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DUAL EFFECT OF ADRENALIN ON SUGAR TRANSPORT IN RAT DIAPHRAGM MUSCLE

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Summary

The effect of adrenalin on the membrane transport of the non-metabolized sugar, 3-methylglucose, was studied in isolated "intact" rat hemidiaphragms and related to simultaneously occurring changes in the internal levels of Na^+, ATP, glucose-6-*P*, glycerol formation and ^{45}Ca uptake and loss. Basal sugar transport was inhibited by low (10^{-8}—10^{-5} M) concentrations of adrenalin; this was antagonized by propranolol and practolol. High concentrations (10^{-4}—10^{-3} M) stimulated sugar transport, and this was blocked by propranolol and butoxamine and was dependent on external Ca^{2+}. These results suggest interaction with two different classes of adrenergic receptors, possibly of β_1 and β_2 types. Both low and high concentrations increased Na^+ and K^+ gradients by a practolol-sensitive effect. Isoproterenol behaved identically but phenylephrine had only the two practolol-sensitive effects on sugar and ion transport. Insulin did not interfere with inhibition of sugar transport and decrease in internal Na^+ but prevented stimulation of sugar transport. Under anoxia adrenalin had no effect on sugar transport but led to greater Na^+ gain by tissue. Addition of 3.0 mM palmitate decreased inhibition of sugar transport without changing receptor specificity. ATP was decreased and lipolysis enchanged by high adrenalin but glucose-6-*P* was increased by the low concentration as well. Influx of ^{45}Ca was decreased by low and increased by high adrenalin; ^{45}Ca efflux was also differentially affected. The results indicate that inhibition and stimulation of sugar transport depend on different receptors and that the latter response may override the former. The data are consistent with the earlier postulated regulatory role of sarcoplasmic Ca^{2+} on sugar transport in muscle, with adrenalin affecting Ca^{2+} fluxes and distribution both directly and indirectly.

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Abbreviation: EGTA, ethyleneglycol-bis(β-aminoethylether)-*N,N'*-tetraacetic acid.

Introduction

Membrane transport of glucose is the initial rate-limiting step in its utilization by muscle, and many of the factors known to influence glucose utilization are known to affect this step [1]. One such factor of physiological importance is adrenalin and decreased peripheral glucose utilization is thought to play an important role in this hyperglycemic effect (for review see ref. 2). In contrast to its metabolic effects, e.g. on lipolysis and glycogenolysis, the effect of adrenalin on the membrane transport of glucose is still controversial and based largely on indirect data.

We have recently demonstrated that low concentrations of adrenalin inhibit whereas high concentrations stimulate the transport of a non-metabolized glucose analog in resting isolated atrial muscle [3]. In the soleus, a red or slow skeletal muscle, only the inhibitory effect was seen [4]. The present study demonstrates a dual effect of adrenalin in diaphragm muscle and attempts to relate its two phases to other effects of adrenalin on metabolism and ion transport. A portion of this work has been reported previously in abstract form [5].

Methods

Fed young male Sprague-Dawley rats (Canadian Breeding Farms, St. Constant la Prairie, Quebec) weighing 50—70 g were killed by cervical dislocation and "intact" hemidiphragms were isolated [6]. Each hemidiaphragm was incubated for 30 min at 37°C with gentle shaking in 4.0 ml Krebs-Henseleit buffer, pH 7.4, containing a mixture of ^{14}C-labelled and unlabelled 3-*O*-methyl-D-glucose (total concentration, 5.0 mM) and tracer amounts of ^{3}H-labelled inulin, serving as extracellular marker. Ascorbic acid, 1.0 mg/ml, was added to prevent the oxidation of adrenalin [3], and 4% bovine serum albumin ("fatty acid poor", Miles Research Laboratories) was included in experiments with palmitate and the corresponding controls. Incubation with sugar was usually preceded by a 20 min period of preincubation in the absence of sugar; drugs and other additions were present in both preincubation and incubation media. All media were saturated with 95% O_2/5% CO_2; in anoxic experiments 95% N_2/5% CO_2 was used. After incubation the muscles were treated and analyzed for radioactivity and ion content as described before [7].

The same procedure was followed in measurements of Ca^{2+} uptake, except that trace amounts of $^{45}CaCl_2$ were added instead of [^{14}C]methylglucose.

For the experiments shown in Fig. 1, rat soleus muscles were isolated [8] and incubated and treated as described above.

ATP and glucose-6-*P* [9] and glycerol [10] were determined by enzymatic methods. Na^+ and K^+ were determined by flame photometry, and the results are expressed as millimolar concentrations in the intracellular water. Data for sugar are in "percent penetration", i.e. the concentration of sugar in the intracellular water space is expressed as a percentage of the final concentration in the incubation medium. The rationale for using this semiquantitative parameter of transport is given in Discussion. Results are usually presented as means of paired differences (±S.E.), i.e. as increments or decrements in the

value of percent sugar penetration or of ion or metabolite concentrations observed between the treated and contralateral control muscles. Statistical evaluation was done by Student's paired *t*-test.

Crystalline zinc insulin was obtained from Connaught Laboratories, Toronto. 3-*O*-[*Me*-^{14}C]glucose was obtained from ICN Corp. and generally labelled [^{3}H] inulin and $^{45}CaCl_2$ from New England Nuclear Corp. Other chemicals were of reagent quality and were obtained from a variety of commercial sources.

Results

The dose vs. response curve shown in Fig. 1 indicates that the effect of adrenalin on sugar transport in diaphragm muscle is a dual one. Whereas concentrations of adrenalin above about 10^{-4} M elicited a clearcut stimulatory effect, lower concentrations significantly inhibited sugar transport. This is analogous to the dual response observed in resting atria [3]. For comparison, the exclusively inhibitory effect in soleus muscle is shown.

In Table I the effect of two selected adrenalin concentrations, a low inhibitory and a high stimualtory one, are shown in more detail. The effects on sugar transport were significant and of opposite sense. In contrast, both the high and low adrenalin concentrations decreased internal Na^+ and increased internal K^+, an effect consistent with stimulation of the Na^+ pump.

To characterize the nature of the adrenoreceptors involved, the effects of the two adrenalin concentrations were determined in the presence of specific adrenergic antagonists. It was found (not shown) that in the absence of adrenalin, none of the antagonists affected sugar transport or Na^+ and K^+

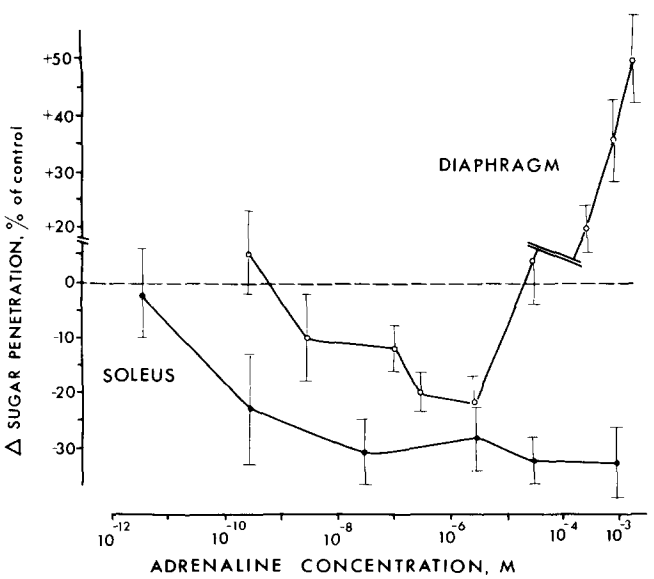

Fig. 1. Effets of L-adrenalin on the transport of 5.0 mM 3-methylglucose in rat hemidiaphragm and soleus muscles. Note that in this experiment only the ordinate shows the mean (±S.E.) change as percent of the paired control value. $n = 6 - 12$ pairs in each group.

TABLE I

EFFECTS OF HIGH AND LOW ADRENALIN CONCENTRATIONS ON PERCENT SUGAR PENETRATION AND ON INTRACELLULAR LEVELS OF Na^+ AND K^+

The data are means ± S.E., with the Nos. of paired experiments in parentheses. For details and definitions, see Methods.

	Sugar penetration (%)	Na^+ (intracellular, mM)	K^+ (intracellular, mM)
Control	19.9 ± 1.1 (16)	23.3 ± 1.0 (11)	155.5 ± 1.9 (18)
Adrenaline ($3 \cdot 10^{-7}$ M)	12.8 ± 1.3 (16)	16.8 ± 0.9 (11)	164.3 ± 2.1 (18)
	$\Delta = -7.1 \pm 1.0$ (16)	$\Delta = -6.5 \pm 0.7$ (11)	$\Delta = +8.8 \pm 1.7$ (18)
	$P < 0.001$	$P < 0.001$	$P < 0.001$
Control	18.9 ± 0.6 (17)	21.3 ± 1.3 (12)	153.9 ± 1.6 (12)
Adrenaline ($6 \cdot 10^{-4}$ M)	24.3 ± 1.1 (17)	16.3 ± 1.3 (12)	160.4 ± 1.4 (12)
	$\Delta = +5.4 \pm 0.7$ (17)	$\Delta = -5.0 \pm 1.1$ (12)	$\Delta = +6.5 \pm 1.3$ (12)
	$P < 0.001$	$P < 0.001$	$P < 0.001$

levels. The control levels thus remaining unchanged, the data in Table II include, therefore, only the means of paired differences in the values for sugar penetration and intracellular ion content due to the addition of adrenalin in the presence of the various antagonists. The results show that both the inhibitory and the stimulatory effect are unaffected by the α blocker phentolamine but abolished the β blocker propranolol. However, the response to more specific β inhibitors was different in the two cases. The inhibitory effect was blocked by practolol, believed to be a specific β_1 inhibitor [11] but unaffected by butoxamine which is predominantly a β_2 inhibitor [12]. The opposite was true for the stimualtory effect of the high adrenalin concentration. It may be noted that even a 10-fold increased concentration of practolol (340 μM) failed to antagonize the stimulation of sugar tranport by high adrenalin. Therefore, as far as sugar transport regulation in the rat diaphragm is concerned, the two effects of adrenalin appear to be mediated by two different classes of β receptors.

The decrease in Na^+ levels and the reciprocal increase in K^+ levels, caused by both high and low adrenalin concentrations, was inhibited by propranolol and practolol but was unaffected by butoxamine.

In the reverse experimental design (not shown) the various antagonists were added to one of each pair of tissues treated with adrenalin and analogous results were obtained. Propranolol largely abolished both the inhibition of sugar transport by $3 \cdot 10^{-7}$ M adrenalin and the stimulation by $6 \cdot 10^{-4}$ M adrenalin, as well as the decrease in intracellular Na^+ levels. The addition of phentolamine was ineffective.

The above conclusions are supported by the data in Table III which show that isoproterenol, a pure β-adrenergic agonist had a duel effect like adrenalin. The inhibitory phase was blocked by practolol, whereas the stimulatory phase was blocked by butoxamine. It may be noted that even the very high concentration of butoxamine used here, 100 μM did not antagonize the inhibitory phase of the isoproterenol effect.

As shown in Table IV, sugar transport was also inhibited by a low concentration of phenylephrine, an α agonist known to have some β activity [13]. The

TABLE II

EFFECTS OF α- AND β-ADRENERGIC ANTAGONISTS

The data are differences in sugar penetration or in intracellular ion content (means ± S.E.) due to the addition of adrenaline to one of each pair of tissues incubated in the presence of the adrenergic antagonists indicated. Absolute values for sugar penetration and ion content in the absence of antagonists are shown in Table I.

Addition to control and experimental	Adrenalin addition to experimental (M)	Sugar penetration (%)		Na^+ (intracellular, mM)		K^+ (intracellular, mM)	
		Δ	*P*	Δ	*P*	Δ	*P*
—	$3 \cdot 10^{-7}$	−7.1 ± 1.0 (16)	<0.001	−6.4 ± 0.7 (11)	<0.001	+8.8 ± 1.7 (18)	<0.001
Phentolamine, 50 μM	$3 \cdot 10^{-7}$	−7.9 ± 1.4 (6)	<0.005	−6.5 ± 1.0 (6)	<0.005	+7.1 ± 1.2 (6)	<0.005
Propranolol, 75 μM	$3 \cdot 10^{-7}$	−1.3 ± 1.7 (6)		+1.7 ± 3.5 (6)		−3.3 ± 3.4 (6)	
Practolol, 34 μM	$3 \cdot 10^{-7}$	+0.8 ± 1.1 (6)		−2.3 ± 1.8 (6)		+3.2 ± 2.4 (6)	
Butoxamine, 10 μM	$3 \cdot 10^{-7}$	−5.5 ± 0.9 (12)	<0.001	−11.1 ± 2.1 (12)	<0.001	+11.6 ± 2.0 (8)	<0.001
—	$6 \cdot 10^{-4}$	+5.4 ± 1.4 (17)	<0.001	−5.0 ± 1.1 (12)	<0.001	+6.5 ± 1.3 (12)	<0.001
Phentolamine, 75 μM	$6 \cdot 10^{-4}$	+5.3 ± 0.7 (11)	<0.001	−6.7 ± 1.2 (10)	<0.001	+8.7 ± 1.2 (12)	<0.001
Propranolol, 75 μM	$6 \cdot 10^{-4}$	+0.2 ± 0.4 (20)		−1.2 ± 3.3 (6)		+2.7 ± 2.8 (6)	
Practolol, 34 μM	$6 \cdot 10^{-4}$	+5.5 ± 0.7 (9)	<0.001	−1.6 ± 1.5 (6)		±2.4 ± 1.9 (6)	
Practolol, 340 μM	$6 \cdot 10^{-4}$	+5.9 ± 1.3 (6)	<0.02				
Butoxamine, 10 μM	$6 \cdot 10^{-4}$	+0.2 ± 1.0 (9)		−8.9 ± 2.3 (6)	<0.02	+7.9 ± 2.0 (6)	<0.02

TABLE III

EFFECTS OF ISOPROTERENOL AND THEIR INHIBITION BY ADRENERGIC ANTAGONISTS

The data are differences in the values for percent sugar penetration (means ± S.E.) due to the addition of isoproterenol to one of each pair of tissues incubated in the presence of the antagonists indicated. The control (basal) percent sugar penetration was 21.7 ± 0.8 (21) and was not altered by the antagonists alone.

Addition to control and experimental	Isoproterenol addition to experimental (M)	Sugar penetration (%)	
		Δ	*P*
–	$7.5 \cdot 10^{-5}$	−5.6 ± 1.1 (12)	<0.001
Propranolol, 75 μM	$7.5 \cdot 10^{-5}$	+0.9 ± 1.2 (5)	
Practolol, 34 μM	$7.5 \cdot 10^{-5}$	+0.6 ± 1.6 (9)	
Butoxamine, 100 μM	$7.5 \cdot 10^{-5}$	−4.5 ± 0.7 (5)	<0.005
–	$1 \cdot 10^{-3}$	+5.2 ± 0.9 (9)	<0.001
Propranolol, 75 μM	$1 \cdot 10^{-3}$	−0.8 ± 0.9 (4)	
Practolol, 34 μM	$1 \cdot 10^{-3}$	+6.5 ± 1.4 (12)	<0.001
Butoxamine, 100 μM	$1 \cdot 10^{-3}$	+0.8 ± 1.3 (5)	

data indicate that as far as this particular effect is concerned, it appears to behave as a β_1 agonist. In contrast to adrenalin and isoproterenol, higher concentrations of phenylephrine were devoid of any effect.

Table V shows the effect of adrenalin when sugar transport was stimulated by insulin or anoxia. In the presence of 0.25 munit/ml, a dose causing submaximal stimulation of sugar transport [14], $3 \cdot 10^{-7}$ M adrenalin inhibited sugar transport but no stimulation above that by insulin alone was seen with $6 \cdot 10^{-4}$ M; the decrease in internal Na^+ with both adrenalin concentrations was unchanged in the presence od insulin. In conctrast, under anoxic conditions neither concentration of adrenalin affected sugar transport but both caused an additional rise in internal Na^+, additive to that caused by anoxia alone.

The influence of external Ca^{2+} on the effect of adrenalin was studied by incubation in Ca^{2+}-free medium containing 0.1 mM EGTA; earlier experiments

TABLE IV

EFFECT OF PHENYLEPHRINE AND ITS INHIBITION BY ADRENERGIC ANTAGONISTS

For details, see Table III. The control (basal) percent sugar penetration was 20.8 ± 0.8 (18)

Addition to control and experimental	Phenylephrine addition to experimental (M)	Sugar penetration (%)	
		Δ	*P*
–	$7.5 \cdot 10^{-5}$	−5.4 ± 0.8 (6)	<0.005
Phentolamine, 50 μM	$7.5 \cdot 10^{-5}$	−5.4 ± 0.9 (12)	<0.001
Propranolol, 75 μM	$7.5 \cdot 10^{-5}$	+2.5 ± 1.7 (11)	
Practolol, 34 μM	$7.5 \cdot 10^{-5}$	+1.0 ± 1.3 (6)	
Butoxamine, 100 μM	$7.5 \cdot 10^{-5}$	−4.8 ± 0.8 (10)	<0.001
–	$3 \cdot 10^{-4}$	−0.2 ± 1.2 (6)	
–	$1 \cdot 10^{-3}$	−0.8 ± 2.3 (6)	

TABLE V

EFFECTS OF ADRENALIN IN THE PRESENCE OF 0.25 munit/ml INSULIN OR ANOXIA

The data show differences in the values for percent sugar penetration and intracellular Na^+ levels (mean ± S.E.) due to the addition of adrenalin to one of each pair of muscles incubated under the conditions shown. Control experiments show basal values and the effects of insulin and N_2 alone.

Addition to control and experimental	Addition to experimental	Sugar penetration		Intracellular Na^+	
		%	Δ	mM	Δ
--		23.2 ± 1.2 (16)		24.2 ± 2.1 (14)	
	Insulin	58.9 ± 2.0 (10)	+35.2 ± 2.0 (10) $P < 0.001$	25.5 ± 1.0 (8)	−1.5 ± 2.5 (8) $P < 0.001$
	N_2	55.7 ± 2.3 (6)	+32.6 ± 2.2 (6) $P < 0.001$	76.5 ± 7.2 (6)	+52.1 ± 7.5 (6) $P < 0.001$
Insulin	$3 \cdot 10^{-7}$ M adrenalin		−8.2 ± 1.0 (14) $P < 0.001$		−8.4 ± 1.3 (10) $P < 0.001$
	$6 \cdot 10^{-4}$ M adrenalin		−2.2 ± 1.3 (22)		−4.1 ± 1.1 (10) $P < 0.005$
N_2	$3 \cdot 10^{-7}$ M adrenalin		+1.5 ± 1.1 (9)		+15.8 ± 2.0 (13)
	$6 \cdot 10^{-4}$ M adrenalin		−0.8 ± 1.8 (15)		+16.8 ± 1.8 (5) $P < 0.001$

[15] indicated that this medium was highly effective in decreasing the stimulation of sugar transport by insulin. The inhibitory effect of low adrenalin ($3 \cdot 10^{-7}$ M) remained unchanged but the stimulation by high adrenalin ($6 \cdot 10^{-4}$ M) was abolished in Ca^{2+}-free medium with EGTA. Sugar penetration (%) was 23.4 ± 1.7 and 23.9 ± 2.0, without and with adrenalin, respectively, Δ = +0.6 ± 1.6 (6). The effect of adrenalin to decrease internal Na^+ remained unchanged in Ca^{2+}-free medium at both adrenalin concentrations.

Table VI shows the effect of the two representative adrenalin concentrations on several metabolic parameters. The data indicate that only the high adrenalin concentration, $6 \cdot 10^{-4}$ M, caused a significant drop in ATP content and an

TABLE VI

EFFECT OF ADRENALIN ON GLYCEROL PRODUCTION AND THE CONTENT OF ATP AND GLUCOSE 6-PHOSPHATE

The values (mean ± S.E.) are in μmol/g wet tissue weight and refer to tissue content of ATP and glucose-6-*P* and to the sum of tissue and medium content of glycerol. Zero time tissue content of glycerol was 0.14 ± 0.03 (6) μmol/g. $n = 6$ pairs in each group.

	Glycerol	ATP	Glucose-6-*P*
Control	0.53 ± 0.08	3.26 ± 0.10	0.46 ± 0.06
Adrenaline, $3 \cdot 10^{-7}$ M	0.46 ± 0.05	3.02 ± 0.13	1.25 ± 0.05
	Δ = −0.07 ± 0.07	Δ = −0.24 ± 0.19	Δ = +0.79 ± 0.05 $P < 0.001$
Control	0.57 ± 0.06	3.09 ± 0.10	0.82 ± 0.09
Adrenalin, $6 \cdot 10^{-4}$ M	1.05 ± 0.12	2.57 ± 0.13	1.44 ± 0.4
	Δ = +0.48 ± 0.10 $P < 0.001$	Δ = −0.52 ± 0.10 $P < 0.001$	Δ = +0.62 ± 0.10 $P < 0.001$

TABLE VII

INFLUENCE OF PALMITATE ON THE EFFECT OF ADRENERGIC AGONISTS

The data are paired differences (mean ± S.E.) in sugar penetration due to the addition of the agents indicated. The control values in the absence and presence of 3.0 mM palmitate are indicated for comparison.

	Sugar penetration			
	%	Δ	%	Δ
	—		3.0 mM palmitate	
Control	20.2 ± 0.6 (60)		21.0 ± 0.8 (26)	
Adrenaline				
$3 \cdot 10^{-7}$ M		−7.1 ± 1.0 (16)		+0.8 ± 3.0 (6)
$6 \cdot 10^{-4}$ M		+5.4 ± 1.4 (17)		+7.7 ± 0.6 (5)
Isoproterenol				
$7.5 \cdot 10^{-5}$ M		−5.6 ± 1.1 (12)		−3.2 ± 0.7 (5)
$1 \cdot 10^{-3}$		+5.2 + 0.9 (9)		+5.3 ± 0.3 (5)
Phenylephrine				
$7.5 \cdot 10^{-5}$ M		−5.4 ± 0.8 (6)		−1.3 ± 0.9 (6)

increase in lipolysis, as measured by glycerol formation. The level of glucose-6-*P* was equally and significantly elevated by both adrenalin concentrations.

The ability of free fatty acids to modulate the effect of adrenalin was investigated in view of a recent report [16] that the addition of palmitate converts a β receptor-dependent inhibition of glucose uptake in the diaphragm into α receptor-dependent stimulation. The data in Table VII confirm that in the presence of palmitate to albumin the inhibitory effect of adrenalin, isoproterenol and phenylephrine on sugar transport appears to be decreased or abolished, as though the drug concentration were increased. The stimulatory effect of the high concentrations of adrenalin and isoproterenol was not significantly altered but it is conceivable that augmentation could be seen under different conditions. No change in the receptor specificity of the response was observed: In the presence of 3 mM palmitate 75 μM propranolol antagonized both the inhibitory effect of $3 \cdot 10^{-7}$ M adrenalin ($\Delta = +5.3 \pm 1.1\%$ sugar penetration (6), $P < 0.01$) and the stimulation by $6 \cdot 10^{-4}$ M ($\Delta = -4.2 \pm 0.9$ (5), $P < 0.01$). Phentolamine, 50 μM, was ineffective in both instances ($\Delta = -1.0 \pm 1.2$ (5) and $\Delta = -0.1 \pm 1.0$ (6), respectively. In the presence of palmitate the increase in glucose-6-*P* content caused by $3 \cdot 10^{-7}$ M adrenalin remained unchanged ($\Delta = +1.19 \pm 0.10$ μmol/g (4), $P < 0.005$), and so did its failure to decrease the ATP level ($\Delta = -0.17 \pm 0.09$ μmol/g (6), $P < 0.10$).

As the effects of adrenalin on Ca^{2+} fluxes in normal resting diaphragm muscle have not been reported, a limited number of uptake and efflux studies with ^{45}Ca were done. The figures in Table VIII show 30 min uptake. These values are still far below steady-state levels (Bihler, I. and Sawh, P.C., unpublished data) and they probably reflect net uptake, as some ^{45}Ca efflux is bound to take place. The data show that uptake was significantly decreased by low adrenalin and increased by high adrenalin, paralleling the effects on sugar

TABLE VIII

EFFECTS OF ADRENALIN ON ^{45}Ca UPTAKE

The data show percent ^{45}Ca penetration and means of paired differences (±S.E.) due to the addition of adrenalin to one of each pair of tissues. For other details see Methods.

Addition to control and experimental	Adrenalin addition to experimental (M)	^{45}Ca penetration		
		%	Δ	P
—	—	48.6 ± 2.8 (12)		
	$3 \cdot 10^{-7}$	36.2 ± 3.4 (6)	−12.2 ± 3.2	<0.01
	$6 \cdot 10^{-4}$	60.4 ± 3.0 (6)	+12.0 ± 2.6	<0.01
Propranolol, 75 μM	—	46.3 ± 2.3 (12)		
	$3 \cdot 10^{-7}$	45.6 ± 2.0 (6)	−1.8 ± 2.6	
	$6 \cdot 10^{-4}$	46.3 ± 4.6 (6)	+1.1 ± 0.7 (6)	

transport. These effects were blocked by propranolol, indicating their β-adrenergic nature. Efflux from preloaded tissues was estimated by measuring ^{45}Ca loss during the period of 20—30 min of washout in unlabelled medium (of normal Ca^{2+} content). The ^{45}Ca loss, expressed as percent of ^{45}Ca present in paired control muscles at the 20 min point was 8.6 ± 1.9 (8) under basal conditions, 31.9 ± 3.5 (6) with $3 \cdot 10^{-7}$ M adrenalin ($P < 0.001$) and 18.3 ± 4.0 (7) with $6 \cdot 10^{-4}$ M adrenalin ($P < 0.05$, for difference from basal or from $3 \cdot 10^{-7}$ M adrenalin). The stimulation of efflux was clearly greater with the low adrenalin concentration.

Discussion

Equilibration of transport substrates and extracellular markers in the interstitial space of solid tissues incubated in vitro requires considerable time. It is not feasible, therefore, to determine unidirectional initial transport rates in such preparations. For this reason we have used as a semiquantitative measure of transport the percent penetration which reflects net uptake over a 30 min period. The use of this parameter has been discussed previously [14].

The effect of adrenalin to inhibit glucose utilization in mammalian skeletal muscle has long been recognized and has been linked to alterations in the rate of glycogenolysis and glucose phosphorylation [17,18]. Such changes, resulting in elevated intracellular glucose levels, may be expected to decrease net glucose uptake even if the activity of the transport system remains unaltered. Glucose uptake or utilization are, therefore, unsatisfactory as indicators of membrane transport. Indeed, the transport of non-metabolized glucose analogs in the diaphragm was reported not to be changed by adrenalin [19] or increased [20]. Adrenalin was also shown to increase glucose uptake and membrane transport in a variety of other tissues including isolated heart, white adipose tissue, frog sartorius and avian erythrocytes (for review see ref. 20).

We have recently demonstrated in resting isolated atria a dual effect of adrenalin on sugar transport, inhibitory at low concentrations and stimulatory

at higher concentrations; this was separate from the contraction-dependent increase described previously [22]. We have also observed a similar dual effect of adrenalin in the detrusor, a smooth muscle [23]. The experiments reported here (Fig. 1 and Table I) show that in rat diaphragm adrenalin also had a dual effect; the concentrations required were significantly higher than those effective in the isolated resting atrium but very similar to those effective in the detrusor. In agreement with earlier reports [24,25], both low and high adrenalin concentrations caused a decrease in intracellular Na^+ levels (Tables I, II, V), suggesting apparent stimulation of the Na^+ pump.

The data on receptor specificity (Tables II, III and IV) indicate that the effects on sugar and on ion transport of adrenalin and related agents are β-adrenoceptor interactions; this is consistent with earlier reports on the nature of adrenoceptors in skeletal muscle [26] and on the specificity of adrenergic effects on Na^+ transport [25]. Moreover, it appears that inhibition of sugar transport and apparent stimulation of the Na^+ pump are β_1 effects, whereas stimulation of sugar transport by the higher concentrations of adrenalin and isoproterenol are β_2 effects. Adrenergic activation of phosphorylation in diaphragm muscle has recently also been identified as a β_2 effect [27]. The effect of phenylephrine appears to be purely β_1, as no butoxamine-sensitive stimulation of sugar transport was observed at concentrations up to 10^{-3} M. The above conclusions depend, of course, on whether the antagonists used are as specific as currently believed. But, even if this receptor classification is not strictly applicable in the present case, the data show that two different classes of receptors are involved. Accepted at face value, they lend support to the concept that the (β_1) effect on sugar transport of low concentrations may be overcome by an additional (β_2) effect at higher agonist concentrations, whereas the (β_1) effect on ion transport remains unaffected. The β_2 effect was absent in the soleus, perhaps because of the difference in metabolic pattern discussed below.

We have earlier drawn attention to the striking correlation between stimulation of sugar transport and increased internal Na^+ levels in a variety of situations [15]. In the present experiments there was such a parallelism only at low adrenalin concentrations where both sugar transport and internal Na^+ levels were depressed. In contrast, at high adrenalin concentrations sugar transport was increased despite the decrease in internal Na^+ levels, indicating that additional mechanism(s) come into play, overshadowing the inhibition of sugar transport but not affecting ion transport. Moreover, under anoxic conditions (Table V) neither high nor low concentrations of adrenalin had any effect on sugar transport whereas intracellular Na^+, already raised by anoxia, was further increased.

The stimulation of sugar transport by high concentrations of adrenalin coincides with an increase in lipolysis and a drop in ATP levels (Table VI). The same three effects were seen in adipocytes, and it has been pointed out [28] that such a combination of effects would result from the intracellular accumulation of free fatty acids which may act as uncouplers of oxidative phosphorylation. The effect of added exogenous fatty acids to shift the response of sugar transport as though adrenalin levels were increased (Table VII) is consistent with this notion. Also, the antilipolytic agent nicotinic acid significantly depressed adrenalin-stimulated sugar transport in isolated

rat atria [3]. The failure of adrenalin at concentrations as high as $3 \cdot 10^{-3}$ M to stimulate sugar transport in rat soleus [4] is not inconsistent with such a mechanism: The soleus is a red muscle with relatively high capacity for oxidative metabolism and hence presumable better able to rapidly oxidize fatty acids and prevent their accumulation.

Although neither of these two mechanisms is sufficient to explain both phases of the dual effect of adrenalin on sugar transport the data are consistent with each of them playing a role in either the inhibitory or the stimulatory phase. The present observations also do not support two other mechanisms proposed earlier. The concept of a primary regulatory role for ATP [29] is inconsistent with the observation that ATP levels remained unchanged while sugar transport was depressed by low concentrations of adrenalin. The suggestion [30] that accumulation of intracellular glucose should enhance the influx of a non-metabolized sugar by countertransport cannot explain the inhibition of sugar transport by low concentrations of adrenalin which in fact raised glucose-6-*P* levels and presumably intracellular glucose levels as well [2]. At best, these two mechanisms could contribute to the stimulatory effect at high adrenalin concentrations. As indicated above, the two phases of the adrenalin effect on sugar transport appear to be mediated by separate receptors and may depend on separate mechanisms of action.

Another mechanism whereby adrenalin could influence sugar transport in skeletal muscle is through a more direct action on Ca^{2+} fluxes and distribution, similar to that in the heart [31] and denervated diaphragm [32]. The data in Table VIII show that ^{45}Ca influx was indeed decreased and increased by low and high concentrations of adrenalin, respectively. ^{45}Ca efflux was also differentially affected, being significantly more accelerated by low adrenalin than by the high concentration. These data suggest that adrenalin may affect both sarcolemmal fluxes of Ca^{2+} and its sequestration into storage pools (sarcoplasmic reticulum and mitochondria), thus affecting the amount available for efflux. There are at present no direct data on how adrenalin influences the distribution of Ca^{2+} between various intracellular compartments in skeletal muscle.

Clausen [33] and ourselves [1.15] have suggested that regulation of sugar transport may be mediated by sarcoplasmic calcium, presumably through its binding to specific regulatory sites; there is increasing evidence that the effect of many regulatory factors, including insulin, metabolic inhibition and alterations in intracellular Na^+ may be mediated in this manner [1,15,33]. The dual effect of adrenalin described here would be compatible with such a mechanism, and future experiments should establish which of the various direct and indirect ways of influencing Ca^{2+} fluxes and distribution are involved.

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