CALL FOR PAPERS | Fetal Physiological Programming

Increased blood pressure in the offspring of diabetic mothers

Rogerio B. Wichi, 1,2 Silvia B. Souza, 1,2 Dulce E. Casarini, 1 Mariana Morris, 3 Maria Luiza Barreto-Chaves, 4 and Maria Claudia Irigoyen 4
1Nephrology Department, Federal University of São Paulo-UNIFESP, São Paulo, Brazil; 2Hypertension Unit and Heart Institute of Medical School-University of São Paulo, São Paulo, Brazil; 3Wright State University School of Medicine, Department of Pharmacology & Toxicology, Dayton, Ohio; and 4University of São Paulo, Department of Anatomy, São Paulo, Brazil

Increased blood pressure in the offspring of diabetic mothers. Am J Physiol Regul Integr Comp Physiol 288: R1129–R1133, 2005. First published January 20, 2005; doi:10.1152/ajpregu.00366.2004.—Studies were conducted in rats to determine the effect of maternal diabetes and the consequent hyperglycemia on cardiovascular function in the offspring. Diabetes was induced in pregnant Wistar rats through streptozotocin injection (50 mg/kg). Cardiovascular parameters were measured in 2-mo-old offspring animals of diabetic (OD, n = 12) and control rats (OC, n = 8). Arterial pressure (AP), heart rate (HR), baroreflex sensitivity, and vascular responsiveness to phenylephrine (PH) and sodium nitroprusside (SN) were measured. Angiotensin-converting enzyme (ACE) activity in heart, kidney, and lung was determined. OD rats exhibited increases in systolic AP (138 ± 8 vs. 119 ± 6 mmHg, OD vs. OC), with no change in HR (342 ± 21 vs. 364 ± 39 beats per minute (bpm), OD vs. OC). The reflex tachycardia elicited by SN was reduced in OD rats, as indicated by the slope of the linear regression (−2.2 ± 0.4 vs. −3.6 ± 0.8 bpm/mmHg, OD vs. OC). Vascular responsiveness to PH was increased 63% in OD rats compared with OC. OD rats showed increases in ACE activity in heart, kidney, and lung (1.13 ± 0.24, 3.04 ± 0.86, 40.8 ± 8.9 vs. 0.73 ± 0.19, 1.7 ± 0.45, 28.1 ± 6 nmol His-Leu·min−1·mg protein−1, OD vs. OC). Results suggest that diabetes during pregnancy affects cardiovascular function in offspring, seen as hypertension, baroreflex dysfunction, and activation of tissue renin-angiotensin system.

EVIDENCE SUGGESTS THAT THE mother’s lifestyle may have long-lasting effects on offspring. For example, smoking, alcohol consumption, and presence of disease states such as diabetes and hypertension during pregnancy all affect fetal development (8, 27). Alterations in fetal development could contribute to pathologies in the adult. With regard to diabetes, even when treated, the maternal disease has profound consequences on the fetus, seen as changes in brain, pancreas, lung, kidney, and heart (2, 14, 21, 44).

To study diabetes in the experimental setting, chemical toxins have been widely used. Streptozotocin (STZ) is a pancreatic toxin that induces hyperglycemia, hypoinsulinemia, polyuria, and weight loss in rats (9, 28, 38, 45). When STZ-induced hyperglycemia is present during pregnancy, there are changes in fetal development and metabolism, including growth restriction and abnormal pancreatic function (5, 22, 36). Epidemiological and animal studies have shown that low birth weight increases the incidence of cardiovascular disease and diabetes mellitus in adulthood (3). Intraternal growth restriction produced by placental insufficiency in pregnant rats was associated with marked elevation in blood pressure of the offspring (1). Adult offspring of STZ-diabetic rats also showed signs of cardiovascular dysfunction, seen as a reduced response to endothelium-dependent vasodilators and enhanced norepinephrine-induced vasoconstriction (23).

There is evidence for an association between the development of diabetes and the activation of the renin-angiotensin system (RAS). Experimental (18, 30, 39) and clinical studies (7, 17, 19, 42) have demonstrated that angiotensin-converting enzyme (ACE) inhibitors and ANG II receptor type 1 (AT1) antagonists may be used for treatment and may retard the development of cardiac disease in diabetes mellitus. A large clinical trial showed that long-term treatment with losartan lowers the risk of developing diabetes (33). In animal studies, evidence exists that ACE levels are increased in diabetic models and that angiotensin-converting enzyme inhibitor (ACEI) and angiotensin receptor blockers (ARBs) lower blood pressure and improve kidney function. Using genetically modified mice, Huang et al. (26) showed that interactions occur between ACE and the pathological consequences of renal function and blood pressure. A general role for RAS in diabetes-induced hypertension is supported by the depressor effects of ACEI and ARB in rats (25, 29). However, less information is available on the developmental effects of maternal diabetes, in particular, its relation to the RAS.

Experiments were conducted to explore the mechanisms by which maternal diabetes in rats affects the cardiovascular system in the offspring. Using STZ-diabetic rats, we measured metabolic and cardiovascular parameters and tissue ACE levels in the male offspring of diabetic mothers.

MATERIALS AND METHODS

Animals and diabetes induction. Male and female Wistar rats were obtained from a breeding colony at the University of São Paulo (São...
R1130  HYPERTENSION IN OFFSPRING OF DIABETIC RATS

Paulo, Brazil). The animals had free access to food and water and were housed in temperature-controlled rooms (22°C) under a 12:12-h light-dark cycle. The offspring used in the study (n = 20) were produced from diabetic (n = 4) or control (n = 2) pregnant female rats. The Experimental Animal Use Committee of the University of São Paulo approved all animal protocols.

Female and male Wistar rats (250–300 g) were mated. On the 7th day of pregnancy, rats were fasted for 6 hr, and STZ (50 mg/kg, 10 mM citrate buffer, pH 4.5) was injected into the tail vein. Blood was collected from a tail cut on days 14 and 21 of gestation. Blood glucose was measured using the ACCU-CHEK system (Roche Diagnostics, Mannheim, Germany), which is based on bioamperometry. Results confirmed a hyperglycemia, blood glucose levels between 250 and 400 mg/dl. Pregnant controls were injected with a citrate buffer. After parturition, blood glucose and body weight were measured in diabetic offspring (OD; n = 12, 3 animals from each diabetic pregnant rat) and control rats (OC, n = 8; 4 animals from each control pregnant rat) on postnatal days 3, 15, 30, and 60.

Cardiovascular measurements. At ~60 days of age, male offspring from diabetic and control mothers were anesthetized (ketamine and xylazine 80/40 mg/kg ip), and carotid artery and jugular vein catheters were inserted for direct measurements of arterial pressure (AP) and drug administration, respectively. All cardiovascular measurements, including baroreflex testing, were performed in conscious, freely moving rats (1 day after surgery). The arterial cannula was connected to a transducer (Narco Bio-Systems Miniature Pressure Transducer RP 1500, Austin, TX), and pressure signals were recorded for a 20-min period using a microcomputer equipped with an analog-to-digital converter (CODAS, 2-kHz sampling frequency, Dataq Instruments, Akron, OH). To assess baroreflex control of heart rate (HR) and vascular responsiveness, phenylephrine (PH; 0.5 to 8.0 µg/kg) or sodium nitroprusside (SN; 5 to 80 µg/kg) was injected into the venous catheter. The drugs were given intravenously as bolus injections (0.1 ml), resulting in pressor responses from 10 to 40 mmHg. Subsequent injections were given only after HR and AP had returned to basal levels. The data were analyzed on a beat-to-beat basis to quantify changes in AP and HR. Baroreflex sensitivity was reported as values derived from the slope of the linear regression line for each animal, and the relationship between change in HR and AP was shown. Vascular responsiveness to constriction and dilation were evaluated by the maximum change in AP for each dose of PH and SN, respectively.

Measurement of ACE activity. ACE activity was determined in neonate (3 day) and adult (60 day) rats using the fluorimetric assay described by Oliveira et al. (37). Heart, kidney, and lung were quickly harvested, rinsed, blotted and homogenized in 0.4 M sodium borate buffer (BB), pH 7.2. Supernatants from homogenized tissues (20 µl) were incubated with 490 or 480 µl of assay buffer containing 5 mM Hip-His-Leu in 0.4 M sodium BB and 0.9 M NaCl, pH 8.3 for 15 or 30 min at 37°C. The reaction was stopped by the addition of 1.2 ml of 0.34 M NaOH. The product, His-Leu, was measured fluorimetrically at 365-nm excitation and 495-nm emission with a fluorescence spectrometer (Shimadzu, RF 1501, Japan). γ-phthalaldehyde (100 µl, 20 mg/ml) in methanol was added, and after 10 min, the solution was acidified with 200 µl 3 N HCl and centrifuged in at 3,000 rpm for 10 min at room temperature. To correct for the intrinsic fluorescence of the tissues, time zero blanks were prepared by adding tissue after NaOH. All samples were assayed in duplicate. The sensitivity of the assay is less than or equal to 0.02 nmol·mg tissue·min⁻¹, the fluorescence intensity is linear with the concentration of His-Leu generated from 0.02 to 15 nmol·mg⁻¹·min⁻¹. The results are expressed as nmol His-Leu·min⁻¹·mg⁻¹ of protein. Protein was measured with Bradford’s method (4) using bovine serum albumin as the standard.

Statistical analyses. Data are expressed as means ± SD. A Student’s unpaired t-test was used to compare the two groups. ANOVA for repeated measures was used for multiple times or drug doses followed by Tukey’s post hoc comparison. Significance level was established at P < 0.05.

RESULTS

STZ treatment on the 7th day of pregnancy, induced hyperglycemia at 14 and 21 days of gestation (313 ± 26 and 347 ± 58 mg/dl) compared with controls (114 ± 9 mg/dl) (Table 1). Diabetic dams gave birth to fewer pups than the controls (6 ± 3 vs. 11 ± 2 pups/litter; P < 0.05). ANOVA analysis showed an overall difference in body weight between the groups with a decrease seen only at 30 days of age (Table 1). Blood glucose levels were changed in OD rats. There was a reduction of ~50% at 3 days and an increase of 23% at 15 days (Table 1). The blood glucose level was not different between the groups at 30 and 60 days of age.

Cardiovascular parameters were evaluated in adult offspring rats (>60 days). Systolic and mean arterial pressure (MAP) were increased in the diabetic group (Table 2) with no change in HR. The reflex tachycardia elicited by SN was reduced in diabetic offspring, as indicated by the slope of the linear regression relating changes in HR to changes in MAP (~2.2 ± 0.4 vs. ~3.6 ± 0.8 bpm/mmHg in OC group). The bradycardia elicited by phenylephrine remained unchanged (Fig. 1).

Vascular responsiveness was evaluated by the AP responses to SN and PH (Fig. 2). SN induced similar reductions in AP in both groups. The pressor response to PH was greater in OD rats than in controls.

ACE activity was evaluated in heart, lung, and kidney in neonates (3 days) and adults (60 days) (Table 3). There were no group changes in neonates, whereas in adult OD rats, there were increases in all tissues compared with controls. There was a significant correlation between systolic arterial pressure (SAP) and lung ACE in adult rats (linear regression slope = 0.65, r = 0.86 and r² = 0.75). There were also age-related changes, seen as lower ACE in heart and higher ACE in lungs in adults compared with neonates.

DISCUSSION

Results show that hyperglycemia during pregnancy produces long-lasting hypertension in the male offspring associated with changes in plasma glucose and tissue ACE activity. These data emphasize that metabolic changes in the mother may produce severe consequences for the developing fetus, leading to long-term changes in the adult.

Hyperglycemia is known to be a pathogenic factor in the long-term complications of diabetes mellitus. Even during fetal life, high glucose poses a risk. For example, Dabelea et al. (8) demonstrated a higher prevalence of diabetes in offspring of women with hyperglycemia during pregnancy. The effect of glucose per se was evaluated in an animal study in which

<table>
<thead>
<tr>
<th>Days</th>
<th>Body Weight, gm</th>
<th>Blood Glucose, mg/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OC</td>
<td>OD</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>80</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>OC</th>
<th>OD</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.6±0.6</td>
<td>5.7±0.09</td>
<td>61±6</td>
</tr>
<tr>
<td>22.8±0.8</td>
<td>16.5±1.3</td>
<td>56±5</td>
</tr>
<tr>
<td>81±3</td>
<td>65±5*</td>
<td>86±10</td>
</tr>
<tr>
<td>231±9</td>
<td>208±17</td>
<td>44±5</td>
</tr>
</tbody>
</table>

Data are reported as means ± SD of offspring control (OC, n = 8) and offspring diabetic rats (OD, n = 12). *P < 0.05 compared with the OC group to ANOVA for multiple groups, followed by a Tukey post hoc comparison.
Table 2. Resting heart rate, diastolic, mean, and systolic arterial pressure of control and offspring diabetic rat

<table>
<thead>
<tr>
<th></th>
<th>HR, beats/min</th>
<th>DAP, mm Hg</th>
<th>MAP, mm Hg</th>
<th>SAP, mm Hg</th>
</tr>
</thead>
<tbody>
<tr>
<td>OC</td>
<td>342±23</td>
<td>93±11</td>
<td>107±7</td>
<td>120±6</td>
</tr>
<tr>
<td>OD</td>
<td>375±60</td>
<td>100±9</td>
<td>119±9*</td>
<td>138±9*</td>
</tr>
</tbody>
</table>

Data are reported as mean ± SD for offspring control (OC, n = 8) and offspring diabetic (OD, n = 12). HR, heart rate; DAP, diastolic arterial pressure; MAP, mean arterial pressure; SAP, systolic arterial pressure. *P < 0.05 vs. OC, using Student’s unpaired t-test.

glucose was infused during the last week of pregnancy (16). The results showed evidence for glucose intolerance and impaired insulin secretion in the rat offspring of the dams treated with glucose. This is consistent with data in the STZ model in our study and a previous publication (20). Hyperglycemia in pregnancy resulted in insulin resistance, changes in glucose metabolism, and vascular dysfunction in the diabetic offspring.

In vitro studies of ventricular myocytes showed that hyperglycemic media impaired relaxation (10). Mesangial cell exposure to high glucose resulted in significant increases in intracellular renin activity and ANG II generation (47). Thus much evidence supports a role for hyperglycemia in the development of cardiovascular changes and detrimental complications.

Cardiovascular pathologies in adult life are associated with low birth weight and nutritional and metabolic status. Barker et al. (3) reported that men and women with the lowest birth weight had the highest blood pressure as adults. Protein restriction during pregnancy in rats induces fuel consumption and hypertension in the adult offspring (24). Canavan et al. (5) demonstrated that diabetic pregnancy was associated with suppression in fetal growth, related to reduced protein synthesis. Our results showed that the body weight of diabetic offspring was not reduced at birth or in neonates. However, at 30 days of life, body weight was significantly lower in the diabetic offspring, suggesting that the diabetic fetal environment compromised development.

This is the first report to show sustained hypertension in the offspring of diabetic rats. In adult rats (2 mo age), MAP and SAP were increased by 12 and 18 mmHg, respectively. Using a similar protocol, Holemans et al. (23) observed a decrease in

Table 3. Angiotensin-converting enzyme activity of kidney, lung, and heart of neonatal and adults control and offspring diabetic rats

<table>
<thead>
<tr>
<th></th>
<th>ACE activity, nmol His-Leu/min·mg protein·cell protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kidney</td>
<td></td>
</tr>
<tr>
<td>Neonatal</td>
<td>OC: 2.36±0.34</td>
</tr>
<tr>
<td></td>
<td>OD: 2.76±0.48</td>
</tr>
<tr>
<td>Adult</td>
<td>OC: 1.77±0.45</td>
</tr>
<tr>
<td></td>
<td>OD: 3.04±0.86</td>
</tr>
</tbody>
</table>

Data are reported as means ± SD to offspring control (OC, n = 6) and offspring diabetic rats (OD, n = 6). ACE, angiotensin-converting enzyme. *P < 0.05 compared with OC group and †P < 0.05 compared with the neonatal group to ANOVA for multiple groups, followed by a Tukey post hoc comparison.

This is the first report to show sustained hypertension in the offspring of diabetic rats. In adult rats (2 mo age), MAP and SAP were increased by 12 and 18 mmHg, respectively. Using a similar protocol, Holemans et al. (23) observed a decrease in

Fig. 1. Baroreflex sensitivity as measured by dose-response of HR to depressor (nitroprusside) and pressor (phenylephrine) stimuli. Graph shows the regression analyses for the tachycardia and bradycardia responses in the control offspring (OC, n = 8) and the diabetic offspring rat (OD, n = 12). Data are reported as mean ± SD. *P < 0.05 for slope vs. OC group, using the Student’s unpaired t-test.

Fig. 2. Vascular responsiveness to sodium nitroprusside (A) and phenylephrine (B) in offspring control (OC, ■, n = 8) and offspring diabetic rats (OD, ▲, n = 12). Data are reported as mean ± SD. *P < 0.05 compared with the OC group to ANOVA for multiple groups, followed by a Tukey post hoc comparison.
heart rate, but no change in blood pressure in female STZ-diabetic rat offspring. However, this study was complicated by the method used for cardiovascular recording. The measurements were made in conscious rats, but on the same day as the surgical procedures. Furthermore, blood pressure levels were high in both controls and diabetic offspring, with a SAP of more than 170 mmHg. This is compared with control levels of 120 mmHg in our study, consistent with normal, nonstress levels. Studies in humans also suggest a relationship between maternal diabetes and hypertension in the offspring (6, 41), similar to what is observed in our study.

It is also important to note that the offspring of diabetic rats showed changes in baroreflex sensitivity and vascular responsiveness to vasoactive drugs. Indeed, baroreflex dysfunction has been observed in some complex diseases such as hypertension and diabetes. Mancia et al. (35) observed depressed function in baroreceptors from hypertensive humans. Maeda et al. (34) demonstrated impairment of tachycardic and bradycardic response in STZ-diabetic rats. The present study demonstrated a reduced reflex tachycardic response in offspring of diabetic rats. This impairment of baroreceptor function may contribute to the hypertensive state in adult animals. Considering that hypertension is usually associated with sympathetic overactivity, the reduced reflex tachycardia may be due to a lower sympathetic reserve. The vagal withdrawal seems to be normal because there were no changes in the bradycardia response induced by increasing arterial pressure. These changes in the baroreflex function may also be occurring at other sites of the reflex arc, as in the baroreceptor endings or the central nervous system (9). In fact, the demonstration of increasing insulin binding in the nucleus of the tractus solitarius observed in the adult rats from hyperglycemic mothers, indicates that central control of cardiovascular centers could be involved in the baroreflex impairment in the present study (32). In addition, PH produced a greater maximal vasoconstrictor response, while the vasodilator response to SN was not different. Data from in vitro studies using mesenteric arteries from offspring from diabetic dams also showed an enhanced constriction in response to norepinephrine (23). The lack of change in vasodilatation is consistent with the study of Holemans et al. (23). However, in this study, the offspring of diabetic rats had a reduction in relaxation after ACh and bradykinin stimulation, suggesting impaired synthesis of endothelium-derived vasodilators, like prostacyclin and nitric oxide synthesis. Similar changes were observed in arteries of STZ-diabetic rats, in which enhanced vascular sensitivity to norepinephrine and an impairment in ACh-induced vasodilatation were demonstrated (43). Furthermore, saturated fat fed during the pregnancy in diabetic rats induces impairment in endothelium-dependent dilation and enhanced constrictor responses to norepinephrine in femoral arteries in 15-day-old diabetic offspring (31). All these data suggest that in utero related to the maternal condition may induce vascular disease in later life. The progress of cardiovascular disease may be correlated with smooth muscle and endothelium dysfunction through metabolic (insulin resistance, hyperglycemia, and hypercholesterolemia), hormones (renin angiotensin, catecholamine), and neural changes (sympathetic and parasympathetic) (15). Consequently, vascular sensitivity enhanced by vasoconstrictor agents may be contributing to the increase in blood pressure in offspring of diabetic rats.

Tissue ACE activity was measured to provide information on the relationship between diabetes and the RAS. All components of RAS have been identified in the heart, kidney, liver, and lung (12, 13). Results showed that ACE activity was enhanced in the heart, kidney, and lung of the offspring of diabetic rats. There were also global, age-related decreases in ACE, consistent with results in humans and other animal species (11). Although circulating ACE is critical in the formation of ANG II and blood pressure regulation, there is less information on the tissue ACE systems. Some evidence showed the positive relationship between the development of hypertension and activity of local ACE in tissues. For example, Sharifi et al. (40) demonstrated increased ACE activity in kidney, heart, lung and aorta during the development of two-kidney, one-clipped hypertension in rats, suggesting an important role of local ACE in the development of hypertension. Furthermore, STZ-diabetic rats showed enhanced blood pressure accompanied by increases in serum ACE activity (46). Huang et al. observed that diabetes produced an increase in blood pressure, specifically, in mice with genetically enhanced ACE activity (26). Our results have also demonstrated a positive correlation between systolic arterial pressure and lung ACE. Because the lung is an important site for angiotensin metabolism, the alterations in ACE may be involved in the blood pressure changes. It would be important in future studies to measure circulating ACE activity.

In summary, our study suggests that hyperglycemia in pregnancy has long-lasting effects on fetal physiological programming, inducing cardiovascular diseases like hypertension, baroreflex dysfunction, and activation of RAS in adult life.

ACKNOWLEDGMENTS

This study was supported Fundação de Amparo à Pesquisa do Estado de São Paulo (01/07632-5 to R. Wich) Fundação Zerbini (to M. C. Irigoyen), National Institute of Health and Research-O1-HL-69319 (to M. Morris).

REFERENCES


