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Research Article

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ABSTRACT The impact of diabetes on cyclic nucleotide-associated mechanisms regulating skeletal muscle protein and amino acid metabolism was assessed using epitrochlaris preparations from streptozotocin-induced diabetic rats. 1 nM epinephrine inhibited alanine and glutamine release from control preparations, but no inhibition was observed from diabetic preparations with <0.1 mM. 10 nM epinephrine stimulated lactate production from control muscle but stimulation in diabetic preparations was observed only at 0.1 mM. Serotonin inhibited amino acid release and stimulated lactate production equally in control and diabetic muscle. 0.1 mM epinephrine increased cyclic (c)AMP levels by 360% in control muscles, but these levels were increased only 83% in diabetic muscle. Basal-, fluoride-, and serotonin-stimulated adenylyl cyclase activities were equal in membrane preparations of diabetic and control muscle, but epinephrine-stimulated adenylyl cyclase was reduced by 60% in diabetic muscle. Carbamylcholine stimulation of alanine and glutamine release was blunted in diabetic preparations. Carbamylcholine increased cGMP levels in control but not in diabetic muscle. In diabetic muscle, guanylyl cyclase activity was 65% of control and the stimulation of cyclase activity by sodium azide was less in diabetic than control preparations. Added cGMP stimulated alanine and glutamine release from control, but not from diabetic muscle. These data suggest a loss of adrenergic and cholinergic responsiveness in diabetic muscle. Because amino acid release also showed a decreased responsiveness to added cAMP and cGMP, the presence of other derangements in the mechanism(s) of cyclic nucleotide regulation of muscle amino acid metabolism also seems likely.

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INTRODUCTION

Glucose overproduction is a fundamental contributor to hyperglycemia in diabetes mellitus (1). This may result in part from increased gluconeogenesis as suggested by an increased hepatic fractional extraction of alanine, the principal gluconeogenic amino acid in man (2). Because alanine production occurs in peripheral tissues such as skeletal muscle (3), an accelerated rate of alanine delivery is essential for continuing alanine availability in diabetes mellitus (4, 5). Increased alanine release has been observed from muscle preparations of experimentally diabetic rats (4, 6). Although the amino groups for net alanine formation from glutamate and pyruvate are most probably derived from the deamination of other amino acids, the source of carbon for alanine formation is unclear. This may be contributed by the catabolism of other, protein-derived amino acids, by pyruvate formed by glycolysis, or by a combination of both processes (4, 7–13). Because insulin promotes muscle protein synthesis and also inhibits muscle protein degradation (14–16), increased alanine release would be expected in an insulin deficiency state such as diabetes mellitus. However, the impact of the diabetic state on other, noninsulin-dependent mechanisms that regulate muscle protein and amino acid metabolism has not been investigated in detail. Agonists that alter muscle cyclic nucleotide levels also provide important control mechanisms for the regulation of skeletal muscle protein and amino acid metabolism. Physiologic levels of adrenergic and serotonergic agonists, acting in association with increased intracellular levels of cyclic (c)AMP,[1] inhibit skeletal muscle alanine and glutamine formation and release (17–20); physiologic levels of cholinergic agonists acting in association with increased intracellu-

[1] *Abbreviation used in this paper*: cAMP, cGMP, cyclic AMP, GMP.

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lar levels of cGMP, stimulate skeletal muscle alanine and glutamine formation and release (21, 22). The impact of experimental, streptozotocin-induced diabetes on the actions of these agonists on skeletal muscle cyclic nucleotide metabolism and on the formation and release of alanine and glutamine has been investigated using intact rat epitrochlaris skeletal muscle preparations. The implications of these findings are discussed.

METHODS

Alanine aminotransferase (EC 2.6.1.2), glutamate dehydrogenase (EC 1.4.1.3), glutaminase (EC 3.5.1.2), lactate dehydrogenase (EC 1.1.1.27), carbamylcholine, epinephrine (HCl), serotonin (5-hydroxy-triptamine), and other biochemical reagents were obtained from Sigma Chemical Co. (St. Louis, Mo.). [α-^{32}P]ATP (29.5 Ci/mol sp act), [α-^{32}P]GTP (14.2 Ci/mol sp act), [^{3}H]cAMP (26 Ci/mol sp act), and [^{3}H]cGMP (21 Ci/mol sp act) were purchased from New England Nuclear (Boston, Mass.). [^{125}I]2-O-succinyl cAMP and cGMP tyrosine methyl esters were from Collaborative Research, Inc. (Waltham, Mass.) Crystalline glucagon-free insulin was a gift of Dr. J. Galloway (Eli Lilly & Co., Indianapolis, Ind.).

As described previously (1), epitrochlaris preparations were obtained from streptozotocin-treated (65 mg/kg i.v.) and control Sprague-Dawley rats (Texas Inbred, Houston, Tex.). These preparations remained viable in vitro as judged by phosphocreatine and ATP levels that were not different from levels in vivo. Experimental diabetes in the streptozotocin-treated animals was assured by the presence of 2% glucosuria (Diastix, Ames Co., Elkhart, Ind.) for a minimum of 10 d before sacrifice of the animals. Semimicro- and microfluorometric enzymatic techniques were used to determine levels of alanine, glutamate, and glutamine in perchloric acid extracts of skeletal muscle and in the incubation media as described previously (17, 19, 20). cAMP and cGMP were determined in trichloroacetic acid extracts of skeletal muscle by double antibody radioimmunoassays using ^{125}I-labeled tyrosine methyl esters of succinylated cyclic nucleotides (23–25). Partial purification of cyclic nucleotides from trichloroacetic acid extracts was obtained by double chromatography over Dowex 50 columns and aluminum oxide (Dow Chemical Co., Midland, Mich.) as described by Birnbaumer (26) for simultaneous separations of cGMP and cAMP. Tritiated cyclic nucleotides were added to each muscle homogenate to quantitate sample recovery. Although cyclic nucleotide levels were determined after 2, 5, 10, 20, and 60 min of incubation with each agonist, only the data for 2-min incubations with epinephrine and 10-min with serotonin and carbamylcholine are presented because these periods produced the greatest observed increments in cyclic nucleotide levels. Estimations of adenylyl and guanylyl cyclase activities in skeletal muscle homogenates were based on the determination of the rate of production of [^{32}P]cAMP and [^{32}P]cGMP from [α-^{32}P]ATP and [α-^{32}P]GTP, respectively. The cAMP formed was isolated by a minor modification (27) of the method of Krishna and Krishnan (28). Both isolation methods consist of an initial chromatography over Dowex 50 (AG-X4, 100–200 mesh, H^+ form) followed by chromatography of the cyclic nucleotide containing eluate from the Dowex 50 column over neutral aluminum oxide. Yields of cyclic nucleotides (as assessed by recovery of tritium-labeled cyclic nucleotides added to each individual sample) were between 60 and 75% for cAMP and 40 and 50% for cGMP. Reaction blanks were between 2 and 4 cpm, ^{32}P behaving as cyclic nucleotide per 10^6 cpm of added labeled substrate. Skeletal muscle homogenates in which adenylyl cyclase activities were determined were prepared by Polytron homogenization (Brinkmann Instruments, Inc. Westbury, N. Y.) as described previously (22) except that 27% (wt/wt) sucrose prepared in 1.0 mM EDTA, 10 mM Tris HCl, was used as homogenization medium. Activities were determined on 10-μl aliquots containing 50–80 μg protein. Protein in the homogenate and membrane preparations was determined using the method of Lowry (29). Statistical assessments of the data were made using Student's t test or analysis of variance as appropriate (30).

RESULTS

Potential impairments of cyclic nucleotide-associated mechanisms controlling protein and amino acid metabolism were investigated in epitrochlaris preparations of control and streptozotocin-diabetic rats. The effect of epinephrine on skeletal muscle alanine and glutamine release was first studied (Fig. 1). Concentrations of epinephrine as low as 1 nM inhibited alanine and glutamine release from control muscle preparations ($P < 0.05$), and a 50% inhibition was produced by 10 μM epinephrine. In contrast, significant inhibition of alanine or glutamine formation and release in diabetic preparations was observed only with the highest concentration of epinephrine studied (0.1 mM), which reduced alanine and glutamine release 21 and 14% ($P < 0.05$ each), respectively. Muscle lactate production was also insensitive to epinephrine. 10 nM epinephrine stimulated ($P < 0.05$) lactate production from control preparations. On the other hand, basal levels of lactate production from muscles of diabetic animals were 88% that of control rats ($P < 0.05$), and this was increased only minimally with epinephrine (0.1 mM). In contradistinction, the effects of serotonin, another autacoid that inhibits muscle proteolysis and amino acid release (4), were of relatively equal magnitude in muscles of diabetic and control rats (Fig. 2). Serotonin inhibited alanine and glutamine release and also stimulated lactate production to an equal extent in control and diabetic skeletal muscle. With either preparation, 0.1 mM serotonin increased lactate production by 70%. This difference between epinephrine and serotonin action on alanine and glutamine release from diabetic muscle is even more apparent in studies of the total content of alanine and glutamine in tissues plus media after incubation (Table I). In control studies, both epinephrine (10 μM) and serotonin (10 μM) reduced total alanine and glutamine equally in preparations from normal rats. However, with preparations from streptozotocin-diabetic rats, epinephrine produced virtually no change in total alanine and glutamine.

Because the action of both epinephrine and serotonin on muscle amino acid formation appears to be mediated by increased intracellular levels of cAMP (17, 19), the effect of incubation with these agonists on muscle cAMP levels was studied for periods of

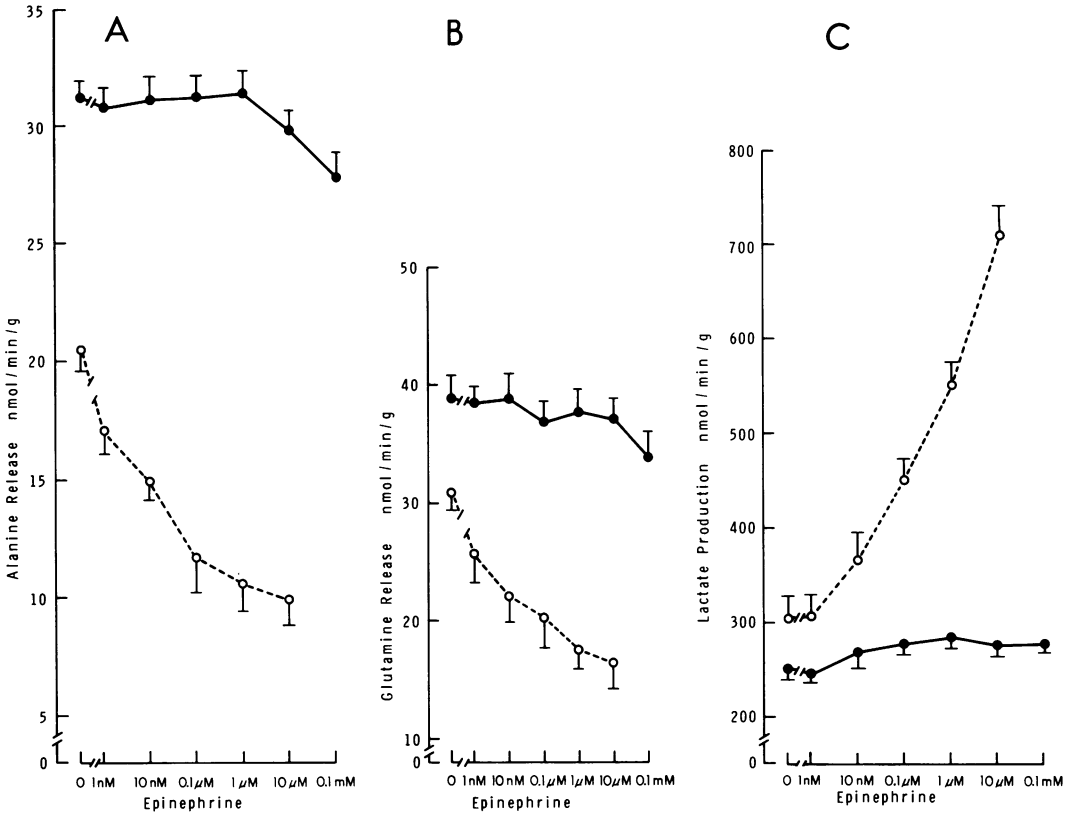

FIGURE 1 Effects of epinephrine on amino acid and lactate release from control and diabetic muscle. Epitrochlaris preparations from control (○) and streptozotocin-diabetic rats (●) were obtained and incubated for 1 h in Krebs-Henseleit media (pH 7.4) containing glucose (5 mM) and varying concentrations of epinephrine as indicated. Release of alanine (A), glutamine (B), and lactate (C) to the media was determined enzymatically as outlined in Methods. Values shown are the means (±SEM) for at least 12 experiments.

2, 5, 10, 20, 30, and 60 min. However, only the data for 2-min incubations with epinephrine and 10-min incubations with serotonin are reported because these times were associated with the greatest changes in cyclic nucleotide levels. In control muscle, epinephrine produced a concentration-dependent increase in cAMP levels with concentrations as low as 10 nM, significantly increasing cAMP levels ($P < 0.05$) (Fig. 3). Larger increases of 360% were observed at 0.1 mM epinephrine. In diabetic muscle preparations, significant increments in cAMP levels were not observed with concentrations of epinephrine <1 μM; 0.1 mM epinephrine increased cAMP levels by only 83%. Thus, the increment produced by 0.1 mM epinephrine was 333% greater in control as compared with diabetic preparations ($P < 0.01$). Conversely, serotonin produced increased cAMP levels in muscle of both diabetic and control rats. At 0.1 mM serotonin, levels of cAMP in either control or diabetic preparations were increased 120%. The basis for the relative lack of effectiveness of epinephrine as compared with serotonin in increasing cAMP levels in muscle of diabetic rats was next investigated. As shown in Table II, basal- and fluoride-stimulated adenylyl cyclase activities were not different in membranes of diabetic as compared with control preparations. Fluoride-stimulated adenylyl cyclase activity was 400% greater than basal cyclase activity ($P < 0.01$ each). Serotonin increased adenylyl cyclase activity of membranes from control and diabetic muscle by 93 and 132%, respectively ($P < 0.01$ each). In contrast, epinephrine (10 μM) stimulated adenylyl cyclase 356% in membranes from control preparations, but only 85% in membranes from diabetic preparations. These data also provide the first demonstration of a serotonin-stimulated adenylyl cyclase in rat skeletal muscle.

To determine whether the diminished epinephrine-stimulated adenylyl cyclase in membranes of diabetic muscle was the result of the single concentration of epinephrine studied, adenylyl cyclase activity was determined in the presence of a broad range of epinephrine levels (1 nM through 1 mM) (Fig. 4). At every concentration of epinephrine studied, adenylyl cyclase activity was 100–250% greater in membranes prepared

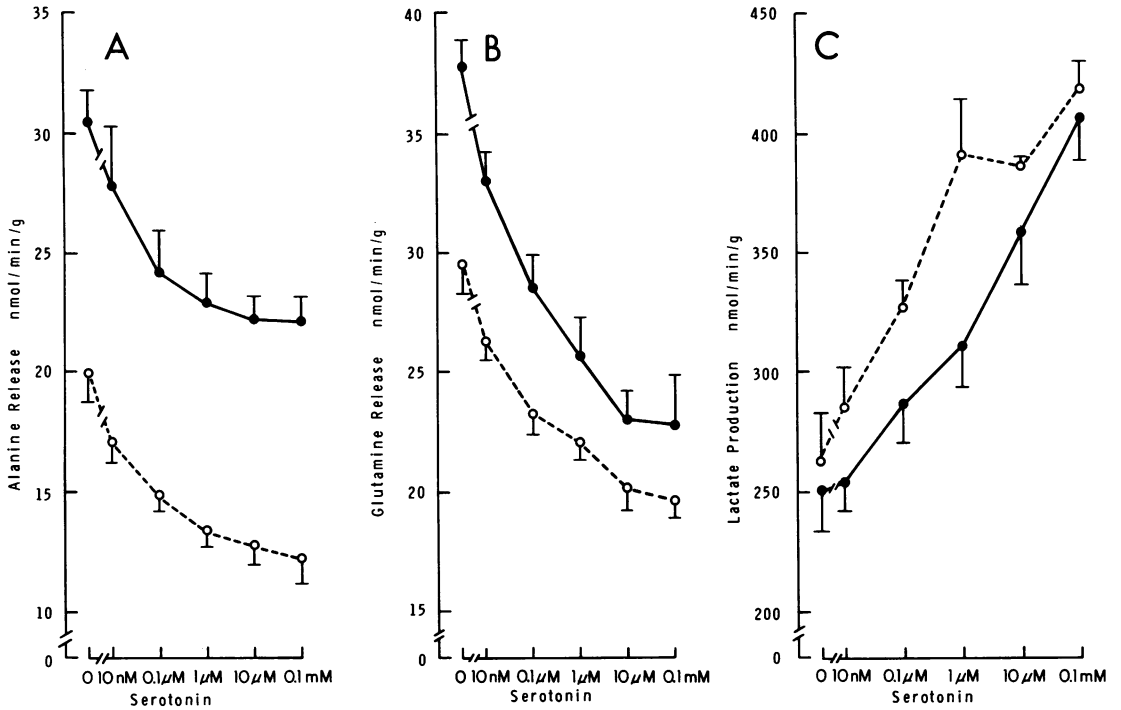

FIGURE 2 Effects of serotonin on amino acid and lactate release from muscle of control and diabetic rats. Epitrochlaris preparations from control (○) and streptozotocin-diabetic rats (●) were obtained and incubated for 1 h in Krebs-Henseleit bicarbonate buffer (pH 7.4) containing glucose (5 mM) and varying concentrations of serotonin as indicated. Release of alanine (A), glutamine (B), and lactate (C) to the media was determined enzymatically as outlined in Methods. Values shown are the means (±SEM) for at least 12 experiments.

from control as compared with diabetic muscle ($P < 0.02$ each). Peak adenylyl cyclase stimulation was observed at 1 μM epinephrine for both preparations. Half-maximal stimulation above basal activity was observed at 0.2 μM epinephrine in diabetic membranes and at 0.4 μM epinephrine in control membranes. Similar studies were also performed for the serotonin-stimulated adenylyl cyclase. No significant difference could be ascertained in the stimulation of adenylyl cyclase activity by serotonin with muscle membranes of either control or diabetic rats. The greatest stimulation of adenylyl cyclase was observed at 0.5 μM serotonin with both preparations. The effect of added cAMP on amino acid release from diabetic and control

TABLE I

Effects of Epinephrine and Serotonin on the Net Balance of Alanine and Glutamine in Muscle of Control and Diabetic Rats

Animal treatment	Addition	Concentration	Alanine				Glutamine			
			Tissue level	Amount released	Total	Difference	Tissue level	Amount released	Total	Difference
		μM	*nmol/g muscle, wet wt*							
Control	None		1,412±183	1,207±48	2,721±139	—	3,406±212	1,776±156	5,188±283	—
	Epinephrine	10	1,083±132	617±46	1,698±146	(−)1,023	2,699±203	1,009±104	3,704±261	(−)1,484
	Serotonin	10	1,142±121	718±53	1,858±152	(−)863	2,553±196	1,202±137	3,749±218	(−)1,439
Streptozotocin diabetes	None		1,830±180	1,980±81	3,820±134	—	3,852±350	2,351±183	6,196±263	—
	Epinephrine	10	1,766±161	1,932±120	3,718±133	(−)102	3,949±392	2,220±146	6,163±251	(−)33
	Serotonin	10	1,460±110	2,360±209	2,837±160	(−)983	2,723±663	1,372±182	4,112±421	(−)2,084

Epitrochlaris preparations of control and streptozotocin-diabetic rats were obtained and incubated for 1 h in media containing epinephrine (10 μM) or serotonin (10 μM). After incubation, muscles were rinsed, blotted, and frozen in liquid nitrogen. Alanine and glutamine in the incubation media and in the neutralized perchloric acid extracts of each muscle were determined enzymatically as outlined in Methods. Values shown are the means (±SEM) for at least 10 experiments and are expressed as nanomoles per gram muscle, wet weight.

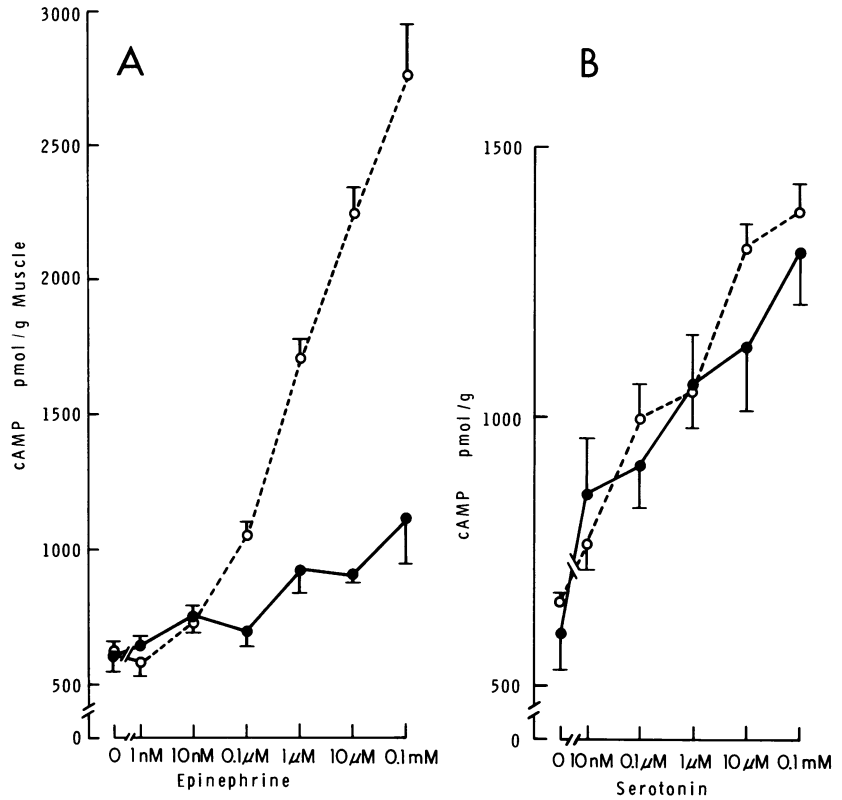

FIGURE 3 Effects of epinephrine and serotonin on cAMP levels in skeletal muscle of control and diabetic rats. Epitrochlaris preparations of control (●) and streptozotocin-diabetic rats (○) were obtained and incubated in Krebs-Henseleit buffer (pH 7.4) for 2 min with varying concentrations of epinephrine (A), or 10 min with varying concentrations of serotonin (B) as indicated. cAMP was determined in trichloroacetic acid extracts of each muscle using double antibody radioimmunoassay methods as outlined in detail in Methods. Values shown are the means (±SEM) for at least 12 experiments.

TABLE II

Effect of Epinephrine, Serotonin, and Sodium Fluoride on Adenylyl Cyclase Activity in Muscle of Control and Diabetic Rats

Addition	Concentration	Adenylyl cyclase activity	
		Control	Streptozotocin diabetes
		pmol cAMP/min/mg protein	
Basal	—	6.44±0.65	5.55±0.59
Serotonin	1 μM	12.4±0.64	12.9±0.56
Epinephrine	10 μM	29.2±0.52	10.3±0.62
Sodium fluoride	10 mM	31.9±1.8	30.1±1.13

Epitrochlaris preparations from control and streptozotocin-diabetic rats were obtained and membrane fractions prepared by differential centrifugation. Adenylyl cyclase activity was assayed as outlined in Methods with serotonin, epinephrine, or sodium fluoride added at the concentrations indicated. Values shown for cyclase activity are the means (±SEM) for at least 12 experiments and are given as picomoles of cAMP formed per minute per milligram protein.

muscle preparations was also investigated. Dibutyryl cAMP (1 mM) but not butyric acid (data not shown) reduced alanine and glutamine release from control muscle preparations by 51 and 44%, respectively ($P < 0.001$ each). Similarly, dibutyryl cAMP reduced alanine and glutamine release from preparations of diabetic rats by 42 and 40%, respectively ($P < 0.001$). Nevertheless, alanine and glutamine release from diabetic muscle remained 93 and 39% greater than from control muscle ($P < 0.01$ each).

Cholinergic agonists, acting by a nicotinic cholinergic receptor and in association with increased cGMP levels, accelerate alanine and glutamine formation and release from muscle (5, 6). With control preparations (Fig. 5), carbamylcholine (1 μM) increased alanine release by 50% and glutamine release by 41% ($P < 0.01$ each). Significant stimulation was observed with concentrations as low as 1 nM carbamylcholine ($P < 0.05$). However, carbamylcholine had no additional effect on the increased rates of alanine and glutamine formation and release in diabetic prepara-

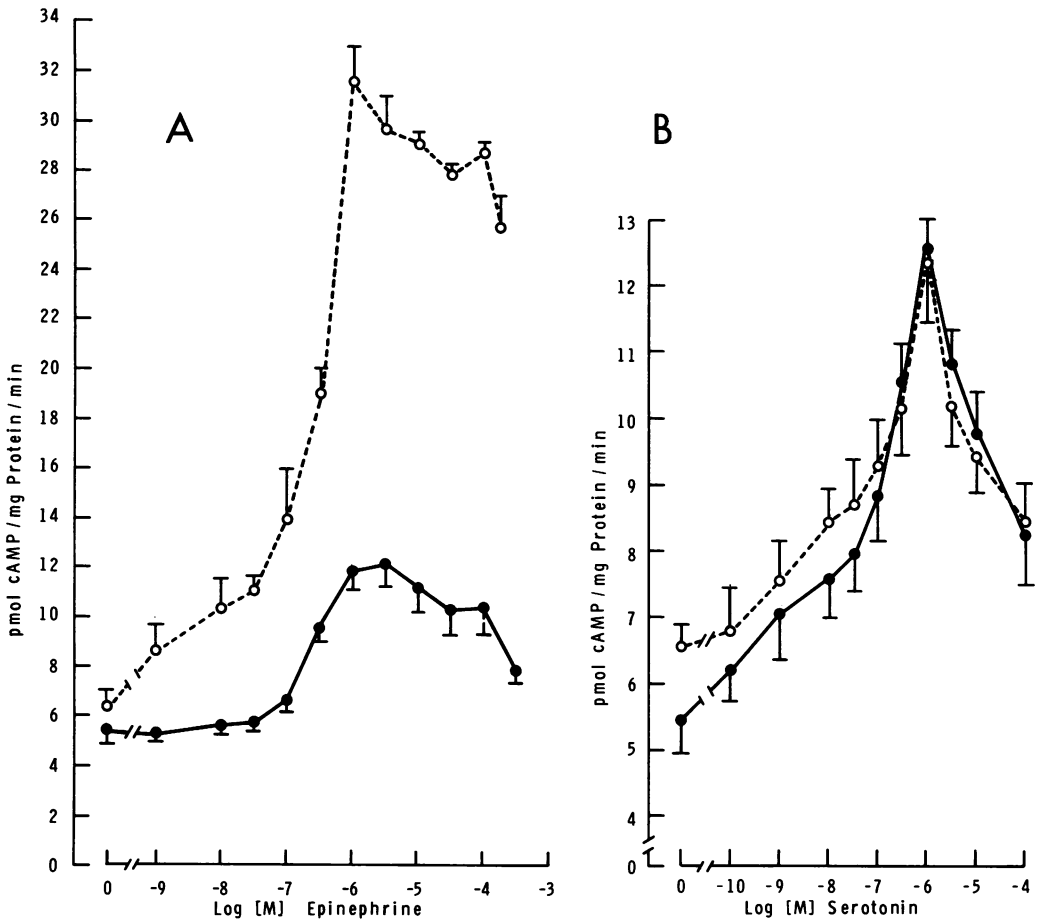

FIGURE 4 Effects of epinephrine and serotonin on adenylyl cyclase activity from muscle of control and diabetic rats. Membrane preparations from control (●) and streptozotocin-diabetic rats (○) were prepared and incubated with varying concentrations of epinephrine (A) or serotonin (B) as outlined in Methods. Adenylyl cyclase activity was estimated by the appearance of ^{32}P label in cAMP from [α-^{32}P]ATP. Values shown are the means (±SEM) for at least 12 experiments.

tions. Carbamylcholine-stimulated rates of alanine and glutamine formation with control preparations were not different from basal rates of amino acid release from diabetic preparations in the absence of carbamylcholine.

The basis for the loss of responsiveness to carbamylcholine in diabetic muscle was next investigated. As shown in Fig. 6, cGMP levels were 40% lower in skeletal muscle of diabetic as compared with control rats. Carbamylcholine produced a concentration-dependent increase in cGMP levels of control muscle; 10 μM carbamylcholine increased cGMP levels by 56% ($P < 0.01$). However, carbamylcholine had no effect on cGMP levels in diabetic muscle. Guanylyl cyclase activity was determined in muscle membrane preparations of control and diabetic rats. As seen in Table III, 40,000 g soluble guanylyl cyclase activity was 33% lower and the particulate activity was 37% lower in diabetic as compared with control skeletal muscle ($P < 0.01$ each). Sodium azide has been shown to stimulate guanylyl cyclase activity in other tissues (31). In muscle of control rats, preincubation for 10 min at 32.5°C with sodium azide (5 mM) increased soluble guanylyl cyclase activity by 33% and the particulate activity by 51%. In comparison, the effect of sodium azide on the enzyme activities from diabetic muscle was minimal. The results of cGMP addition on skeletal muscle alanine and glutamine formation and release were also investigated. cGMP increased alanine and glutamine release from muscle of control rats by 40 and 30%, respectively ($P < 0.02$ each), but did not affect amino acid release from preparations of diabetic rats. cGMP at the same concentration did not alter alanine or glutamine release from muscle of either control or diabetic rats.

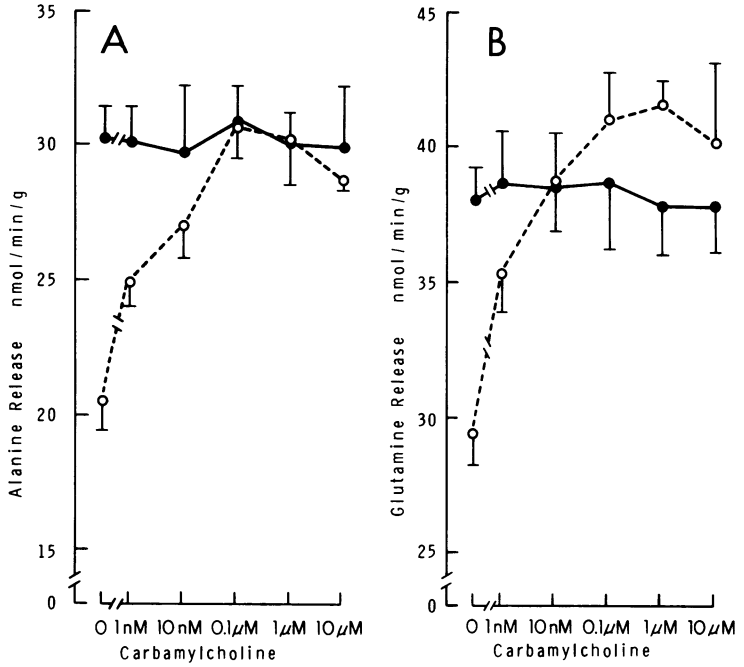

FIGURE 5 Effects of carbamylcholine on alanine and glutamine release from skeletal muscle of control and diabetic rats. Preparations from control (○) and diabetic rats (●) were obtained and incubated for 1 h in Krebs-Henseleit media (pH 7.4) containing glucose (5 mM) and varying concentrations of carbamylcholine as indicated. The release of alanine (A) and glutamine (B) to the incubation media was determined enzymatically using techniques outlined in Methods. Values shown are the means (±SEM) for at least six experiments.

DISCUSSION

Alanine and glutamine formation and release are increased 60 and 40%, respectively, in skeletal muscle of streptozotocin-diabetic rats. This may result in part from an accelerated degradation of one or more classes of skeletal muscle proteins, from a diminished rate of protein resynthesis, or from a combination of both processes (10, 15, 16). Although insulin influences both protein synthesis and protein degradation in skeletal muscle and abnormalities of both pathways have been described in experimental diabetes mellitus, potential abnormalities of other factors regulating protein and amino acid metabolism in diabetic muscle have not been fully explored. Catecholamines acting through a β-adrenergic receptor and in association with increased intracellular levels of cAMP are potent inhibitors of alanine and glutamine formation and release from skeletal muscle (17–19). Skeletal muscle of diabetic animals showed a marked loss of responsiveness to these effects of catecholamines, because 1,000- to 10,000-fold greater concentrations of epinephrine were required to alter amino acid release or lactate production with diabetic as compared with control muscles (Fig. 1). These findings are in contrast to the unaltered effects of serotonin on amino acid release and lactate production from diabetic skeletal muscle (Fig. 2). These differences in responsiveness to epinephrine and serotonin do not derive from an alteration of amino acid transport (Table I), or from altered amino acid reutilization by oxidation to CO_2 or by reincorporation into protein because the latter pathways are not quantitatively substantial compared with amino acid release (20). Thus, it seems likely that the diminished responsiveness of amino acid release to epinephrine derives from a failure to inhibit alanine and glutamine formation in diabetic muscle. Because this effect of epinephrine appears to be mediated by increased cAMP levels in normal muscle, the relative inability of epinephrine to stimulate cAMP accumulation in diabetic muscle (Fig. 3) may account in part for the insensitivity of amino acid formation.

The diminished epinephrine stimulation of cAMP accumulation in diabetic muscle most probably derives from the reduced epinephrine-stimulated adenylyl cyclase activity in membranes of diabetic muscles (Table II). Although basal-, fluoride-, and serotonin-stimulated adenylyl cyclase activities were approximately equal in membranes of diabetic as compared with control muscle (Table II, Fig. 4), epinephrine stimulation of adenylyl cyclase activity was 80% less in diabetic membranes. The dose-response curve of

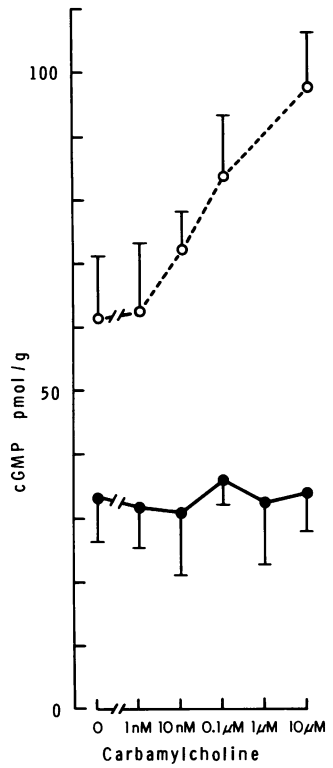

FIGURE 6 Effect of carbamylcholine on cGMP levels in skeletal muscle of control and diabetic rats. Epitrochlaris preparations from streptozotocin-induced diabetic (●) and control (○) rats were obtained and incubated for 10 min in Krebs-Henseleit media containing glucose (5 mM) and carbamylcholine added at the concentrations shown. After incubation, muscles were rapidly removed, rinsed, blotted, and frozen in liquid nitrogen. Levels of cGMP were determined on the trichloracetic extracts of each muscle preparation using double antibody radioimmunoassay techniques as outlined in Methods. Values shown are the means (±SEM) for at least eight experiments.

adenylyl cyclase stimulation by epinephrine was blunted and shifted 3 log-unit intervals, which corresponds reasonably well to the shift of the dose-response curve of cAMP accumulation in the intact muscle (Fig. 3). Furthermore, preliminary assessments of cAMP phosphodiesterase activities revealed no difference between control and diabetic muscle.[2] These observations support the concept that a diminished epinephrine-stimulated adenylyl cyclase activity may account primarily for the decreased epinephrine-induced cAMP accumulation in diabetic muscle. However, the data do not provide a precise mechanism for this reduction. Although basal-, fluoride-, and serotonin-stimulated adenylyl cyclase activities were unchanged, a uniform reduction in the amount of adenylyl cyclase enzyme or the presence of an acquired inhibitor appears to be somewhat unlikely based upon these data using an in vitro enzyme assay system. Because serotonin stimulation of adenylyl cyclase was unaltered in diabetic muscle, abnormal nucleotide regulation of the basic adenylyl cyclase system, or an abnormal capacity of adenylyl cyclase to couple to receptors in diabetes also appears unlikely, assuming that the mechanism of epinephrine and serotonin stimulation of adenylyl cyclase are similar. A decreased number of adrenergic receptors, an altered receptor affinity for catecholamines, or an altered capacity of the β-adrenergic receptor to couple to an otherwise normal adenylyl cyclase appear to be the most probable reasons for the diminished epinephrine-stimulated adenylyl cyclase activity. These results may be interpreted to suggest a loss of adrenergic responsiveness or desensitization (20, 32, 33); this may be similar to the desensitization found in the pseudodiabetic azotemic state (20), in which abnormalities of both serotonin- and epinephrine-stimulated adenylyl cyclase were observed. Although the data of the present study do not provide insight into the means by which adrenergic but not serotonergic responsiveness is lost in diabetic

[2] Garber, A. J., M. L. Entman, E. P. Bornet, and W. J. Thompson. Unpublished observations.

TABLE III
Guanylyl Cyclase Activity in Skeletal Muscle of Control and Diabetic Rats

		Guanylyl cyclase activity			
		Soluble		Particulate	
Addition	Concentration	Normal	Diabetic	Normal	Diabetic
	mM	*pmol/min/mg protein*			
Control	—	22.2±0.40	13.7±0.67	8.28±0.10	5.21±0.23
NaN_3	5	29.5±0.47	16.3±0.79	12.5±0.87	6.01±0.17

Epitrochlaris preparations of rat skeletal muscle were obtained and fractionated by differential centrifugation into a 40,000 *g* pellet and supernatant fraction. Guanylyl cyclase activity was assayed as outlined in Methods. Values shown are the means (±SEM) for at least 10 experiments and are expressed as picomoles of cGMP formed per minute per milligram protein.

muscle, high levels of plasma catecholamines have been observed in poorly controlled diabetic patients (34), and these may contribute to the loss of adrenergic responsiveness via homologous desensitization.

In diabetic muscle, the dose-responsive curve of amino acid release to catecholamines was shifted 5 log-unit intervals, whereas cAMP accumulation and adenylyl cyclase stimulation were shifted 3 log units. Furthermore, neither serotonin nor dibutyryl cAMP completely normalized alanine and glutamine release from diabetic as compared with control muscle under the same incubation conditions. Although the absolute reductions in alanine and glutamine release produced by serotonin or dibutyryl cAMP were similar, amino acid release from diabetic muscle remained elevated by 50–100%. At any level of cAMP, higher rates of amino acid formation and release were observed in diabetic as compared with control preparations. Factors in addition to the loss of adrenergic responsiveness must also participate in the accelerated alanine and glutamine formation in diabetic muscle. Nevertheless, at concentrations of epinephrine and serotonin producing equal stimulation of cAMP levels, serotonin did cause slightly greater inhibition of alanine and glutamine output from diabetic muscle. Thus, the data of the present study do not exclude differences in cAMP compartmentation or in cAMP-dependent protein kinase regulation between serotonin and epinephrine action in diabetic as contrasted to control muscle. In the past we have shown that muscle glutamine and alanine formation can be increased by nicotinic cholinergic stimulation associated with elevation of cGMP levels (21, 22). Nevertheless, the abnormal cholinergic responsiveness observed in diabetic muscle does not account for the increased alanine and glutamine release from diabetic muscle. Although carbamylcholine did not further stimulate alanine and glutamine release in diabetic muscle (Fig. 5), decreased cGMP levels were found and these levels were unresponsive to carbamylcholine, presumably a result of the diminished guanylyl cyclase activity. Based on these results, it is not possible to account for the increased alanine and glutamine release from diabetic muscle because decreased rather than increased rates of amino acid release would have been anticipated as the result of the lower cGMP levels. Although these abnormalities of guanylyl cyclase in diabetic muscle are similar to an abnormality found in diabetic liver (35), the basis for that effect is not apparent from the data of the present study.

Diabetes mellitus is characterized by a relative or absolute insulin deficiency that may result in accelerated muscle proteolysis and amino acid release (4, 8, 16). The findings of the present study demonstrate abnormalities of adrenergic and cholinergic regulation of protein and amino acid metabolism in diabetic muscle. Increased as well as decreased cAMP levels and catecholamine-stimulated adenylyl cyclase activities have been observed in a number of tissues obtained from a variety of diabetic models (36–39). The present finding of a subsensitivity of muscle amino acid release to physiologic levels of catecholamines provides a mechanism in addition to insulin deficiency that may thereby cause glucose overproduction in diabetic man, because physiologic levels of catecholamines (0.1–10 nM) would be ineffective in restraining the accelerated rates of muscle alanine delivery in vivo as a result of this desensitization process (34, 40).

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