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# Captopril does not affect reflex increases in adrenal or lumbar sympathetic nerve activity to hypoglycemia in rats

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**Abstract**

Blockade of angiotensin II (ANGII) receptors or converting enzyme inhibition attenuates reflex increases in epinephrine during insulin-induced hypoglycemia. Because ANGII receptors are found in several sites within the central nervous system, the aim of this study was to examine whether acute captopril attenuates the reflex increase in adrenal preganglionic sympathetic nerve activity (SNA) induced by hypoglycemia. We infused vehicle (control) or insulin (30 U/kg IV) in anesthetized rats or in rats pretreated with captopril (Cap-insulin; 2.5 mg/kg, then 1 mg/kg per hour IV) while measuring hemodynamics and SNA from adrenal preganglionic, adrenal postganglionic, and lumbar sympathetic nerves. Hypoglycemia elicited similar adrenal preganglionic SNA increases in insulin-treated (260% ± 31% from 100% baseline) and Cap-insulin–treated (255% ± 34%) rats. Likewise, increases in adrenal postganglionic SNA and lumbar SNA were equivalent in the insulin and Cap-insulin groups. Hypoglycemia also elicited a tachycardia in insulin-treated rats that was attenuated in Cap-insulin–treated rats, and corresponding blood pressure decreases in insulin rats were enhanced in Cap-insulin–treated rats. Thus, blockade of ANGII formation by captopril did not affect hypoglycemia-induced activation of adrenal preganglionic SNA, indicating that the renin-angiotensin systems in the brain and spinal cord do not modulate increases in adrenal SNA during hypoglycemia.


## 1. Introduction

The ability to initiate appropriate counterregulation against hypoglycemia is impaired in many individuals with type 1 diabetes [1]. These patients are predisposed to severe episodes of hypoglycemia, and this problem has been exacerbated by the recent practice of more aggressive therapies [2]. In this setting, hypoglycemia is now recognized as a primary limitation in the effective treatment of type 1 diabetic patients [3].

Angiotensin-converting enzyme (ACE) inhibitors are frequently given to diabetic patients for the treatment of cardiovascular side effects that commonly affect these individuals. However, the use of these drugs has been associated with increased risk of hypoglycemic episodes during treatment with insulin or oral antidiabetic drugs [4,5]. As a possible explanation of hypoglycemia during ACE inhibition, previous studies found that these agents caused enhanced glucose disposal from muscle tissues to result in increased insulin sensitivity [6,7]. As an additional mechanism, ACE inhibitors may impair the release of epinephrine during hypoglycemia, which is considered one of the most important counterregulatory hormones during periods of severe depressions in blood glucose. In favor of this, hypoglycemia-induced elevations in epinephrine were inhibited by anti–angiotensin I (ANGI) antibodies [8], angiotensin II (ANGII) antagonism with saralasin [8,9], inhibition of ACE [8-11], and inhibition of $AT_1$ and $AT_2$ receptors [11,12] in most but not all [13] studies.

Despite the clinical importance of hypoglycemic episodes associated with ACE inhibition, it is unclear how and at what level renin-angiotensin blockade acts to attenuate elevations in epinephrine during hypoglycemia. Insulin-induced decreases in blood glucose activate glucose-sensitive neurons in the central nervous system (CNS), which generate increases in adrenal sympathetic nerve activity (SNA) to result in 50-fold elevations in plasma epinephrine levels [14]. Importantly, the renin-angiotensin system is represented at all levels of this pathway, including brain, spinal cord, sympathetic ganglia, and adrenal chromaffin cells [15-17]. Given this background, it is

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possible that ANGII will potentiate hypoglycemia-induced increases in adrenal SNA through an action of ANGII receptors acting at several sites within the CNS.

The primary aim of this study was to examine whether acute captopril, which has been shown to inhibit ACE activity behind the blood-brain barrier [18-21], attenuates reflex increases in adrenal preganglionic SNA induced by hypoglycemia. To determine whether ACE inhibition has generalized attenuating effects on other sympathetic nerves, we set a secondary goal, which was to test whether captopril attenuates hypoglycemia-induced increases in adrenal postganglionic SNA, which may be involved in steroid production or regulation of blood flow in the adrenal cortex [22], and in lumbar SNA, which, at the L3 through L5 level, is almost entirely postganglionic in composition [23].

## 2. Methods

### 2.1. Animals

Male Sprague-Dawley rats, weighing 200 to 225 g, were purchased from Harlan (Indianapolis, IN). The rats were housed in a temperature-controlled colony room illuminated on a 12:12 light-dark cycle. All procedures were performed in accordance with the Lehman College and National Institutes of Health guidelines for the care and use of experimental animals.

### 2.2. Surgical procedure

One week after arrival, overnight-fasted rats were anesthetized and prepared for cardiovascular monitoring during captopril and insulin infusion. Anesthesia was induced with thiopental (40 mg/kg IP, Henry Schein, Mellville, NY) and was maintained with urethane (0.6 g/kg IV, followed by supplemental doses of 0.1-0.2 g/kg as needed for a final dose of 0.6 to 0.8 g/kg, Sigma, St. Louis, MO), and body temperature was kept near 37.5°C using a temperature-controlled surgical table and heating lamp. The trachea was cannulated for spontaneous respiration of room air to prevent upper respiratory tract obstruction and hypoxia. Blood pressure was measured from a catheter in the left femoral artery using a pressure transducer (Statham P23XL, Astro-Med, West Warick, RI) connected to a PowerLab data acquisition system and a Macintosh computer. Heart rate (HR) was calculated from the blood pressure pulse using the PowerLab system. Two catheters were inserted into the left femoral vein for infusion of insulin and captopril, respectively. A final catheter was inserted into the tail artery to obtain samples for blood glucose.

Multifiber recordings of lumbar SNA were obtained as previously described [24]. Briefly, a midline abdominal incision was made and a lumbar sympathetic nerve was isolated, and its cut central end was placed on a bipolar platinum-iridium electrode (Cooner Wire, Chatsworth, CA) and covered with dental impression material (Bisico S4, Bielfeld, Germany). Nerve activity was led through a Grass model HIP511 high-impedance probe (Astro-Med Grass), amplified (×3000 to ×20 000), filtered (30-3000 Hz) with a Grass preamplifier (Model P511), and led to an oscilloscope (Model 54600A, Hewlett-Packard, Colorado Springs, CO), an audiomonitor (Grass model AM8), and an integrator (Grass model 7P3) for display on the PowerLab system. The time constant for the 7P3 was set at 0.2 second, and rectification was set so that both positive and negative signals were integrated. SNA was corrected for postmortem background activity to ensure that electrical noise was excluded in the assessment of sympathetic outflow. For multifiber recordings of adrenal SNA, a left adrenal nerve branch was exposed through a flank incision, a bipolar platinum-iridium electrode was attached, and SNA was recorded as described above. Adrenal nerves were determined to be primarily pre- or postganglionic by the method of Carlsson et al [22]. Briefly, the rats received an intravenous bolus injection of trimethaphan (10 mg/kg, Hoffmann-La Roche, Basle, Switzerland), a ganglionic blocker, and values were followed for 20 minutes until blood pressure and adrenal SNA returned to control levels. A decrease in adrenal SNA indicated that the nerve was primarily postganglionic, whereas an increase in SNA indicated that the nerve contained primarily preganglionic fibers. Similar injections given to lumbar SNA preparations confirmed that lumbar nerves at the L3 through L5 level were almost entirely postganglionic in composition.

### 2.3. Experimental procedure

The goal of the protocol was to determine the effects of insulin-induced hypoglycemia (insulin group) on lumbar SNA (n = 9), adrenal preganglionic SNA (n = 7), and adrenal postganglionic SNA (n = 5), as well as the effects of captopril followed by hypoglycemia (Cap-insulin group) on lumbar SNA (n = 8), adrenal preganglionic SNA (n = 7), and adrenal postganglionic SNA (n = 5). In control experiments (control group), we determined the effects of the vehicles for captopril and insulin on lumbar SNA (n = 10), adrenal preganglionic SNA (n = 2), and adrenal postganglionic SNA (n = 2).

During surgical preparation, all rats received saline through the venous catheter at a rate of 0.3 mL/h. After surgery completion, the rats were allowed to equilibrate for 45 minutes before the experimental protocol. Trimethaphan was then given, followed by a 20-minute stabilization period. After this, basal levels of mean arterial pressure (MAP), HR, lumbar or adrenal SNA, and blood glucose (Accu-Check Advantage portable glucometer, Boehinger Mannheim, Indianapolis, IN) were recorded during a 15-minute baseline period (Fig. 1, baseline period I). At the end of this period, Cap-insulin–treated rats received a bolus intravenous injection of captopril (2.5 mg/kg) followed by continuous captopril infusion (1 mg/kg per hour) using an infusion pump (model 11, Harvard Apparatus, Holliston, MA). This dose of intravenous captopril has been previously shown to abolish blood pressure increases to bolus injections of