Cardiopulmonary Reflex Impairment in Experimental Diabetes in Rats

Vera Lucia Longo Oliveira, Edson Dias Moreira, Vera de Moura Azvedo Farah, Fernanda Consolim-Colombo, Eduardo Moacyr Krieger, Maria Claudia Irigoyen

Abstract—The aim of the present study was to evaluate the sensitivity of the cardiopulmonary receptors in experimental diabetes induced by streptozotocin by the use of 2 different methods: (1) administration of increasing doses of serotonin to analyze peak changes of arterial pressure and heart rate for each given dose in conscious intact normal and diabetic rats; (2) expanding blood volume with the use of dextran (6%) to produce similar increases in left ventricular end-diastolic pressure to quantify the arterial pressure, heart rate, and renal sympathetic nerve activity in sinoaortic, denervated, anesthetized normal and diabetic rats. Blood samples were collected to measure blood glucose. Diabetic rats showed hyperglycemia (22±0.7 versus 7±0.2 mmol/L), reduced body weight (226±12 versus 260±4 g) and heart rate (294±14 versus 350±10 bpm), and similar arterial pressure (104±4 versus 113±4 mm Hg) when compared with control rats. Serotonin induced significant bradycardia and hypotension, which were similar and proportional to the dose injected in both groups. Mean arterial pressure and heart rate decreases in response to volume overload were significantly lower in diabetic than in control rats. The reflex reduction of the renal sympathetic nerve activity as expressed by percentage changes in nerve activity in response to increasing left end-diastolic pressure was abolished in diabetic animals (1.9±0.8% versus −14±4%/mm Hg in controls). These results showed an impairment of cardiopulmonary reflex control of circulation in diabetes during acute volume expansion. The normal responses to serotonin administration indicated that the cardiopulmonary reflex is still preserved in diabetic rats. (Hypertension. 1999;34[part 2]:813-817.)

Key Words: cardiac function ■ diabetes mellitus ■ blood pressure ■ renal nerve ■ reflex

Clinical studies indicate that insulin-dependent diabetes mellitus is associated with alterations of the autonomic nervous system control of cardiovascular function. Beat-to-beat heart rate (HR) variation is reduced at rest and during deep breathing, suggesting that parasympathetic nervous control of the heart is diminished in diabetics.1-2 On the other hand, a blunted chronotropic response to exercise and subnormal plasma catecholamine levels3 suggest an impairment of sympathetic activity in these individuals.4

In experimental studies, administration of streptozotocin (STZ) is a well-established method for the induction of diabetes in rats. This model has been commonly used to demonstrate the occurrence and to study the pathogenic mechanisms of various complications such as changes in fluid balance and blood volume homeostasis.5

Recent results from our laboratory suggested that reflex tachycardic response elicited by the reduction of arterial pressure (AP) is attenuated in short-term diabetes,6 whereas the reflex bradycardia in response to an AP increase has been reported to be normal. In contrast, baroreflex-mediated bradycardia is impaired in alloxan-induced diabetic rabbits, suggesting that changes in baroreflex function in experimen-

Received May 9, 1999; first decision June 22, 1999; revision accepted July 8, 1999.
From the Experimental Division and Hypertension Unit, Heart Institute, University of São Paulo, Brazil.
Correspondence to Maria Claudia I. Irigoyen, Experimental Division and Hypertension Unit, Heart Institute, University of São Paulo, Rua Dr Eneas de Carvalho Aguiar 44, São Paulo, SP, 05403-000, Brazil. E-mail: hipirigoyen@incor4.incor.usp.br
© 1999 American Heart Association, Inc.
Hypertension is available at http://www.hypertensionaha.org

813
food, and maintained in a room with a constant temperature (23°C) on a 12-hour light/dark cycle. All surgical procedures and protocols used are in accordance with the Guidelines for Ethical Care of Experimental Animals and were approved by the Institutional Animals Care and Use Committee.

Rats were made diabetic by a single injection of STZ (50 mg/kg IV, Sigma Chemical Co) dissolved in 10 mmol/L citrate buffer, pH 4.5, administrated 15 days before the experiments. The rats were fasted for 8 hours before STZ injection.

After protocols were performed, the hearts of the rats were excised and weighed. The ratio of the heart weight to the body weight was determined and compared between groups.

Catheterization and AP Recording
One day before the experiments, arterial and venous catheters were placed in the right femoral artery and vein with rats under sodium pentobarbital anesthesia (40 mg/kg IP, added Hypnol 3%, Cristalia, Itapira) for direct measurements of AP and for drug administration. The catheters were exteriorized through the back of the neck.

According to each protocol, blood pressure and left ventricular end-diastolic pressure (LVEDP) were recorded continuously by catheters inserted into the femoral artery and left ventricle, respectively, and connected to a strain-gauge transducer (P23Db, Gould-Statham). The signal from this transducer was fed into an amplifier (GPA-4 model 2, Stemtech, Inc) and a 16-channel digital analog converter (Stemtech, Inc) and from this to a microcomputer (Gateway 2000, 4DX2-66V). HR and AP were analyzed with a microcomputer (IBM-AT/CODAS) on a beat-to-beat basis at 3000-Hz frequency.

The diabetic group (n=8, DG) and the control group (n=6, CG) were submitted to 2 different protocols to evaluate the cardiopulmonary reflex on 2 consecutive days.

Protocol 1: Bezold-Jarish Reflex
The responses to stimulation of chemosensitive cardiopulmonary receptors (Bezold-Jarish reflex) were determined in conscious, unrestrained animals. After recording 15 minutes of resting AP and HR, successive bolus injections of 5-HT (2, 4, 8, and 16 μg/kg serotonin, Sigma Chemical Co) were given to the animals while the mean arterial pressure (MAP) and HR were recorded. For data analysis, control and peak changes of MAP and HR, for each given dose, were analyzed with a microcomputer (IBM-AT/CODAS). Injections were not repeated until the recorded parameters had returned to preinjection levels. The changes in MAP and HR produced by 5-HT were expressed as percent changes to the control level.

Protocol 2: Volume Expansion
After resting AP and HR were recorded, the animals were anesthetized by sodium pentobarbital anesthesia (40 mg/kg IP) (Hypnol 3%, Cristalia, Itapira) for the evaluation of cardiopulmonary reflex control of RSNA by volume expansion.13 A polyvinyl catheter inserted into the left ventricle through the right common carotid monitored LVEDP.

The left kidney was identified by a left retroperitoneal incision so that the RSNA could be recorded. A thin, bipolar, stainless steel electrode (0.03-mm diameter) was placed around a branch of the renal nerve and carefully insulated with silicone rubber (Wacker SIL GEL 604). RSNA was recorded with an electrode cable and preamplified with the use of a high-input impedance differential amplifier. To test the validity of the recording, we administered a bolus dose of phenylephrine to produce a large increase in AP (~40 mm Hg), which elicited a reflex decrease in mean rectified renal nerve activity. The multifen fiber RSNA was expressed as a relative change in each rat before and after volume expansion. Resting RSNA before sinoaortic denervation (SAD) was quantified, as described previously.16 Baseline RSNA after SAD was termed 100%. After a control recording period of 15 minutes, all rats underwent SAD, leaving the cardiopulmonary afferents intact. The effectiveness of SAD was confirmed by demonstrating that phenylephrine-induced increase (~40 mm Hg) in AP produces a decrease in HR no greater than 10 to 15 bpm, when the normal rate should be 60 to 80 bpm.

Volume expansion to cardiopulmonary stimulation was performed by injecting dextran 70 (Braun) into the femoral vein at 4 steps: 0.5, 1.0, 2.0, and 3.0 mL. Each step lasted 30 seconds. The parameters of AP, HR, LVEDP, and RSNA were continuously recorded before and during volume expansion.

To evaluate the reflex effects of volume expansion, LVEDP and RSNA were taken in the control condition and after each step of a graded volume expansion. RSNA responses were expressed as percent changes with respect to the control level. Cardiopulmonary baroreflex (CPBR) gain was calculated as the ratio of the RSNA (%) to the change in LVEDP (mm Hg) occurring after each step. In each animal, gain values were obtained for each of the steps in the graded volume expansion and the average of these values was defined as the CPBR of the animal.

Statistical Analysis
Data are reported as mean±SEM. Statistical analysis was performed by 2-way ANOVA. A multiple-comparison Bonferroni test or the unpaired Student’s t test was used when appropriate. Differences were considered to be significant at P<0.05.

Results
All rats given STZ (DG) developed severe significant hyperglycemia (22±0.7 versus 7±0.2 mmol/L in the CG) (P<0.05). The body weight was significantly lower in the DG compared with the CG (226±0.7 versus 237±0.9 g, P<0.05). The DG showed a significant increase in the cardiac weight index (3.4±0.14 versus 2.6±0.03 mg/g for the CG) (P<0.05). No differences were found in absolute heart weight of the DG (1±0.09 g) compared with the CG (1±0.03 g).

Protocol 1: Bezold-Jarish Reflex
At baseline, the resting MAP was similar in both the CG (113±4 mm Hg) and the DG (104±4 mm Hg), but the HR was significantly reduced in the DG (294±14 versus 350±10 bpm for the CG) (P<0.05).

Serotonin induced a significant bradycardia and hypotension that were proportional to the doses injected in both groups (Figure 1). The bradycardic response to the first dose of serotonin (2 μg/kg) was higher in the DG compared with the CG (13±2% versus 6±0.8%, respectively) (P<0.05). For the doses of serotonin of 4, 8, and 16 μg/kg, the decrease in
Mean Arterial Pressure, Heart Rate, and Renal Sympathetic Nerve Activity in Control and Diabetic Rats Before and After Sinoaortic Denervation

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Before SAD</th>
<th>After SAD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MAP, mm Hg</td>
<td>129±1</td>
<td>161±8*</td>
</tr>
<tr>
<td>HR, bpm</td>
<td>373±34</td>
<td>423±10</td>
</tr>
<tr>
<td>RSNA, %</td>
<td>50±13</td>
<td>63±2</td>
</tr>
<tr>
<td>Diabetic group</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MAP, mm Hg</td>
<td>121±2</td>
<td>151±7*</td>
</tr>
<tr>
<td>HR, bpm</td>
<td>292±9†</td>
<td>330±19†</td>
</tr>
<tr>
<td>RSNA, %</td>
<td>36±2</td>
<td>47±4*</td>
</tr>
</tbody>
</table>

Values are expressed as mean±SEM.
*P<0.05 before vs after SAD; †control group vs diabetic group.

HR was similar in both groups (CG 12±4.5%, 69±4.9%, and 79±5.3% versus DG 27±7.8%, 61±3.5%, and 79±4.2%).

The hypotension response to serotonin (decrease in MAP) was lower at the first dose (2 μg/kg) in the DG compared with that in the CG (5±0.7% versus 10±1.2%, respectively). For the other doses of serotonin, 4, 8, and 16 μg/kg, the decreases in MAP were similar in both groups (CG 8±0.8%, 24±1.6%, and 29±4.5% versus DG 8±2.1%, 21±2.1%, and 33±6.3%).

Protocol 2: Volume Expansion

Effect of SAD on MAP, HR, and RSNA

The anesthesia per se caused a small, similar increase in MAP but not in HR in both groups. Before SAD, MAP was similar in the 2 groups (129±1 versus 121±2 mm Hg in the CG and DG, respectively). The HR in the DG (292±9 bpm) was significantly lower than that of the CG (373±4 bpm) (P<0.05). RSNA was 50±13% in the CG and 36±1.9% in the DG (Table).

After SAD, MAP showed a significant and similar increase in both groups (CG 161±8 and DG 151±7 mm Hg). The HR had no significant increase in both groups but compared with the CG (423±10 bpm), the HR of the DG was lower (330±19 bpm) (P<0.05). The RSNA was 63±4% and 47±4% in control and diabetic rats, respectively.

Responses to Volume Expansion on Cardiovascular Variables

Volume expansion increased LVEDP to a similar level in both groups (4.5±0.7 versus 6±1 mm Hg in the DG). The MAP showed significant decreases that were proportional to the volume infused in both groups (Figure 2A). However, the decreases observed in the MAP of the DG (3±9%, 6±3%, 22±3%, and 19±8%) were significantly smaller than those of the CG (10±12%, 23±3%, 41±9%, and 30±4%) (P<0.05). Also, the HR responses were different between the groups (Figure 2B). The HR decreases observed in the DG (31±21%, 20±12%, 47±31%, and 23±9%) were significantly blunted compared with the CG (78±59%, 114±47%, 101±59%, and 112±54% (P<0.05).

The RSNA response was completely different between the groups. The volume infusion caused reflex decreases in RSNA in the CG proportional to the volume applied but did not change the RSNA in the DG (Figure 2C). Accordingly, the calculated CPBR gain in the CG was −14±4% RSNA/mm Hg and only 1.9±0.8% RSNA/mm Hg in the DG.

Discussion

As previously demonstrated,6,10 STZ-induced diabetes was associated with a decrease in the body weight, in the resting

![Figure 2](http://hyper.ahajournals.org/)

Figure 2. Responses to acute volume expansion by 6% dextran (0.5, 1, 2, and 3 mL) in the CG (n=6) and the DG (n=8) A, Hypotension; B, bradycardia; C, RSNA; and D, CPBR. Values are reported as mean±SEM (*P<0.05, CG vs DG).
arterial blood pressure, and in the HR of conscious rats. The cardiac weight index of the diabetic rats was larger, probably because of the loss of body weight after STZ treatment rather than because of changes in heart morphology.

There are many studies in the literature indicating that the vagal cardiopulmonary receptors play a major role in the control of sympathetic outflow to renal and splanchic vascular beds.\(^{18-20}\) Stimulation of either mechanosensitive or chemosensitive receptors elicits reflex inhibition of sympathetic nerve activity. This reflex sympathoinhibition has been observed with nonselective stimuli such as volume loading and chemical administration\(^{21-23}\) and with different stimuli that are selective for ventricular receptors.\(^{24,25}\) The control rats in the current study had the expected response to stimulation of cardiopulmonary receptors in both protocols. However, the diabetic rats behaved differently in the 2 protocols: (1) preserved MAP and HR reflex responses to serotonin administration and (2) blunted MAP, HR, and RSNA responses to volume expansion.

**Protocol 1: Bezold-Jarish Reflex**
The stimulation of the cardiopulmonary receptors by injection of serotonin caused a similar bradycardia and hypotension (Bezold-Jarish effect) in both the CG and DG, except for the first lower dose (2 \( \mu \text{g/kg} \)). With this dose, the bradycardia was larger and the hypotensive response was less intense in the DG compared with the CG. Therefore the lower hypotensive response in STZ-diabetic rats suggests a lower withdrawal of peripheral sympathetic activity in the DG. On the other hand, it is possible that an attenuation of cardiovascular reflexes caused by subthreshold stimulation of serotonin 5-HT3 receptors have occurred in controls, contributing to the difference observed between diabetics and controls. It was demonstrated that subthreshold stimulation of cardiopulmonary receptors by the 5-HT3 receptor agonist phenylbiguanide may attenuate cardiovascular reflexes.\(^{26}\) The cardiovascular responses to stimulation of chemosensitive cardiac receptors were grossly preserved in diabetic rats. The interpretation of these results needs some considerations: (1) most ventricular afferents exhibit some degree of both mechanosensitivity and chemosensitivity. Coleridge and Coleridge\(^{27}\) consider that there is a spectrum of activity from receptors ranging from those that are strongly mechanosensitive and have a cardiac rhythm at normal levels of pressure to those that have no cardiac rhythm even at high pressure but can be stimulated chemically. Although there may be a spectrum of sensitivities, it was demonstrated that most ventricular receptors increase their activity in response to at least 1 chemical stimuli.\(^{28}\) (2) On the other hand, serotonin injected intravenously could have central effects modulating the CPBR. Although serotonin does not cross the blood-brain barrier,\(^{29}\) it was well demonstrated that local application on the nucleus tractus solitarii alters baroreflex integration.\(^{30}\) (3) The arterial baroreflex in this protocol was preserved and could have a participation in the cardiovascular response because there is redundancy in the projection of afferent inputs to the central nervous system.\(^{31}\) Therefore, by using a potent stimulus such as serotonin injection, we demonstrated that the cardiopulmonary reflex arc is maintained in diabetic rats.

**Protocol 2: Volume Expansion**
It is established that volume expansion causes reflex sympathoinhibition (bradycardia and vasodilatation) mediated by vagal cardiopulmonary receptors in normal rats. The stimulation of cardiopulmonary receptors results in decreases in renal vascular resistance and in efferent renal nerve activity.\(^{25}\) However, volume expansion is also a stimulus to different cardiac receptors and to arterial baroreceptors and chemoreceptors.\(^{32}\) Therefore the SAD was performed to exclude any possible influence of baroreflex on the cardiovascular system during volume expansion.\(^{15}\) Indeed, Minisi and Thames\(^{33}\) showed that impaired responses of infarcted dogs to changes in filling pressure were apparent only after denervation of the sinoaortic baroreceptors. As previously demonstrated in our laboratory, the SAD was associated with an acute increase in ABP, HR, and RSNA.\(^{16}\) In the current study, both groups demonstrated a similar increase in these variables. It should be stressed that volume expansion increased LVEDP to a similar level in both groups. However, the DG had a significantly lower response of MAP and HR and a completely abolished RSNA response. In the study of Patel and Zhang,\(^{34}\) the RSNA inhibition in diabetic rats was lower than that observed in controls. In the current experiment, the volume expansion did not modify resting RSNA. The differences between those and our results may be due to different methodological approaches. In the study by Patel and Zhang, not only were the rats not sinoaortically denervated, but the protocol to produce volume expansion was longer lasting (40 minutes).

Our results indicate that with a stimulus that selectively activates the cardiopulmonary receptors (in a range that can be considered physiological), an impairment of CPBR can be demonstrated. These data in diabetic rats are in accordance with previous clinical and experimental studies in the literature that showed an important attenuation in cardiovascular reflexes in diabetes.\(^ {1,6,7,9,10}\)

The physiopathological relevance of the reflex RSNA impairment in diabetic rats may be associated with the role of the kidneys in the salt and water balance (by direct modulation on sodium excretion)\(^ {35}\) and in renin secretion.\(^ {31}\) Clinical studies demonstrate that sodium retention occurs in subjects with diabetes with short duration\(^ {36}\) and during volume expansion induced by water immersion.\(^ {37}\) Similar reduction in sodium excretion as well as reduced renal sympathoinhibition to a volume expansion were observed in STZ-diabetes.\(^ {34}\) Indeed, it was demonstrated that STZ-diabetic rats (2 weeks) have elevated plasma levels of angiotensin II under basal conditions.\(^ {38}\) It was described that the angiotensin II system is involved with the increase of sympathetic outflow by its action on the central nervous system. It is possible that the larger increase in AP observed in our study after SAD in the diabetic rats is associated with an increased baseline-activated renin-angiotensin system. The finding that renal denervation decreases tubular glucose reabsorption (20% to 25%), leading to an increase in glucose urinary excretion,\(^ {39}\) indicates that RSNA modulates the glucose tubular transport.

In the current study we were not able to determine the site in the cardiopulmonary reflex arc that was responsible for the impairment in the cardiopulmonary reflex. It is well estab-
lished that diabetes may alter the nerves of the somatic and autonomic nervous systems. These changes depend on the time course of the physiopathological process and are associated with metabolic disorders such as hyperglycemia or insulinopenia. We can speculate that there is a diffuse involvement of the afferent and efferent pathways related to the same damage typically observed in diabetes. The blunted response of RSNA to acute expansion of volume may be related to hyperglycemia or to reduced plasma insulin; it has been demonstrated that insulin treatment reverses or improves not only glucose homeostasis but also cardiovascular function in STZ-diabetic rats.

In conclusion, the results of the current study clearly indicate that STZ-induced diabetic rats had an impairment of CPBR when evaluated by reduced MAP and HR response and an abolished RSNA response during acute volume expansion. The normal MAP and HR responses to the injection of serotonin indicated that the CPBR arc is still preserved in diabetic rats. The physiological importance of these differential responses may be related to the modulation of RSNA by the cardiopulmonary receptor impairing the buffering system that regulates pressor stimuli of volume management in diabetes.

References


Cardiopulmonary Reflex Impairment in Experimental Diabetes in Rats
Vera Lucia Longo Oliveira, Edson Dias Moreira, Vera de Moura Azevedo Farah, Fernanda Consolim-Colombo, Eduardo Moacyr Krieger and Maria Claudia Irigoyen

Hypertension. 1999;34:813-817
doi: 10.1161/01.HYP.34.4.813

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/34/4/813

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at:
http://hyper.ahajournals.org/subscriptions/