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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

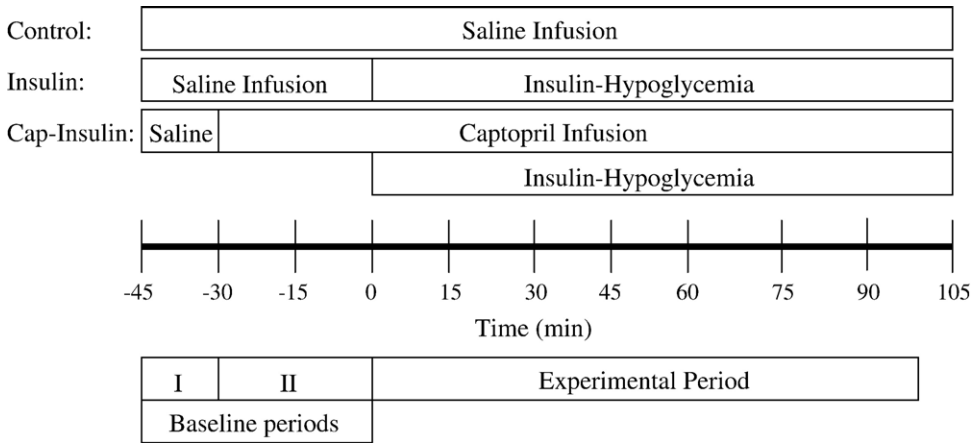

Fig. 1. The experimental protocol for the control, insulin, and Cap-insulin groups. MAP, HR, blood glucose, and SNA measurements were performed throughout the 2 baseline periods and the experimental period.

angiotensin I, indicating a complete blockade of converting enzyme activity [25,26]. Control and insulin-treated rats received an equal volume of the vehicle. All rats were then monitored during an additional 30-minute baseline period (Fig. 1, baseline period II). At the end of the 2 baseline periods, regular pork insulin (Iletin, Eli Lilly, Indianapolis, IN) in isotonic saline (20 U/mL) was administered through the femoral vein as a single bolus (30 U/kg) in the insulin and Cap-insulin groups, and all parameters were monitored during 105 minutes.

2.4. Statistical analysis

Basal MAP, HR, and SNA values were collected at 40 samples per second and then averaged over the final 10 minutes of baseline period I to obtain a single value for this period and over the final 10 minutes of baseline period II to obtain a single value for this period. Means of lumbar SNA and of adrenal SNA measured during baseline period II were not different among the 3 experimental groups (Table 1). Therefore, SNA from baseline period II was taken as 100%, and the following values were expressed as a percentage of this baseline level. During the 105-minute experimental period, averages of 5-minute samples of MAP, HR, and SNA were obtained beginning at 10, 25, 40, 55, 70, 85, and 100 minutes into infusion. Because control adrenal preganglionic and control adrenal postganglionic activities did not differ during the protocol, they were collapsed into a single adrenal control group (n = 4) that is presented in Figs. 5 and 6. For the analyses of MAP, HR, and blood glucose, values from the separate nerve groups (lumbar, adrenal preganglionic, and adrenal postganglionic) were collapsed into single insulin (n = 21), Cap-insulin (n = 20), and control (n = 14) groups. All data were analyzed using appropriate single measure or repeated measures analysis of variance (ANOVA) and presented as means ± SEM. Post hoc comparisons were made using Fisher least significant difference tests when the global F ratio was significant. Differences between groups were considered significant at the $P < .05$ level.

3. Results

3.1. Baseline period I: before captopril

Before captopril or insulin injection, basal MAP, HR, and lumbar SNA were equivalent in the control, insulin, and Cap-insulin groups (Table 1). Basal adrenal preganglionic SNA was significantly lower in the Cap-insulin group than in the insulin group, and basal adrenal postganglionic SNA was lower in the Cap-insulin group than in the control and insulin groups (Table 1). Initial blood glucose levels were not different among the control (143 ± 9 mg/dL), insulin (140 ± 10 mg/dL), and Cap-insulin (133 ± 8 mg/dL) groups.

Table 1
Baseline MAP, HR, and SNA in the 3 experimental groups

Measure	Control		Insulin		Cap-insulin	
	Baseline period I	Baseline period II	Baseline period I	Baseline period II	Baseline period I	Baseline period II
MAP (mm Hg)	105 ± 5	103 ± 4	103 ± 3	100 ± 3	100 ± 3	79 ± 3*
HR (bpm)	367 ± 10	367 ± 11	357 ± 7	356 ± 7	373 ± 6	401 ± 7*
Lumbar SNA (mV/s)	1.00 ± 0.08	1.17 ± 0.10	0.98 ± 0.11	1.03 ± 0.09	0.79 ± 0.15	1.27 ± 0.24
Pre-adrenal SNA (mV/s)	0.57 ± 0.34	0.61 ± 0.24	0.97 ± 0.10	1.01 ± 0.08	0.44 ± 0.14†	1.16 ± 0.24
Post-adrenal SNA (mV/s)	0.85 ± 0.03	0.66 ± 0.05	0.87 ± 0.13	0.91 ± 0.12	0.29 ± 0.05*	0.79 ± 0.23

All values are means ± SEM. Pre-adrenal indicates adrenal preganglionic; post-adrenal, adrenal postganglionic.

* $P < .05$, compared with control and insulin, same period.

† $P < .05$, compared with insulin, same period.

3.2. Baseline period II: during captopril

Administration of captopril in the Cap-insulin group caused a decrease in MAP and an elevation in HR during the second baseline period, which contrasted with no change in the other 2 groups (Table 1). Captopril also caused increases in lumbar SNA, adrenal preganglionic SNA, and adrenal postganglionic SNA (Table 1). Analyses of these data using 3 (groups) by 2 (baseline period I vs baseline period II) ANOVAs revealed significant interactions for lumbar SNA ($P < .01$), for adrenal preganglionic SNA ($P < .05$), and for adrenal postganglionic SNA ($P < .05$), indicating that SNA increased more in the Cap-insulin group than in the control and insulin groups during baseline period II. Despite these increases, post hoc analyses during baseline period II showed that means of lumbar SNA and adrenal preganglionic and postganglionic SNA were not different among the 3 experimental groups. Blood glucose values remained equivalent in the control, insulin, and Cap-insulin groups (Fig. 2).

3.3. Responses to insulin-induced hypoglycemia

During the 105-minute experimental period, bolus injection of insulin produced a rapid and sustained hypoglycemia that was similar in the insulin and Cap-insulin groups, but which contrasted with no change in the control group (Fig. 2). Analysis of variance of these data revealed a group by repeated measures interaction ($P < .0001$), and a separate analysis comparing the insulin to the Cap-insulin group showed no difference, indicating that the evolution of hypoglycemia over time was equivalent in these 2 groups. HR in control rats showed little change during the experimental period, contrasting with substantial HR increases in the 2 insulin-infused groups (Fig. 3). Intravenous administration of captopril attenuated these HR increases. The overall ANOVA of the data revealed a group by repeated measures interaction ($P < .001$), and a separate analysis comparing the insulin group to the Cap-insulin group showed a significant group by repeated measures interaction ($P < .001$), indicating that captopril significantly blunted the rise in HR caused by hypoglycemia. Recordings of MAP revealed that bolus injection of insulin caused an initial and transitory blood pressure decline (approximately 15 mm Hg) in the 2 insulin-infused groups (Fig. 4). Following this, MAP recovered to preinjection levels in the insulin group, but pressures remained lower than preinjection values in the Cap-insulin group (Fig. 4). The overall ANOVA again

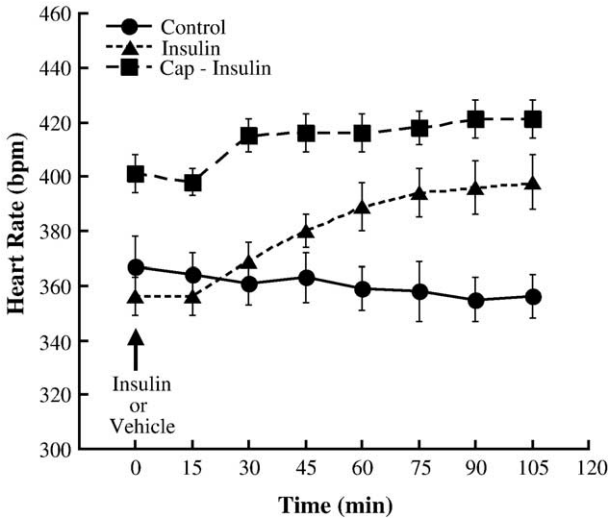

Fig. 3. HR changes to vehicle or insulin-hypoglycemia in the control (n = 14), insulin (n = 21), and Cap-insulin (n = 20) groups. The data point at time = 0 minute corresponds to the average of the final 10 minutes of baseline period II. Values are means ± SEM.

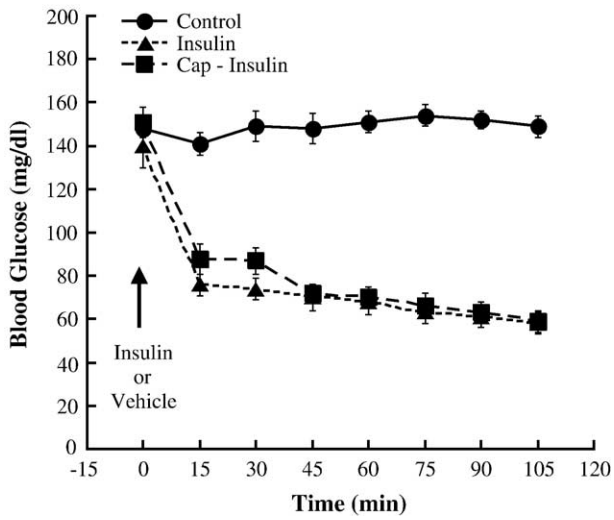

Fig. 2. Blood glucose changes to vehicle or insulin-hypoglycemia in the control (n = 14), insulin (n = 21), and Cap-insulin (n = 20) groups. The data point at time = 0 minute corresponds to the average of the final 10 minutes of baseline period II. Values are means ± SEM.

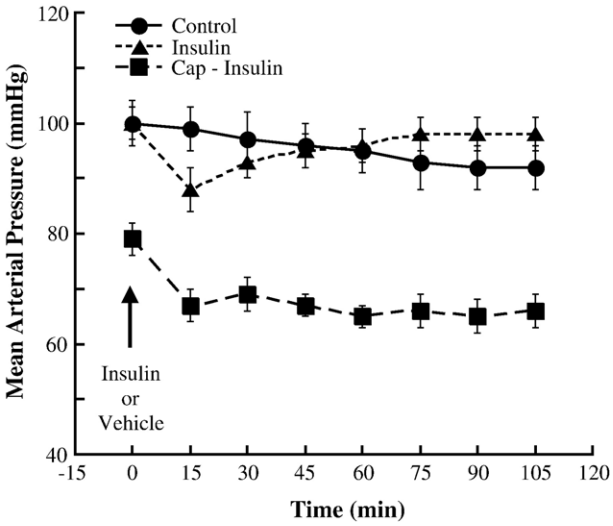

Fig. 4. Changes in MAP to vehicle or insulin-hypoglycemia in the control (n = 14), insulin (n = 21), and Cap-insulin (n = 20) groups. The data point at time = 0 minute corresponds to the average of the final 10 minutes of baseline period II. Values are means ± SEM.

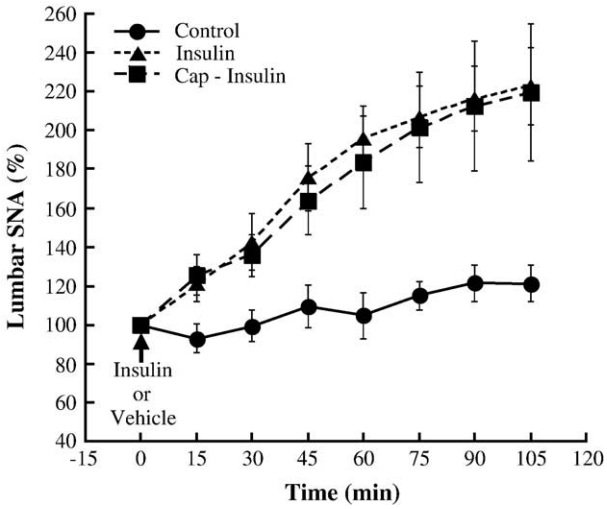

Fig. 5. Changes in lumbar SNA from baseline period II (time = 0) to vehicle or insulin-hypoglycemia in the control (n = 10), insulin (n = 9), and Cap-insulin (n = 8) groups. Basal values from baseline period II were taken as 100%, and lumbar SNA responses were expressed as a percentage of this baseline level. Values are means ± SEM.

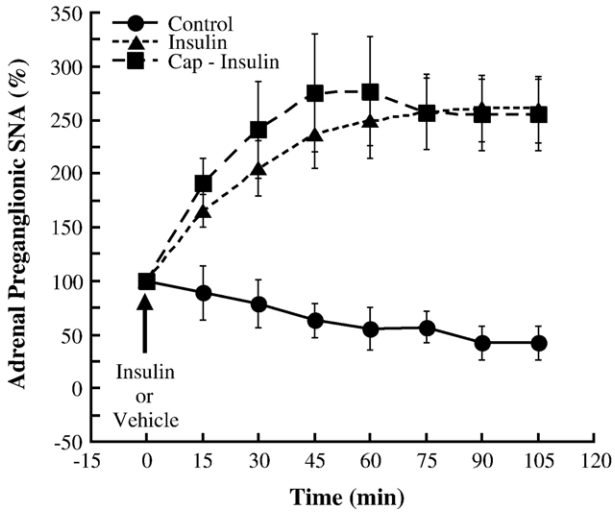

Fig. 7. Changes in adrenal preganglionic SNA from baseline period II to vehicle or insulin-hypoglycemia in the control (n = 4), insulin (n = 7), and Cap-insulin (n = 7) groups. Basal values from baseline period II were taken as 100%, and adrenal SNA responses were expressed as a percentage of this baseline level. Values are means ± SEM.

revealed a group by repeated measures interaction ($P < .001$), and a separate analysis comparing the insulin group to the Cap-insulin group demonstrated a significant group by repeated measures interaction ($P < .001$), indicating that MAPs remained lower in the Cap-insulin group during the latter portion of the experimental period.

During measurement of lumbar SNA, control rats displayed a mild increase in SNA, contrasting with rapid and large SNA increases in the 2 insulin-infused groups (Fig. 5). Administration of captopril had no effect on lumbar SNA increases to hypoglycemia. During the measurements of adrenal postganglionic SNA, insulin-induced hypoglycemia caused increases in sympathetic activity that were not different between the insulin and Cap-insulin groups (Fig. 6). Finally, and in agreement with previous studies [22], hypoglycemia produced even larger elevations in adrenal preganglionic SNA; however, these increases were again not different between the insulin and Cap-insulin groups (Fig. 7).

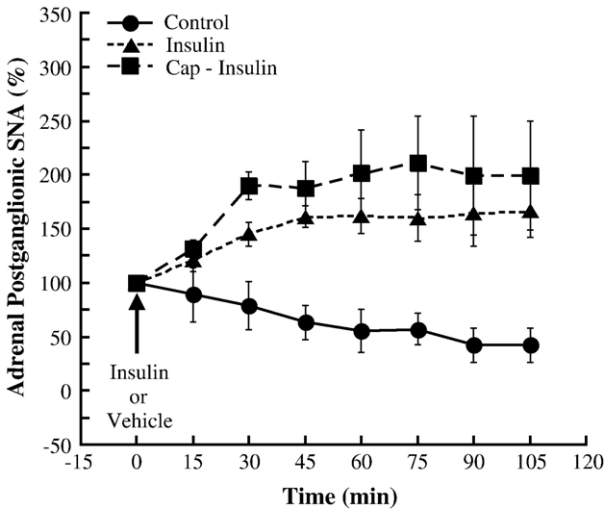

Fig. 6. Changes in adrenal postganglionic SNA from baseline period II to vehicle or insulin-hypoglycemia in the control (n = 4), insulin (n = 5), and Cap-insulin (n = 5) groups. Basal values from baseline period II were taken as 100%, and adrenal SNA responses were expressed as a percentage of this baseline level. Values are means ± SEM.

4. Discussion

The key finding of the present study is that intravenous captopril did not affect hypoglycemia-induced increases in adrenal preganglionic SNA. Because acute systemic administration of captopril in previous studies has been shown to block converting enzyme activity in brain areas behind the blood-brain barrier and in the cerebrospinal fluid [18-21], the present findings indicate that ANGII formation in the brain and spinal cord is not necessary for mediating elevations in adrenal SNA to hypoglycemia. They further suggest that ANGII facilitation of epinephrine release may not occur in the CNS or spinal cord, but rather at the adrenal preganglionic sympathetic terminal, on chromaffin cells, or on both sites. In agreement with our findings, Worck and colleagues [27] recently demonstrated that intracerebroventricular ANGII receptor antagonism did not affect hypoglycemia-induced elevations in plasma epinephrine, which further indicated that the CNS renin-angiotensin system is not essential for reflex regulation of epinephrine release during hypoglycemia. In favor of a more distal interaction, earlier work showed that electrical stimulation of nerves to the adrenal medulla elicited increases in epinephrine that were potentiated by ANGII and attenuated by captopril and saralasin [17,28]. As a potential explanation for these previous findings, it is

possible that ANGII potentiates acetylcholine secretion from adrenal preganglionic terminals because angiotensin receptors have been localized on nerve tracts innervating the bovine adrenal medulla [29]. As an alternate possibility, chromaffin cells may be directly activated because ANGII stimulates catecholamine release from cultured adrenal medullary cells [30], and both AT_1 and AT_2 receptor subtypes have been identified on adrenal chromaffin cells, with the AT_2 receptor comprising 70% of the binding sites [31].

As a second goal for the present study, we examined whether captopril affects increases in lumbar or adrenal postganglionic SNA produced by hypoglycemia. We found that sympathetic activation in these 2 nerves was not affected by captopril. Thus, our overall finding that neither pre- nor postganglionic SNA increases were altered by converting enzyme inhibition again suggests that the interaction between ANGII and sympathetic output may occur along more distal segments of the sympathetic nerves. In agreement with a distal interaction, electrical stimulation of sympathetic nerves in previous studies produced elevations in plasma norepinephrine that were attenuated by pretreatment with captopril [32,33]. In explanation to this, ANGII has been shown to act at postganglionic prejunctional sites to facilitate norepinephrine synthesis and release and to inhibit norepinephrine reuptake [17]. Finally, captopril has been shown to attenuate vascular reactivity to exogenous norepinephrine, suggesting a possible interaction between ANGII and adrenergic receptors [25,33].

Insulin-induced hypoglycemia typically produces increases in systolic blood pressure accompanied by decreases in diastolic pressure, which together result in either no change or a small decrease in MAP [13,34-36]. In the present study, we also found a transitory MAP decrease in insulin-treated rats followed by a recovery to levels that were not different from preinjection values. In contrast, captopril-treated rats showed no recovery in MAP after insulin, resulting in lower blood pressure values throughout the experimental period. In agreement with these findings, Worck et al [37] also found that hypoglycemia-induced decreases in MAP in humans were exacerbated by pretreatment with losartan. The enhanced blood pressure decreases in our captopril-treated rats may be partly explained by the blunted HR increases in this group (Fig. 2), which suggest that the drug may have attenuated hypoglycemia-induced increases in cardiac output. As an additional possibility, our captopril-treated rats may have experienced normal increases in SNA, but a reduced transduction of this activity into vasoconstriction to result in enhanced blood pressure decreases during hypoglycemia. The importance of sympathetic activation in maintaining blood pressure during hypoglycemia was highlighted by previous studies showing that hypoglycemia-induced decreases in diastolic blood pressure and elevations in forearm blood flow were both enhanced by blockade of α_1 receptors [36,38].

While monitoring HR changes, we found that hypoglycemia stimulated a powerful tachycardia and that pretreatment with captopril attenuated these increases by about half. As a mechanism to explain these findings, hypoglycemia found in previous studies generated increases in HR that were mediated entirely by activation of cardiac β_1-adrenergic receptors [36,39]. Part of this tachycardia may be because of increases in circulating epinephrine and part may be because of elevations in sympathetic neural drive to the heart. In this setting, the attenuated HR increases by captopril in the present study may have been secondary to reduced epinephrine release, to a blunted cardiac SNA activation, or both mechanisms. In favor of a neural mechanism, studies in animals have shown that bilateral carotid occlusion produced sympathetically mediated increases in HR that were largely attenuated by pretreatment with either losartan or captopril [25,40]. Furthermore, captopril has been shown to reduce norepinephrine spillover caused by stimulation of the left stellate ganglion in isolated rat hearts [32], again indicating that ANGII facilitates norepinephrine release from postganglionic sympathetic terminals. As an alternative explanation that is not related to ACE inhibition per se, captopril pretreatment may have increased HR to near maximal levels, which in turn may have resulted in the observed blunting of the HR increases to hypoglycemia in the Cap-insulin group.

A limitation to our studies was that hypoglycemia was induced by insulin. Because insulin administration during euglycemic clamp conditions can stimulate increases in adrenal and lumbar SNA [41], it is not possible in the present study to determine whether the SNA increases were more because of hypoglycemia or because of stimulation by insulin per se. Nevertheless, the current findings are relevant to clinical hypoglycemia in diabetic patients, which under most conditions are induced by administration of insulin. As a second limitation, the current studies were carried out in urethane-anesthetized rats. Although all anesthetics are known to alter cardiovascular regulation, urethane has the special ability to induce a surgical plane of anesthesia without affecting neurotransmission in the peripheral nervous system or altering autonomic reflex responses [42,43].

In summary, we found that blockade of ANGII formation by captopril did not affect reflex increases in adrenal preganglionic SNA to insulin-induced hypoglycemia in anesthetized rats. These findings indicate that the renin-angiotensin systems in the CNS do not modulate increases in adrenal nerve activity during hypoglycemia. Because previous reports have shown that converting enzyme inhibitors and ANGII receptor blockers reduce hypoglycemia-induced epinephrine release, the present findings indicate that the interaction between ANGII and epinephrine release probably occurs at the adrenal preganglionic terminal or on the chromaffin cells themselves.

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