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J Clin Invest. 1978;62(3):633-641. <https://doi.org/10.1172/JCI109170>.

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SUBSENSITIVITY OF ADENYLATE CYCLASE AND AMINO ACID RELEASE TO EPINEPHRINE AND SEROTONIN

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ABSTRACT The mechanism of the increased alanine and glutamine formation and release from skeletal muscle in experimental uremia was investigated using epitrochlearis preparations from control and chronically uremic rats. In uremic muscle, insensitivity to epinephrine or serotonin suppression of alanine and glutamine release was observed. With control muscles, 1 nm or greater, epinephrine inhibited alanine and glutamine release, whereas with uremic muscles, epinephrine concentrations $<1 \mu\text{M}$ did not alter amino acid release. Decreased alanine and glutamine release with 1 nM serotonin was observed in control muscles, but no inhibition was observed with concentrations $<1 \mu\text{M}$ in uremic muscle. Muscle amino acid levels were the same in control and uremic muscles in the presence or absence of epinephrine or serotonin. The reutilization of released alanine by protein synthesis or oxidation to CO_2 was not differentially affected by epinephrine in uremic muscles as compared with control muscle. Dibutylryl-cAMP inhibited amino acid release equally in uremic and control muscles. Epinephrine or serotonin increased cAMP levels two- to fourfold or more in control than in uremic muscle. Basal and fluoride-stimulated adenylate cyclase activities were equal in uremic and control muscle homogenates and in membrane fractions, but $10 \mu\text{M}$ epinephrine-stimulated adenylate cyclase was reduced 30–60% with uremia. At any concentration of epinephrine (0.001–100 μM), the stimulation of membrane adenylate cyclase activity was one- to twofold greater with control membranes than with uremic muscle

membranes. With either control or uremic muscle, peak adenylate cyclase activity was observed at $1 \mu\text{M}$ epinephrine.

These data indicate that skeletal muscle in chronic uremia acquires an insensitivity to the metabolic action of epinephrine or serotonin. This insensitivity may be attributable in part to the diminished increments in muscle cAMP levels produced by adrenergic and serotonergic agonists. The decreased cAMP levels may derive in turn from a decreased activity or subsensitization of the agonist-stimulated adenylate cyclase in uremic muscle.

INTRODUCTION

A broad range of abnormal blood amino acid levels have been reported in uremic man (1–4). Blood alanine levels have been observed to be unaltered, increased, or decreased (5, 6). These disparate observations prompted an investigation of alanine and glutamine metabolism and release from skeletal muscle of experimentally uremic rats. In the accompanying study, we found increased alanine and glutamine formation and release from skeletal muscle of experimentally uremic rats.¹ Although resistance to the action of insulin has been postulated in the chronically uremic state (7–12), insulin insensitivity appeared not to account for the increased muscle amino acid release.¹ β -Adrenergic agonists acting through adenylate cyclase and intercellular levels of cAMP appear to modulate the rate of protein degradation and amino acid release from skele-

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Received for publication 28 February 1977 and in revised form 24 April 1978.

¹Garber, A. J. 1978. Skeletal muscle protein and amino acid metabolism in experimental chronic uremia in the rat. Accelerated alanine and glutamine formation and release. *J. Clin. Invest.* 62: 623–632.

tal muscle (13). In this study, we have investigated the influence of uremia on adrenergic and serotonergic mechanisms controlling alanine and glutamine formation and release from skeletal muscle. The results of this study have been presented previously (14) and have appeared in abstract form (15, 16).

METHODS

Alanine aminotransferase (EC 2.6.1.2), glutamate dehydrogenase (EC 1.4.1.3), glutaminase (EC 3.5.1.2), and other biochemical reagents were obtained from the Sigma Chemical Co. (St. Louis, Mo.). [^{14}C]alanine, [$\alpha\text{-}^{32}\text{P}$]ATP, and [^3H]cAMP were purchased from New England Nuclear (Boston, Mass.). Crystalline glucagon-free insulin was a gift of Dr. J. Galloway (Eli Lilly and Co., Indianapolis, Ind.).

Epitrochlearis preparations were obtained and incubated as described previously (17). After incubation, the muscles were removed rapidly, briefly rinsed in cold buffer, blotted, and then frozen in liquid nitrogen. Frozen muscle preparations were weighted while maintained at -20°C , and then were rapidly homogenized in ice-cold perchloric acid, and subsequently centrifuged. The resulting supernate was neutralized with 3 M potassium hydroxide, 0.4 M imidazole, and 0.7 M potassium chloride, and the potassium perchlorate precipitate removed by further centrifugation (18).

Studies of protein synthesis were carried out in incubation flasks using continuous gassing with 95% O_2 -5% CO_2 for 1- to 4-h periods. The incubation media contained a mixture of 16 essential and nonessential amino acids and insulin (100 mU/ml). 1.0 $\mu\text{Ci/ml}$ [^{14}C]alanine was also added. Labeled amino acid incorporation into protein was determined as described previously.¹ Studies of alanine oxidation by intact epitrochlearis preparations were carried out with alanine as the only added amino acid (final concentration 0.4 mM), and [^{14}C]alanine was included at a 1- $\mu\text{Ci/ml}$ final concentration. After a 2-h incubation, [^{14}C]CO₂ was trapped and counted as described previously.¹ Calculations of the metabolic flux of alanine were performed using mean alanine specific activities as determined in aliquots of the incubation media before and after incubation.

Semimicro- and microfluorometric enzymatic techniques were used to determine levels of intermediates and substrates in perchloric acid extracts of skeletal muscle and in the incubation media. Alanine (19), aspartate, glutamate, and glutamine (20) were assayed using coupled enzymatic assays. Cyclic AMP was determined in trichloroacetic acid extracts of muscle by double-antibody radioimmunoassay (21). Adenylate cyclase activities were determined in a 50- μl final volume containing (unless otherwise specified) 3.0 mM [$\alpha\text{-}^{32}\text{P}$]ATP (2-4 μM), 5.0 mM MgCl_2 , 1 mM EDTA, 1 mM [^3H]cAMP ($\approx 10,000$ cpm), 24 mM bis-tris-propane-HCl buffer (pH 7.5), and an ATP-regenerating system consisting of 20 mM creatine phosphate, 0.2 mg/ml creatine phosphokinase, and 0.02 mg/ml myokinase and up to 20 μl of homogenate of 10,000 g particles 50-75 μg protein). When present, epinephrine was 10 μM and NaF was 10 mM. Incubations were at 37°C for 15 min and were terminated by the addition of 100 μl of a "stop solution" containing 10 mM cAMP, 40 mM ATP, and 1% sodium dodecyl sulfate, followed by immediate boiling for 3.5 min. Reaction blanks were prepared by incubating all reagents in the absence of adenylate cyclase containing material and adding the homogenate or membrane particles after the stop solution and before the boiling step. The ^{32}P -labeled cAMP formed during the incubations was isolated by a modification of the method of Salomon et al. (22). This method, consisting of

double, sequential chromatography on Dowex 50-X4 (200-400 mesh, H^+ form) (The Dow Chemical Co., Midland, Mich.) and aluminum oxide, yields low blanks (≈ 2 -4 cpm of ^{32}P -labeled cAMP-like material for each 1,000,000 cpm of [$\alpha\text{-}^{32}\text{P}$]ATP added). Recoveries of cAMP ranged from 60 to 85% as assessed individually for each sample by a determination of the [^3H]cAMP added to the reaction mixtures as the recovery marker. Using 150 μg of homogenate or 10,000 g particulate fractions, the above described assay conditions yielded linear time curves for up to 20 min, and resulted in preservation of $>80\%$ of the initially added [$\alpha\text{-}^{32}\text{P}$]ATP as such. The amount of ^{32}P in ATP at the end of incubations for adenylate cyclase activity was determined by chromatography of a small, 1- μl aliquot of unboiled incubation mixture on plastic-backed polyethylene-imine-cellulose TLC plates (Brinkmann Instruments, Inc., Wesbury, N. Y.), using 1 M LiCl as developing solvent. Under these conditions, ^{32}P -labeled breakdown products of ATP (ADP, AMP, cAMP, PP_i , and P_i) are separated from ATP and are quantitated by liquid scintillation counting (23). Using this approach, it was determined that neither normal nor uremic muscle homogenates or 10,000 g particles had any effect on the maintenance of ATP levels throughout the incubation for adenylate cyclase activity. Furthermore, these preparations had no effect on the linearity of cAMP formation as a function of time. Protein in the homogenate and membrane preparations was determined using the method of Lowry et al. (24). Statistical assessments were made using Student's *t* test (25). Chronically uremic rats were produced surgically by unilateral nephrectomy and segmental infarction of the contralateral kidney after ligation of two of the renal arterial branches (26). Supplemental feedings of casein, dextrose, and water were given. Control rats were pair-fed. Serum urea nitrogen was 59.1 ± 6.8 and 18.3 ± 2.7 mg/dl (mean \pm SE) in the uremic and control groups, respectively.

RESULTS

The effect of increasing concentrations of exogenous epinephrine on alanine release from skeletal muscle of control and chronically uremic rats was studied (Fig. 1). Concentrations of epinephrine as low as 1 nM produced inhibition of alanine release from control preparations, but with uremic preparations alanine release was unaffected by epinephrine concentrations $< 1 \mu\text{M}$. With uremic muscle, the inhibition produced by 10 μM epinephrine was only 15% of the basal release rate and the decrease in alanine release (5 nmol/min per g muscle) with uremic preparations was about one-half of that produced with control preparations (9.5 nmol/min per g muscle). Similar data were obtained with the effects of epinephrine on glutamine release from control and uremic muscle (Fig. 2). With control preparations, 1 nM epinephrine reduced glutamine release, and a maximal inhibition (40%) of glutamine release was observed at 10 μM . In preparations obtained from chronically uremic rats, inhibition of glutamine release was not observed with levels of epinephrine $< 10 \mu\text{M}$, and at this concentration, the inhibition of glutamine release was only 15% of basal. The amount of the decrease in glutamine release in uremic muscle was less than one-half of that observed with control muscle. Similar data (not shown) with

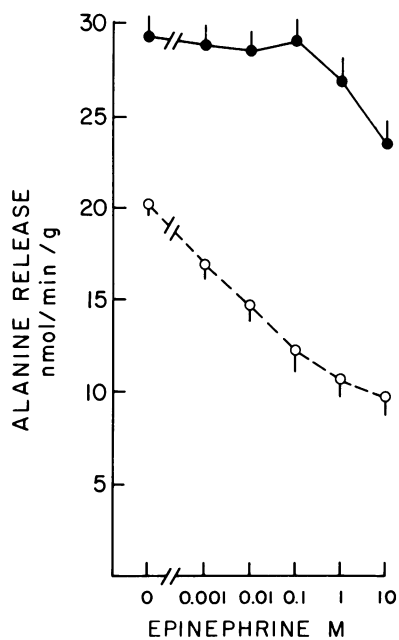


FIGURE 1 The effect of epinephrine on alanine release from skeletal muscle of control and chronically uremic rats. Epitrochlearis preparations from control (○) and chronically uremic (●) rats were obtained and incubated for 1 h in Krebs-Henseleit buffer (pH 7.4) containing 5 mM glucose and epinephrine at the concentrations indicated. After incubation, the preparations were removed and frozen in liquid nitrogen. Alanine levels in the media were determined enzymatically. Values shown are the means (\pm SEM) for at least eight experiments.

control and uremic preparations were observed in studies of glutamate release.

Serotonin (5-hydroxytryptamine), acting through a D-serotonergic receptor and cAMP, can also modulate alanine and glutamine formation and release in skeletal muscle (27). The effect of serotonin on alanine and glutamine formation and release in preparations from control and chronically uremic rats was therefore investigated. As shown in Fig. 3, serotonin produced a concentration-dependent decrease in alanine release from preparations of control rats. At 10 μ M, serotonin decreased alanine release from control preparations by 40%. In contrast, alanine release from preparations of uremic rats were not inhibited by concentrations of serotonin <1 μ M, and at 10 μ M, serotonin produced only an \approx 20% inhibition of alanine release. Similar effects of serotonin on glutamine release were also found (Fig. 4). A progressive inhibition of glutamine release from control preparations was observed with increasing concentrations of serotonin between 0.001 and 10 μ M. At the latter concentration, glutamine release was reduced 33%. With preparations from uremic animals, however, significant inhibition of glutamine release did not occur with

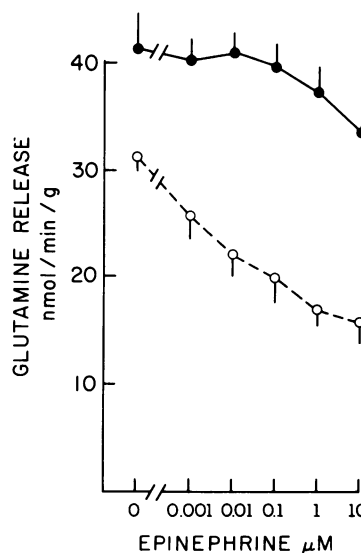


FIGURE 2 Effect of epinephrine on glutamine release from skeletal muscle of control and chronically uremic rats. Epitrochlearis preparations from control (○) and chronically uremic (●) rats were obtained and incubated with epinephrine added at the concentrations indicated. After incubation, the muscles were removed, rinsed, blotted, and frozen in liquid nitrogen. Glutamine in the media was determined enzymatically. The values shown are the means (\pm SEM) for at least eight experiments.

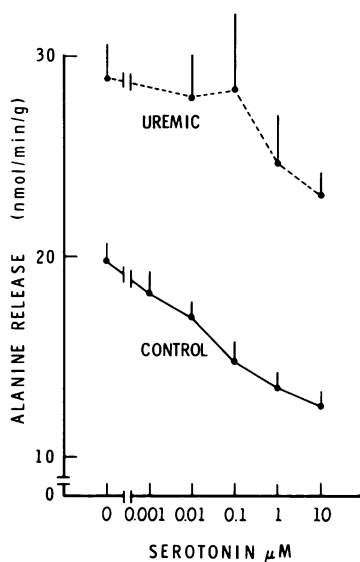


FIGURE 3 Effect of serotonin on alanine release from epitrochlearis preparations of control and chronically uremic rats. Epitrochlearis preparations from control (○) and chronically uremic (●) rats were obtained and incubated with serotonin at the concentrations indicated. After a 1-h incubation, the preparations were removed and frozen in liquid nitrogen. Alanine levels were determined enzymatically. Values shown are the means (\pm SEM) for at least six experiments.

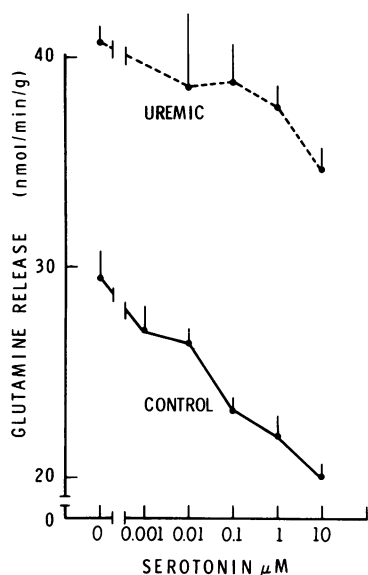


FIGURE 4 Effect of serotonin on glutamine release from skeletal muscle of control and chronically uremic rats. Epitrochlearis preparations from control (○) and chronically uremic (●) rats were incubated in Krebs-Henseleit buffer containing serotonin at the concentrations indicated. Glutamine in the media after incubation was determined enzymatically. The values shown are the means (\pm SEM) for at least six experiments.

concentrations $<10 \mu\text{M}$ ($P < 0.05$), and the extent of this inhibition was only $\approx 15\%$. Levels of alanine and glutamine in skeletal muscles after incubation with $10 \mu\text{M}$ epinephrine or serotonin were not significantly different in muscle preparations from uremic as compared to control animals (Table I).

The effect of epinephrine or serotonin on medium alanine reutilization was also investigated (Table II). In these studies, 100 mU/ml insulin was added to the media so that the transport of labeled alanine might not be limiting for an assessment of reutilization. Rates of added $[\text{U-}^{14}\text{C}]$ alanine oxidation to $[\text{C}^{14}]\text{CO}_2$ were low in uremic muscle. The addition of either $10 \mu\text{M}$ epinephrine or serotonin to the incubation media did not alter substantially these rates of alanine oxidation. With uremic, but not control preparations, the incorporation of added, extracellular alanine into muscle proteins *in vitro* was reduced 25% by epinephrine.

Because epinephrine and serotonin action on muscle amino acid formation and release appears to be associated with increased intracellular levels of cAMP, and because the action of these agonists can be reproduced by dibutyryl-cAMP (14), the effects of dibutyryl-cAMP addition on alanine, glutamate, and glutamine release were investigated with control and uremic muscle preparations (Table III). Either $10 \mu\text{M}$ epinephrine or 1 mM dibutyryl-cAMP decreased alanine

TABLE I
Effect of Incubation with Epinephrine or Serotonin on Levels of Alanine, Glutamate, and Glutamine in Muscles from Control and Chronically Uremic Rats

Addition	Pre-treatment	Alanine	Glutamate level	Glutamine
None	control	1.59 ± 0.11	0.69 ± 0.11	3.52 ± 0.17
	uremic	1.70 ± 0.12	0.68 ± 0.03	3.53 ± 0.29
Epinephrine	control	1.34 ± 0.11	0.65 ± 0.07	3.43 ± 0.34
	uremia	1.37 ± 0.12	0.58 ± 0.08	3.19 ± 0.25
Serotonin	control	1.51 ± 0.11	0.52 ± 0.04	3.29 ± 0.20
	uremic	1.50 ± 0.06	0.63 ± 0.05	3.25 ± 0.15

Epitrochlearis preparations from control and chronically uremic rats were obtained and incubated for 1 h in Krebs-Henseleit bicarbonate buffer (pH 7.4) containing $10 \mu\text{M}$ epinephrine or serotonin, as indicated. After incubation, the muscles were removed, rinsed, blotted, and frozen in liquid nitrogen. For purposes of comparison, unincubated preparations were freeze-clamped *in situ*. Levels of alanine and glutamine in neutralized perchloric acid extracts of each muscle were determined enzymatically. Values shown are the means (\pm SEM) for at least 10 experiments.

release from control preparations by 50%. With uremic preparations, epinephrine reduced alanine release by 20%, but dibutyryl-cAMP reduced alanine release by nearly 70%. Rates of alanine release with added dibutyryl-cAMP were equal with either type of

TABLE II
Effect of Epinephrine and Serotonin on Alanine Oxidation to CO_2 and Alanine Incorporation into Protein by Skeletal Muscle of Control and Chronically Uremic Rats

Addition	Oxidation to $[\text{C}^{14}]\text{CO}_2$		Incorporation into trichloroacetic acid precipitable protein	
	Control	Uremic	Control	Uremic
$\text{nmol/min/g muscle, wet wt}$				
None	0.135 ± 0.008	0.171 ± 0.038	0.606 ± 0.086	0.674 ± 0.039
Epinephrine	0.169 ± 0.014	0.130 ± 0.035	0.627 ± 0.071	0.417 ± 0.036
Serotonin	0.126 ± 0.010	0.118 ± 0.006	0.595 ± 0.116	0.486 ± 0.027

Epitrochlearis preparations from control and chronically uremic rats were obtained and incubated in Krebs-Henseleit bicarbonate buffer containing $10 \mu\text{M}$ epinephrine or serotonin as indicated. For studies of alanine oxidation to CO_2 , $1.0 \mu\text{Ci}$, 0.4 mM $[\text{U-}^{14}\text{C}]$ alanine was added to the incubation media. For studies of alanine incorporation into muscle protein, a mixture of 17 essential and nonessential amino acids containing $1.0 \mu\text{Ci}$, 0.4 mM $[\text{U-}^{14}\text{C}]$ alanine and 100 mU/ml insulin was added to the incubation media. After incubation, muscles were removed from the media, rinsed, blotted, and frozen in liquid nitrogen. Labeling of protein, CO_2 , and acid soluble fractions of muscle was determined as described in Methods. For purposes of rate calculations, alanine specific activities in media were determined before and after incubation. Values shown are the means (\pm SEM) for at least six experiments.

TABLE III

The Effects of Epinephrine and N_6, O_2' -Dibutyryl cAMP on Amino Acid Release from Skeletal Muscle of Control and Chronically Uremic Rats

Addition	Treatment	Amino acid release		
		Alanine	Glutamate	Glutamine
<i>nmol/min/g muscle</i>				
None	control	20.2±1.1	10.2±0.8	30.8±2.7
	uremia	29.9±2.3	15.8±1.3	42.3±3.1
Epinephrine	control	10.7±0.9	10.8±0.9	17.1±1.5
	uremia	24.0±1.1	15.8±1.3	34.1±3.0
Dibutyryl cAMP	control	10.4±1.2	11.1±0.9	16.8±2.0
	uremia	11.8±1.7	16.9±1.1	18.7±2.3

Epitrochlearis preparations from control and chronically uremic rats were obtained and incubation for 1 h with 10 μ M epinephrine or 1 mM dibutyryl cAMP as indicated. After incubation, the muscles were removed, rinsed, blotted, and frozen in liquid nitrogen. Alanine, glutamate, and glutamine in the incubation media was determined enzymatically. Values shown are the means (\pm SEM) for at least eight experiments.

preparation. Similarly, with preparations from control rats, epinephrine reduced glutamine release \cong 45% and dibutyryl-cAMP reduced glutamine release 46%. With preparations from chronically uremic rats, epinephrine reduced glutamine release by only 20%, but dibutyryl-cAMP inhibited glutamine release 56%. Thus, rates of glutamine release with added dibutyryl-cAMP were nearly equal in both muscle preparations.

The effect of epinephrine on cAMP levels in muscle preparations of control and uremic rats was next examined (Table IV). After a 2-min incubation with increasing concentrations of epinephrine, preparations were rapidly frozen in liquid nitrogen and levels of cAMP were determined by radioimmunoassay. In an independent series of experiments (data not shown), a 2-min incubation period with epinephrine resulted in maximal increments in cAMP. 10 μ M epinephrine produced a 268% increase in cAMP levels in control muscles, but only a 101% increase in uremic muscles. Significantly increased levels of cAMP in control preparations were observed with 10 nM epinephrine ($P < 0.02$). In uremic preparations, significantly increased levels of cAMP were observed only with epinephrine concentrations of 1 μ M or greater ($P < 0.01$). Decreased stimulation of skeletal muscle cAMP levels with serotonin were also observed with preparations from uremic rats as compared with control rats (Table V). In contrast to the optimal 2-min incubation period with epinephrine, the optimal period with serotonin was found to be 5 min. With preparations from control rats, 10 μ M serotonin increased

TABLE IV

Effect of Epinephrine on Steady-State Levels of cAMP in Skeletal Muscle Preparations of Control and Chronically Uremic Rats

Epinephrine addition	cAMP level	
	Control	Chronic uremia
μ M	<i>pmol/g muscle, wet wt</i>	
0	609.8±39.4	542.5±30.9
0.001	577.8±50.5	546.4±40.0
0.01	726.2±32.1	595.0±40.1
0.1	1053.2±50.7	675.2±73.8
1	1710.0±71.2	793.9±61.3
10	2245.8±95.2	1091.2±84.0

Epitrochlearis preparations from control and uremic rats were incubated with varying concentrations of epinephrine as indicated. After a 2-min incubation, the preparations were rapidly removed from their media, rinsed, blotted, and frozen in liquid nitrogen. Levels of cAMP were determined in ether-washed trichloroacetic acid extracts of muscle using a sensitive double-antibody radioimmunoassay. Values shown are the means (\pm SEM) for at least 10 experiments.

levels of cAMP 114%, whereas with preparations from chronically uremic rats, serotonin increased cAMP levels 17%.

To assess the capacity of these muscle preparations to generate cAMP, adenylate cyclase activity was determined in whole homogenates and in membrane fractions of epitrochlearis preparations from control and uremic animals. As shown in Table VI, basal adenylate cyclase activity was approximately equal in either homogenates or membrane fractions of control and uremic muscles. Adenylate cyclase activity was stimulated sevenfold by 10 mM NaF in both kinds of muscle preparations. 10 μ M epinephrine

TABLE V

Effect of Serotonin on Steady State Levels of cAMP in Skeletal Muscle Preparations from Control and Chronically Uremic Rats

Serotonin concentration	cAMP level	
	Control	Chronic uremia
μ M	<i>pmol/g muscle, wet wt</i>	
0	644±25	544±24
0.1	995±65	649±40
10	1381±45	734±18

Muscle preparations from control and uremic rats were incubated with varying concentrations of serotonin as indicated. After a 5-min incubation, the muscles were rapidly removed from their media, rinsed, blotted, and frozen in liquid nitrogen. Levels of cAMP were determined using a double-antibody radioimmunoassay. Values shown are the means (\pm SEM) for at least eight experiments.

TABLE VI
Adenylate Cyclase Activity in Skeletal Muscle of Control and Chronically Uremic Rats

Addition	Adenylate cyclase activity			
	Whole homogenate		Membrane fraction	
	Control	Uremic	Control	Uremic
	<i>pmol/min/mg protein</i>			
None	5.88±0.44	6.85±0.78	6.39±0.67	5.60±0.25
Epinephrine, 10 μ M	40.27±2.89	18.57±1.38	29.52±0.46	18.90±0.88
NaF, 10 mM	47.67±2.98	46.04±4.35	47.29±3.41	45.58±2.58

Epitrochlearis preparations from control and chronically uremic rats were homogenized and membrane fractions prepared by differential centrifugation. Homogenates and skeletal muscle membrane preparations were assayed for adenylate cyclase activity in the presence of 10 μ M epinephrine, 10 mM NaF, or under basal conditions. Adenylate cyclase activity was estimated as the rate of appearance of 32 P-label in cAMP from [α - 32 P]ATP. Values shown are the means (\pm SEM) for at least 12 determinations.

stimulated adenylate cyclase activity in control muscle homogenates or membrane fractions was approximately four- to sixfold greater than basal activity. However, with muscle preparations obtained from chronically uremic rats, adenylate cyclase activity was increased only about twofold by epinephrine.

To investigate further the basis for the decreased epinephrine-stimulated adenylate cyclase activity in uremic muscle, the dependence of cyclase activity on epinephrine concentrations was determined. With membranes from control or uremic muscle, maximal stimulation of cyclase activity was observed with an epinephrine concentration of 1 μ M epinephrine. At each concentration of epinephrine studied, the increments in adenylate cyclase activity above basal were 100–200% greater in muscle membrane preparations from control rats as compared with chronically uremic rats.

DISCUSSION

In epitrochlearis skeletal muscle preparations from chronically uremic rats, alanine and glutamine formation and release are increased 30–50% compared with preparations from pair-fed control rats.¹ This increased amino acid release may derive in part from an increased degradation of one or more classes of skeletal muscle proteins, or from diminished protein synthesis, or both. Although insulin is an important hormonal modulator of protein and amino acid metabolism in skeletal muscle (28–30), no evidence was found to support the hypothesis that an acquired insensitivity or resistance to insulin action is a consequence of the chronically

uremic state. To the contrary, the extent of insulin inhibition of alanine and glutamine release from uremic muscle was greater than the inhibition observed with control muscle. In addition to insulin, other agents have been described which may modulate amino acid release from skeletal muscle. Catecholamines, acting through a β -adrenergic receptor and cAMP, are potent inhibitors of alanine and glutamine formation and release in skeletal muscle (13, 31). Similarly, serotonin (5-hydroxytryptamine), acting through a D-serotonergic receptor and intracellular cAMP, can also modulate alanine and glutamine formation and release from skeletal muscle (27). The data of Figs. 1–4 demonstrate clearly that skeletal muscle from uremic rats acquires an insensitivity to the inhibitory actions of epinephrine and serotonin on muscle alanine and glutamine release. The amount of the decrease in amino acid release and the fractional inhibition produced by epinephrine or serotonin were less with uremic preparations as compared with control preparations. The diminished effect of epinephrine or serotonin on muscle amino acid release did not result from an alteration in muscle amino acid transport because tissue levels of alanine and glutamine in the presence of epinephrine or serotonin were virtually identical in control and uremic preparations (Table I). Because pathways of alanine reutilization from the media, such as by resynthesis to protein or by oxidation to CO₂, were not affected by epinephrine differently in control muscles as compared with uremic muscles (Table II), the insensitivity of amino acid release to epinephrine may reflect a relative failure of suppression of alanine and glutamine formation in uremic muscle.

The diminished epinephrine and serotonin inhibition of muscle alanine and glutamine formation and release in chronic uremia appears to result, at least in part, from an impaired generation by these agonists of intracellular cAMP. This conclusion is based upon three major lines of evidence. First, the inhibition of alanine and glutamine formation by dibutyryl cAMP was similar in skeletal muscle preparations of uremic and control rats. This suggests that the mechanism of epinephrine or serotonin action beyond the point of cAMP generation may be unaltered in chronically uremic muscle (Table III). Second, increments in cAMP levels produced by epinephrine (Table IV) or serotonin (Table V) were lower in uremic muscle as compared with control muscle. Third, epinephrine-stimulated adenylate cyclase activity was diminished 40–50% in skeletal muscle of uremic rats as compared with control rats. Decreased cyclase activity was observed in whole muscle homogenates and in muscle membrane preparations (Table VI). Although the lower cAMP levels produced by epinephrine in uremic muscle could have resulted from either diminished

cAMP generation or from accelerated cAMP disposal, the data on adenylate cyclase activity provide good evidence that an impaired generation of cAMP from exogenous epinephrine is at least one mechanism accounting for the lower cAMP levels observed in uremic muscle. Factors other than the decreased activity of epinephrine-stimulated adenylate cyclase may also be involved in the mechanism(s) accounting for the diminished cyclic AMP levels found in skeletal muscle of uremic animals, because there does not appear to be a 1:1 correspondence in the effect of uremia on epinephrine activation of adenylate cyclase and epinephrine stimulation of cAMP levels. Thus, the dose-response curve of adenylate cyclase to epinephrine is shifted ≈ 10 -fold to higher concentrations of the amine in uremia (Fig. 5), whereas the dose-response curve of cAMP accumulation is shifted at least 100-fold in uremic muscles (Table IV). This observation suggests that an accelerated rate of cAMP removal operative only in the intact muscle, perhaps involving an increased activity of cAMP phosphodiesterase, may also be a concomitant abnormality in uremic muscle. Alternatively, the lack of strict correspondence between epinephrine stimulation of adenylate cyclase,

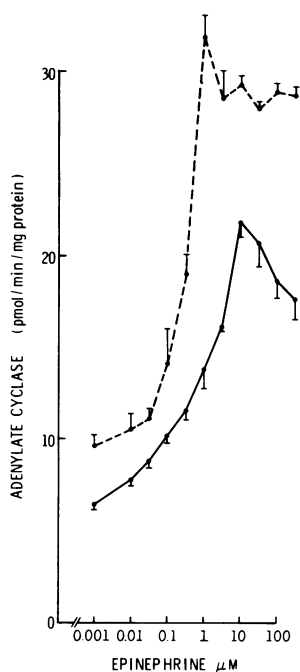


FIGURE 5 Effect of epinephrine concentrations on skeletal muscle membrane adenylate cyclase from muscles of control and chronically uremic rats. Skeletal muscle membrane fractions from epitrochlearis preparations of control (○) and chronically uremic (●) rats were prepared by differential centrifugation of muscle homogenates. Aliquots of these membrane preparations were assayed for adenylate cyclase activity. Values shown are the means (\pm SEM) or at least six experiments.

the observed increments in cAMP levels and the resulting inhibition of alanine and glutamine release may reflect intracellular compartmentation of cAMP within the muscle cell. Only a small fraction of the measured increments in cAMP may be metabolically active with respect to inhibition of alanine and glutamine release. Adenylate cyclase regulation in uremic muscle may also be abnormal, but these abnormalities may not be evident under the conditions of the *in vitro* enzyme assay technique used in this study.

Although the results of this study do not delineate the basis for the reduced epinephrine-stimulated adenylate cyclase activity in uremic muscle, several possibilities can be excluded. Adenylate cyclase activities in the basal state and in the presence of fluoride were equal in uremic and control muscle, suggesting that the amount of adenylate cyclase enzymatic activity *per se* in uremic muscle was not uniformly reduced. These data also appear to exclude the likelihood of an acquired inhibitor of adenylate cyclase activity in uremia. Because a decrease in only epinephrine- and serotonin-stimulated adenylate cyclase activities was found, this may indicate a primary abnormality of adrenergic and serotonergic receptors, or a defect in their linkage to adenylate cyclase or abnormal nucleotide regulation of adenylate cyclase in uremia. Similar curves of epinephrine stimulation of membrane adenylate cyclase activities in preparations of control and chronically uremic rats were found (Fig. 5). With membrane preparations, one-half maximal stimulation was found at $0.2 \mu\text{M}$ epinephrine in control and uremic preparations. These data may be interpreted to suggest a state of adrenergic desensitization in uremia (31, 32). Exposure to high levels of adrenergic effectors have been shown to result in a diminished number of available adrenergic receptors, which in turn causes a decreased activity of the agonist-stimulated adenylate cyclase (33). However, subtle alterations of catecholamine receptors or of muscle membrane structure cannot be excluded by these data.

The results of this study also demonstrate that abnormalities of factors other than cyclic nucleotide metabolism must also participate in the mechanism of the increased alanine and glutamine formation and release observed in muscle from chronically uremic rats. This conclusion is based on the finding that, in the absence of added epinephrine, increased rates of alanine and glutamine release from uremic muscle were observed despite the presence of nearly identical cAMP levels in control and uremic muscle. Furthermore, $10 \mu\text{M}$ epinephrine increased cyclic AMP levels in uremic muscle to the same level as that produced by $0.1 \mu\text{M}$ epinephrine with control preparations, but alanine and glutamine release from uremic preparations with $10 \mu\text{M}$ epinephrine remained ele-

vated when compared with rates of release from control preparations in the presence of 0.1 μ M epinephrine. These data indicate that a strict correspondence cannot be observed between control and uremic muscles with respect to cAMP levels in muscle and absolute rates of alanine and glutamine formation and release. The nature of those other factors accounting for the increased amino acid release from uremic muscles are not evident from the results of this or the accompanying study.

The observations of insensitivity to catecholamine suppression of alanine and glutamine formation and release from skeletal muscle in chronic uremia have important physiologic implications. In normal and uremic man, circulating levels of epinephrine range between 1.0 and 10 nM (34, 35). In uremic man, rates of alanine production as determined by isotope dilution techniques are increased 190% compared with normal volunteers (35). The data of the present study demonstrate that physiologic levels of epinephrine in vivo could potentially influence alanine and glutamine release only in muscle of normal man. Based on the experimentally uremic rat model, these same in vivo concentrations of epinephrine (1–10 nM) would be expected to have no effect on alanine and glutamine release from skeletal muscle in uremic man. In order for epinephrine to influence alanine and glutamine release in uremia, levels which are 100-fold greater than that observed in vivo would be required. It therefore seems likely that the regulation of amino acid release from skeletal muscle by at least those mechanisms associated with adrenergic and serotonergic agonists may well be impaired in patients with chronic uremia. It is also probable that abnormalities of other regulatory mechanisms must also be present to account fully for the increased skeletal muscle amino acid release observed in chronic uremia. Nevertheless, derangements of the adenylate cyclase–cAMP system in muscle appear to play a significant role in the abnormal muscle amino acid metabolism in chronic uremia.

ACKNOWLEDGMENTS

We are indebted to Jannie Corbin, Yael Harari, Carol Maillet, and Nurit Shimoni for their excellent technical assistance. The advice and commentary of Dr. Wadi Suki and Dr. Lutz Birnbaumer are gratefully acknowledged.

These studies were supported, in part, by contract NO1-AM-4-2215 from the Artificial Kidney–Chronic Uremia Program of the National Institute of Arthritis, Metabolism, and Digestive Diseases and by a grant from the Muscular Dystrophy Association of America.

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