Biochemical Pharmacology, Vol. 28, pp. 807–813.

0006-2952/79/0315-0807 $02.00/0

EFFECTS OF EPINEPHRINE AND THE CYCLIC AMP PHOSPHODIESTERASE INHIBITOR SQ 20009 ON GLUCOSE AND GLYCOGEN METABOLISM IN SKELETAL MUSCLE*

SANDRA DAVIDHEISER,† ELLA S. HAUGAARD and NIELS HAUGAARD

Department of Pharmacology, School of Medicine, University of Pennsylvania, Philadelphia, PA 19104, U.S.A.

(*Received* 25 *August* 1977; *accepted* 23 *August* 1978)

Abstract—In rat diaphragm incubated *in vitro*, epinephrine and isoproterenol inhibited glucose uptake and the incorporation of radioactive glucose into glycogen. The metabolic effects of epinephrine were inhibited by the beta-adrenergic blocking agents, propranolol and sotalol, but not by the alpha-adrenergic blocking drug, phentolamine. The phosphodiesterase inhibitor, 1-ethyl-4-(isopropylidine-hydrazino)-1H-pyrazolo-(3,4-b)-pyridine-5-carboxylic acid, ethyl ester, hydrochloride (SQ 20009), also decreased glucose uptake and glycogen synthesis. In contrast to the action of epinephrine, exogenous calcium ions were required for the effects of SQ 20009 on phosphorylase activation, increased tissue glucose-6-phosphate levels and lactate production. It was concluded that the inhibition of glucose uptake that occurred when tissue cyclic AMP levels were increased by beta-adrenergic drugs or phosphodiesterase inhibitors was not due directly to accumulation of glucose-6-phosphate from glycogenolysis but was a result of a cyclic AMP-mediated inhibition of glycogen synthesis.

It is well known that catecholamines increase phosphorylase activity and stimulate glycogenolysis in skeletal muscle [1–4]. The activation of phosphorylase by epinephrine appears to be a beta-adrenergic effect of the drug since it has been shown to be abolished by beta-adrenergic blockade and unaffected by phentolamine [4]. The inhibition by epinephrine of net glucose utilization by skeletal muscle, a phenomenon originally discovered by Walaas [5], has been studied much less extensively than the action of epinephrine on glycogenolysis. In a recent study [6] we reported that the decrease in utilization of glucose by rat diaphragm *in vitro*, produced by epinephrine and by phosphodiesterase inhibitors, was equal in extent to the inhibition of incorporation of [U-^{14}C]glucose into glycogen and that the catecholamine had no effect on the rate of transport of 3-O-methylglucose into the cell. These findings, together with the observations that epinephrine decreases the activity of glycogen synthase in skeletal muscle [7, 8], indicate strongly that the decrease in glucose utilization in the presence of the hormone is due specifically to an inhibition of the glycogen synthetic pathway.

In the investigation presented here, we have studied in more detail the mechanisms involved in this important metabolic action of epinephrine and phosphodiesterase inhibitors.

* The studies reported here were supported by a grant from the Heart, Lung and Blood Institute of the National Institutes of Health (HL-01813) and a grant from the Muscular Dystrophy Association of America.

† Predoctoral trainee supported by USPHS Training Grant 5T01 GM 00474. Part of the work presented here was taken from a thesis submitted by S.D. in partial fulfillment of the requirement for the degree of Ph.D in Pharmacology, University of Pennsylvania. Present address: Department of Animal Biology, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA 19104.

MATERIALS AND METHODS

Materials. L-Epinephrine bitartrate was obtained from CalBiochem, propranolol hydrochloride from Ayerst Laboratories, D,L-isoproterenol hydrochloride from the Sterling Drug Co., sotalol from the Regis Chemical Co. and phentolamine from the Ciba Pharmaceutical Co. ^{14}C-labeled glucose and [^{3}H]cyclic AMP were obtained from the ICN Corp. and Schwarz/Mann respectively. The phosphodiesterase inhibitor, SQ 20009 [1-ethyl-4-(isopropylidine-hydrazino)-1H-pyrazolo-(3,4-b)-pyridine-5-carboxylic acid, ethyl ester, hydrochloride] was kindly supplied by the late Dr. Sidney Hess of the Squibb Medical Research Institute, Princeton, NJ [9]. Glucose oxidase was purchased from the Worthington Biochemical Corp.

Tissue incubations and metabolite analyses. Male Wistar strain rats weighing from 125 to 150 g were fasted overnight and killed by decapitation. Hemidiaphragms were dissected out and placed in ice-cold 0.15 M NaCl. The tissues were then blotted lightly on filter paper and weighed on a torsion balance. The results of metabolic measurements were expressed per g wet weight of tissue.

In some experiments the tissues were preincubated for 15 or 20 min at 37° in 25-ml Erlenmeyer flasks containing 2 ml medium of the following composition: 0.04 M HEPES (*N*-2-hydroxyethyl piperazine-*N*-ethane sulfonic acid) buffer neutralized with NaOH to pH 7.2, 0.005 M KCl, 0.005 M $MgCl_2$ and 0.108 M NaCl. In other experiments 0.006 M sodium succinate was added to the preincubation medium (to maintain tissue ATP content) and the NaCl concentration was reduced, to maintain isosmolarity. The flasks were gassed with oxygen. After the preincubation, the tissues were lightly blotted on filter paper and transferred to new flasks containing media of the same composition with either 0.006 M [U-^{14}C]glucose or 0.006 M non-radioactive glucose. The flasks were

gassed with oxygen and incubated for various times, as indicated. In some of the experiments hemidiaphragms were incubated directly in media containing glucose, without preincubation.

Aliquots of the medium were deprotenized with $ZnSO_4$ and $Ba(OH)_2$ and the supernatant fluid was analyzed for glucose using the glucose oxidase method. Lactate was measured by the procedure of Hohorst [10]. Total tissue glycogen was determined by the method of Montgomery [11] or by the measurement of glucose by the glucose oxidase method after hydrolysis of glycogen in 2 N H_2SO_4 for 1 hr and neutralization. Incorporation of [U-^{14}C]glucose into glycogen was determined by the method of Thomas *et al.* [12]. All measurements of radioactivity were made using a Packard liquid scintillation counter. For the determination of glucose-6-phosphate, the tissues were ground in chilled mortars with ice-cold 0.625 N perchloric acid (PCA). Aliquots of the PCA extracts were neutralized with 0.3 M triethanolamine, 0.67 M K_2CO_3 and glucose-6-phosphate determined in the supernatant fluid by the method of Slein [13].

Determination of phosphorylase activity. Tissues were ground in chilled mortars in buffer containing 0.02 M NaF, 0.001 M EDTA and 0.05 M Tris (HCl), pH 6.8. Measurement of phosphorylase *a* and total phosphorylase activity was done by a modification [14] of the method of Cori and Illingworth [15] and was expressed as per cent phosphorylase *a*.

Determination of cyclic AMP. In experiments in which cyclic AMP was determined, the hemidiaphragms were frozen in liquid N_2 and then ground in chilled mortars with frozen 5% trichloroacetic acid (TCA). The extracts were centrifuged and the TCA was extracted from the supernatant fluid with ether. Cyclic AMP in the samples was measured by the method of Gilman [16].

RESULTS

Effects of adrenergic blocking agents on epinephrine-mediated changes of carbohydrate metabolism. The effects of epinephrine in the presence and absence of the beta-adrenergic antagonist, propranolol, on glucose utilization and net incorporation of [^{14}C]glucose into glycogen were studied in rat diaphragms incubated *in vitro*. The tissue contents of cyclic AMP at the end of 60 min were also determined in separate experiments; the results are presented in Table 1. The marked decreases in glucose utilization, net incorporation of glucose into glycogen, and total glycogen caused by epinephrine were readily apparent. In another experiment, similar in design to those reported above, D,L-isoproterenol was also found to inhibit glucose utilization. In four paired experiments the mean glucose utilization was 16.90 ± 0.61 μmoles/g/60 min in control hemidiaphragms and 12.73 ± 0.64 μmoles/g/60 min in the presence of 6 μM D,L-isoproterenol ($P < 0.001$ by paired *t*-test). Propranolol alone, as seen in Table 1, had no significant effect but abolished the metabolic changes produced by epinephrine. At the end of a 60-min incubation the tissue content of cyclic AMP when epinephrine was present was approximately twice that of control tissues while no increase in cyclic AMP was produced by epinephrine in the presence of propranolol.

The results presented in Table 2 provide additional evidence for the beta-adrenergic-mediated action of epinephrine on carbohydrate metabolism. The concentration of epinephrine in these experiments was decreased from 30 to 6 μM but the inhibitory effects of the hormone on glucose utilization and incorporation of glucose into glycogen were similar for both doses. The beta-adrenergic antagonist, sotalol, alone produced no significant change in glucose metabolism but, like propranolol, blocked the decrease in glucose utilization and glycogen synthesis caused by epinephrine. The alpha-adrenergic blocking agent, phentolamine, did not prevent the inhibitory effects of epinephrine on either glucose utilization or glycogen synthesis but the drug by itself significantly increased glucose utilization of the muscles.

Relation of epinephrine effects on glucose metabolism to tissue elevation of cyclic AMP. We have shown

Table 1. Effects of epinephrine and propranol on cyclic AMP content, glucose utilization and glycogen metabolism in rat hemidiaphragms incubated *in vitro**

Additions	Cyclic AMP (nmoles/g at 60 min)	Glucose utilization (μmoles/g/60 min)	Net incorporation of [U-^{14}C]glucose into glycogen (μmoles/g/60 min)	Total glycogen (μmoles/g at 90 min)	Lactate formation (μmoles/g/90 min)
Control	0.439 ± 0.033 (N = 3)	14.98 ± 0.78 (N = 11)	5.33 ± 0.42 (N = 7)	13.71 ± 1.53 (N = 4)	16.60 ± 1.62 (N = 4)
Epinephrine (30 μM)	0.851 ± 0.093† (N = 4)	10.43 ± 0.46† (N = 8)	2.65 ± 0.20† (N = 8)	6.29 ± 0.15† (N = 4)	23.96 ± 1.49† (N = 4)
Propranolol (100 μM)	0.330 ± 0.023 (N = 4)	14.04 ± 0.56 (N = 12)	4.87 ± 0.18 (N = 8)	15.40 ± 2.63 (N = 4)	14.78 ± 0.74 (N = 4)
Epinephrine and propranolol	0.337 ± 0.016‡ (N = 4)	13.25 ± 0.61‡ (N = 8)	5.13 ± 0.41‡ (N = 8)	13.05 ± 2.77§ (N = 4)	19.41 ± 1.78§ (N = 4)

* Rat hemidiaphragms were preincubated without substrate, with or without propranolol (100 μM), for 15 min at 37° followed by incubation for 60 min at 37° with addition of epinephrine or propranolol, as indicated. In experiments for cyclic AMP measurements, 6 mM glucose was present. In other experiments 6 mM [U-^{14}C]glucose was added. All results are given as mean ± SEM. Analysis of variance was done on the pooled values for each group.

† Differences (control vs epinephrine, $P < 0.01$).

‡ Differences (epinephrine vs epinephrine and propranolol, $P < 0.01$).

§ Differences (epinephrine vs epinephrine and propranolol, $P < 0.05$).

Table 2. Effects of phentolamine and sotalol on the action of epinephrine on glucose and glycogen metabolism in skeletal muscle*

Addition	Glucose utilization (μmoles/g/90 min)	Net incorporation of [U-^{14}C] glucose into glycogen (μmoles/g/90 min)
Control	16.87 ± 1.19 (N = 6)	5.03 ± 1.01 (N = 6)
Epinephrine (6 μM)	9.59 ± 0.58† (N ± 6)	1.63 ± 0.59† (N = 6)
Phentolamine (100 μM)	21.30 ± 1.27 (N = 5)	5.78 ± 0.71 (N = 6)
Epinephrine and phentolamine	15.52 ± 0.90‡ (N = 5)	1.60 ± 0.18‡ (N = 6)
Sotalol (100 μM)	18.51 ± 1.33 (N = 5)	6.00 ± 0.71 (N = 4)
Epinephrine and sotalol	16.47 ± 0.92 (N = 5)	6.08 ± 0.56 (N = 4)

* Rat hemidiaphragms were incubated for 90 min at 37° in media containing 6 mM [U-^{14}C] glucose. Analysis of variance was done on the pooled values of each group.

† Difference (epinephrine vs control, $P < 0.001$).

‡ Difference (epinephrine and phentolamine vs phentolamine, $P < 0.001$).

that the phosphodiesterase inhibitors, theophylline and SQ 20009, like epinephrine, cause a marked decrease in glucose utilization and incorporation of glucose into glycogen in rat diaphragms incubated *in vitro* [6]. In order to determine the relationships of these metabolic effects to the tissue concentrations of cyclic AMP, we incubated rat hemidiaphragms for various periods of time with epinephrine (30 μM) and SQ 20009 (31 μM) in media identical in composition to those used in the experiments on glucose metabolism. The cyclic AMP tissue contents at the end of the incubations were measured; the results are summarized in Fig. 1. Both epinephrine and SQ 20009 alone produced large increases in cyclic AMP content during the first few minutes of the incubation. After 10 min, cyclic AMP concentrations fell but still remained significantly elevated above control levels at the end of a 60-min incubation period. When both epinephrine and SQ 20009 were present in the medium the cyclic AMP content of the tissue rose to very high levels (note break in ordinate to accommodate first three values) that were maintained during the entire 60-min incubation period. However, this does not lead to any additional inhibition of glucose utilization or glycogen synthesis [6]. These findings indicate that, if cyclic AMP is implicated in the inhibition of glucose utilization by epinephrine or a phosphodiesterase inhibitor, a relatively small increase in the concentration of the cyclic nucleotide is sufficient to produce the maximal effect.

Reversal of the metabolic effects of epinephrine by addition of propranolol. The results of experiments designed to test whether the inhibition of glucose utilization by epinephrine could be overcome by the addition of propranolol after administration of epinephrine are presented in Table 3. The tissues were preincubated for 15 min without substrate in the presence and absence of SQ 20009. They were then transferred to media containing 6 mM glucose and were incubated for 70 min in media with or without epinephrine. SQ 20009, when added, was present during both the preincubation and the incubation period with glucose. Propranolol was added to the medium 10 min after the start of the incubation period. It was evident that the initial rise in the tissue cyclic AMP levels produced by epinephrine during the first 10 min of the incubation (Fig. 1) was not sufficient to measurably inhibit glucose utilization. It appeared that, in order for epinephrine to be effective, the drug had to be acting during the entire incubation period. As expected, in tissues incubated with the phosphodiesterase inhibitor cyclic AMP concentrations should have remained elevated despite the

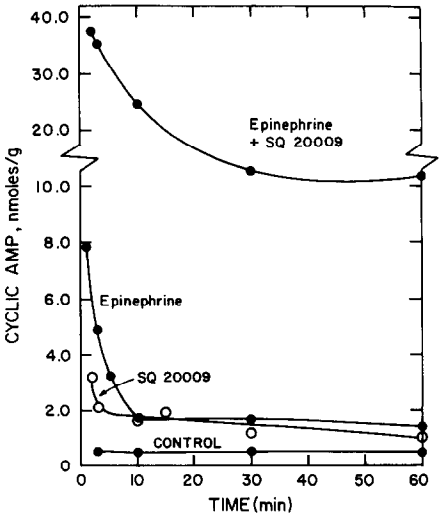

Fig. 1. Effects of epinephrine and SQ 20009 on the tissue content of cyclic AMP of rat hemidiaphragms. Muscles were preincubated for 15 min at 37° in glucose-free HEPES medium and then transferred to media containing 6 mM glucose and either epinephrine (30 μM), SQ 20009 (31 μM) or both epinephrine and SQ 20009. Cyclic AMP tissue concentrations were determined after 0, 1–5, 10, 15, 30 and 60 min of incubation. Values represent means ± S.E.M. (N = 3–5).

Table 3. Effect of propranolol on glucose utilization of rat hemidiaphragm after a 10-min incubation with epinephrine or epinephrine and SQ 20009*

Additions to media			
Preincubation	Incubation, 0 time	Incubation, after 10 min	Glucose utilization (μmoles/g/70 min)
0	Control	0	19.74 ± 1.22 (N = 4)
		Propranolol (100 μM)	17.24 ± 1.24 (N = 6)
0	Epinephrine (30 μM)	0	12.63 ± 0.96† (N = 4)
		Propranolol (100 μM)	17.55 ± 0.85‡ (N = 8)
SQ 20009 (31 μM)	Epinephrine and SQ 20009 (31 μM)	0	11.57 ± 0.58† (N = 5)
		Propranolol (100 μM)	11.61 ± 0.52† (N = 5)

* Hemidiaphragms were preincubated for 15 min at 37° in glucose-free media in the presence and absence of 31 μM SQ 20009. Tissues were then transferred to media with 6 mM glucose, with or without epinephrine (30 μM) or SQ 20009 (31 μM), and incubated for 70 min. Propranolol (100 μM), when present, was added after 10 min of the second incubation. SQ 20009, when added, was present during both the preincubation and the 70-min incubation for measurement of glucose utilization. Analysis of variance was done on the pooled values of each group.

† Difference from control, $P < 0.01$.

‡ Difference (epinephrine vs epinephrine and propranolol, $P < 0.01$).

block of beta-adrenergic receptors by propranolol and hence utilization of glucose was inhibited.

Effects of SQ 20009 on glucose utilization and incorporation of glucose into glycogen: the role of calcium ions. From the results presented here and in our previous paper [6], SQ 20009 and epinephrine had apparently similar effects on glucose metabolism. However, unlike epinephrine, SQ 20009 did not increase lactate production (Table 4). This was an unexpected finding, since a drug that increases tissue cyclic AMP concentration would be expected to stimulate glycogenolysis and the formation of lactate. Since calcium ions have been shown to be necessary for the stimulation of phosphorylase *b* kinase [17, 18], we decided to see whether addition of $CaCl_2$ to the incubation medium would alter the effect of SQ 20009 or epinephrine on glucose metabolism. It was clear from the results presented in Table 4 that the presence of added calcium ions in the incubation medium was not required for the inhibitory effects of SQ 20009 or epinephrine on glucose uptake and glycogen synthesis. Although calcium ions alone appeared to inhibit glucose utilization and glycogen synthesis to a small extent, the inhibitory effects of SQ 20009 and epinephrine were still appar-

Table 4. Role of calcium ions in the actions of SQ 20009 and epinephrine on glucose metabolism of rat hemidiaphragm incubated *in vitro**

	No $CaCl_2$ added		$CaCl_2$ (2 mM)		
	Control	+ SQ 20009 (31 μM)	Control	+ SQ 20009 (31 μM)	+ Epinephrine (30 μM)
Glucose utilization (μmoles/g/60 min)	12.61 ± 1.57 (N = 9)	8.78 ± 0.68† (N = 11)	10.21 ± 0.42 (N = 25)	5.53 ± 0.25† (N = 24)	6.43 ± 0.42† (N ± 16)
Net incorporation of [U-^{14}C]glucose into glycogen (μmoles/g/60 min)	6.30 ± 0.33 (N = 10)	2.52 ± 0.25† (N = 11)	4.56 ± 0.48 (N = 4)	1.51 ± 0.10† (N = 4)	1.17 ± 0.24† (N = 4)
Lactate production (μmoles/g/60 min)	8.90 ± 0.47 (N = 10)	9.39 ± 0.57 (N = 9)	9.42 ± 0.32 (N = 21)	10.78 ± 0.36† (N = 20)	14.67 ± 0.73† (N = 8)

* Hemidiaphragms were preincubated for 15 min at 37° in medium containing 6 mM Na succinate as substrate. The tissues were then transferred to media containing 6 mM [U-^{14}C]glucose and incubated for 60 min at 37°. Analysis of variance was done on the pooled values of each group.

† Effects of SQ 20009 or epinephrine, $P < 0.01$.

ent. A small but significant increase in lactate production by SQ 20009 was seen only when $CaCl_2$ was added to the medium.

Effects of epinephrine and SQ 20009 on phosphorylase activity in the presence and absence of added extracellular calcium. To study the relationships between the inhibitory effect of epinephrine and/or SQ 20009 on glucose utilization and glycogen synthesis and the activation of phosphorylase, rat hemidiaphragms were incubated with epinephrine and SQ 20009 for various periods of time in the presence and absence of 2 mM $CaCl_2$, and phosphorylase *a* was measured as a percentage of total phosphorylase. The results are presented in Fig. 2. It was seen that calcium ions or SQ 20009 alone produced a small but significant increase in phosphorylase *a* activity. Epinephrine caused a marked rise in phosphorylase activity that was greater and more sustained when calcium ions had been added to the medium. Surprisingly, the addition of SQ 20009 caused a much greater increase in phosphorylase activity in the presence, than in the absence, of extracellular calcium. It was clear from these experiments that the inhibition of glucose utilization and glycogen synthesis was not well correlated with the activation of phosphorylase. The addition of calcium ions to the medium caused an activation of phosphorylase that was equal to that produced by SQ 20009, but only the latter produced a significant decrease in glucose utilization and glycogen synthesis. Epinephrine and SQ 20009 inhibited glucose metabolism equally in the absence and in the presence of added $CaCl_2$, despite the fact that phosphorylase activation was greatly potentiated by calcium ions.

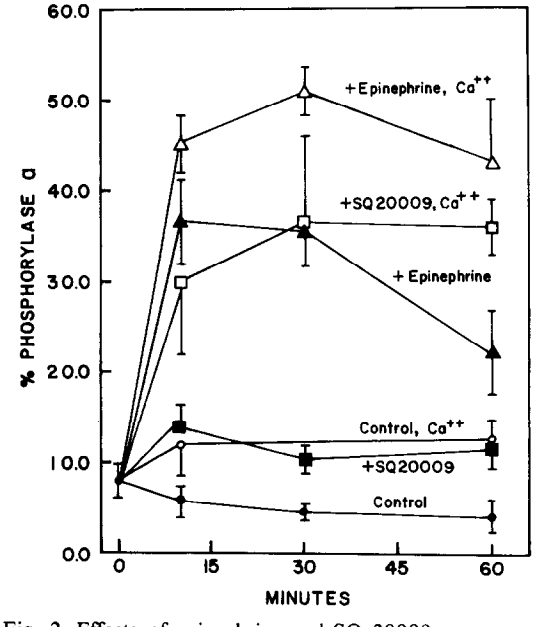

Fig. 2. Effects of epinephrine and SQ 20009 on per cent activation of phosphorylase *a* in rat hemidiaphragms incubated in medium ± calcium (2 mM). Tissues were preincubated for 15 min at 37° in glucose-free HEPES medium and then transferred to ± calcium-containing media with 6 mM glucose and either epinephrine (30 μM) or SQ 20009 (31 μM). The percentage of phosphorylase *a* was determined after 0, 10, 30 and 60 min of incubation. Values represent means ± S.E.M. (N = 3–10).

Relation of glucose-6-phosphate content of muscle to inhibition of glucose metabolism. Increased glycogenolysis in skeletal muscle is associated with an increase in the tissue content of glucose-6-phosphate [19, 20]. Since this metabolite has been shown to inhibit hexokinase activity [21], the decreased glucose utilization produced by epinephrine or SQ 20009 could result from inhibition of this enzyme. Therefore, we determined the effects of epinephrine and SQ 20009 on the tissue content of glucose-6-phosphate in rat diaphragms incubated for 1 hr at 37°. The results are given in Table 5. It was seen that addition of 2 mM $CaCl_2$ to the incubation medium had no effect on the final tissue concentration of glucose-6-phosphate when no drug was added. In the presence of epinephrine there was a large increase in glucose-6-phosphate in the tissue that was uninfluenced by calcium ions. In contrast, the increase in glucose-6-phosphate produced by SQ 20009 was significant only in the medium containing added $CaCl_2$. Since SQ 20009 was equally effective in inhibiting glucose utilization and glycogen synthesis in the presence and absence of calcium, it appeared that tissue glucose-6-phosphate content was not a major factor in causing inhibition of glucose metabolism by the phosphodiesterase inhibitor.

DISCUSSION

The evidence presented in this study supports the view that the metabolic actions of epinephrine on glucose metabolism in skeletal muscle are mediated by an increase in the tissue concentration of cyclic AMP through a beta-adrenergic activation of adenylate cyclase. Our experiments confirm the observation made by Abramson and Arky [22] that propranolol prevents the decrease in glucose uptake in the rat diaphragm caused by epinephrine. We also have shown that the decrease in glycogen synthesis and total tissue glycogen and the increase in cyclic AMP and lactate formation are blocked effectively by this drug. The inhibition of glucose utilization persists in the presence of phentolamine, indicating that the hormone effect is not mediated by alpha receptors. It is evident that tissue cyclic AMP levels must be increased either through stimulation of adenylate cyclase or by inhibition of phosphodiesterase in order to observe these changes in glucose

Table 5. Effects of epinephrine and SQ 20009 on tissue content of glucose-6-phosphate in the presence and absence of added $CaCl_2$*

	Glucose-6-phosphate (μmoles/g)	
Conditions	No $CaCl_2$ added	$CaCl_2$ (2 mM)
Control	0.130 ± 0.018 (N = 8)	0.112 ± 0.025 (N = 6)
Epinephrine (30 μM)	0.430 ± 0.034† (N = 4)	0.487 ± 0.052† (N = 4)
SQ 20009 (31 μM)	0.171 ± 0.020 (N = 4)	0.316 ± 0.069† (N = 4)

* Hemidiaphragms were incubated for 60 min at 37° in a medium containing 6 mM glucose, and tissue glucose-6-phosphate was determined. Analysis of variance was done on the pooled values for each group.

† Difference of epinephrine or SQ 20009 from control, $P < 0.01$.

and glycogen metabolism. However, the large rapid initial increase in cyclic AMP (Fig. 1) that is observed following exposure of the muscle to epinephrine is not sufficient to maintain the effect on glucose utilization during a prolonged subsequent incubation period after addition of propranolol unless phosphodiesterase is inhibited (Table 3).

The enzyme, phosphorylase *b* kinase, that converts phosphorylase *b* to *a* in skeletal muscle needs calcium ions for activity [17, 18]. It is apparent from our results that the phosphodiesterase inhibitor, SQ 20009, does not increase phosphorylase activity unless calcium ions are present in the incubation medium, even though this drug significantly raises the tissue concentration of cyclic AMP. Hess *et al.* [23] also found that theophylline did not affect phosphorylase *a* activity in rat diaphragm incubated in phosphate-buffered medium without calcium ions. The findings that epinephrine increases phosphorylase *a* and stimulates glycogenolysis in tissues incubated in a medium containing no calcium indicates that this hormone could be raising the intracellular concentration of calcium and thus increasing phosphorylase *b* kinase activity. Possible sources of this calcium could be the membranes of the sarcolemma, t-tubules, or sarcoplasmic reticulum. The observation that epinephrine is capable of increasing the twitch tension of fast-contracting skeletal muscle [24] and prolonging its active state [25] is suggestive of a greater availability of intracellular calcium in the presence of epinephrine. Therefore, the stimulation of phosphorylase *a* activity produced by epinephrine in muscles incubated without calcium ions may not be due simply to the elevation of the tissue concentration of cyclic AMP but may also involve translocation of calcium ions.

Several investigators have judged the catecholamine-mediated inhibition of glucose utilization in skeletal muscle to be a secondary consequence of the increased glucose-6-phosphate concentration from glycogenolysis [5, 26], since this metabolite has been found to decrease hexokinase activity in tissue extracts [21]. However, the observation that SQ 20009 causes a marked decrease in glucose utilization under conditions where glycogenolysis is not stimulated does not support this hypothesis. In earlier experiments we have found that the inhibition of glucose utilization by SQ 20009 and by epinephrine is equal to the decrease in glycogen synthesis from glucose and that this effect does not seem to be due to inhibition of the glucose transport system [6]. The results of the experiments reported here show clearly that the inhibition of glucose uptake that occurs when the cyclic AMP level of the muscle increases is not correlated with an increase in tissue glucose-6-phosphate. For example, in the absence of calcium ions in the incubation medium, SQ 20009 and epinephrine inhibited glucose utilization to the same extent. However, epinephrine markedly increased the glucose-6-phosphate content of the muscles while SQ 20009 had no significant effect (Table 5).

The contribution of a lowered utilization of glucose in skeletal muscle to the epinephrine-induced hyperglycemia found *in vivo* has been difficult to evaluate due to the effects of this hormone on several organ systems [27]. Chatonnet *et al.* [28] did demonstrate a significant role of the peripheral action of epinephrine in producing hyperglycemia in adrenal-demedullated dogs exposed to cold ambient temperatures. However, they attributed the decreased glucose utilization to an epinephrine-mediated inhibition of insulin secretion although they did not measure blood insulin concentration. In recent studies by Shikama and Ui [29] the effect of epinephrine on the turnover rate of blood glucose in rats receiving a glucose load was determined. Epinephrine caused a decrease in peripheral glucose utilization without inhibiting glucose-induced insulin secretion. This effect of epinephrine was also demonstrated in rats treated with 5-methoxyindole-2-carboxylic acid to inhibit gluconeogenesis. In other experiments, these investigators showed that the hyperglycemia caused by epinephrine in normal rats could be blocked completely by administration of phentolamine and propranolol. However, in rats treated with either 5-methoxyindole-2-carboxylic acid or antiinsulin serum, epinephrine-induced hyperglycemia was blocked by propranolol alone, but not by phentolamine [30]. Thus, there is considerable evidence that epinephrine does decrease peripheral glucose utilization by a direct beta-adrenergic action, an effect that could be important in the regulation of blood glucose levels, especially under conditions of stress.

Our observation that the decrease in glucose utilization of muscle produced by epinephrine or a phosphodiesterase inhibitor is always associated with an inhibition of net incorporation of [^{14}C]glucose into glycogen (Table 4), even in the absence of a large increase in phosphorylase activity (Fig. 2), indicates that inhibition of glycogen synthesis contributes to the depletion of muscle glycogen *in vivo* when tissue cyclic AMP levels are elevated.

REFERENCES

1. C. F. Cori, *Physiol. Rev.* **11**, 143 (1931).
2. E. W. Sutherland and T. W. Rall, *Pharmac. Rev.* **12**, 265 (1960).
3. W. H. Danforth, E. Helmreich and C. F. Cori, *Proc. natn. Acad. Sci. U.S.A.* **48**, 1191 (1962).
4. H. I. El, S. Ali, A. Antonio and N. Haugaard, *J. Pharmac. exp. Ther.* **145**, 142 (1964).
5. E. Walaas, *Acta physiol. scand.* **35**, 109 (1955).
6. E. S. Haugaard, S. Davidheiser and N. Haugaard, *Biochem. Pharmac.* **25**, 439 (1976).
7. J. W. Craig and J. Larner, *Nature, Lond.* **202**, 971 (1964).
8. L. C. Shen, C. Villar-Palasi and J. Larner, *Physiol. Chem. Physics* **2**, 536 (1970).
9. M. Chasin, D. N. Harris, M. B. Phillips and S. M. Hess. *Biochem. Pharmac.* **21**, 2443 (1972).
10. H. J. Hohorst, in *Methods of Enzymatic Analysis* (Ed. H. U. Bergmeyer), pp. 266–69. Academic Press, New York (1963).
11. R. Montgomery, *Archs Biochem.* **67**, 378 (1957).
12. J. A. Thomas, K. K. Schlender and J. Larner, *Analyt. Biochem.* **25**, 486 (1968).
13. M. W. Slein, in *Methods of Enzymatic Analysis* (Ed. H. U. Bergmeyer), pp. 117–23. Academic Press, New York (1963).
14. R. S. Horn, C. E. Aronson, M. E. Hess and N. Haugaard, *Biochem. Pharmac.* **16**, 2109 (1967).
15. G. T. Corl and B. Illingworth, *Biochim. biophys. Acta* **21**, 105 (1956).
16. A. Gilman, *Proc. natn. Acad. Sci. U.S.A.* **67**, 305 (1970).
17. W. L. Meyer, E. H. Fischer and E. G. Krebs, *Biochemistry* **3**, 1033 (1964).

18. C. O. Broström, F. L. Hunkeler and E. G. Krebs, *J. biol. Chem.* **246**, 1961 (1971).
19. O. Walaas and E. Walaas, *J. biol. Chem.* **187**, 769 (1950).
20. E. Walaas, *Acta physiol. scand.* **35**, 126 (1955).
21. R. K. Crane and A. Sols, *J. biol. Chem.* **203**, 273 (1953).
22. E. A. Abramson and R. A. Arky, *Diabetes* **17**, 141 (1968).
23. M. E. Hess, D. Hottenstein, J. Shanfeld and N. Haugaard, *J. Pharmac. exp. Ther.* **144**, 274 (1963).
24. W. C. Bowman and M. W. Nott, *Pharmac. Rev.* **21**, 27 (1969).
25. M. Goffart and J. M. Ritchie, *J. Physiol., Lond.* **116**, 357 (1952).
26. E. W. Sutherland, in *Phosphorus Metabolism* (Eds W. P. McElroy and B. Glass), Vol II, pp. 577–93. The Johns Hopkins Press, Baltimore (1952).
27. J. Himms-Hagen, *Pharmac. Rev.* **19**, 367 (1967).
28. J. Chatonnet, Y. Minaire, A. Pernod and J. C. Vincent-Falquet, *J. appl. Physiol.* **32**, 170 (1972).
29. H. Shikama and M. Ui, *Am. J. Physiol.* **299**, 955 (1975).
30. H. Shikama and M. Ui, *Am. J. Physiol.* **299**, 962 (1975).