux negligible. Under these conditions, rate measurements are a close approximation of initial velocities. As seen in Figure 3, AIB influx is approximately twice as rapid in the presence of Na^+ (0.29 mμmoles per ml cell water per minute) as in its absence (0.14 mμmoles per ml cell water per minute).

Efflux was studied by preloading the cut diaphragm with AIB, then resuspending the tissues in Krebs-HCO_3 or Tris-choline chloride buffer, neither of which contained amino acid, and measuring the rate of loss of AIB from the tissue. The cut diaphragm was selected for these studies because *a*) AIB preloading was accomplished with greater ease in this preparation than in the

TABLE V

*Effect of Na^+ on the temperature coefficient (Q_{10}) of AIB penetration in the presence and absence of insulin**

Medium	Na^+	Intracellular AIB				Q_{10}	
		Control		Insulin			
		27°	37°	27°	37°	Control	Insulin
	mEq/L	*mμmoles/ml*					
Krebs-phosphate	137	3.6 ± 0.3	8.3 ± 0.3	7.4 ± 0.4	17.1 ± 0.1	2.3	2.3
Tris-choline	0	2.8 ± 0.4	5.4 ± 0.3	3.1 ± 0.1	5.8 ± 0.4	1.9	1.9

* Intact diaphragms were incubated in AIB-1-C^{14}, 1×10^{-5} M, for 60 minutes. Each value represents the mean ± SE or the mean of 3 to 5 experiments.

TABLE VI

*Effect of Na^+ and insulin on the active transport and diffusion of AIB into the cut rat diaphragm**

Medium	Na^+	$\frac{\text{Intracellular}}{\text{Extracellular}}$ AIB ratio			
		Control	Insulin	Control	Insulin
		AIB, 1×10^{-6} M		AIB, 1×10^{-2} M	
	mEq/L				
Krebs-phosphate	137	1.82 ± 0.58	3.32 ± 0.69	0.54 ± 0.18	0.55 ± 0.04
Tris-choline	0	1.07 ± 0.03	1.10 ± 0.08	0.61 ± 0.11	0.58 ± 0.10

* Cut rat diaphragms were incubated at 37° C for 60 minutes in AIB, 1×10^{-6} M, and 20 minutes in AIB, 1×10^{-2} M. All values represent the mean ± SD for 3 to 4 experiments.

intact diaphragm, and *b*) incubation volume/intracellular volume ratios could be kept extremely large (> 4,000), thereby maintaining extracellular content of AIB at negligible levels throughout the incubation. Efflux was not influenced by Na^+; $k = 0.008$ minute^{-1} in the presence or absence of Na^+ (Figure 4). In addition, efflux both in the presence and absence of Na^+ was characterized by a Q_{10} of 1.2.

Effect of strophanthin K on sugar and AIB transport. Strophanthin K, 1×10^{-3} M, had no effect on the basal penetration of either 2DG or galactose or their insulin response but inhibited AIB penetration in the presence and absence of insulin (Figure 5). At a concentration of 1×10^{-5} M, strophanthin K did not depress the active transport of AIB (Table VII). In the absence of Na^+, when active transport was no longer observed, strophanthin K, 1×10^{-3} M, had no inhibitory effect on AIB penetration.

Differential effect of strophanthin K on AIB and Na transport. Strophanthin K, 1×10^{-3} M, not only inhibited AIB transport but also impaired the Na^+ pump as indicated by a marked increase in the intracellular concentration of Na^+ (Table VII). At lower concentrations, 1×10^{-5} M, the glycoside had no significant effect on AIB penetration even though it markedly depressed Na^+ pump activity.

The intracellular concentration of ATP is maintained by the intact rat diaphragm preparation

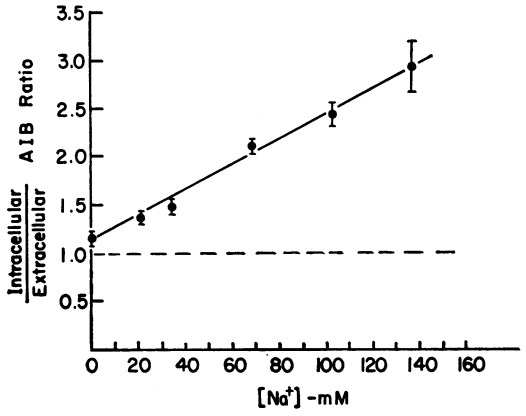

FIG. 2. EFFECT OF Na^+ ON THE ACTIVE TRANSPORT OF AIB IN THE RAT DIAPHRAGM. Cut diaphragms were incubated for 1 hour in the presence of insulin (0.4 U per ml) in varying concentrations of Na^+. NaCl was replaced by choline chloride in these studies, isosmolarity being maintained at 280 to 300 mOsm per L. AIB concentration, 1.5×10^{-5} M. Each point represents the mean ± SE of the mean of at least three experiments.

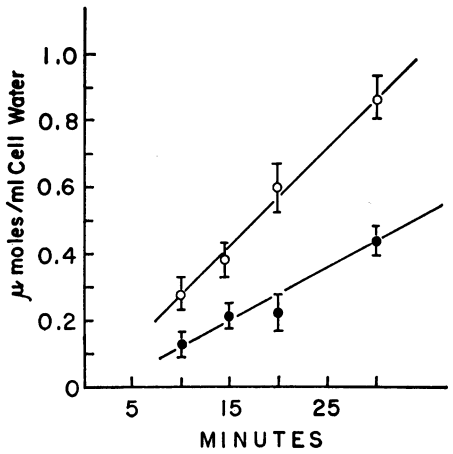

FIG. 3. RATE OF AIB-1-C^{14} PENETRATION INTO THE INTACT DIAPHRAGM IN THE PRESENCE AND ABSENCE OF Na^+. Diaphragms were incubated in Tris-choline (●——●) or Krebs-phosphate (○——○) buffer in the presence of insulin (0.4 U per ml) for 10 to 30 minutes. AIB concentration, 1×10^{-5} M. Each point represents the mean ± SE of the mean of three experiments.

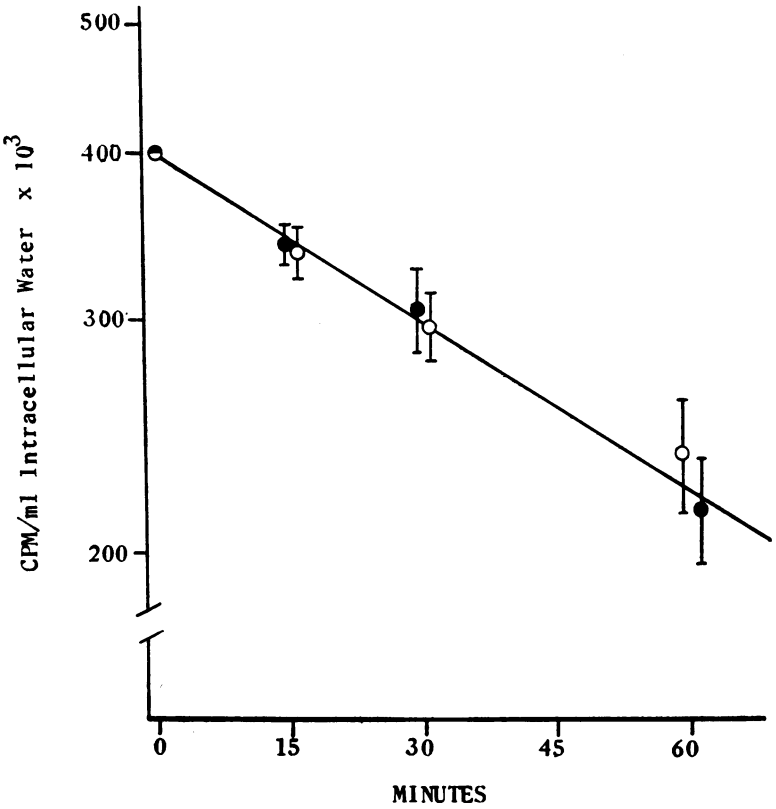

FIG. 4. EFFECT OF Na^+ ON AIB EFFLUX FROM RAT DIAPHRAGM. Cut diaphragms were preloaded with AIB-1-C^{14} by incubating in Krebs-bicarbonate buffer containing AIB-1-C^{14} for 30 minutes. They were then washed and incubated for varying periods in a large volume (300 ml) of either Tris-choline (open circles) or Krebs-bicarbonate (closed circles) buffer, containing no AIB. Each point represents the mean ± SE of the mean of three experiments.

when incubated aerobically in the absence of exogenous substrate and is not affected by strophanthin K even at concentrations of 1×10^{-3} M (Table VIII). The possibility that strophanthin K interferes with phosphorylating reactions (e.g., hexokinase-mediated phosphorylation of 2DG) or oxidative phosphorylation was also examined by determining tissue ATP content and the rate of 2DG phosphorylation after incubating the dia-

TABLE VII

*Effect of strophanthin K on AIB transport and Na^+ and ATP content of the intact rat diaphragm**

Experimental conditions	Intracellular content of		
	AIB	Na^+	ATP
	mμmoles/ml	*μmoles/ml*	*μmoles/ml*
Control			
Before incubation		23.2±1.8	2.66±0.38
After incubation	42.4±1.4	24.0±1.2	2.58±0.05
Strophanthin K			
1×10^{-5} M	40.6±2.5	44.4±0.6	2.65±0.11
1×10^{-3} M	24.0±1.6	55.9±4.4	2.54±0.07

* Intact rat diaphragms were incubated at 37° C for 60 minutes in AIB, 1.5×10^{-6} M. All values represent the mean ± SE of the mean intracellular content of 3 to 6 experiments.

TABLE VIII

*Effect of strophanthin K (1×10^{-3} M) on tissue content of ATP and on phopshorylating capacity of intact rat diaphragm**

Conditions	Intracellular content of	
	ATP	2DG-6-P
Control	1.7 ± 0.16	10.1 ± 1.4
Strophanthin K	1.65 ± 0.26	11.5 ± 2.0

* Intact rat diaphragms were incubated for 90 minutes at 37° C in Krebs-phosphate buffer without exogenous substrate in the presence and absence of strophanthin K, 1×10^{-3} M. They were then transferred to Krebs-phosphate medium containing 0.01 M 2DG and insulin, 0.4 U per ml, with and without strophanthin K, and incubated an additional 30 minutes. Values represent mean ± SEM of the mean of 3 to 6 experiments.

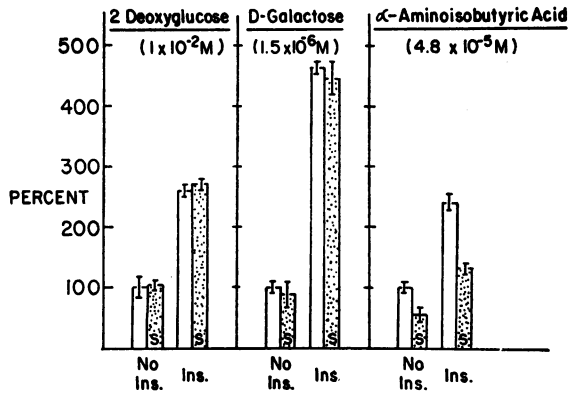

FIG. 5. EFFECT OF STROPHANTHIN K ON SUGAR AND AMINO ACID TRANSPORT IN INTACT RAT DIAPHRAGM. Strophanthin concentration was 1×10^{-3} M. Penetration in the absence of insulin and strophanthin is arbitrarily set at 100%. Incubations were carried out at 37° C for 60 minutes in Krebs-phosphate buffer. Insulin when present was at a concentration of 0.4 U per ml. Each value represents the mean ± SE of the mean of five to six experiments.

phragm for 120 minutes in the presence of strophanthin K, 1×10^{-3} M. Strophanthin K did not affect the control level of ATP, and furthermore, phosphorylation of substrate was not blocked. By using the quantity of sugar phosphorylated as an index of ATP generation by oxidative phosphorylation, these results would also indicate that the glycoside does not interfere with this parameter of cell metabolism.

Discussion

The concept of a carrier mechanism forms the basis of most operational models for nondiffusion transport across biological membranes. Carrier-mediated transport, whether active or nonactive, is characterized by saturation kinetics, exchange diffusion, competitive inhibition by structurally related analogues, and high temperature coefficients (17). The recent demonstration that Na^+ is required for the active transport of a variety of inorganic and organic solutes (1, 3, 6) raises the question as to whether Na^+ is essential for carrier-mediated transport systems in general. The results of this study, in which both types of mediated transport have been examined in the same tissue, indicate that nonactive carrier-mediated transport, exemplified by 2DG penetration in muscle, is not Na^+ dependent nor is the response of this system to insulin affected by Na^+ privation. In contrast, active carrier-mediated AIB transport and its stimulation by insulin are Na^+ dependent.

In the absence of Na^+, AIB is not transported against an electrochemical gradient, although its penetration still appears to be carrier-mediated. The apparent conversion of an active transport process to a nonactive one either by the removal of Na^+ or the addition of strophanthin K suggests that active transport systems are composed of at least two components: *1*) a carrier mechanism that determines the structural specificity of the transport system and *2*) a coupling mechanism that links energy-producing system(s) within the cell to the carrier in such a way as to convert metabolic energy to osmotic work. According to this scheme, the carrier system would not be Na^+ dependent or affected by cardiac glycosides, whereas the coupling mechanism would be affected by both. This would account for the lack of effect of strophanthin K on AIB transport in the absence of Na^+ and the lack of Na^+ dependence or strophanthin K inhibition of 2DG transport. A similar hypothesis has been proposed recently by Csaky (18) based on the observation that active sugar transport across the small intestine is Na^+ dependent and blocked by cardiac glycosides, whereas nonactive carrier-mediated sugar transport (e.g., that transport observed when the sugar concentration at the mucosal surface is very high) is unaffected by either.

The recent demonstration by Skou (19) and Post, Merritt, Kinsolving, and Albright (20) of a membrane localized, Na^+- and K^+-dependent, glycoside-inhibited ATPase raises the intriguing possibility that this enzyme complex may represent the coupling component in the proposed scheme of an active transport system. Since this specific type of ATPase activity has been demonstrated in practically every kind of mammalian tissue (21–23), its presence or absence would not appear to be the critical factor that determines whether transport is active or nonactive. Two other possible explanations that deserve further comment are that *1*) the ATPase complex must be structurally coupled to a specific carrier system in order to enable active transport to occur and *2*) there are multiple types of Na^+- and K^+-dependent ATPase complexes, each of which functions with a specific carrier system. The observation by Allfrey, Meudt, Hopkins, and Mirsky of a Na^+-

dependent active amino acid transport system in the thymocyte nucleus (24) and the fact that active amino acid transport in the human reticulocyte diminishes considerably or disappears concomitant with the loss of subcellular organelles known to contain Na^+- and K^+-dependent ATPase activity (e.g., nucleus and endoplasmic reticulum) (25, 26) are findings consistent with the suggestion that the ATPase complex must be structurally coupled to a specific carrier system. On the other hand, the ability to markedly depress Na^+ transport with small quantities of strophanthin K (1×10^{-5} M) without a concomitant inhibition of AIB penetration is consistent with the possibility that there are multiple specific forms of Na^+- and K^+-activated ATPase. Obviously, additional information is required in order to decide whether either or both of these alternative possibilities have merit or whether there is still another explanation. The observation that active Na^+ transport can be markedly inhibited without concomitant inhibition of active AIB transport is also evidence against the suggestion that active Na^+ transport per se is essential for the active transport of solutes whose penetration is Na^+ dependent (5).

The membrane-localized, Na^+- and K^+-dependent ATPase does not appear to participate in either the generation of ATP by oxidative phosphorylation or the phosphorylation of substrate by phosphokinases. For example, the intracellular content of ATP in the intact rat diaphragm was not affected by strophanthin K (1×10^{-3} M) after aerobic incubation for 120 minutes even in the absence of exogenous substrate. Furthermore, when 2DG was added to these diaphragms, greater than 10 μmoles per ml per 30 minutes was phosphorylated by hexokinase. This is the same rate of ATP generation by oxidative phosphorylation from endogenous substrate seen in intact diaphragms just excised and incubated aerobically in the absence of strophanthin.

These studies also indicate that many of the basal diffusion characteristics of the muscle cell membrane are not acutely affected by the extracellular content of Na^+, providing isosmolarity is maintained. Thus, the basal penetration of galactose (e.g., in the absence of insulin), which is primarily a diffusion process, was unaffected by the extracellular content of Na^+. Furthermore, neither the distribution of normally nonpermeant solutes such as sucrose and inulin nor tissue water content was affected by the presence or absence of Na^+. In addition, AIB efflux, a diffusion process, was also not influenced by Na^+.

The results obtained with insulin are of interest. If the hormone acts only by altering the behavior of a carrier system, it is difficult to explain either the ability of the hormone to stimulate galactose penetration or the failure of the hormone to accelerate carrier-mediated AIB entry in the absence of Na^+. On the other hand, if insulin action is dependent upon some interaction with the Na^+- and K^+-activated ATPase system, one would not expect to demonstrate an insulin effect on either galactose or 2DG entry in the absence of Na^+ or presence of high concentrations of glycoside; yet marked stimulation of sugar entry is observed under these conditions. The ability of insulin to stimulate the penetration of such a diverse group of solutes as sugars, amino acids, and electrolytes (27, 28), would suggest that some general characteristic of membrane structure and function as yet undefined is affected by the hormone. Whether this is a consequence of direct interaction between the hormone and a membrane component or is subsequent to some hormonally responsive intracellular process is unresolved.

Summary

The effects of Na^+ on sugar and amino acid transport were studied in the cut and intact rat diaphragm preparations and frog sartorius muscle. Amino acid transport was measured with the nonmetabolizable amino acid analogue α-aminoisobutyric acid (AIB); sugar transport was studied with 2-deoxyglucose and galactose. AIB is transported by an active carrier-mediated process and by diffusion, whereas 2-deoxyglucose is transported by the same nonactive carrier-mediated system responsible for glucose movement. Galactose penetration, in the absence of insulin, appears to be a process similar to diffusion.

The results of the present study indicate that *1*) Na^+ is necessary for the active transport of AIB and its stimulation by insulin, *2*) sugar transport and its insulin stimulation are not dependent on Na^+, *3*) strophanthin K inhibits the amino acid transport system and does so independently from its inhibition of Na^+ transport, and *4*) the kinetics of AIB penetration in the absence of Na^+ are those

of a nonactive carrier-mediated mechanism similar to that involved in sugar transport.

These studies suggest that the active transport process consists of two components: *1*) a carrier system responsible for the structural specificity of the process and *2*) a mechanism responsible for the linkage of metabolic energy and osmotic work.

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References

1. Ricklis, E., and J. H. Quastel. Effects of cations on sugar absorption by isolated surviving guinea pig intestine. Canad. J. Biochem. 1958, **36**, 347.
2. Csaky, T. Z., and L. Zollicoffer. Ionic effect on intestinal transport of glucose in the rat. Amer. J. Physiol. 1960, **198**, 1056.
3. Csaky, T. Z. Significance of sodium ions in active intestinal transport of nonelectrolytes. Amer. J. Physiol. 1961, **201**, 999.
4. Crane, R. K., D. Miller, and I. Bihler. The restrictions on possible mechanisms of intestinal active transport of sugars *in* Membrane Transport and Metabolism, A. Kleinzeller and A. Kotyk, Eds. New York, Academic Press, 1961, p. 439.
5. Bihler, I., and R. K. Crane. Studies on the mechanism of intestinal absorption of sugars. V. The influence of several cations and anions on the active transport of sugars, *in vitro,* by various preparations of hamster small intestine. Biochim. biophys. Acta (Amst.) 1962, **59**, 78.
6. Kleinzeller, A., and A. Kotyk. Cations and transport of galactose in kidney-cortex slices. Biochim. biophys. Acta (Amst.) 1961, **54**, 367.
7. Kipnis, D. M., and C. F. Cori. Studies of tissue permeability. V. The penetration and phosphorylation of 2-deoxyglucose in the rat diaphragm. J. biol. Chem. 1959, **234**, 171.
8. Helmreich, E., and D. M. Kipnis. Amino acid transport in lymph node cells. J. biol. Chem. 1962, **237**, 2582.
9. Akedo, H., and H. N. Christensen. Nature of insulin action on amino acid uptake by the isolated diaphragm. J. biol. Chem. 1962, **237**, 118.
10. Gemmill, C. L. The effect of insulin on the glycogen content of isolated muscles. Bull. Johns Hopk. Hosp. 1940, **66**, 232.
11. Kipnis, D. M., and C. F. Cori. Studies of tissue permeability. III. The effect of insulin on pentose uptake by the diaphragm. J. biol. Chem. 1957, **224**, 681.
12. Battaglia, F. C., and P. J. Randle. Regulation of glucose uptake by muscle. 4. The specificity of monosaccharide—transport systems in rat-diaphragm muscle. Biochem. J. 1960, **75**, 408.
13. Parrish, J. E., and D. M. Kipnis. Transport and phosphorylation of galactose in striated muscle. In preparation.
14. Waravdekar, V. S., and L. D. Saslaw. A sensitive colorimetric method for the estimation of 2-deoxy sugars with the use of the malonaldehyde-thiobarbituric acid reaction. J. biol. Chem. 1959, **234**, 1945.
15. Strehler, B. L., and J. R. Totter. Fire luminesence in the study of energy transfer mechanisms. I. Substrate and enzyme determination. Arch. Biochem. 1952, **40**, 28.
16. Peckham, W. D., and E. Knobil. Increase in amino acid transport by muscle cells in response to injury. Fed. Proc. 1962, **21**, 145.
17. Rosenberg, T., and W. Wilbrandt. Carrier transport uphill. I. General. J. theor. Biol. 1963, **5**, 288.
18. Csaky, T. Z. A possible link between active transport of electrolytes and nonelectrolytes. Fed. Proc. 1963, **22**, 3.
19. Skou, J. C. The influence of some cations on an adenosine triphosphatase from peripheral nerves. Biochim. biophys. Acta (Amst.) 1957, **23**, 394.
20. Post, R. L., C. R. Merritt, C. R. Kinsolving, and C. D. Albright. Membrane adenosine triphosphatase as a participant in the active transport of sodium and potassium in the human erythrocyte. J. biol. Chem. 1960, **235**, 1796.
21. Bonting, S. L., and L. L. Caravaggio. Studies on sodium-potassium-activated adenosinetriphosphatase. V. Correlation of enzyme activity with cation flux in six tissues. Arch. Biochem. 1963, **101**, 37.
22. Bonting, S. L., L. L. Caravaggio, and N. M. Hawkins. Studies on sodium-potassium-activated adenosinetriphosphatase. VI. Its role in cation transport in the lens of cat, calf and rabbit. Arch. Biochem. 1963, **101**, 47.
23. Bonting, S. L., L. L. Caravaggio, and N. M. Hawkins. Studies on sodium-potassium-activated adenosinetriphosphatase. IV. Correlation with cation transport sensitive to cardiac glycosides. Arch. Biochem. 1962, **98**, 413.
24. Allfrey, V. G., R. Meudt, J. W. Hopkins, and A. E. Mirsky. Sodium-dependent "transport" reactions in the cell nucleus and their role in protein and nucleic acid synthesis. Proc. nat. Acad. Sci. (Wash.) 1961, **47**, 907.
25. Winter, C. G., and H. N. Christensen. Migration of amino acids across the membrane of the human erythrocyte. J. biol. Chem. 1964, **239**, 872.
26. Yunis, A. Personal communication.
27. Zierler, K. L. Effect of insulin on potassium efflux from rat muscle in the presence and absence of glucose. Amer. J. Physiol. 1960, **198**, 1066.
28. Kernan, R. P. Stimulation of active transport of sodium from sodium-rich frog muscle by insulin and lactate. Nature (Lond.) 1961, **190**, 347.