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Cardiovascular control in experimental diabetes

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Abstract

Several studies have reported impairment in cardiovascular function and control in diabetes. The studies cited in this review were carried out from a few days up to 3 months after streptozotocin administration and were concerned with the control of the circulation. We observed that early changes (5 days) in blood pressure control by different peripheral receptors were maintained for several months. Moreover, the impairment of reflex responses observed after baroreceptor and chemoreceptor stimulation was probably related to changes in the efferent limb of the reflex arc (sympathetic and parasympathetic), but changes also in the central nervous system could not be excluded. Changes in renal sympathetic nerve activity during volume expansion were blunted in streptozotocin-treated rats, indicating an adaptive natriuretic and diuretic response in the diabetic state. The improvement of diabetic cardiovascular dysfunction induced by exercise training seems to be related to changes in the autonomic nervous system. Complementary studies about the complex interaction between circulation control systems are clearly needed to adequately address the management of pathophysiological changes associated with diabetes.

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Introduction

Diabetes mellitus is one of the most important world health problems, especially in developing countries, where prevalence and incidence rates are highest. Diabetic patients are particularly prone to cardiovascular diseases including hypertension, atherosclerosis, diabetic cardiomyopathy, congestive heart failure, and cardiac autonomic neuropathy (1).

Autonomic neuropathy is a frequent complication of diabetes mellitus associated with high morbidity and mortality in symptomatic patients (2), which affects the autonomic modulation of the sinus node, reducing heart rate variability (HRV) (3). The increased mortality rate may be related to disorders in

cardiovascular control, including impairment of autonomic reflex control leading to orthostatic hypotension, painless myocardial infarction and sudden death (4), the last possibly determined by nocturnal desaturation episodes (5) or arrhythmias. Recently, it was demonstrated that cardiac sympathetic dysfunction in diabetes involves areas of dysinnervation (distal), as well as areas of hyperinnervation (proximal) in the left ventricle, which facilitate malignant arrhythmias by altering electrical stability and/or impairing myocardial blood flow (6).

Abnormalities in the renin-angiotensin-aldosterone-kinin cascade have been implicated in the pathogenesis and clinical expression of these cardiovascular-renal sequelae. Angiotensin II, through its effects on contractility, growth, and the sympathetic nervous system, may play a key role in this pathologic process. Angiotensin-converting enzyme inhibitors and some direct renin inhibitors prevent or reduce the progression of some of these complications (7). In fact, angiotensin-converting enzyme inhibitors have been reported to improve kidney, heart and, to a lesser extent, eye and peripheral nerve function of patients with diabetes mellitus (8).

Experimental diabetes induced by streptozotocin (STZ) has been used by several investigators to study disorders of the autonomic control of the cardiovascular system. Rats treated with STZ display many of the features seen in human subjects with uncontrolled diabetes mellitus, including hyperglycemia, hypoinsulinemia, increased urinary glucose levels and consequently polyuria, as well as weight loss (9-11).

Degenerative changes in autonomic neurons were observed from 3 days (12) to several weeks after STZ administration in rats (13). We observed that rats presented lower arterial pressure and heart rate (HR) 5 to 80 days after diabetes induction with STZ (10,14-16) (Table 1). The mechanisms involved in these alterations are not completely understood. Some investigators have associated bradycardia and hypotension with reduction in intrinsic HR, in vagal tonus and in cardiovascular reflex control (10,14-16), suggesting early autonomic dysfunction. Furthermore, impairment of heart contractility and vascular responsiveness, as well as changes in blood volume caused by osmotic diuresis could be involved in the pathogenesis of these alterations.

In this paper we review the effects of experimental diabetes on the autonomic control of HR and cardiovascular reflexes, as well as the benefits of exercise training for the control of these disorders.

Table 1. Time course of changes in sedentary STZ-induced diabetic rats.

	Control	STZ (5 days)	STZ (15 days)	STZ (80 days)
HR (bpm)	332 ± 2	291 ± 4*	296 ± 11*	279 ± 9*
MAP (mmHg)	117 ± 3	102 ± 2*	99 ± 3*	91 ± 4*
IHR (bpm)	398 ± 6	302 ± 10*	-	284 ± 11*
Serum glucose (mg/dl)	106 ± 15	306 ± 19*	447 ± 49*	479 ± 8*

Values are reported as means ± SEM.
*P<0.05 *vs* sedentary control group (ANOVA). STZ: streptozotocin, HR: heart rate, MAP: mean arterial pressure, IHR: intrinsic heart rate (data taken from Refs. 10,14,16).

Autonomic control of heart rate in experimental diabetes

Studies from our laboratory have shown reduction in vagal tonus and maintenance of sympathetic tonus to the heart evaluated by pharmacological blockade with propranolol and methylatropine, respectively (14,16) (Figure 1), as previously demonstrated by Wegner et al. (17), suggesting the presence of cardiac vagal neuropathy. Resting bradycardia in STZ-diabetic rats has been attributed to changes in the sinoatrial node with a consequent reduction in intrinsic HR (14,16, 18), although functional alterations in the cholinergic mechanism cannot be excluded as a causal factor. These data indicate that vagal function and intrinsic HR are decreased in short- and long-term STZ-induced diabetes in rats.

The bradycardic response to methacholine injection was similar in control and diabetic rats 5 days after STZ administration (14). However, in 15-day STZ-treated rats this response was higher than in normal rats (10), suggesting that the early impairment in vagal tonus may be leading to an adaptive change in muscarinic receptors. Kuntscherova and Vlk (19) found decreased acetylcholine concentrations in isolated auricles of diabetic rats and Tomlinson et al. (11) observed functional defects in cardiac cholinergic nerves in diabetic rats with vagal dysfunction. Indeed, it was demonstrated that coupling of cholinergic receptors to adenylate cyclase is altered in STZ-diabetic rats because the content of Gi proteins in cardiac tissue increased after STZ administration (20). Moreover, the interactions between the sympathetic and parasympathetic system are complex, suggesting a different vagal action at different levels of sympathetic function (21).

HRV may be reduced in diabetic autonomic neuropathy through injury to the parasympathetic fibers. Thus, HRV is a valuable index of cardiac parasympathetic nerve functional integrity (22). Evaluating rats injected with STZ 12-18 weeks before, Fazan et al. (3) observed that the standard deviation of the lengths of adjacent pulse pressures, an index of HRV, was reduced in these animals in comparison to their controls, indicating the presence of functional cardiac parasympathetic neuropathy. Since we studied the same HRV index in 45-90-day STZ-diabetic rats treated with insulin, and did not find differences between them and their controls, we suggest a definitive role of metabolic decompensation in the genesis and maintenance of these autonomic changes. Indeed, the negative correlation between HRV indices and plasma glucose ($r = -0.26$, $P = 0.012$) supports this idea (Schaan BD, Ferlin E and Irigoyen MC, unpublished data). Using biotelemetry techniques, Hicks et al. (23) also demonstrated time-dependent reductions in HRV in STZ-diabetic rats, changes which were reversed, at least in part, by insulin administration.

Functional changes in cholinergic or adrenergic mechanisms cannot exclude morphological changes at this early stage of the diabetic state, since Monckton and Pehowich (12) reported degenerative changes in autonomic neurons of STZ-diabetic rats. These investigators found changes in axons from the sympathetic paravertebral chain within 24 h of STZ injection. Schmidt et al. (13), for instance, found that mesenteric axonopathy was apparent in Sprague-Dawley rats treated with STZ 1.5 to 3 months earlier. Kriel et al. (24) showed degeneration of sympathetic neurons in Wistar rats that had been treated with STZ 1 year earlier. These changes are consistent with decreases in neuronal activ-

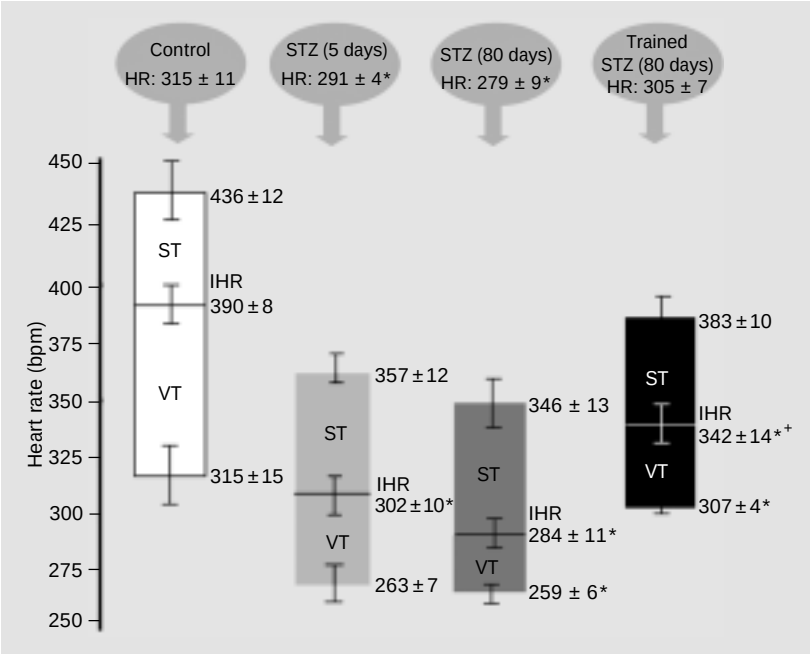

Figure 1. Intrinsic heart rate (IHR), sympathetic tonus (ST) and vagal tonus (VT) of sedentary control rats (control), 5-day-sedentary diabetic rats (STZ - 5 days), 80-day-sedentary diabetic rats (STZ - 80 days), and 80-day-trained diabetic rats (trained STZ - 80 days). IHR was evaluated after simultaneous blockade with propranolol and methylatropine. Sympathetic tonus was determined as the difference between maximum heart rate after methylatropine injection and IHR. Vagal tonus was obtained by the difference between the lowest heart rate after propranolol injection and IHR. Data are reported as means ± SEM. *$P<0.05$ compared to sedentary control rats (ANOVA); $^{+}P<0.05$ compared to 80-day-sedentary diabetic rats (Student *t*-test). HR: basal heart rate, STZ: streptozotocin (data taken from Refs. 14,16).

ity. However, Felten et al. (25) found no change in the pattern of noradrenergic innervation in the hearts of rats with 8 months of STZ-induced diabetes. Sharma and Thomas (26) also could not demonstrate morphological changes in the peripheral nerves of this animal model even after extended periods of diabetes. Probably the pattern of development of autonomic neuropathy depends on the rat strain used, animal age when STZ was given, the use or not of insulin during the experiments, etc. Finally, it cannot be ruled out that STZ *per se* is directly toxic to the nervous system, since it is certainly toxic to pancreatic beta cells (27) and the lesions described in the study of Monckton and Pehowich (12) occurred so soon after STZ use (24 h) that they could not have been determined by diabetes itself.

On the other hand, biochemical changes have been frequently described in STZ-diabetic rats. Schmidt and Plurad (28) found a reduction in dopamine ß-hydroxylase activity in the superior cervical ganglia from STZ-treated rats, although the activity of this enzyme was unaltered in the superior mesenteric ganglia. However, Jobidon et al. (29) found no change in plasma or cardiac noradrenaline levels after treatment with STZ. Obviously, tissue or plasma noradrenaline levels alone provide little information about sympathetic nervous system activity because they depend on a number of factors, including rate of synthesis, uptake, and release of noradrenaline by the tissues. Indeed, Sato et al. (30) reported a decreased contractile response to noradrenaline in left atria isolated from STZ-diabetic rats. Carrier and Aronstam (31) demonstrated that acetylcholinesterase levels were lower in right atria from STZ-diabetic rats. This might increase the effective concentrations of acetylcholine acting on myocardial receptors, therefore contributing to supersensitivity to acetylcholine. These alterations in cardiac cholinergic and noradrenergic function may be involved in the bradycardia presented by diabetic rats.

Baroreflex control in experimental diabetes

The arterial baroreflex system is one of the most influential and rapidly acting mechanisms for blood pressure control. Indeed, the minimization of blood pressure variability by the baroreflex mechanisms is important since a reduced baroreflex is an independent risk factor for sudden death after myocardial infarction (32). Diabetic patients with normal cardiovascular reflexes have a lower incidence of mortality than diabetic individuals with abnormal autonomic reflex function (2). Some studies have shown that the arterial baroreceptor reflex exerts a major influence on both the sympathetic and parasympathetic systems for cardiovascular control. Hence, the disorders in autonomic efferent and afferent neuronal systems could have important consequences for the control of cardiovascular function. Several studies using experimental models have been conducted to investigate the mechanisms of reflex dysfunction of diabetes (10,14-16,33,34).

We have demonstrated that early in the course of experimental diabetes there is an impairment of baroreflex control in STZ rats characterized by reduction of baroreflex-mediated tachycardia, while baroreflex-mediated bradycardia is still maintained (14,15). Later (15 and 30 days after STZ), the baroreflex-mediated bradycardia was also lost in diabetic rats (10), and these changes persisted even 80 days after STZ injection (35) (Figure 2). On the other hand, McDowell et al. (34) observed maintenance of the response to the increase in blood pressure induced by infusion of a vasoconstrictor agent 2 weeks after STZ treatment, but their study was conducted on rabbits. Also in a different animal model, the spontaneously diabetic rat (Bio-Breeding), Krizsan-Agbas and Buñag (36) demonstrated exacerbation of baroreflex-mediated tachycardia, while Eckberg et al. (37) reported a normal tachycardia re-

sponse to decreases of blood pressure in diabetic humans.

Different mechanisms may be acting in the baroreflex control of HR in diabetes (34,37). Moreover, the different data obtained in the cited studies may be attributed to time-dependent changes in HR control. Also, metabolic factors could contribute to the discrepant results, since our data were obtained in rats receiving no insulin 5, 15 and 80 days after STZ administration (10, 14,15,35), and other investigators observed correction of previously abnormal cardiovascular reflexes by treating diabetic rats with insulin (38).

The impaired ability to perform adequate HR regulation during changes in arterial pressure has been attributed to some alterations in cardiac parasympathetic activity (14), although changes in the receptor function or in the central mediation of the baroreceptor reflex cannot be excluded (39). The parasympathetic dysfunction could be due to alterations in cardiac muscarinic receptors (31). Williams et al. (40) reported that the density of cardiac muscarinic receptors was unaltered in STZ-diabetic rats. However, these investigators did not measure the density of atrial muscarinic receptors separately. Carrier et al. (41) demonstrated that there was no difference in muscarinic receptor density in ventricles from STZ-diabetic and age-matched control rats, but the density of muscarinic receptors was reduced in the right and left atria from diabetic rats (31).

Li et al. (42) demonstrated a higher negative chronotropic effect induced by methacholine injection in 6-week STZ-induced diabetic rats. In fact, our studies demonstrated that electric vagal stimulation, as well as myocardial muscarinic receptor stimulation by methacholine injections determined an increase in the bradycardic response of short-term STZ-induced diabetic animals, suggesting efferent pathway impairment in the reflex arc (43). The increased bradycardia induced by methacholine injection could represent hypersensitivity of receptors linked to the characteristic reduction in parasympathetic activity shown by these animals. The up-regulation of muscarinic receptors could be related to reduced vagal function, probably contributing to the reduction of the tachycardic response to arterial pressure falls as described previously in diabetic (14,15) and aged rats (44). These responses characterized by reflex parasympathetic withdrawal and cardiac sympathetic activation suggest that the interactions between the two branches of the autonomic nervous system are complex, with different degrees of modulation at different levels of activity of these systems (21).

Arterial chemoreceptors in experimental diabetes

The arterial chemoreceptors represent an

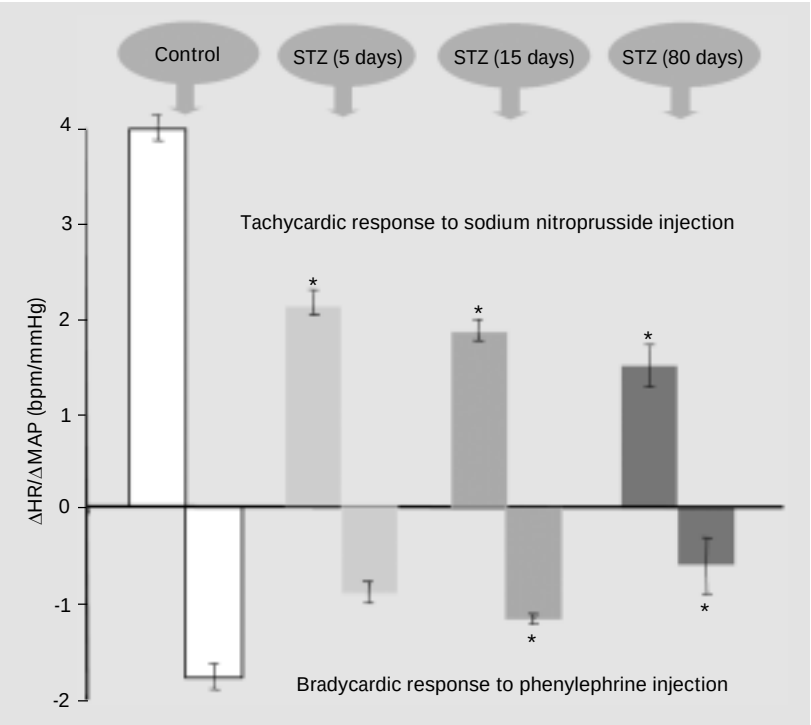

Figure 2. Tachycardic and bradycardic responses to arterial pressure decreases and increases, respectively, in sedentary control rats (control), 5-day-sedentary diabetic rats (STZ - 5 days), 15-day-sedentary diabetic rats (STZ - 15 days), and 80-day-sedentary diabetic rats (STZ - 80 days) *P<0.05 compared to sedentary control rats (ANOVA) (data taken from Refs. 10,14,39). STZ: streptozotocin, HR: heart rate, MAP: mean arterial pressure.

important group of afferences that participate in the control of autonomic function. However, they have been less studied than baroreceptors. Infusion of intravenous potassium cyanide (KCN) stimulates the parasympathetic and sympathetic chemoreceptor pathways (45). Franchini and Krieger (45) showed that stimulation of the chemoreceptors with KCN resulted in bradycardia (cardiac vagal stimulation) and hypertension (sympathetic stimulation) in rats. Using the same methodology in diabetic rats injected 15 days before with STZ, the concomitant evaluation of baro- and chemoreflexes showed that both cardiovascular responses were impaired in this model (10). Indeed, diabetes was associated with hyporesponsiveness of vagal cardiac activation evoked by the chemoreflex, as demonstrated by the reduction of the bradycardic responses produced by stimulation of the ca-

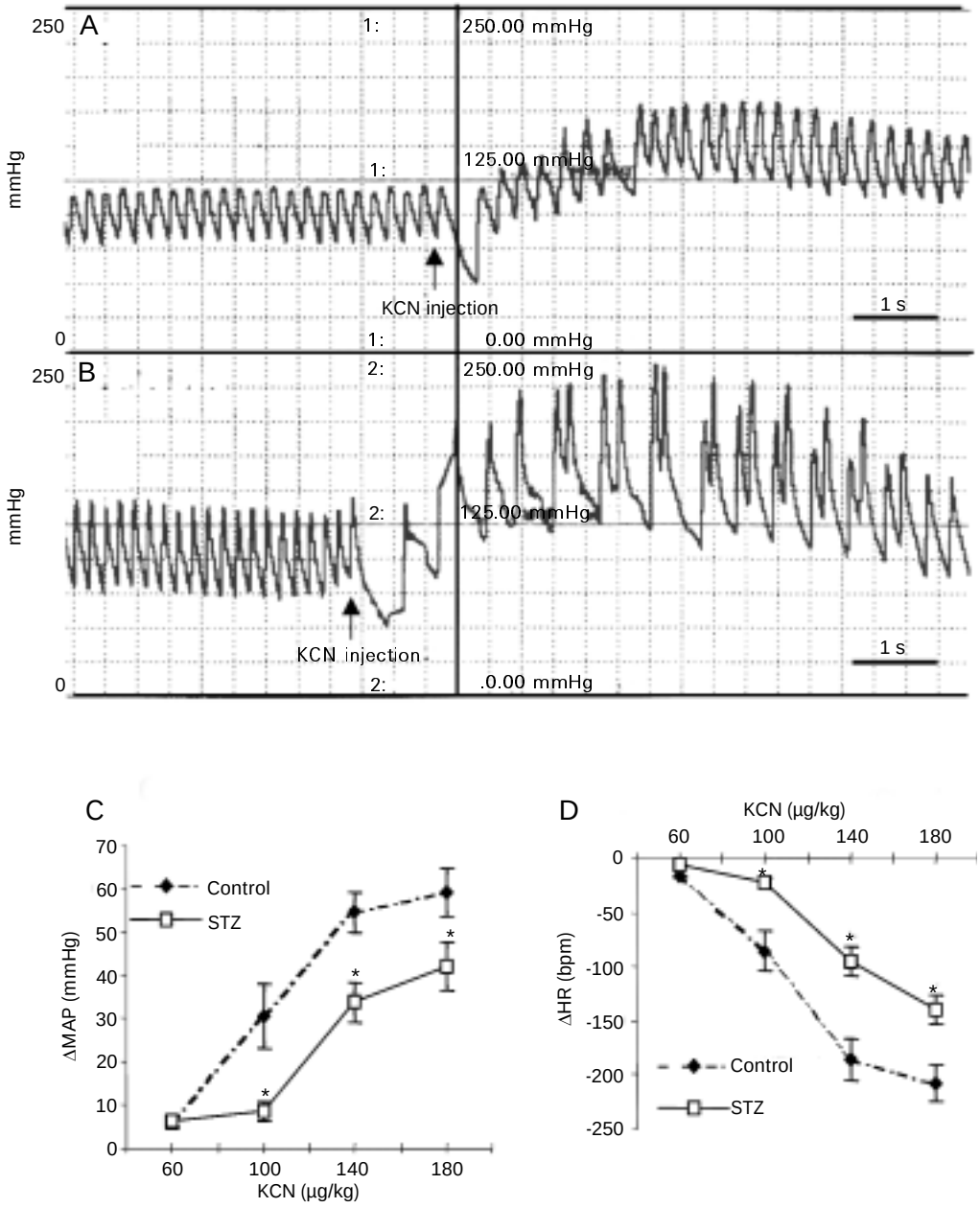

Figure 3. Arterial pressure during KCN injection into a 15-day STZ-induced diabetic rat (A) and a control rat (B). Line graphs showing the effects of STZ-induced diabetes on mean arterial pressure (MAP) responses (C) and heart rate (HR) responses (D) of control and 15-day STZ-diabetic rats to increasing doses of KCN. *P<0.05 compared to control (ANOVA) (data taken from Ref. 10). STZ: streptozotocin.

rotid body (Figure 3). At the same time the pressor response induced by vascular sympathetic activation in response to the chemoreflex stimulation was impaired in STZ-induced diabetic rats (10) (Figure 3).

The impairment of bradycardic responses elicited by chemo- or baroreceptor stimulation may indicate changes in the efferent branch of these reflexes, since pharmacological blockade showed reduced heart parasympathetic function in diabetic rats (10, 14,15). Moreover, baro- and chemoreflexes are integrated by pathways that converge to the same site in the central nervous system (46) that could be involved in the changes demonstrated in both responses in this model of diabetes. Finally, we cannot exclude that changes in the afferent limb of reflex arches may be participating in the depressed responses.

In fact, histological studies performed on spontaneously diabetic rats showed structural changes in carotid body (47) such as axonal swelling and intramyelinic edema suggesting diabetic neuropathy. These histopathological findings could be responsible for the reduced arterial chemoreceptor drive through impairment of nerve conduction. This finding supports data from our laboratory indicating a neurogenic origin of the reduction of chemoreceptor response after intravenous injection of KCN.

Cardiopulmonary reflexes in experimental diabetes

Among the different mechanisms of cardiovascular control, the cardiopulmonary reflex is activated by stimuli located in different structures of the thorax, including atria, ventricles, veins and pulmonary parenchyma, which represent an important source of information about volume and blood pressure variations in the cardiopulmonary region.

The cardiopulmonary receptors have an important participation in the homeostasis of fluid balance, both by modulating the activity of the sympathetic nervous system on the cardiovascular system, and by acting on the kidney (48-50). Intravenous infusion of chemical substances like serotonin or stimulation of cardiopulmonary receptors promoting bradycardia and hypotension are methods used to evaluate the cardiopulmonary receptors (Bezold-Jarisch reflex). The renal response is a reduction of vascular resistance and efferent sympathetic activity (50). The reflex response obtained in our laboratory after the stimulation of cardiopulmonary receptors by injecting serotonin was similar for diabetic and control rats (48), suggesting that the cardiovascular response to stimulation of chemosensitive cardiac receptors is preserved in the STZ-diabetic model. However, the reflex response induced by a similar plasma volume expansion and associated changes in left ventricle end diastolic pressure produced lower bradycardia and hypotension in diabetic than in control rats. Furthermore, the modulation of renal sympathetic activity was abolished in STZ animals. The physiological role of this altered response in diabetes could be associated with renal dysfunction in the balance between sodium and water intake and uptake, changing the natriuretic and diuretic responses in this condition. The reduction in renal sodium excretion associated with a decrease in renal sympathetic activation was also described by Patel and Zhang (49). Indeed, the examination of the various components of the volume reflex in different models of diabetic rats indicated an altered neural component associated with a humoral component of the effector limb probably related to atrial natriuretic factor (50).

Exercise training in experimental diabetes

Cardiovascular, metabolic and autonomic improvement induced by acute and chronic exercise have led many investigators to sug-

gest exercise training as an important non-pharmacological treatment for different pathologies like diabetes, hypertension and coronary artery disease (16,51-54). Exercise influences several aspects of diabetes, including blood glucose concentration, insulin action and cardiovascular risk factors (35,51). Beyond the acute impact of physical activity, long-term exercise behaviors have been repeatedly associated with decreased rates of type 2 diabetes (51,52).

In our laboratory, exercise training applied to young and aged normotensive rats and young hypertensive rats improved the autonomic control of cardiovascular function (53,55,56). Indeed, we have recently demonstrated the benefits of exercise training in diabetes-induced dysfunction (16). The hypotension presented by sedentary STZ rats was not observed in trained diabetic rats (11 weeks of training on a treadmill). This improvement could be related to an increase in cardiac output in diabetic rats (57), since Jackson and Carrier (33) have suggested previously that the decrease in arterial pressure should be the result of a decreased cardiac output in sedentary diabetic rats due to hypovolemia caused by hyperglycemic osmotic diuresis. Moreover, exercise training reverses the changes in the contractile properties of the heart induced by STZ diabetes in rats such as reduced cardiac contractility and relaxation (16,57), suggesting that the improvement in cardiac function could be due to a decrease in the severity of the diabetic state (58). Besides these changes, exercise also improves glucose homeostasis, reducing the glucose/insulin ratio and increasing insulin sensitivity (54,55). We have also demonstrated that the increase in body weight observed in trained diabetic rats seems to indicate an improvement of the metabolic state. Other changes such as reduction in ultrastructural glomerular lesions and albumin excretion observed in a rat model of type 2 diabetes mellitus submitted to aerobic training may be an improved metabolic control and delayed diabetic complications (59).

Moreover, we observed an increase in resting HR in trained diabetic rats that was correlated with changes in intrinsic HR, confirming the important role of the sinoatrial node in HR changes in experimental diabetes (16). In contrast, previous studies have demonstrated that exercise training decreases resting HR in young (55) and aged (56) normotensive rats and humans (60). The decreased intrinsic HR previously observed in our laboratory in trained control rats (55) as well as a decreased sympathetic tonus in spontaneously hypertensive rats (53) after training may be the mechanisms involved in exercise bradycardia. In STZ-induced diabetic rats we did not observe changes in sympathetic tonus between sedentary and trained groups, suggesting that the increase in resting HR in trained diabetic rats may be related to the improvement of intrinsic pacemaker regulation (16) (Figure 1). In contrast to the impairment of vagal function observed in normal rats after exercise training by the reduced bradycardia in response to electrical vagal stimulation (55), exercise training did not modify the reduced parasympathetic function observed in diabetic rats (16,17) (Figure 1). Therefore, changes in reflex control of the circulation related to parasympathetic function may persist after exercise training, as demonstrated previously in trained nitric oxide blockade hypertensive rats (54).

These data show that physical activity delays and improves the hemodynamic and metabolic dysfunction observed in diabetes, and should be considered in the prevention and treatment of this disease.

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