Arginine-induced pancreatic hormone secretion during exercise in rats

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Trabelsi, Fethi, and Jean-Marc Lavoie. Arginine-induced pancreatic hormone secretion during exercise in rats. J. Appl. Physiol. 81(6): 2528–2533, 1996.—The aim of the present investigation was to 1) determine whether arginine-induced pancreatic hormone secretion can be modified during an exercise bout, and 2) verify whether the sectioning of the hepatic branch of the vagus nerve can alter the arginine-induced insulin and glucagon secretion during exercise in rats. To this end, we studied the effects of an intraperitoneal injection of arginine (1 g/kg body mass) during an exercise bout (30 min, 26 m/min, 0% grade) on the pancreatic hormone responses. These effects were determined in one group of sham-operated exercising rats and compared with three control groups: one group of resting rats, one group of saline-injected exercising rats, and one group of hepatic-vagotomized exercising rats. Five minutes after the injection of arginine, significant (P < 0.05) increases in insulin, glucagon, and C-peptide concentrations were observed in exercising as well as in resting rats. These responses were not, however, altered by the hepatic vagotomy and/or by the exercise bout. It is concluded that arginine is a potent stimulator of pancreatic hormone secretion during exercise, even though the sympathoadrenal system is activated. These results also indicate that a hepatic vagotomy does not seem to influence arginine-induced hormonal pancreatic responses and question the role of the putative hepatic arginoreceptors in the control of the pancreatic hormone secretion during exercise.

hepatic vagus nerve; insulin; glucagon; C peptide

AMINO ACIDS have been reported to be very potent stimuli for insulin and glucagon secretion from the pancreas (1, 5). Arginine has been recognized as the most efficacious amino acid for the stimulation of insulin and glucagon secretion (5). Arginine is not metabolized by the B cell and appears to stimulate insulin release by depolarizing the cell membrane because of its transport into the cell in a positively charged form (10). It has been suggested in other reports that arginine-induced insulin release may also be mediated by arginine-derived nitrogen oxides (9). Because of its action, arginine was used as a nonglucose secretagogue to elucidate the B-cell adaptations to training and to evaluate the insulin secretory capacity in humans (3, 11) and rats (6).

Arginine sensors were reported by Tanaka et al. (24) to exist in the liver and to modulate arginine-induced pancreatic hormone secretion. In Tanaka et al. and a subsequent study (19), it was reported that an intraperitoneal injection of arginine enhanced plasma insulin and glucagon concentrations more in hepatic-vagotomized than in sham-vagotomized rats. In a parallel electrophysiological study (26), injection of arginine into the hepatic portal vein caused a reflex inhibition of pancreatic vagus nerve activity. Based on these findings, it was hypothesized that the arginine sensor system modulates insulin and glucagon release through the afferent vagus nerve (19, 25). Similar findings were also reported regarding the existence of hepatic neural units sensitive to glucose (19). There is growing evidence that these hepatic glucoreceptors modulate insulin release through the afferent hepatic branch of the vagus nerve (15, 23, 24). These hepatic glucoreceptors appear to play a regulatory role in different situations, such as food intake (22), insulin-induced hypoglycemia (4, 12), and, more specifically, physical exercise (13, 14). In the latter situation, results from our laboratory have indicated that hepatic vagotomy (HV) in adrenomedullated rats resulted in higher insulin and lower glucagon and catecholamine concentrations during exercise (13). It was concluded that the liver, through neural mediation of the hepatic vagus nerve, may contribute to the hormonal regulation occurring during exercise, particularly the pancreatic hormone secretion. Based on the reported observation that hepatic glucoreceptors are involved in the hormonal regulation during exercise (13, 14), one could suspect that the putative hepatic arginoreceptors may also play a regulatory role in the pancreatic hormone secretion during exercise. Although much is known about hepatic glucoreceptors, no data are available regarding the arginine-induced pancreatic hormone secretion during exercise, as well as the importance of the hepatic vagus nerve to these responses. Therefore, the present study was designed to determine the effects of an arginine injection on insulin, C-peptide, and glucagon responses during exercise and to examine whether the putative hepatic arginoreceptors are functionally involved in the control of these pancreatic hormone responses during exercise.

METHODS

Subjects. Male Sprague-Dawley rats (Charles River), weighing 250–275 g were housed in individual cages and allowed food (normal chow; RMH 4020, Prolab) and water ad libitum for 2 wk after they reached our laboratory. The lights were on from 0700 until 1900, and the room temperature was maintained at 20–23°C. During the second week after their arrival, the rats were progressively run on a motor-driven rodent treadmill beginning with 15 min/day at 15 m/min and increasing to 30 min/day at 26 m/min (0% grade) so that they were well-acclimated to running and being handled.

Surgery. Three to four days before experimentation, all rats were implanted with a chronically indwelling jugular catheter under pentobarbital sodium (40 mg/kg ip) anesthesia as previously described (14). After insertion, the catheters were filled with saline-containing heparin (517 U/ml; Fisher Scientific). After the rats were catheterized, they either underwent an HV or were sham operated. Sectioning of the hepatic branch of the anterior abdominal vagal trunk was conducted...
according to the technique described by Tordoff and Novin (27). General descriptions of the abdominal vagal system of the rat indicate that in most cases only a single hepatic branch of the anterior vagus is apparent (16, 20), although two or three hepatic branches have also been observed (21). After the HV or the sham operation, the abdominal cavity was immediately closed with suture and the animals were allowed 3–4 days to recuperate.

Verification of surgery. At the present time, there is no easy test for verifying the completeness of the HV used here on a case-by-case basis (18, 27). At surgery, we used the most careful techniques to ensure complete destruction of the hepatic vagus nerve. During surgery, great care was taken to ensure that no tissue remained between the liver and esophagus from the esophageal plexus to the cardia of the stomach. When there was any ambiguity concerning the identification of the vagal hepatic branch, the animal was discarded from the experiment. Recent results from Trabelsi et al. (29) and Lee and Miller (15), which show an increase in insulin response to an acute HV in adrenomedullated rats, may be used for further assessments of our surgical technique.

Experimental groups. Rats under all conditions had to recover to their presurgery weights to be used in the experimental protocol. On the day of the experiment, food was removed from cages at 0600 and the experimental procedure was conducted between 0900 and 1230. The rats weighed $332.6 \pm 2.5$ g when they were killed, and the number of animals in each group ranged from 9 to 13. Rats were randomly assigned to one of the four experimental groups. Three groups of rats received an intraperitoneal injection of L-arginine (1 g/kg body mass, pH = 7.4) dissolved in a 25% (wt/vol) solution of 0.9% NaCl and warmed at 37°C before injection. This dose has been shown to stimulate pancreatic hormone secretion in rats (19, 24, 28). The main group of rats (arginine-sham-exercise) injected with arginine were sham operated and submitted to a 30-min exercise period. This group was compared with three other groups: a group of sham-operated rats injected with arginine in the resting condition (arginine-sham-rest), a group of sham-operated rats injected with saline and submitted to the same exercise bout (saline-sham-exercise), and a group of HV rats injected with arginine and also submitted to the 30-min exercise period (arginine-HV-exercise).

Fig. 1. Plasma glucose concentrations at rest (A) and during exercise (EX) in hepatic-vagotomized (HV) (C) and sham-operated (SHM) rats injected with arginine (ARG) or saline (SAL) (B). Values are means ± SE. n = 9–13 Rats in each group. Significantly different at $P < 0.05$ compared with corresponding resting conditions; * between conditions.
intervals during the next 30 min. Collected blood was simultaneously replaced with blood from a donor animal submitted to the same injection. At the end of the 30-min run, the rats were anesthetized via the venous catheter with pentobarbital sodium (20 mg/kg) while they were still running (exercised groups). Immediately, the abdominal cavity was opened and a piece of liver (caudate lobe) was frozen with aluminium block tongs cooled to liquid nitrogen temperature. Nonexercised control rats were treated in the same manner as the exercised rats.

Analyses. Venous blood was collected into a heparinized syringe. Because of the small volume of each blood collection (0.6–1.1 ml), the different metabolic and hormonal parameters were measured at different sampling times. To reduce the amount of blood sampled during the testing period, glucagon concentrations were not measured at minutes 5 and 15. A large portion of the sampled blood was centrifuged, and the plasma was stored for glucose, C-peptide, and insulin analyses. Blood used for plasma glucagon determination (500 µl) was preserved in Trasylol (50 µl) before centrifugation. All tissue and blood samples were stored at –70°C until analyses were performed.

Plasma glucose concentration was determined by the use of a glucose analyzer (YSI 2300, Yellow Springs Instrument). Insulin, glucagon, and C-peptide levels were determined by a commercially available radioimmunoassay (Immunocorp, ICN Biomedicals, and Linco Research, respectively). Liver glycogen contents were determined with the phenolsulfuric acid reaction according to the method of Lo et al. (17). All data are reported as means ± SE.

Statistical comparisons of blood parameters were made with a two-way analysis of variance with repeated-measures design. The effects of arginine-induced pancreatic hormone secretion during exercise were evaluated by comparing the data of this group of rats to three control groups: 1) a resting group (arginine-sham-exercise vs. arginine-sham-rest); 2) a saline-injected group (arginine-sham-exercise vs. saline-sham-exercise), and 3) an HV group (arginine-sham-exercise vs. arginine-HV-exercise). A one-way analysis of variance nonrepeated-measures design was used for comparisons of liver glycogen concentrations. Fisher’s post hoc test was used in the event of a significant (P < 0.05) F-ratio.

RESULTS

As presented in Table 1, liver glycogen concentrations measured at the end of the experimental treatment were significantly (P < 0.01) lower in exercising rats, whether injected with arginine or saline (1.02–1.19 g/100 g liver tissue), than in rats in the resting condition (4.8 ± 0.5 g/100 g liver tissue). The injection of arginine did not have any effect on plasma glucose concentrations in all groups of rats during the first 5 min (Fig. 1). However, plasma glucose significantly (P < 0.05) decreased in all groups injected with arginine from minute 10 to the end of the experimental period. The arginine-induced decrease in plasma glucose was observed in resting as well as in exercising rats (Fig. 1A) and was not affected by HV during exercise (Fig. 1C). The decrease in exercising plasma glucose was not observed in the saline-injected group (Fig. 1B). Plasma insulin concentrations were signifi-

Fig. 2. Plasma insulin concentrations at rest (A) and during EX in HV (C) and SHM rats injected with ARG or SAL (B). Values are means ± SE, n = 9–13 Rats in each group. Significantly different at P < 0.05 † compared with corresponding resting conditions; * between conditions.
cantly (P < 0.05) increased by the arginine injection whether at rest or during exercise (Fig. 2). These increases were similar in resting and exercising conditions (Fig. 2A); they were not observed in the saline-injected group (Fig. 2B) and were not affected by HV (Fig. 2C). The C-peptide response for all groups was the same as that of insulin (Fig. 3), with the exception that the effects of arginine injection were more important at rest than during exercise (minute 10; Fig. 3A). Plasma glucagon concentrations were also significantly (P < 0.05) increased by the injection of arginine (Fig. 4). These increases were similar in resting and exercising conditions (Fig. 4A); they were not observed in the saline-injected group (Fig. 4B) and were not affected by HV (Fig. 4C).

DISCUSSION

In previous exercise studies, arginine-stimulated insulin responses have been used to test the pancreatic islet insulin secretion after exercise (3, 6, 11). This is the first report of the effects of the insulin secretagogue arginine during an exercise bout. The first finding of the present study is that an injection of arginine before exercise resulted in a significant increase in exercising insulin, C-peptide, and glucagon concentrations. This is clearly observed when glucose and hormonal concentrations measured in arginine-injected rats are compared with saline-injected rats. These results are the first to indicate that even if the sympathoadrenal system is activated during exercise, the arginine stimulus still resulted in an increase in insulin secretion. These results are in agreement with those reported in recent studies (24, 26, 28) that were conducted in the resting condition. The most likely explanation for the arginine-induced insulin secretion during exercise is that the mechanisms of action of arginine and exercise in the pancreatic islet cells are independently activated. Exercise is well known to result in an activation of the sympathoadrenal system, which causes a decrease in plasma insulin concentrations (7). This response has been attributed to the action of norepinephrine via the α-adrenergic receptors (8). On the other hand, arginine-induced pancreatic insulin secretion appears to be mediated by a depolarization phenomenon because of its transport into the cell in a positively charged form (10). Recent observations in isolated islets of rats suggest that arginine-induced insulin release might also be modulated by arginine-derived nitrogen oxides (9). Although the present study was not designed to elucidate the mechanism for insulin secretion or inhibition, the present results do indicate that under the present conditions arginine appears to be a more powerful stimulus of insulin response during exercise than the activation of the sympathoadrenal system. The observation that arginine can still stimulate insulin secretion during exercise does not mean...
that exercise did not have any inhibitory effect on insulin secretion. Comparisons of the effects of arginine between resting and exercising situations indicate a similar response for plasma insulin levels (Fig. 2A). However, the arginine-induced elevation in C-peptide levels was significantly lower during exercise than in the resting condition (Fig. 3A). This indicates that insulin secretion was still inhibited by the exercise stimulus. The similarity of the plasma insulin response between rest and exercise might be explained by a greater hepatic insulin removal in the situation (rest) of greater insulin secretion (2).

The second aim of the present study was to test the possibility that the putative hepatic arginoreceptors might be involved in pancreatic hormone secretion during exercise. It has been reported that arginine sensors exist in the hepatic portal system and modulate pancreatic hormone secretion through vagal neural afferents (24, 25). Intraperitoneal injection of arginine (1 g/kg) has been reported to enhance plasma insulin and glucagon concentrations more in hepatic-vagotomized rats than in sham-operated rats (19, 24). There is also electrophysiological evidence showing that injection of arginine into the hepatic portal vein causes a reflex inhibition of pancreatic vagus activity and a reflex activation of the pancreatic sympathetic nerve activity (26). Based on these observations, we postulated that, if the hepatic arginoreceptors are functional during exercise, an HV should result in a change in the arginine-induced pancreatic hormone secretion during exercise. However, our results revealed no effects of the HV on arginine-induced pancreatic hormone secretion during exercise. This observation suggests that, contrary to hepatic glucoreceptors, which may regulate to a certain point insulin and glucagon secretion during exercise (2, 13), hepatic arginosensors do not appear to be involved in the hormonal responses during exercise.

Although an effect of HV on insulin and glucagon response to arginine infusion at rest has been consistently reported by another laboratory (19, 24), we were not able to reproduce these effects in a recent study (28) with the same injection dose of arginine (1 g/kg). This discrepancy was tentatively explained by the different fasting state of the animals in these studies. It is possible that physical exercise is not an appropriate physiological situation for the arginine hepatic sensor system to be activated. It has been hypothesized that hepatic arginine sensors, through afferent vagal nerves, prevent exaggerated pancreatic hormone secretion triggered by a direct stimulation of this amino acid (24, 25).

During exercise, this modulation of hepatic vagus nerve might be difficult to observe, since other regulatory mechanisms, such as the sympathoadrenal system, are in action.

Glucagon secretion has been reported to be increased by arginine (5). In the present study, injection of arginine also resulted in a significant increase in

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Fig. 4. Plasma glucagon concentrations at rest (A) and during EX in HV (C) and SHM rats injected with ARG or SAL (B). Values are means ± SE. n = 9–13 Rats in each group. Significantly different at P < 0.05 * compared with corresponding resting conditions; † between conditions.
plasma glucagon levels. This increase was most likely the result of arginine injection and not the exercise per se, since no increase in glucagon was observed in the saline-injected rats (Fig. 4B). Accordingly, no differences in arginine-induced glucagon secretion were observed between rest and exercise situations (Fig. 4A). Similar to the insulin response, there were no effects of HV on the arginine-induced glucagon response. Overall, these results indicate that arginine can stimulate glucagon secretion during an exercise bout and that this effect is not affected by an HV.

Trabelsi et al. (28) and other investigators (19, 25) have reported that plasma glucose rises in the first 10 min after injection of arginine in food-deprived rats. This increase has been attributed to an increased gluconeogenesis, since the same observation was not made in glucogenesis-inhibited rats (28). Arginine injection in the present resting condition did not result in a significant increase in blood glucose levels (Fig. 1A). This might be attributed to the fact that rats in the present study were evaluated in the fed state, a situation in which gluconeogenesis is not very much activated. However, the combination of exercise and arginine stimuli resulted in a more rapid decrease in blood glucose levels than arginine alone, since a difference in plasma glucose between these two conditions was observed after 10 min of exercise. This may be attributed to a potentiation of the arginine-induced hyperinsulinemia in the exercise situation and/or a larger hepatic glycogenolysis in exercise than in the resting condition (Table 1).

In summary, results of the present study show that an injection of arginine before exercise results in an increase in insulin and glucagon secretion during exercise. This finding suggests that the arginine-induced insulin secretion stimulus overrides the exercise-induced insulin inhibition stimulus. In addition, hepatic arginosensors do not seem to be functional during exercise.

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