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Biological Response of Spontaneously Hypertensive Rats to the Streptozotocin Administration

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ABSTRACT

The sensitivity of adult spontaneously hypertensive rats (SHR) to the diabetogenic effect of streptozotocin (STZ) was studied. The animals were subdivided into three groups: control (citrate buffer), streptozotocin 40 mg/kg or 50 mg/kg, and general biologic parameters were analyzed, in addition to systolic blood pressure, blood glucose and insulin levels determinations. Both doses were able to induce hyperglycemia above 300 mg/dl; however, 50 mg/kg provoked a more pronounced physiological alterations in body weight, diuresis, water and food intake. There was no change on systolic blood pressure with either dose. Results suggested that SHRs did not need doses of streptozotocin above 40mg/kg in order to produce diabetes probably because this strain was much more sensible than normotensive rats. In addition, streptozotocin might be a drug choice to induce diabetes without provoking alterations in the blood pressure which allowed the use of this experimental model in the studies of induced hypertension-diabetes.

Key words: Streptozotocin, SHR, diabetes, blood pressure, insulin

INTRODUCTION

Diabetes and hypertension are both relatively common diseases in the Western countries. The coexistence of both diseases which provoke a myocardium dysfunction leads to an increase of morbidity and mortality and implicates in double risk of death by the cardiovascular disorder (Wold et al., 2001; Feldstein, 2002). Therefore, the experimental research requires animal models which characterize both diabetes and hypertension. The spontaneously hypertensive rats treated with streptozotocin (STZ-SHR) and obese Zucker rats are in general used (Van Zwieten, 1999). Streptozotocin in a wide variety of dosages from 20 to 200 mg/kg of body weight has been used to induce diabetes. In spite of the controversies, it is generally recognized that lower doses associated to hyperlipidic diets lead to diabetes mellitus II. However, type I seems to be induced with doses above 40 mg/kg (Ito et al., 1999; Gao and You, 2001). The cytotoxic action of the streptozotocin is mediated by the reactive oxygen species with a simultaneous massive increase in the cytosolic calcium concentration that provokes a rapid destruction of beta cells (Szkudelski, 2001). There are also reports showing that streptozotocin-induced diabetes in the rat might alter blood pressure. However the results are conflicting (Yamamoto, 1988). A significant decrease in the systolic blood pressure was observed in the STZ-SHR rats (Susic et al. 1990). However, others authors reported an increase of blood pressure followed by 45mg/Kg streptozotocin (Murali and Goyal, 2002). In addition, other findings reported

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an increase in arterial blood pressure after the first week when the streptozotocin was injected (Ramos, 1988). Since STZ might cause a generalized organ damage (Muruganandan et al., 2002), this study was performed in order to identify the optimum dosage able to induce experimental diabetes but did not provoke alterations in the blood pressure and others physiological parameters.

MATERIAL AND METHODS

Animals

Fifteen male spontaneously hypertensive rats (SHR) obtained from colonies maintained by the Federal University of Rio de Janeiro State were studied. Rats aged 20 weeks and weighed 300g, were maintained in metabolic cages in a temperature (21±2°C) and humidity-controlled (60±10%) room submitted to a 12h-dark/light cycle (artificial lights, 7 a.m.–7 p.m.) and air exhaustion cycle (15min/h). All the produceres were carried out in accordance with the "Principles of laboratory animal care" (NIH publication no. 85-23, revised 1985). The experimental protocols used in this study were approved by Ethics Committee for Animal Experimentation at the Federal University of Rio de Janeiro State.

Methodos

After an adaptation period of two weeks the animals were divided into three groups: control (n=5), treated with a dose of 40 mg/kg streptozotocin (n=5) and treated with a dose of 50 mg/kg streptozotocin (n=5). During the study all the rats fed standard rat chow (Nuvilab, Nuvital, Brazil) *ad libitum* and had free access to the water. The diet and water intake was daily monitored as well as the diuresis and rat's healthy conditions. All the animals were weighed weekly throughout the experiment. The systolic blood pressure was determined once a week using the non invasive method of the tail-cuff plethismography in conscious rats (Letica LE 5100, Panlab®). The blood glucose was measured on animals in fasting of six hours once a week, using the glucosimeter Accu-Chek® Advantage (Roche). The blood was collected by vein punction under pentobarbital anesthesia (i.p.) and serum insulin levels were determined by the rat insulin ELISA kit for enzyme immunoassay (EIA) (Kamiya Biomedical Company) after 28 days of assay.

Diabetes Model

The control group received only the vehicle, and the treated groups received a dose of 40mg/kg (n=5) and 50mg/kg (n=5) of streptozotocin (S-0130 Sigma, St. Louis, MO), dissolved in citrate buffer 0.1M, pH 4.4, injected intraperitoneally.

Data analysis

The statistical significance of the differences was assessed by one-way ANOVA and a Newman-Keuls test. In all the cases the $p<0.05$ was considered statistically significant. All analysis were performed using GraphPad Prism® version 4.0 for Windows® (GraphPad Software, San Diego, CA).

RESULTS

Both doses of the streptozotocin did not show variability in response since 100% of rats independent of dose reached 300mg/dl of glycemia. However, there was clearly a difference in time-dose effect (Fig. 1).

The levels of insulin blood decreased sharply in the rats (Fig. 2). The treatment altered the biological parameters: diuresis, food and water intake being the changes more pronounced with 50mg/Kg (Table 1). There was a significant body weight loss (approximately 25%), when compared with control, after 28 days of treatment with streptozotocin, characterizing once more the diabetes model (Fig. 3).

The Fig. 4 demonstrated that all the groups had a systolic blood pressure maintained at similar levels comparing control and STZ-diabetes induced rats groups.

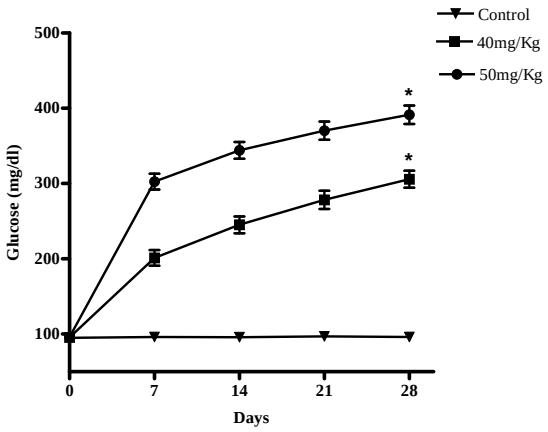

Figure 1 - Mean ± SD of blood glucose of SHR of 15 rats (*p<0.05 when compared with control).

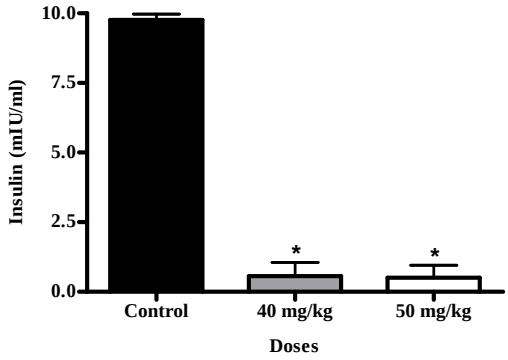

Figure 2 - Mean ± SD of insulin blood levels of SHR (n=15) after diabetes state being confirmed (*p<0.05 when compared with control).

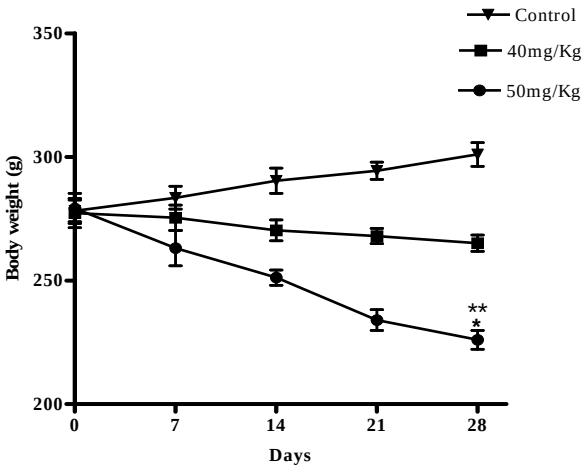

Figure 3 - Mean ± SD of body weight of SHR of 15 rats (*p<0.01 when compared with control; **p<0.05 when compared with 40 mg/kg).

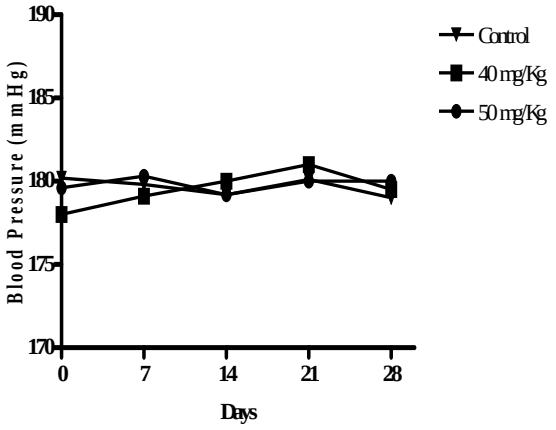

Figure 4 - Mean ± SD of blood pressure of SHR of 15 rats ($p>0.05$).

Table 1 - Mean ± SD of biologic parameters of SHR of 15 rats ([a] $p<0.05$ when compared with control).

	Water Intake (ml)			Food Intake (g)			Diuresis (ml)		
	Control	**40 mg/kg**	**50 mg/kg**	**Control**	**40 mg/kg**	**50 mg/kg**	**Control**	**40 mg/kg**	**50 mg/kg**
0 day	34.5±1.9	33.7±2.0	33.0±1.5	30.5±1.5	29,2±2.0	31,1±2.9	10.2±1.8	10.3±1.9	10.5±1.7
7 days	33.8±2.0	34.5±2.1	38.1±1.9[a]	30.9±1.3	32.1±3.2	33.2±2.5[a]	10.8±2.1	11.9±1.5	14.1±1.4[a]
14 days	32.9±1.9	35.7±2.2	40.0±2.4[a]	31.2±1.4	33.5±2.9	36.0±1.7[a]	10.3±2.6	12.8±1.6	17.5±1.2[a]
21 days	34.0±2.1	36.5±2.5	43.2±3.3[a]	30.1±1.7	35.1±3.3	38.0±1.9[a]	11.0±2.0	15.4±1.3	20.1±1.6[a]
28 days	33.5±1.7	37.0±3.5	45.3±3.5[a]	31.4±2.0	37,2±4.0	40.0±2.2[a]	10.6±2.2	18.1±2.5	25.4±2.8[a]

DISCUSSION

Along the years, the spontaneously hypertensive rats (SHR), have been considered as an excellent model of the experimental hypertension that could serve as a counterpart for the clinical essential hypertension (Okamoto and Aoki, 1963; Trippodo and Frohlich, 1981). There are several reports suggesting a link between the hypertension and diabetes, and SHR could be also a suitable animal model for the investigations of cardiovascular disorders mechanism observed during diabetes (Cooper, 1997). In fact, the precise mechanisms involved in the etiology of cardiovascular complications in diabetes are undefined (Crespo, 2003). However, there is a consensus that diabetes might occur with an oxidative stress, which in turn causes an endothelial dysfunction (De Mattia, 2003). Anyhow, the combination of the hypertension and diabetes results in a severe cardiac pathology than is seen with the either disease alone (Yu and Mcneill, 1991). Experimental and clinical assays have demonstrated that these patients were much more sensible to develop a generalized organ damages and stroke and such occurrence decrease the life expectance and/or compromised the life quality as well (Kaur; Regesta, 2002).

The present study clearly demonstrated that streptozotocin at doses 40 or 50 mg/Kg was able to provoke a sustained hyperglycemia; however, the time course of blood glucose changes was significantly different: 28 days versus 7 days, respectively after a single injection. Streptozotocin either at 40 or 50 mg/kg provoked a significant hypoinsulinemia and induced an insulin-dependent diabetes. On the other hand, some authors reported such low levels of insulin only when high doses of streptozotocin such as 200mg/kg were administered (Ito et al., 1999). Furthermore, a long term stable hyperglycemia here reported was also in contrary to the findings of some authors that only performed such model with doses above 40mg/Kg. However,

those estudies not always were carried out with SHR (Ar'Rajab and Ahren, 1993). In this assay it was demonstrated that both the doses of streptozotocin were effective to induce the diabetes. However, the highest doses were more harmful since it provoked a pronounced body weight loss and an increase of diuresis compromising significantly the clinical status of those animals. In fact, with 50mg/Kg of streptozotocin, the rats reached a decompensate state of diabetes.

Regarding the possible effect of streptozotocin on blood pressure of SHR, the model did not show alteration. However, others authors reported either increase or decrease of systolic blood pressure (Somani et al., 1979; Susic et al, 1990; Sato at al., 1991). Such apparent disagreement might be associated to the differences in methodology including not only different dosage but also via administration of the drug (Dai et al., 1994).

In conclusion, the results confirmed that SHR were more sensitive to the effects of a decrease in the pancreatic cells function provoked by the streptozotocin as previously reported by other authors (Reaven and Ho, 1988), and therefore, there was no need of doses above 40 mg/kg to induce diabetes in this rat. The fact that STZ at 40 mg/kg did not change the systolic blood pressure of SHR, made this dosage more proper to the assays involved in the study of diabetes and hypertensive disorders.

RESUMO

Foi estudada a sensibilidade de ratos espontaneamente hipertensos (SHR) adultos ao efeito diabetogênico da estreptozotocina (STZ). Os animais foram subdivididos em grupos: controle (tampão citrato), 40 mg/kg ou 50 mg/kg de estreptozotocina, sendo analisados parâmetros biológicos gerais, pressão arterial sistólica, níveis sanguíneos de glicose e insulina. Ambas doses foram capazes de induzir hiperglicemia acima de 300 mg/dl, entretanto a dose de 50 mg/kg provocou efeitos fisiológicos mais pronunciados no peso corpóreo, diurese, ingestão hídrica e de ração. Não houve alteração da pressão arterial sistólica em qualquer dose. Nossos achados sugerem que SHRs não necessitam de doses de estreptozotocina acima de 40 mg/kg com para produzir diabetes, provavelmente porque essa cepa é muito mais sensível do que ratos normotensos. A estreptozotocina pode ser a droga de escolha para induzir diabetes sem provocar alterações na pressão arterial, permitindo o uso desse modelo experimental nos estudos da hipertensão induzida pelo diabetes.

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