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Hyperglycemic Action of Angiotensin II in Freely Moving Rats

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MACHADO, L. J. C., I. MIHESSEN-NETO, U. MARUBAYASHI, A. M. REIS AND C. C. COIMBRA. *Hyperglycemic effect of angiotensin II in freely moving rats.* PEPTIDES **16**(3) 479–483. 1995.—Angiotensin II has been implicated in the regulation of liver glycogen phosphorylase. Although it has been suggested that angiotensin II can raise blood glucose levels during hemorrhage, experimental data have not been presented. In the present study, the effect of angiotensin II on blood glucose levels was studied in freely moving rats, divided in three experimental groups: 1) intravenous administration of angiotensin II (0.48, 1.9, or 4.8 nmol) caused a dose-dependence response; 2) intracerebroventricular administration of angiotensin II (1.9 or 4.8 nmol) did not cause any significant change in glycemia compared with saline-treated controls; 3) intravenous administration of [Sar^1,Thr^8]angiotensin II, an antagonist of angiotensin II (750 ng/100 g b.wt. as a bolus plus a continuous injection of 25 ng/100 g b.wt./min over 30 min), greatly attenuated (39.2% lower than controls; $p < 0.01$) the hyperglycemic response to hemorrhage (1.2 ml/100 g b.wt.). These data indicate an in vivo involvement of angiotensin II in blood glucose regulation.

Angiotensin II [Sar^1,Thr^8]Angiotensin II Hyperglycemia Hemorrhage

ANGIOTENSIN II has several actions on the liver. It stimulates gluconeogenesis (10,13,15,18) and glycogenolysis (15,20,31) and inhibits the synthesis of fatty acids in the perfused liver and isolated hepatocytes (13). These actions of angiotensin II seem to be mediated by a specific membrane receptor, as a correlation has been found between angiotensin II binding and stimulation of phosphorylase activity (4,10), and between binding specificity and biological potency (4). The possibility that angiotensin II functions as a hormone influencing hepatic glucose production in certain conditions has been proposed (13,15,18,31,34).

Early reports on the effect of angiotensin II on blood glucose are conflicting. It has been reported to rise (17), to remain unaltered (12,32), or even to decline (27) after injection of angiotensin II. Recently, it has been found (3) that infusion of angiotensin II enhances whole-body glucose utilization. What makes these observations physiologically meaningful are the data indicating that under certain conditions, notably during hemorrhage, angiotensin II concentrations in plasma reach levels that very likely would activate the glycogenolytic and gluconeogenic processes in liver (28,29).

Although it has been suggested that angiotensin II can raise the blood glucose levels during hemorrhage (4,13,18,21,34), experimental data have not been presented. A marked increase of blood glucose after hemorrhage has been repeatedly confirmed in many species (1,16,23,34). The resultant hyperglycemia, which occurs already in the early stages of bleeding, is due in large measure to an increase in both circulating catecholamines of adrenal medullary origin and hepatic sympathetic nerve activity (1,16,23). However, the increase in hepatic venous glucose concentration in response to hemorrhage can be greatly attenuated but not abolished when both adrenal glands and hepatic sympathetic nerves are removed (1,16).

These results suggest that factors other than the adrenal hormones contribute to the blood sugar increase during hemorrhage. In addition, a number of studies indicate that angiotensin II is the mediator that modulates adrenal activity after hemorrhage (5,8,9,25). Moreover, in cats and dogs (5,8), the adrenomedullary response to hemorrhage fails completely after bilateral nephrectomy. The present experiments were designed to reinvestigate the effect of angiotensin II administration on blood glucose concentration and to examine the participation of this peptide in the hyperglycemic response to hemorrhage.

METHOD

Adult male Wistar rats (12–14 weeks) were used in these experiments. Animals were fed with Purina rat chow and water ad libitum and housed in temperature controlled quarters with 14 h of light (0500–1900 h) per day. At the age of 11 weeks, the rats were placed in individual cages and were frequently handled. One week later, they were anesthetized with thionembutal (30 mg/kg b.wt.), and a silastic catheter was inserted through

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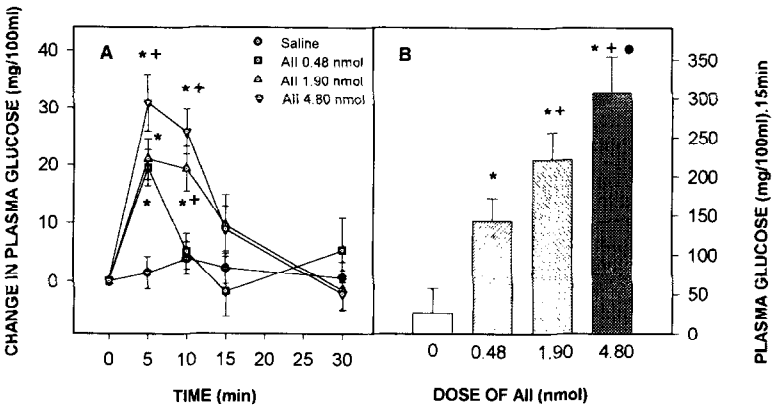

FIG. 1. (A) Effect of IV administration of angiotensin II (ANG II, 0.48, 1.9, or 4.8 nmol) or saline (NaCl 0.15 *M*) on plasma glucose. Values are expressed as changes from preinjection levels. Data are means ± SEM. Preinjection values of plasma glucose (mg/100 ml): group saline, 127 ± 4.7 (n = 7); group ANG II 0.48 nmol, 117 ± 5.3 (n = 8); group ANG II 1.9 nmol, 124 ± 3.4 (n = 8); group ANG II 4.8 nmol, 126 ± 4.4 mg/100 ml (n = 8). *$p < 0.01$ vs. saline group; +$p < 0.01$ vs. ANG II 0.48 nmol group; (B) Integrated area under glucose curve [shown in Fig. 2(A)]. Data are means ± SEM of individual area calculated using the Simpson's rules. *$p < 0.01$ vs. saline group; +$p < 0.01$ vs. ANG II 0.48 nmol group; ●$p < 0.05$ vs. ANG II 1.9 nmol group.

jugular vein into the right atrium for blood sampling by the technique of Harms and Ojeda (11). The sampling catheter was rinsed every 2 day with 1 ml of saline containing 25 μU heparin (Liquemine, Hoffman - La Roche). All animals were allowed a week recovery period before being utilized in the experiments.

Angiotensin II Intravenous Studies

On the day of the experiments the jugular catheter was connected to a peristaltic pump 1 h prior IV infusion of angiotensin II (Sigma, St. Louis, MO) or vehicle control. Throughout the experiment the rats were deprived of food and water, and blood samples were collected at −2, 5, 10, 15, and 30 min, with the animal freely moving in the cage. At time zero, angiotensin II (0.48, 1.9, or 4.8 nmol in 500 μl of NaCl 0.15 *M*) was infused over a period of 120 s. Saline solution (500 μl of NaCl 0.15 *M*) was used as control.

Angiotensin II Intracerebroventricular Studies

In these experiments, beside the jugular catheter insertion as described above, the animals were placed into the stereotaxic apparatus (David Kopf 900), and a unilateral guiding cannula (22 ga) was also implanted in the third cerebral ventricle employing the stereotaxic coordinates of DeGroot (6,7): anteroposterior, 5.4 mm; lateral, 0.05 mm; vertical, 4.0 mm above the base of the skull. This cannula was provided with a mandril and a protective cap. Both cannulae were anchored firmly to the skull with jeweler's screws and fixed with acrylic cement (2). On the day of the experiments, the rats had their venous catether rinsed regularly and connected with a peristaltic pump. An injection needle (30 ga) exceeding the tip of the guiding cannula by 0.3 mm and connected to a Hamilton syringe was introduced into the third cerebral ventricle. The animals were placed back into their home cage 1 h before ICV injection. Throughout the experiment the rats were deprived of food and water. Blood samples were collected at −2, 5, 10, 15, and 30 min, with the rat freely moving in the cage. At time zero, 2 μl of angiotensin II solution (1.9 or 4.8 nmol in NaCl 0.15 *M*) was injected into the third cerebral ventricle. In an attempt to minimize intraventricular pressure changes, the solutions were administered over a period of 120 s. Saline solutions of the same osmolarity of the test solutions were used as controls. Each rat was used at least twice and served as its own control.

Hemorrhagic Hyperglycemia Studies

On the day of the experiments the rats had their venous catether connected with a peristaltic pump 1 h prior to hemorrhage. Thirty minutes before hemorrhage, [Sar^1,Thr^8]angiotensin II (sarthran, Sigma) was administered over 30 min (750 ng/100 g b.wt. as a bolus plus a continuous infusion of 25 ng/100 g b.wt./min). Saline solution of the same volume (0.2 ml as a bolus plus a continuous infusion of 0.007 ml/100 g b.wt./min) was used as controls. The animals were deprived of food and water throughout the experiments. Blood samples were taken −30 min (immediately before sarthran treatment), 0 , 5, 10, 15, and 30 min relative to the start of bleeding. At time zero, the sarthran infusion was stopped and the animals were bled rapidly (1.2 ml/100 g b.wt./2 min of hemorrhage withdraw). Another group of animals was used to determine plasma glucose in the absence of hemorrhage.

Blood samples were kept on ice and centrifuged, and plasma glucose levels were measured by the glucose oxidase method (God-Ana, Labtest, BR).

Differences between groups were checked by ANOVA followed by Newman−Keuls test. Values from samples taken after angiotensin injection or hemorrhage were compared to basal values by the paired t-test.

RESULTS

Effect of Angiotensin II Intravenous Injection to Freely Moving Rats

As illustrated in Fig. 1(A,B), statistically significant increases in plasma glucose levels occurred after IV injection of each of the three angiotensin II solutions utilized. After the injection of 4.8 nmol of angiotensin II, plasma glucose concentration increased rapidly, reaching the highest values 5 min after the in-

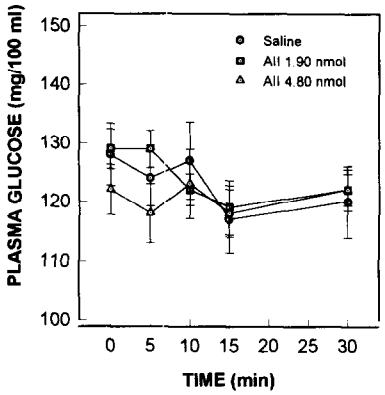

FIG. 2. Plasma glucose levels after ICV injection of angiotensin II (1.9 or 4.8 nmol) or 0.15 *M* NaCl. Each point represents mean ± SEM of $<n = 7-8$ observations.

jection, when the increase was about 25% of the initial values ($p < 0.01$). At 10 min they were still high (20.5% increase, $p < 0.01$), and at 30 min postinjection plasma glucose levels were returning to normal. With the dose of 1.9 nmol, the highest value of plasma glucose also occurred at 5 min postinjection (17% increase, $p < 0.01$). At 10 min they were still 15.7% higher ($p < 0.01$) than the initial values, and at 15 min postinjection plasma glucose levels had returned to preinjection values and remained at this level until the end of the experimental period. Administration of 0.48 nmol of angiotensin II produced a rise in plasma glucose only at 5 min (16.8% higher than the initial values, $p < 0.01$). After IV injection of 0.15 *M* NaCl (control), plasma glucose concentration did not differ significantly from preinjection values at any of the experimental intervals. To compare these experimental situations, 15-min integrated incremental areas over basal surfaces were calculated and are represented in Fig. 1(B). Angiotensin II IV injections produced significant increases in glycemia when compared with saline controls ($p < 0.05$, Newman–Keuls test). Also, the hyperglycemic response was clearly dose dependent.

Effect of Angiotensin II Injection Into Third Cerebral Ventricle to Freely Moving Rats

As illustrated in Fig. 2, ICV injection of 1.9 or 4.8 nmol of angiotensin II did not affect the plasma glucose levels at any of the experimental intervals. The ICV administration of 2 μl of 0.15 *M* NaCl (isosmolar to the angiotensin II solution utilized) was also ineffective (Fig. 2).

Effect of Sarthran Administration on Hemorrhage Hyperglycemia

Following bleeding [Fig. 3(A)], there was an immediate increase of plasma glucose levels. The hyperglycemia induced by hemorrhage was observed throughout the experimental period when compared to initial values or to the control group without hemorrhage (ANOVA, $F = 21.55$, $p < 0.01$). During the infusion of the angiotensin antagonist [Sar^1,Thr^8]angiotensin II (sarthran) the increase of plasma glucose following hemorrhage was greatly attenuated. This effect of sarthran infusion on blood glucose was already evident after 5 min (182 ± 14.5 mg% saline vs. 153 ± 6.6 mg%; $p < 0.05$, Newman–Keuls test) and persisted throughout the experimental period. After 15 min, when the hyperglycemic response to hemorrhage was maximal, values to saline and sarthran-injected animals were, respectively: 230 ± 21.3 mg% vs. 188 ± 10.7 mg% ($p < 0.05$, Newman–Keuls test). Figure 3(B) shows the 30-min integrated area under the incremental glucose curve. There was a significant decrease of 39.2% in the hyperglycemic response in sarthran-treated rats when compared to controls ($p < 0.05$, Newman–Keuls test).

DISCUSSION

The data of the present study show that IV injection of angiotensin II as well as hemorrhage causes a rapid increment of plasma glucose concentration and that the hyperglycemic response to hemorrhage can be depressed by IV infusion of [Sar^1,Thr^8]angiotensin II, a specific angiotensin II competitive antagonist (19,24,26). These findings suggest that angiotensin II may play an important role in blood glucose regulation. Evidence that angiotensin's effect is physiological comes from dose dependence of the hyperglycemic response.

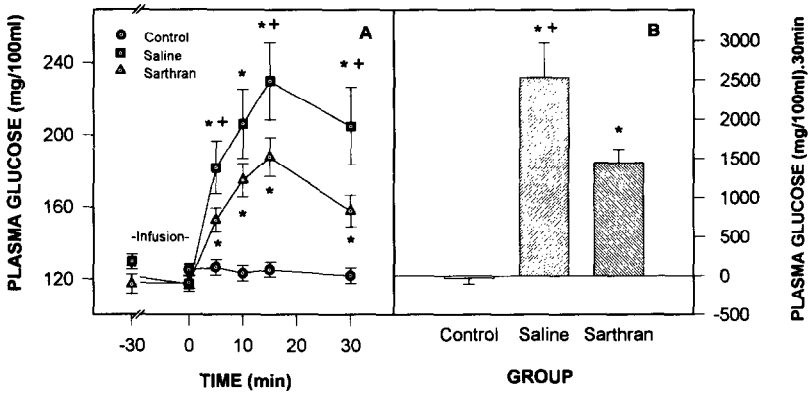

FIG. 3. (A) Effect of pretreatment with sathran (750 ng/100 g b.wt. as a bolus plus a continuous injection of 25 ng/100 g b.wt. over 30 min) or saline (NaCl 0.15 *M*) on the hyperglycemia induced by hemorrhage (rapid bleeding of 1.2 ml/100 g b.wt.). Each point is the mean ± SEM of $n = 9-10$ observations. *$p < 0.01$ vs. control group; +$p < 0.01$ vs. sarthran group. (B) Integrated area under glucose curve [shown in Fig. 3(A)]. Data are means ± SEM of individual area calculated using the trapezoidal rule. *$p < 0.01$ vs. control group; +$p < 0.05$ vs. sarthran group.

Additional evidence for the physiological significance of this effect is suggested by the finding that IV-perfused [Sar^1,Thr^8]angiotensin II substantially reduces the hyperglycemic response to hemorrhage in vivo. To our knowledge, this is the first case where a physiological hyperglycemic response shows some dependence on the renin–angiotensin system. This effect could be attributed to a direct action on liver glycogen phosphorylase, or to a stimulatory effect of angiotensin II on the adrenomedullary secretion. Angiotensin II increases glycogenolysis (10,13,15,18) and gluconeogenesis (15,20,31) of isolated hepatocytes, which contain a high density of high-affinity angiotensin receptors (4,10,22,30). It has also been shown that angiotensin II alters the activity of phosphorylase, glycogen synthase, and pyruvate kinase by increasing their phosphorylation state through a Ca^{2+}-requiring, cyclic AMP-independent mechanism (33).

In analyzing these data, it should be realized that they derive from experiments on isolated hepatocytes incubated in vitro. To assess the relationship between hyperglycemic response and renin–angiotensin system activity, it seemed of interest to us to use physiological stimuli in an in vivo situation. The renin–angiotensin system has been implicated in the increased secretion of adrenal medullary catecholamines that occurs in response to a variety of stimuli. Hemorrhage is known as one of the most powerful stimuli that activate both the renin–angiotensin system (5,8,9,25,28,29) and sympathetic nervous system (1,5,8,9,23,25,34). We have used acute hemorrhage in rats to study if angiotensin II is a cause for increased blood glucose. To assess the role of angiotensin II, we have used sarthran, which has been reported to be a specific and competitive antagonist of angiotensin II (19,24,26). The experiments reported here demonstrate that the hyperglycemic response to hemorrhage in rats can be substantially reduced after [Sar^1,Thr^8]angiotensin II IV infusion.

An interesting observation is that the adrenal release of catecholamines with hemorrhage is reduced by nephrectomy and abolished by adrenal denervation in nephrectomized dogs and cats (5,8). This observation, along with reports of several effects of angiotensin II injected to the brain, led us to the hypothesis that angiotensin support of hyperglycemic response with hemorrhage could be due a central angiotensin action. Corwin et al. (5) suggest that angiotensin II effects on reflexly stimulated adrenal catecholamine releases were due a central angiotensin action. In these experiments by Corwin et al. (5) using the acutely hemorrhaged dogs, ICV infusion of saralasin reduced the adrenal release of catecholamine after hemorrhage. In view of these findings, we considered examining if angiotensin II could induce hyperglycemia in the rat by a central mechanism. The results from all doses of angiotensin II injected ICV, which were unable to produce hyperglycemia, did not support this hypothesis. Intracerebroventricular injection of 1.9 or 4.8 of angiotensin II did not induce a hyperglycemic response, which makes it unlikely that IV-administrated angiotensin II was effective via central action. However, our data do not completely eliminate a central site for angiotensin II action at one of the circumventricular organs that are devoid of blood–brain barrier and are targets for high circulating angiotensin II.

In conclusion, evidence for a peripheral angiotensin II mechanism is presented to explain the hyperglycemic response to IV-injected angiotensin II and the reduced hyperglycemic response after hemorrhage in sarthran-treated rat.

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