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EFFECTS OF EPINEPHRINE AND CYCLIC AMP PHOSPHODIESTERASE INHIBITORS ON THE GLYCOGEN SYNTHETIC PATHWAY AND GLUCOSE CONTENT IN SKELETAL MUSCLE*

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Abstract The actions on carbohydrate metabolism of epinephrine and the cyclic AMP phosphodiesterase inhibitors, theophylline and SQ 20009, were studied using rat hemidiaphragms incubated *in vitro*. The inhibition of glucose uptake produced by epinephrine was almost equal to the decrease in the incorporation of [^{14}C]glucose into glycogen, while incorporation into lactate of ^{14}C from labeled glucose was not influenced by the hormone. Although epinephrine decreased total tissue glycogen, it had relatively little effect on the removal of radioactive glucose incorporated into glycogen during a preincubation in the absence of hormone when the tissue was subsequently incubated with nonradioactive glucose. This indicates that the decrease in the net incorporation of [^{14}C]glucose into glycogen produced by the hormone *in vitro* is caused predominantly by an inhibition of glycogen synthesis and cannot be accounted for by an increase in the turnover rate of glycogen. Theophylline and SQ 20009 produced a dose-dependent inhibition of glucose uptake and glycogen synthesis, similar to that of epinephrine. Incubation of hemidiaphragms with epinephrine or cyclic AMP phosphodiesterase inhibitors in the presence of glucose led to an increased intracellular accumulation of glucose. Possible explanations for this phenomenon are discussed. The uptake of the nonmetabolizable sugar, 3-*O*-methylglucose, by skeletal muscle was not affected by epinephrine, indicating that the hormone influences only the uptake of the natural substrate, glucose, which can be incorporated into glycogen. The alterations in muscle metabolism described here were associated with increases in the tissue content of cyclic AMP and it was concluded that an important action of epinephrine in resting muscle is a cyclic AMP-mediated inhibition of the glucose-to-glycogen synthetic pathway.

It is well established that catecholamines increase the activity of adenylate cyclase in many tissues, leading to accumulation of cyclic AMP in the cell, activation of glycogen phosphorylase and a consequent increase in the rate of glycogenolysis [1–3]. Less well understood is the inhibition of glucose uptake in skeletal muscle exposed to epinephrine [4–7].

From the results of studies of the effects of epinephrine on glucose utilization and net changes in glycogen content of rat diaphragm incubated *in vitro*, Walaas [6] proposed that the hormone inhibited glycogen synthesis. Later, it was shown that epinephrine indeed decreased the proportion of glucose 6-P-independent glycogen synthase in muscle [8, 9] and thus inhibited the activity of an enzyme involved in the formation of glycogen in this tissue.

Epinephrine has also been reported to cause a small accumulation of glucose in muscle [10, 11], but it is not known how this action is related to other metabolic effects of the hormone. It is the purpose of this investigation to study the mechanisms by which epinephrine exerts its many effects on glucose metabolism in muscle and to explore how the different metabolic effects are related. Since most actions of epinephrine on metabolism appear to be secondary to changes in the cell content of cyclic 3′,5′-AMP, the tissue content of this nucleotide was measured and the effects of cyclic AMP phosphodiesterase inhibitors on glucose metabolism in the diaphragm were also studied.

MATERIALS AND METHODS

Materials. The phosphodiesterase inhibitor, SQ 20009 [12] (1-ethyl-4-(isopropylidine-hydrazino)-1H-pyrazolo-(3, 4-b)-pyridine-5-carboxylic acid, ethyl ester, hydrochloride), was kindly supplied by Dr. Sidney Hess of Squibb Medical Research Institute, N.J. L-Epinephrine bitartrate was obtained from Sterling–Winthrop Co. or Calbiochem and ^{14}C-labeled substrates were obtained from ICN Corp.

Tissue incubations and metabolite analyses. Male Wistar strain rats weighing from 125 to 150 g were used. The animals were fasted overnight and killed by decapitation. Hemidiaphragms were carefully dissected out and collected in ice-cold 0·15 M NaCl. They were blotted lightly on filter paper and weighed on a torsion balance. Results of metabolic measurements were expressed per g wet weight of tissue.

The tissues were preincubated for 15 min at 37° in 25-ml Erlenmeyer flasks containing 2-ml medium of the following composition: 0·040 M HEPES (*N*-2-

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hydroxyethyl piperazine-*N*-ethane sulfonic acid) buffer neutralized with NaOH to pH 7·2, 0·005 M $MgCl_2$ and 0·113 M NaCl. After the preincubation, the tissues were lightly blotted on filter paper and transferred to new flasks containing media of the same composition but with 6 mM unlabeled glucose or [U-^{14}C]glucose and 0·110 M rather than 0·113 M NaCl. The flasks were then gassed with oxygen and incubated for various times for metabolic measurements. Glucose disappearance from the medium was determined by the glucose oxidase method after deproproteinization with $ZnSO_4$ and Ba $(OH)_2$. Total tissue glycogen was measured by the method of Montgomery [13] or by determination of glucose by glucose oxidase, following hydrolysis in 2 N H_2SO_4 and neutralization. Incorporation of [U-^{14}C]glucose into glycogen was determined by the method of Thomas *et al.* [14] and the lactate analyses were carried out by the procedure of Hohorst [15]. In some experiments lactate from the medium was separated by ion-exchange chromatography as employed by LaNoue *et al.* [16] for the separation of organic acids. The resin used was Bio-Rad, Ag 1-× 8, 200–400 mesh, in the formate form, and dilute formic acid of gradually increasing concentrations was used for elution.

In experiments in which the accumulation of glucose and glucose 6-phosphate in the tissue was studied, two hemidiaphragms were used for each incubation at 37° in the presence and absence of epinephrine or a cyclic AMP phosphodiesterase inhibitor. The tissues were then rinsed rapidly at 2° in glucose-free HEPES medium or saline, blotted and washed successively for 2 min at 2° in glucose-free HEPES medium. The tissue content of glucose and glucose 6-phosphate was determined after successive 2-min washings as indicated in Results. At the end of the washings the hemidiaphragms were ground in chilled mortars with 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 (pH 7·0) and glucose and glucose 6-phosphate determined in samples of the supernatant fluid by the method of Slein [17]. All measurements of radioactivity were made with a Packard scintillation counter.

Uptake of 3-O-*methylglucose.* Hemidiaphragms were preincubated for 15 min at 37° in HEPES medium without substrate. The tissues were then incubated for 5, 10, 15 or 30 min in HEPES medium with 6 mM [^{14}C-3-*O*]methylglucose in the absence or presence of epinephrine. The hemidiaphragms were dipped four times in 10-ml volumes of 0·15 M NaCl at 2° and blotted on filter paper. They were then washed successively 10 to 12 times in 3-ml volumes of HEPES medium at 2°. The radioactive 3-*O*-methylglucose appearing in the media during 40 min of repeated washings was determined, and the radioactivity remaining in the tissue at the end of the washings was measured following digestion with KOH. The tissue content of 3-*O*-methylglucose was calculated and the logarithm of this value plotted as a function of the time of washing. The intercept on the *y*-axis was taken as a measure of the 3-*O*-methylglucose present intracellularly at the end of the incubation with this substance.

In order to convert these values to intracellular concentrations of 3-*O*-methylglucose, we determined total tissue content of water and the extracellular space using tritiated mannitol as a marker. Total tissue water was found to be 79·6 per cent of the wet weight and extracellular water was 27·9 per cent of total water. This means that 57·4 per cent of the wet weight of the diaphragm could be accounted for as intracellular water. This value was used to calculate the intracellular concentration of 3-*O*-methylglucose.

Determination of cyclic AMP. In experiments in which cyclic AMP was determined, the diaphragms were frozen in liquid nitrogen and the frozen tissue was ground with solid 5% TCA. After centrifugation of the extracts, the TCA was removed by extraction with ether. Cyclic AMP in the supernatant fluids was determined by the method of Gilman [18].

RESULTS

Effects of epinephrine and cyclic AMP phosphodiesterase inhibitors on glucose metabolism

Glucose metabolism. The inhibition of glucose uptake and glycogen synthesis by epinephrine is illustrated in Fig. 1. In the presence of epinephrine there was a significant decrease in the rate of utilization of glucose. This was accompanied by a lowering of the incorporation of radioactive glucose into glycogen, which was seen during the entire period of incubation. Between 40 and 90 min little, if any, additional radioactive glucose was incorporated into glycogen, in contrast to the steady *de novo* formation of glycogen in the control hemidiaphragm incubated in the absence of epinephrine.

Additional data were obtained from experiments in which diaphragms were incubated for 1 hr. Changes in total glycogen and lactate output were also determined. These results are presented in Table 1. It is seen clearly that the inhibition of glucose utilization

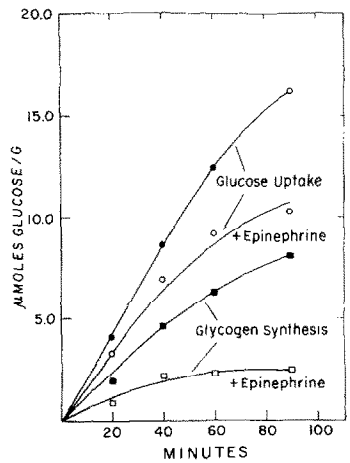

Fig. 1. Effects of epinephrine on glucose uptake and incorporation of [^{14}C]glucose into glycogen by rat diaphragm. Paired rat hemidiaphragms were preincubated for 15 min in glucose-free HEPES medium. The tissues were then transferred to incubation media containing 6 mM [U-^{14}C]glucose (ca. 30,000 cpm/μmole) ± 30 μM epinephrine. Disappearance of glucose from the media and incorporation of [^{14}C]glucose into tissue glycogen were determined after 20, 40, 60 and 90 min of incubation. See Methods for details.

Table 1. Effects of epinephrine on glucose metabolism of rat hemidiaphragms incubated *in vitro* *

	Control (μmoles/g/hr)	+30 μM Epi (μmoles/g/hr)	Difference†
Glucose uptake	13·27 ± 0·39	9·11 ± 0·49	−4·16 ± 0·63
Net incorporation of [^{14}C]glucose into glycogen	6·46 ± 0·18	2·57 ± 0·19	−3·89 ± 0·26
Change in total glycogen (as glucose)‡	6·86 ± 0·79	−1·41 ± 0·86	−8·27 ± 1·16
1/2 Lactate formation	6·91 ± 0·28	9·04 ± 0·34	+2·13 ± 0·44

* Paired rat hemidiaphragms were preincubated for 15 min at 37° in the absence of substrate, followed by incubation (± epinephrine) with 6 mM [U-^{14}C]glucose (ca. 30000 cpm/μmole) for 60 min at 37°. Glucose utilization, total glycogen, incorporation of [^{14}C]glucose into glycogen and lactate formation were determined (N = 11). See Methods for details.

† $P < 0{\cdot}001$.

‡ For each individual experiment final and initial values of tissue glycogen were obtained so that paired values for the change in glycogen content could be calculated. Two small pieces, one from each hemidiaphragm, were combined and used for the analysis of initial glycogen. The mean initial glycogen value for the 11 experiments was 13·8 ± 1·06 μmoles glucose/g.

produced by epinephrine is, within experimental error, equal to the decrease in the incorporation of radioactive glucose into glycogen. In the absence of epinephrine, the glycogen content was increased by an amount about equal to the net entrance of [^{14}C]glucose into glycogen. When epinephrine was present, the total glycogen content decreased and the effect of the hormone on breakdown of glycogen was accompanied by an increase in the production of lactate, reflecting the expected activation of glycogenolysis by epinephrine.

Since the metabolic effects of epinephrine described above are most likely a consequence of the accumulation of cyclic AMP in the cell, we also studied the effects of the cyclic AMP phosphodiesterase inhibitors, theophylline and SQ 20009 [12], on glucose utilization and incorporation of [^{14}C]glucose into glycogen in diaphragms incubated *in vitro*. The results of these experiments are recorded in Table 2.

These substances had the same action as epinephrine in that they markedly decreased glucose utilization and the incorporation of glucose into glycogen. SQ 20009 was much more potent in this respect than theophylline. When the effects of the two compounds on glycogen synthesis were plotted as a function of the logarithm of the dose of drug, linear curves were obtained and SQ 20009 was found to be about 125 times as potent as theophylline. For example, it was calculated that 5 mM theophylline and 0·04 mM SQ 20009 were needed to produce 50 per cent inhibition of glycogen synthesis.

To see whether the new drug, SQ 20009, caused an elevation of the tissue content of cyclic AMP, we measured this nucleotide in diaphragms incubated *in vitro* under conditions similar to those of Table 2. After 15 min of preincubation with 0·153 mM SQ 20009, the cyclic AMP concentration in rat hemidiaphragms was 2·7 ± 0·21 nmoles/g (N = 7), at the end of 60-min incubation, the level was 1·3 ± 0·24 nmoles/g (N = 4). The cyclic AMP values obtained in control hemidiaphragms were 0·54 ± 0·07 (N = 4) and 0·45 (N = 2) nmoles/g at the start and at the end of the 60-min incubation respectively.

Turnover of glycogen. Our results strongly indicate that epinephrine causes an almost complete cessation of glycogen synthesis in rat diaphragm. However, the possibility remains that a very rapid turnover of newly formed glycogen in the presence of the hormone occurs, resulting in a constant level of ^{14}C-labeled glycogen.

In order to study this further, another series of experiments was carried out. Hemidiaphragms were incubated for 30 min in a medium containing 6 mM radioactive glucose, followed by incubation for 30 or

Table 2. Effects of cyclic AMP phosphodiesterase inhibitors on glucose utilization and glycogen synthesis by rat hemidiaphragms incubated *in vitro**

Addition	Glucose utilization (μmoles/g/hr)	Net incorporation of [^{14}C]glucose into glycogen (μmoles/g/hr)
None	13·39 ± 0·55	6·43 ± 0·28
Theophylline, 0·5 mM	11·06 ± 0·64	5·14 ± 0·12
Theophylline, 1·0 mM	10·54 ± 0·94	4·59 ± 0·20
Theophylline, 2·0 mM	9·85 ± 0·35	4·02 ± 0·19
None	13·78 ± 0·37	7·25 ± 0·39
SQ 20009, 0·015 mM	11·27 ± 0·33	4·55 ± 0·37
SQ 20009, 0·031 mM	10·44 ± 0·74	4·07 ± 0·39
SQ 20009, 0·153 mM	7·59 ± 0·30	2·52 ± 0·25

* Paired hemidiaphragms were preincubated (± drugs) for 15 min at 37° in the absence of glucose. This was followed by an incubation with 6 mM [^{14}C]glucose (± drugs) for 60 min at 37°. Values are means ± S. E. M. (N = 7–12).

Table 3. Turnover of radioactive glycogen in hemidiaphragms preincubated with [U-^{14}C]glucose*

	Values at the end of 30 min with [U-^{14}C]-glucose (N = 32)	Incubation with 6 mM nonradioactive glucose			
		30 min		60 min	
		Control (N = 11)	+30 μM Fpi (N = 10)	Control (N = 6)	+30 μM Fpi (N = 5)
Total glycogen (μmoles glucose/g)	18·16 ± 0·62				
Change in total glycogen† (μmoles glucose/g)		+2·48 ± 0·45	−1·74 ± 0·67	+5·33 ± 0·98	−3·62 ± 0·82
[^{14}C]glucose in glycogen (μmoles/g)	3·66 ± 0·15	3·06 ± 0·26	2·71 ± 0·38	3·25 ± 0·43	2·28 ± 0·54

* Hemidiaphragms were preincubated at 37° for 30 min in HEPES medium containing 6 mM [U-^{14}C]glucose. After rinsing in 0·15 M NaCl at 20°, one of each pair of tissues was taken for glycogen analysis and the other was incubated for 30 or 60 min at 37° in HEPES medium containing 6 mM nonradioactive glucose.

† Values obtained from paired experiments.

60 min in a medium containing nonlabeled glucose at the same concentration. The results are summarized in Table 3.

Very little turnover of glycogen occurred in the absence of epinephrine, indicating that the rate of glycogenolysis was low. In the presence of epinephrine, there was a greater loss of radioactivity from glycogen, but even under these conditions about 62 per cent of the radioactivity remained after the 60 min of incubation with nonradioactive glucose. If the decrease in glycogen content of the tissue were due only to increased glycogenolysis, and not to inhibition of the synthesis of glycogen, it would be expected that most, if not all, of the radioactive glucose units would be replaced during a prolonged incubation, during which there is a decrease in total glycogen content.

Action of epinephrine on incorporation of [U-^{14}C] glucose into lactate. Hemidiaphragms were incubated for 60 min with [U-^{14}C]glucose in the presence and absence of epinephrine, and the lactate in the medium was isolated and counted (Table 4). It is striking that only about 10 per cent of the lactate that appeared in the medium was derived from the radioactive glucose taken up by the tissue. This indicates that there is little formation of lactate from newly synthesized radioactive glycogen, in agreement with the results of the experiments in which the breakdown of labeled glycogen was determined directly (Table 3). While epinephrine tended to decrease the specific activity of the lactate in the medium as would be expected with an increase in degradation of preformed glycogen, there was no marked effect of the hormone on the amount of lactate formed from radioactive glucose.

These experiments show conclusively that the marked diminution of glucose uptake produced by epinephrine, amounting to 4·6 μmoles/g, is in no way reflected in corresponding changes in lactate formation. All of the findings presented are consistent with the conclusion that the inhibition of glucose uptake, observed *in vitro* under the conditions employed here, is the result of a decrease in the rate of glycogen synthesis.

Accumulation of tissue glucose. From the results of a previous investigation in our laboratory [19] we concluded that in the synthesis of glycogen from glucose by the rat diaphragm the glucose, in the pathway toward glycogen, did not mix to a significant extent with the pool of intracellular glucose. Since the observations reported here indicate that it is the glucose-to-glycogen synthetic pathway that is inhibited by epinephrine and cyclic AMP phosphodiesterase in-

Table 4. Conversion of [U-^{14}C]glucose into lactate by rat diaphragm incubated *in vitro**

Expt. No.	Epinephrine 30 μM	Lactate formed (μmoles/g)	Specific activity (cpm/μmole)	Total activity in lactate† (cpm/g)	Glucose metabolized to lactate‡ (μmoles/g)
1	0	8·77	8,705	76,343	0·95
	+	12·56	6,446	80,962	1·01
2	0	9·30	9,498	88,331	1·01
	+	11·66	7,636	89,036	1·13
3	0	11·29	7,787	87,915	1·10
	+	13·83	7,806	107,957	1·35
4§	0	12·79	11,105	142,033	1·78
	+	19·65	5,886	115,660	1·45

* Paired hemidiaphragms from 2 rats were preincubated and incubated as described in footnote to Table 1. At the end of the 60-min incubation with [^{14}C]glucose, the amount of lactate in the medium was measured and the specific activity of the lactate determined. See Methods for details.

† Value = specific activity × μmoles lactate in medium/g of tissue.

‡ Specific activity of medium glucose = 80,000 cpm/μmole.

§ Fed rat.

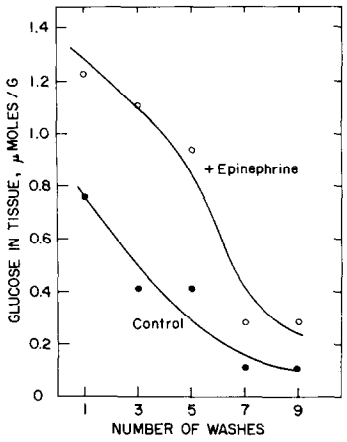

Fig. 2. Effects of epinephrine on accumulation of glucose in rat diaphragm. Paired hemidiaphragms from two rats were preincubated for 15 min at 37° in the absence of glucose. The tissues were then incubated (± 30 μM epinephrine) for 60 min at 37° in a medium containing 6 mM glucose. After two rapid rinses in cold glucose-free medium, the muscles were successively washed for 2-min periods at 2°. Glucose content is plotted as a function of the number of washings.

hibitors, we considered the possibility that in the presence of these substances some of the glucose taken up by the cell, instead of being converted to glycogen, would accumulate as intracellular glucose.

The results of experiments in which paired hemidiaphragms were incubated with 6 mM glucose for 60 min, in the absence and presence of 30 μM epinephrine, are illustrated in Fig. 2. The tissues were rinsed and washed successively as described in Methods and in the legend to Fig. 2.

It can be seen that after each washing the tissue content of glucose was higher in the hemidiaphragms that had been incubated with epinephrine than in controls, indicating that epinephrine promoted the accumulation of glucose in the cell under conditions during which uptake is inhibited. Similar results were obtained when hemidiaphragms were incubated with SQ 20009 or theophylline.

Results of experiments designed to correlate the effects of epinephrine and epinephrine + SQ 20009 on tissue glucose with other metabolic changes are reported in Table 5. In these experiments rat hemidiaphragms were first preincubated for 15 min in the absence of substrate and then incubated for 1 hr in the presence of 6 mM glucose. Glucose utilization and the tissue contents of glucose, glucose 6-phosphate and cyclic AMP were measured at the end of the incubation. Cyclic AMP in the tissue was determined also after only 1 min of incubation.

It is evident that the inhibition of glucose utilization produced by epinephrine, and the concomitant increases in tissue glucose and glucose 6-phosphate, are associated with an increase in the cellular content of cyclic AMP. The additional presence of the cyclic AMP phosphodiesterase inhibitor, SQ 20009, caused a further increase in tissue content of cyclic AMP, but no other significant changes were observed.

Effect of epinephrine on uptake of 3-O-*methylglucose.* In order to determine whether conditions leading to accumulation of intracellular cyclic AMP would alter the rate of transport of carbohydrate into the cell, we measured uptake of 3-*O*-methylglucose by diaphragm in the presence and absence of epinephrine. The results of these experiments are given in Fig. 3.

Hemidiaphragms were incubated in media containing 6 mM [^{14}C-3-*O*]methylglucose for 5, 10, 15 or 30 min, and the intracellular concentration of the sugar for each time period was determined by the washout procedure described in Methods. The left panel of Fig. 3 shows washout curves for tissues incubated for 5 min with and without epinephrine. The lack of effect of the hormone on the slow component is clearly seen. In the right panel we have recorded the calculated results from all of the experiments. It is seen that epinephrine had no effect on the intracellular concentration of 3-*O*-methylglucose at any of the time periods studied. Thus there is no evidence from these experiments that the hormone influences the rate of entrance or the intracellular concentration at equilibrium of this nonmetabolizable sugar. Additional experiments were done to determine whether the presence of epinephrine and SQ 20009 together,

Table 5. Effects of epinephrine and epinephrine + SQ 20009 on glucose utilization and accumulation of G-6-P, glucose and cyclic AMP in rat diaphragm*

	Control	15 μM Epinephrine	15 μM Epinephrine + 77 μM SQ 20009
Glucose uptake (μmoles/g/hr)	12·93 ± 0·86	9·35 ± 0·49	8·71 ± 0·91
Glucose-6-phosphate (μmoles/g at 60 min)	0·092 ± 0·013	0·171 ± 0·028	0·198 ± 0·013
Tissue glucose (μmoles/g at 60 min)	0·209 ± 0·041	0·587 ± 0·024	0·584 ± 0·013
Cyclic AMP (nmoles/g at 1 min)	0·54 ± 0·07	6·2 ± 2·1	42;33
Cyclic AMP (nmoles/g at 60 min)	0·39, 0·50	1·4 ± 0·13	12·1;10·6

* Paired hemidiaphragms from 2 rats were preincubated for 15 min at 37° (± 77 μM SQ 20009) in glucose-free medium. Tissues were transferred to a medium containing 6 mM glucose and incubated for 60 min at 37° with additions as indicated below. Hemidiaphragms were rinsed twice in cold saline and washed 7 times at 2° in glucose-free medium as described in text. Cyclic AMP accumulation in the tissue was determined in separate experiments identical to those above, except that washings of the tissue were omitted. Number of experiments = 4, except when individual values are given.

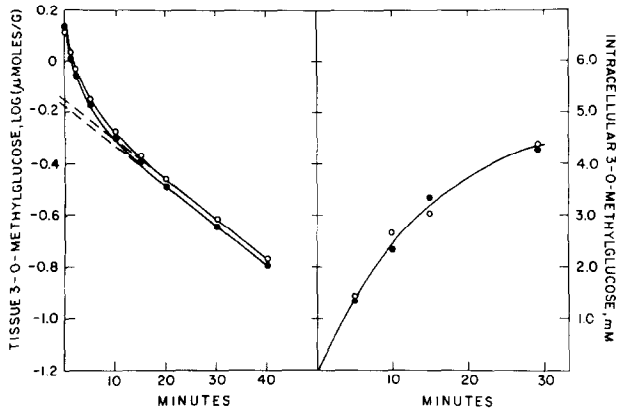

Fig. 3. Effect of epinephrine on 3-*O*-methylglucose transport in rat diaphragm. Paired rat hemidiaphragms were preincubated for 15 min at 37° in substrate-free medium and incubated for 5, 10, 15, or 30 min at 37° in a medium containing 6 mM [^{14}C-3-*O*]methylglucose (± 15 μM epinephrine). Left panel illustrates individual washout curves after 5-min of incubation with 6 mM 3-*O*-methylglucose. Right panel shows the accumulation of the sugar intracellularly as a function of time of incubation. Filled circles are controls, open circles are from experiments with epinephrine. The 5-min values are means of three determinations; others are results from individual experiments.

at the concentrations used for the experiments reported in Table 5, would influence the accumulation of 3-*O*-methylglucose in the rat hemidiaphragms. Despite the fact that the tissue content of cyclic AMP increased more than 20-fold under these conditions, there was no change in sugar transport.

DISCUSSION

The ability of epinephrine to decrease glucose utilization in mammalian skeletal muscle was first reported by Walaas and Walaas [4] and the phenomenon has been studied subsequently by a number of investigators [5, 7, 20, 22]. This action of the hormone has been found to be abolished at pH 8 [7] and to be decreased at 6·8 [21]. In our experiments the pH of the medium was maintained at 7·2, and the inhibitory effects of epinephrine on glucose uptake and glycogen synthesis were very consistent.

The results of our investigation show that the effect of epinephrine on glucose uptake of muscle is closely related to the action of the hormone on glycogen synthesis. There is a remarkable correlation between the inhibitory effects of epinephrine on glucose uptake and on incorporation of glucose into glycogen in a resting muscle preparation, such as the rat diaphragm incubated *in vitro*. It is possible that a high capacity for glycogen synthesis in a tissue is necessary for an inhibitory effect of epinephrine on glucose uptake to become apparent. Thus, in adipose tissue, which contains only a small amount of intracellular glycogen, epinephrine has been observed to increase the rate of glucose uptake [23]. In a contracting perfused-heart preparation, epinephrine administration has also been found to increase the metabolism of glucose [24].

Our experiments on the action of epinephrine on the uptake of 3-*O*-methylglucose by the diaphragm provide no support for the view that epinephrine affects sugar transport itself. Only the uptake on the natural carbohydrate substrate, glucose, is affected by epinephrine under our experimental conditions. Chambaut *et al.* [25] studied the action of dibutyryl cyclic AMP on rat diaphragm and found that this compound had no effect on galactose and glucosamine transport in this tissue. These findings lend further strength to the hypothesis that it is the glucose-to-glycogen synthetic pathway in muscle that is particularly susceptible to inhibition by a catecholamine.

Recently we have reported [19] that glycogen derived from extracellular glucose in rat diaphragm is not diluted by intracellular glucose, as would be expected if there were only one pool of intermediates between glucose and glycogen. We proposed that there were two pathways of glycogen synthesis from glucose that were distinguishable because of their presence at different sites in the cell; one catalyzed the formation of glycogen from extracellular glucose, the other was involved in the synthesis of glycogen from glucose that had already entered the cell.

We are speculating that the enzymes involved in the synthesis of glycogen from extracellular glucose in muscle are localized in cellular membranes, possibly the sarcotubular system, and that homones, directly or indirectly, can influence glycogen synthesis at this site. In fact, Bailey *et al.* [26] have observed that glycogen synthase activity is high in a particulate fraction of muscle homogenates originating from the sarcoplasmic reticulum. It is extremely interesting, in this connection, that these tissue fractions also had a high content of adenylate cyclase and cyclic AMP phosphodiesterase. Meyer *et al.* [27] isolated protein glycogen particles from rabbit skeletal muscle and demonstrated with electron microscopy that these particles were found in the same fractions as vesicles arising from fragments of the sarcoplasmic reticulum.

The significance of the accumulation of glucose in the cell seen with epinephrine is not clear at present. There are several mechanisms that should be consi-

dered. For example, the extra glucose could originate from the glucose in the medium and its accumulation could be a consequence of inhibition of the glycogen synthetic pathway, possibly at the hexokinase step. The observations of Kipnis and Cori [28] bear on this problem. These authors found that the uptake of 2-deoxy-glucose by the intact diaphragm was not influenced by epinephrine but that the phosphorylation of this metabolite was inhibited, leading to accumulation of the unphosphorylated sugar in the cell. Another possibility is that the intracellular glucose produced in response to epinephrine originates from glycogen. This could come about by the action of amylo-1,6,-glucosidase on the limit dextrins formed following phosphorylase action on glycogen [29]. We are currently carrying out experiments designed to clarify the mechanisms of this action of epinephrine.

One of the metabolic effects of epinephrine in muscle is an increase in glucose 6-phosphate caused by the activation of phosphorylase. Since glucose 6-phosphate has been found to inhibit preparations of hexokinase [30], it is possible that the increased tissue concentrations of glucose 6-phosphate seen in diaphragms exposed to epinephrine may contribute to the metabolic effects of the hormone in this tissue. However, it should be pointed out that an elevation of glucose 6-phosphate is not always associated with a decrease in glucose uptake and glycogen synthesis. For example, in diaphragms exposed to insulin there is an increase in the glucose 6-phosphate concentration even though glucose metabolism is enhanced [31, 32].

Although it cannot be ruled out that product inhibition of hexokinase plays some role in the inhibitory effects of epinephrine on glycogen synthesis, it is likely that an action of cyclic AMP is more directly involved. It has been well established that protein kinase activity is increased in extracts of muscles exposed to epinephrine [9] and that the rise in kinase activity results from transformation of the enzyme from a form requiring cyclic AMP to one independent of this nucleotide [9, 33]. This event subsequently leads to activation of phosphorylase and to formation of the less active form of glycogen synthase requiring glucose 6-phosphate for activity. Whether the lowered activity of glycogen synthase can explain the inhibitory effects of epinephrine on the glucose-to-glycogen synthetic pathway demonstrated in our experiments, or whether other sites of protein phosphorylation may be involved is a subject for further investigation.

Among the important questions raised by this investigation is the extent to which the metabolic effects of epinephrine observed *in vitro* reflect actions of the hormone in the intact animal. If epinephrine influences glucose uptake and glycogen synthesis by skeletal muscle *in vivo* in a manner similar to that *in vitro*, such an action could account for a significant portion of the hyperglycemic effect of the hormone.

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