Effects of acute epinephrine treatment on skeletal muscle Sarcoplasmic Reticulum Ca^{2+} ATPase

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Abstract

This investigation examined the effects of metabolic stress, induced by acute epinephrine treatment on skeletal muscle sarcoplasmic reticulum (SR) Ca^{2+} ATPase function. In the first study, Sprague-Dawley rats were given two injections of epinephrine (1.0 and 0.5 $\mu g \cdot g^{-1}$, ip) at 30 min intervals. Sixty min after the initial injection, animals were sacrificed and the gastrocnemius muscles were removed. In the second study, rat plantaris muscles were incubated in epinephrine (1μM and 1mM) for 60 min. In the intact animals, epinephrine reduced whole muscle glycogen by 24% and increased glucose and glucose-6-phosphate content. The decline in muscle glycogen was associated with a 28% reduction in SR glycogen content. Ca^{2+} ATPase activity and peak rate of SR Ca^{2+} uptake by isolated SR vesicles was reduced by 18-20%. Following incubation, *in vitro*, muscle glycogen content was reduced by 30-48%. In addition, Ca^{2+} uptake and ATPase activities were depressed by 25-38% at both epinephrine concentrations. These results show that metabolic stress induced by acute epinephrine treatment depresses SR Ca^{2+} uptake and ATPase activity. These findings are also consistent with the idea that metabolic stress encountered during prolonged exercise alters SR function.

Keywords: Ca^{2+} uptake, exercise, fatigue, glycogen, metabolic stress

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Prolonged exercise places a severe metabolic stress on the active musculature. Energy substrates are rapidly degraded to produce the ATP necessary to meet the increased energy demands. In addition, metabolic byproducts accumulate within the cell and are increased in the plasma. This increased energy demand also elicits several physiological changes such as increased muscle blood flow and hormonal changes designed to enhance the delivery of energy substrates. Because of the dramatic metabolic changes that occur in the active muscles, some have postulated that the development of fatigue is the result of substrate depletion and/or metabolite accumulation (for review see [6, 26]).

A number of groups have shown that fatiguing muscular activity is associated with alterations in intracellular Ca^{2+} handling. Allen, Westerblad and colleagues used intact fibers to show that peak intracellular [Ca^{2+}] during contraction is reduced during fatigue, a change resulting from diminished SR Ca^{2+} release [1, 32]. In addition, changes in the rates of sarcoplasmic reticulum Ca^{2+} uptake and release by isolated SR have been shown to occur following prolonged exercise and electrical stimulation [4, 19, 31, 36]. Previously, our group and others suggested that there is a link between muscle metabolism and the changes in SR Ca^{2+} handling [33, 35]. In fact, we proposed that the reductions in SR function may actually protect the muscle from energy depletion by reducing contractile apparatus activation and lowering the energy demand placed on the muscle fiber [35]. Some suggest that the reductions in SR Ca^{2+} uptake and release result from the direct actions of metabolite accumulation [33]. However, it is unlikely that the changes in SR function associated with fatigue are entirely due to the direct effects of metabolite accumulation. Changes in SR function persist when the SR is removed from its fatigued environment and placed in one that more closely mimics that of a rested muscle [4, 19, 31, 36]. Thus, it is unclear if increased metabolism directly affects SR Ca^{2+} handling and accounts for the "dysfunction" that accompanies fatiguing activity.

In this investigation, we attempted to impose a metabolic stress on skeletal muscle in the absence of contractile activity. We utilized acute administration of epinephrine, applied both *in vivo* and *in vitro*. Epinephrine plays an important role in increasing

muscle metabolism during exercise, as it is known to increase as a function of both the duration of exercise and the intensity [10]. The primary role for epinephrine is to stimulate glucose release from the liver and to promote glycogen breakdown within the muscle. In addition, epinephrine acts with other signals to increase respiration and heart rate during exercise (for reviews see [16, 21]). Previous studies that have utilized acute epinephrine administration have shown that it mimics many of the metabolic and hormonal changes that occur during exercise. Epinephrine decreases muscle glycogen content, elevates plasma glucose and lactate, decreases plasma insulin and stimulates both glucose uptake and oxygen uptake [2, 9, 13, 22, 25]. Thus, from a qualitative standpoint, epinephrine provides a metabolic stress that mimics many of the characteristics of prolonged exercise.

If there is a link between increased metabolism during exercise and reductions in SR function, then application of a metabolic stress in the absence of contractile activity should affect SR Ca^{2+} handling. Thus, the purpose of investigation was to examine the effects of acute epinephrine administration on the SR Ca^{2+} pump.

Materials and Methods

All procedures used in this study were approved by the Virginia Tech Animal Use and Care Committee. For all experiments, male Sprage-Dawley rats (200-300g) were used. They were housed two per cage and allowed free access to standard rodent chow and water.

Study 1: In Vivo Epinephrine Treatment

On the morning of each experiment, rats were given an initial injection of epinephrine ($1 \mu g \cdot g^{-1}$, *ip*) while control animals were injected with an equal volume of sterile saline. Thirty minutes later, a supplemental injection of epinephrine ($0.5 \mu g \cdot g^{-1}$, *ip*) or saline was given. Thirty minutes after the second injection, animals were anesthetized with sodium pentobarbital ($60 mg \cdot kg^{-1}$, *ip*) and both gastrocnemius muscles were removed.

One muscle was blotted dry, weighed, placed in 5 volumes of perchloric acid and homogenized. These samples were then used for measurements of muscle glycogen, glucose and glucose-6-phosphate (G-6-P). The contralateral muscle was used for SR vesicle preparation [17]. For the SR vesicle preparation, muscles were homogenized in ice-cold homogenizing buffer which contained 20mM HEPES, 0.2% sodium azide, 0.2mM PMSF, and 1mM EDTA (VirtiShear, 3 x 15s). Homogenates were centrifuged at 8,000x*g* for 15 min. at 4°C. The supernatant was filtered through four layers of gauze, 600mM KCl was added and centrifuged at 12,000xg for 45 min. The supernatant was centrifuged again at 49,000xg for 90 minutes. The resulting pellet was re-suspended in storage buffer (homogenization buffer containing 300mM sucrose and 150mM KCl) and stored at -80°C for later analysis of SR Ca^{2+} uptake and ATPase activity. In a separate group of animals, SR vesicles were stored in storage buffer containing no sucrose. These samples were used to determine the amount of glycogen associated with the SR.

Study 2: In Vitro Epinephrine Treatment

To verify that the effects of *in* vivo administration of epinephrine were due to direct actions on the muscle, plantaris muscles were incubated in epinephrine and SR function of a homogenate fraction were analyzed [31]. The homogenate approach was used given the small amount of tissue available. For these experiments, two concentrations of epinephrine were used; 10μM, a physiological concentration and 1mM, a concentration known to evoke twitch potentiation in isolated muscle [34]. Each plantaris muscle was surgically removed and tied, at resting length, to glass capillary tubes. They were then incubated, for 60min in physiological saline solution which contained 120.5mM NaCl, 4.8mM KCl, 1.2mM $MgSO_4$, 20.4 mM $NaHCO_3$, 1.6mM $CaCl_2$, 1.2mM NaH_2PO_4, 1.0mM pyruvate and 5.0mM dextrose (95% O_2 / 5% CO_2, 37°C). One muscle of each pair was incubated in the above solution containing epinephrine (10μM or 1mM). After the incubation period, muscles were removed, placed in ice-cold homogenizing buffer and homogenized as described above. The homogenate was then centrifuged at 1600xg for 15 min. The supernatant was then stored at -80°C for later analysis of SR Ca^{2+} uptake and ATPase activity. In a separate group of animals, muscles were incubated then homogenized in perchloric acid and used for measurements of muscle glycogen, glucose and G-6-P.

Biochemical Analyses

SR Ca^{2+} handling was determined by measuring the rates of Ca^{2+} uptake and Ca^{2+} ATPase activity [31, 36]. For the uptake experiments, SR vesicles (25μg) or homogenate fraction (250μg) were placed in a buffer containing 100mM KCl, 20mM HEPES, 5mM $MgCl_2$, 5mM KH_2PO_4, 2mM ATP and 2μM fura-2 (pH. 7.0, 37°C). Uptake was initiated by adding 1.2 $\mu mol \cdot mg^{-1}$ $CaCl_2$ and was allowed to continue until free [Ca^{2+}] in the cuvette declined to a plateau. A Jasco CAF-110 fluorometer was used with excitation wavelengths of 340 and 380 and an emission wavelength of 500. Fura-2 signals were converted into free [Ca^{2+}] as described by Grynkiewicz et al. [11]. The rates of uptake were also confirmed in some samples using an Aligent diode array spectrophotometer and antiparylazo III as the Ca^{2+} indicator.

Ca^{2+} ATPase activity was determined using the enzyme-linked assay described by Luckin et al. [19]. Briefly, basal or Mg^{2+}-stimulated activity was recorded for 3 min. Total activity was then recoded after increasing free Ca^{2+} to 500nM (3 min) and 2 μM, to elicit peak activity (3 min) by adding $CaCl_2$ from stock. Ca^{2+} stimulated activity was determined by subtracting basal from total activities.

For SDS-PAGE, SR vesicle samples (10 μg total protein) were loaded onto a large 4% stacking, 7.5%

separating SDS-polyacrylamide gel (PROTEAN II Slab Cell, BioRad). They were run at 13mA until the tracking dye reached the separating gel, then the current was increased to 18mA. Gels were stained with Coomassie Brilliant Blue to identify protein bands.

Muscle glycogen, glucose and G-6-P levels were determined by the glucoamylase method described by Keppler and Decker [15]. SR glycogen was determined using an approach modified from the total glycogen measurement methods as described by Lees et al. [17]. In both cases, glycogen was hydrolyzed by glucoamylase (EC 3.2.1.3). Glucose was then measured fluorometrically using the production of NADPH.

Statistics

The treatment effects on each dependent variable was determined by Student's t-tests. The effects of epinephrine incubation on each dependent variable was determined using analyses of variance, adjusted for repeated measures made on contralateral muscles. In addition, Pearson's correlations were used to determine associations between variables. The level of significance was established at $p<.05$.

Results

After the epinephrine and sham injections, all animals remained quiet and inactive. However, rats treated with epinephrine showed hyperventilation and a modest increase in body temperature. Although body temperature was not measured in this study, it was apparent that the epinephrine-treated rats were somewhat warmer to the touch than were the control animals.

Wet masses of the gastrocnemius muscles did not differ between control and epinephrine injected groups (1.98 ± 0.13 and 1.91 ± 0.11g, respectively, $p>.05$). Likewise dry masses were not different between the two groups (0.53 ± 0.03 and 0.55 ± 0.04g, $p>.05$).

Table 1. *The effects of* in vivo *epinephrine treatment on gastrocnemius muscle glycogen, glucose, glucose-6-phosphate (G-6-P) and SR glycogen contents.*

Parameter	Control	Epinephrine
Glycogen (µmol·g^{-1} wet mass)	**32.90 ± 3.20**	**25.08 ± 2.38***
Glucose (µmol·g^{-1} wet mass)	**2.94 ± 0.49**	**5.97 ± 0.64***
G-6-P (µmol·g^{-1} wet mass)	**1.00 ± 0.50**	**4.02 ± 0.46***
SR Glycogen (µg·mg^{-1} protein)	**688.05 ± 33.88**	**492.44 ± 43.16***

Values are expressed as mean ± SEM. *$p<.05$ between conditions, n=10 for each group.

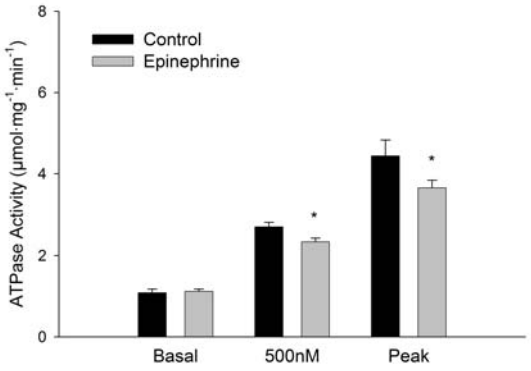

Figure 1. *ATPase activities of SR vesicles obtained from control and epinephrine injected animals. Values presented for 500nM free [Ca^{2+}] and peak are Ca^{2+}-stimulated values. *p<.05 versus control, n=10 for each group.*

As expected, *in vivo* epinephrine treatment affected carbohydrate status of the gastrocnemius muscle. Following treatment, whole muscle glycogen was reduced by 24% (Table 1). In addition, there was a 2-fold increase in muscle glucose and a 4-fold increase in glucose-6-phosphate (G-6-P). Glycogen associated with the SR was reduced to approximately the same extent as whole muscle glycogen (28%).

Epinephrine injection also affected Ca^{2+} pump function in isolated SR vesicles. Ca2+-stimulated ATPase activity was decreased by 18% in the treated animals (Figure 1). Basal ATPase activity (Mg^{2+}-stimulated), however, was not affected. Peak rate of Ca^{2+} uptake and the rate determined at 500nM free [Ca^{2+}] were both reduced by approximately 20% (Figure 2). Whole muscle glycogen content of the contralateral muscle was significantly correlated with both SR Ca^{2+} uptake and ATPase rates (r = 0.682 and 0.601, respectively, $p<.05$).

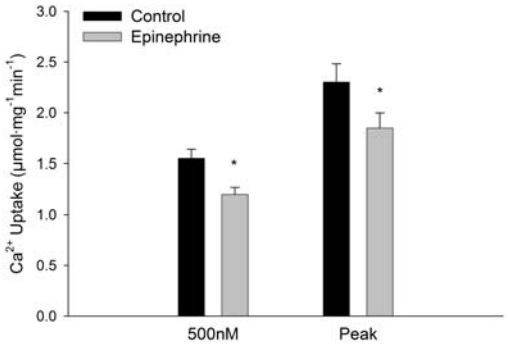

Figure 2. *Rates of SR Ca^{2+} uptake in vesicles obtained from control and epinephrine injected animals. Peak rates (left) and rates determined at 500nM free [Ca^{2+}] (right) are shown. *p<.05 versus control, n=10 for each group.*

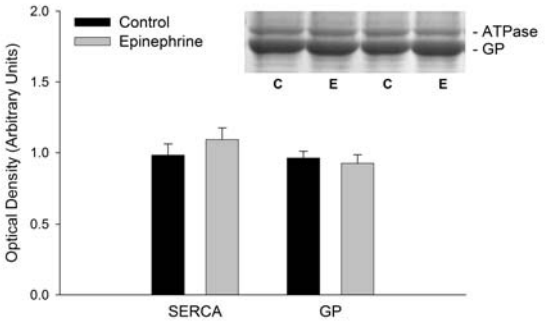

Figure 3. *Mean optical densities of the bands associated with SERCA and GP. Values are expressed in arbitrary units.* Inset: *Commassie-stained SDS-polyacrylamide gel showing the bands associated with these proteins. C, control; E, epinephrine treated gastrocnemius samples.*

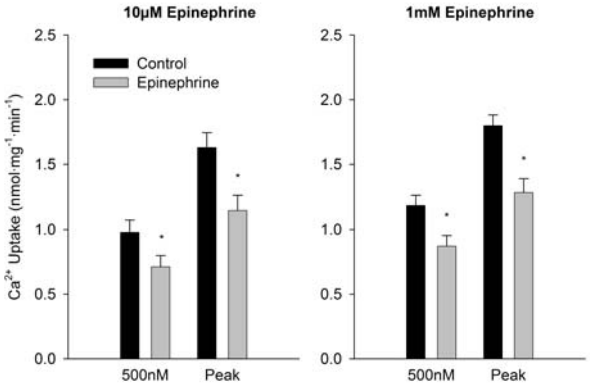

Figure 5. *Peak rates of Ca^{2+} uptake from muscles in 10μM and 1mM epinephrine.* *p<.05 *versus control, n=8 for each group.*

In vivo epinephrine treatment did not significantly alter the protein composition of the SR vesicle preparations. SR vesicle total protein yields for control and treated animals were not significantly different (0.827 ± 0.075 and 0.894 ± 0.073 mg/g, respectively, $p>.05$). Nor did the treatment appear to significantly alter the SERCA or glycogen phosphorylase (GP) content of our samples. SDS-PAGE of the samples showed no changes in the optical densities of the bands associated with these proteins (Figure 3).

In vitro epinephrine treatment also reduced plantaris muscle glycogen. At 10μM and 1mM, glycogen was by 30 and 48%, respectively, compared to the control muscles.

Table 2. *The effects of* in vitro *epinephrine treatment on plantaris muscle glycogen, glucose and glucose-6-phosphate (G-6-P) contents.*

	10 μM Epinephrine		1 mM Epinephrine	
Parameter	Control	Epinephrine	Control	Epinephrine
Glycogen (μmol·g^{-1} wet mass)	**36.18 ± 3.45**	**25.46 ± 2.89***	**32.13 ± 3.06**	**16.85 ± 3.87***
Glucose (μmol·g^{-1} wet mass)	**3.11 ± 0.87**	**6.53 ± 0.72***	**3.32 ± 0.61**	**6.30 ± 0.58***
G-6-P (μmol·g^{-1} wet mass)	**0.82 ± 0.21**	**3.44 ± 0.50***	**0.78± 0.32**	**5.51 ± 0.52***

Values are expressed as mean ± SEM. *$p<.05$ between conditions, n=6 for each epinephrine concentration.

In addition, epinephrine incubation increased glucose by 2-3 fold and elevated G-6-P content by 4-7 fold (Table 2). Incubation of plantaris muscle in epinephrine had qualitatively similar effects on SR function, as did the *in vivo* treatment. Ca^{2+}-stimulated ATPase activity was reduced 25 and 38% by 10μM and 1mM, respectivelywhereas basal activities were not altered (Figure 4). Peak Ca^{2+} uptake rates were reduced 29% by both epinephrine concentrations (Figure 5). Rates recorded at 500nM free [Ca^{2+}] were reduced to a similar extent.

Discussion

The overall goal of this investigation was to examine the effects of metabolic stress on SR function. Our results show that acute epinephrine treatment significantly reduced muscle glycogen and the amount of glycogen associated with the SR as well as increased intracellular glucose and G-6-P contents. In addition,

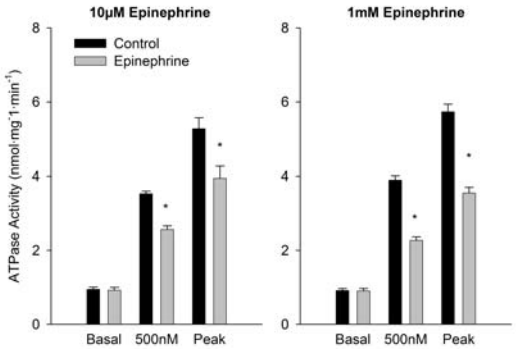

Figure 4. *ATPase activities obtained from muscles incubated in 10μM and 1mM epinephrine. Values presented for 500nM free [Ca^{2+}] and peak are Ca^{2+}-stimulated values.* *p<.05 *versus control, n = 8 for each group.*

epinephrine affected SR function as both Ca^{2+} transport and Ca^{2+}-stimulated ATPase activity was depressed. The changes in muscle glycogen and SR Ca^{2+} pump function occurred following both *in vivo* and *in vitro* treatment. This finding suggests a direct action of epinephrine on the skeletal muscle rather than an effect secondary to whole-body changes such as altered blood flow or elevated body temperature.

The reduction in muscle glycogen was similar to that found by others using epinephrine injections to impose metabolic stress [2, 9, 22] and with incubation of rat fast muscle in epinephrine [13]. Compared to prolonged muscular activity, however, the reductions in both muscle and SR glycogen caused by the epinephrine treatments were substantially less and were not accompanied by significant changes in amount of GP associated with the SR. Lees et al. [17] found that 30min of contractile activity lead to 77 and 95% reductions in muscle and SR glycogen, respectively. Here, the reductions were more modest, 25-40%. Nevertheless, the loss of glycogen and the increase in G-6-P content suggests that epinephrine did indeed induce metabolic stress in the muscles studied, albeit less than muscular activity.

Acute epinephrine treatment was associated with reduced rates of SR Ca^{2+} uptake as well as Ca^{2+}-stimulated ATPase activity. This occurred with both *in vivo* and *in vitro* treatments. Earlier studies examining the direct effects of epinephrine on contractile function show that epinephrine increases twitch force and slows relaxation of fast mammalian muscle but depresses twitch force and increases relaxation rate in slow muscle [3, 30, 34]. The rat gastrocnemius is generally considered a "mixed" muscle containing both fast (type II) and slow (type I) fibers. However, this muscle contains predominantly the fast isoform of the Ca^{2+} ATPase (SERCA1) [14, 23] (J.H. Williams & S.J. Lees, unpublished observations). Given this, the reduced Ca^{2+} uptake rates reported here are consistent with the slowing effects of epinephrine on relaxation of fast muscle contractile function. While relaxation is complex involving both Ca^{2+} sequestration and contractile protein interactions, our data suggest that the slowed relaxation observed in the earlier studies is due, in part, to reduction in the activity of the SR Ca^{2+} ATPase.

The depression in SR function associated with acute epinephrine treatment is qualitatively similar to that which occurs in peripheral muscle following prolonged exercise. A number of studies show that strenuous exercise and electrical stimulation results in depressions of SR vesicle Ca^{2+} uptake by 20-50% (for review see [35]). Our results are consistent with the idea that there is some link between increased metabolism and changes in SR function that occur during prolonged exercise. During maximal contractile activity, the rate of ATP hydrolysis clearly exceeds the rate of ATP re-synthesis. If left unchecked, this would lead to a depletion of ATP and irreversible cell injury. SR Ca^{2+} ATPase activity accounts for 30-40% of total energy consumption during contraction [24]. From a teleological standpoint, it is reasonable to suggest that metabolic stress would initiate a reduction in the rate of SR Ca^{2+} transport and, consequently, a reduction in ATP hydrolysis rate. Such a mechanism would better match ATP utilization and production. Our data are consistent with the idea that metabolic stress, in the absence of contractile activity can evoke changes in SR Ca^{2+} pump function similar to those caused by exercise.

Unfortunately, the nature of a connection between increased metabolism and SR function during exercise is unclear. While our results do not rule out the possibility that metabolite accumulation is responsible for a portion of the decline in SR function during either exercise or epinephrine treatment, we feel that it is unlikely that they are the sole factor responsible. The changes in Ca^{2+} pump function following activity and epinephrine exposure remain when the SR is removed from the muscle and examined under conditions that mimic a rested cell. It is possible that changes in the structural or functional aspects of the SR-glycogenolytic complex [7] during metabolic stress affect SR Ca^{2+} transport. The loss of glycogen and glycolytic enzymes associated with the SR could impair Ca^{2+} uptake directly or secondary to altered compartmentation of ATP production. For example, Cuenda et al. [5] show that glycogen phosphorylase and the Ca^{2+} ATPase directly interact and that glycogen associated with the SR can be used to support Ca^{2+} transport. However, Lees & Williams. [18] recently demonstrated that extraction of SR glycogen appears to increase Ca^{2+} uptake and ATPase activity when measured under conditions similar to those used in this study (i.e. exogenously added ATP). Thus, it is unlikely that the reduction in SR glycogen found here directly affected SR Ca^{2+} pump function. Schertzer et al. [28] reported that thermal stress, similar to that encountered during exercise, results in depressed SR Ca^{2+} uptake. It is possible that the likely increase in muscle temperature following *in vivo* epinephrine treatment could have affected the Ca^{2+} pump. However, the changes in function were similar to those that occurred following *in vitro* treatment where muscle temperature was more closely controlled. Oxidative stress and reactive oxygen species can directly inhibit the SR Ca^{2+} ATPase or alter function secondary to oxidation of protein that influence Ca^{2+} uptake [8, 12, 27, 29]. It is possible that increased metabolism resulting from epinephrine lead to the production of compounds that caused changes in the Ca^{2+} ATPase that persisted after the structure was removed from the intact cell. Interestingly, Luckin et al. [19] showed that prolonged exercise elicits structural modifications in the Ca^{2+} ATPase that contribute to depressed enzyme activity. Also, Matsunaga et al. [20]

report that exhaustive exercise leads to oxidation of the Ca^{2+} pump and depressed Ca^{2+} uptake rate. While the present data suggest the metabolism and SR function during exercise are coupled, it is clear that more work is needed to understand how these two processes interact.

In summary, our results show that metabolic stress induced by acute epinephrine administration alters skeletal muscle SR Ca^{2+} transport. The reductions in SR Ca^{2+} uptake and ATPase activity are consistent with the changes in SR Ca^{2+} pump function observed following contractile activity. Thus, the results support the idea of a functional link between increased metabolism and altered SR function during muscular activity.

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