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Streptozotocin Diabetes-Induced Changes in Aorta, Peripheral Nerves and Stomach of Wistar Rats

R. Sotníková, V. Nosáľová, M. Štefek, S. Štolc, A. Gajdošík, A. Gajdošíková

Institute of Experimental Pharmacology, Slovak Academy of Sciences, Bratislava, Slovakia

Abstract. In rats with diabetes induced by streptozotocin (STZ), we studied the reactivity of the aorta in response to vasoconstrictor and vasorelaxant agents, changes in conduction velocity in the sciatic nerve, and glutathion (GSH) content in the gastric mucosa as well as the occurrence of spontaneous gastric lesions. STZ-induced diabetes was found to be accompanied by endothelial injury, exhibited by diminished endothelium-dependent relaxation and by increased noradrenaline- and H_2O_2-induced contraction. Conduction velocity in the nerves from STZ-treated animals was significantly lower compared to that in nerves from control animals. Moreover, gastric hyperaemia, occasional gastric lesions, and a significant depletion of GSH in the gastric mucosa were observed in STZ-treated rats. Our experiments confirmed the suitability of Wistar rats for the model of STZ-induced diabetes.

Key words: Hyperglycaemia — Endothelium — Vascular reactivity — Action potential conduction — Gastric damage

Introduction

It is known that hyperglycaemia leads to glycation of proteins and oxidative stress (Baynes 1992). In diabetes, oxidative stress was found to be due to an increased production of free radicals and a sharp reduction of antioxidant defenses. Vascular impairment accounts for the majority of clinical complications of diabetes mellitus. Changes in local control of vascular tone, such as imbalance in endothelial production of relaxing and contracting factors, may be related to the initiation and maintenance of abnormal vascular reactivity characteristic of diabetic vascular complications.

Diabetic polyneuropathy with different symptomatology is one of the frequently occurring complications in diabetes mellitus. An increase in oxygen free radical activity in diabetic individuals resulting in vascular complications including impairment of perfusion of the nerve endoneurium was suggested to be the major cause of nerve fiber dysfunction (Cameron and Cotter 1995; Cotter et al.

Correspondence to: RNDr. Ružena Sotníková, CSc., Institute of Experimental Pharmacology SASc, Dúbravská 9, 842 16 Bratislava, Slovakia. E-mail: exfarosa@savba.sk

1995). This hypothesis was supported by the fact that some antioxidants and free radical scavengers exhibited a beneficial effect on peripheral nerve conduction velocity impairment in experimental diabetes (Cotter and Cameron 1995; Cotter et al 1995)

Gastrointestinal disorders have often been reported in diabetic patients and in animal models of diabetes (O'Reilly and Long 1987). In addition to disturbed motility, spontaneous gastric lesions as well as an increased susceptibility of the gastric mucosa to damage were described Endogenous sulfhydryls, mainly reduced glutathione (GSH), play a major role in gastric cytoprotection (Szabo et al. 1981) GSH is importantly involved also in the protection against oxidant- and free-radical-mediated cell injury, which is directly implicated in the mechanism of the overall diabetes-induced damage.

In Wistar rats with diabetes induced by streptozotocin (STZ) we therefore studied a) the reactivity of the aorta in the response to vasoconstrictor and vasorelaxant agents, b) changes in conduction velocity in the sciatic nerve, and c) GSH content in the gastric mucosa as well as the occurrence of spontaneous gastric lesions

Materials and Methods

Diabetes was induced by a single intravenous injection of streptozotocin (STZ) in doses of 40, 50, and 60 mg/kg in citrate buffer to 8-week-old male rats, weighing 200-230 g For the general procedure see the report by Gajdošík et al (1999) published in this issue Diabetes was confirmed by elevated blood glucose concentration (> 20 mmol/l), which was determined before and 12 days after STZ injection and then monthly The experiments were performed 20 weeks after STZ injection Rats were not treated with exogenous insulin Mortality in the group of 40, 50 and 60 mg/kg STZ was 0, 12 5 and 25 %, respectively Rats fasting overnight with free access to water were anaesthetised with thiopental (50 mg/kg i p) After excision of both sciatic nerves, the animals were sacrificed by cervical cord transsection and exsanguinated Other tissue samples were taken immediately

As our preliminary experiments showed that doses of STZ lower than 60 mg/kg did not induce any functional changes in the thoracic aorta and ischiadic nerves, in the experiments with these preparations we used diabetic rats treated with 60 mg/kg of STZ only

Isolated rat thoracic aorta

The thoracic aorta from control and STZ-(60 mg/kg) treated rats was removed, cleaned of adherent tissue, cut into 2-mm rings and mounted in water-jacketed ($37\,^{\circ}\mathrm{C} \pm 0\,5$) chambers containing physiological saline solution (PSS) bubbled with a mixture of 95% O_2 and 5% CO_2 at pH 7 4 The composition of PSS was (in mmol/l) NaCl (118 0), KCl (4 7), K_2PO_4 (1 2), $MgSO_4$(1 2), $CaCl_2$(2 5), $NaHCO_3$ (25 0) and glucose (11 0) The preparations were stretched to optimal resting tension of 20 mN Isometric contractions were recorded on a line-recorder

After the stabilization period (60 min), the rings were precontracted with 10^{-6} mol/l of noradrenaline (NA) In the plateau of the contraction acetylcholine (AC) (10^{-9} to 10^{-4} mol/l) was applied in a cumulative manner Then the rings were washed 3 times in the period of 20 minutes and responses to 20–100 μmol/l H_2O_2 were tested The rings

were washed 3 times again and after 20 minutes NA was applied, and in the plateau of contraction the response to sodium nitroprusside (10^{-12} to 10^{-8} mol/l) was recorded

Isolated sciatic nerve

Both ischiadic nerves were dissected free in sections of maximal possible length One of the nerves was placed in the experimental chamber and electrophysiological assessment was carried out immediately. The other nerve was stored in an incubation chamber filled with oxygenated Krebs solution for further analysis made approximately 2 hours later The composition of the Krebs solution was (in mmol/l) NaCl (136), KCl (5 6), $CaCl_2$ (2 2), $MgCl_2$ (1 2), glucose (4 9), HEPES (5 0), pH was adjusted to 7 3 by NaOH

  The experimental "wet"-type chamber ventilated with oxygen saturated with water vapours contained a system of platinum wire stimulation, grounding and registration electrodes The typical sequential distance between the first and second stimulation electrode, the grounding electrode, the first and second recording electrode was 7, 12 5, 12 5, and 7 mm, respectively Action potential was evoked by supramaximal square wave pulses Action potential conduction velocity (m/s) was estimated in the population of axons most evidently present in the preparation and was calculated from the time delay between stimulation artifact and the peak of the action potential evoked, and the distance between stimulation and recording electrodes The measurements were repeated 3 times in 5 min intervals and were carried out at 22–24 °C

Reduced glutathione and gastric damage

The stomach was excised, opened along the greater curvature and rinsed in ice-cold saline The length of gastric lesions was measured under a dissecting microscope and samples of gastric mucosa were scraped off and weighed GSH content was measured according to a slightly modified method of Sedlak and Lindsay (1968)

Data analysis

The results are expressed as means $\pm$ S E M Student's *t*-test was used for statistical analysis

Results

Isolated rat thoracic aorta

In functional experiments the thoracic aorta isolated from STZ-treated (60 mg/kg) rats was found to respond to AC with significantly lower relaxation than did the control aortas (Fig. 1). NA 10^{-6} mol/l (Fig 2) and H_2O_2 (20–200 μmol/l) (Fig. 3) induced higher contraction in STZ-treated aortas in comparison with control ones. However, neither contractile responses to 100 mmol/l KCl ($n = 24$) nor relaxant responses to 10^{-8} mol/l sodium nitroprusside ($n = 12$) were influenced by STZ-induced diabetes.

Isolated sciatic nerve

Condution velocity of the action potential was ranging from 13.5 to 32.8 m/s in the population of axons dominant in the nerves assessed. The values obtained in series of the first nerve in each animal (i e. in the nerve assessed immediately after dissection) were systematically lower than in those measured in the second nerve
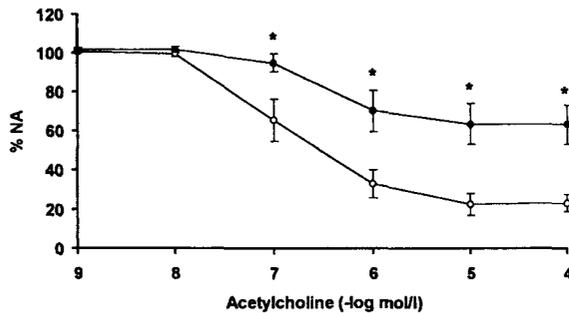
Figure 1. Responses of rat thoracic aorta from control (open circles) and STZ (60 mg/kg) – diabetic (full circles) animals to acetylcholine. Preparations were precontracted with 10^{-6} mol/l noradrenaline (NA). Values are means $\pm$ S.E.M. of 12 experiments. $* p < 0.05$ versus control group.
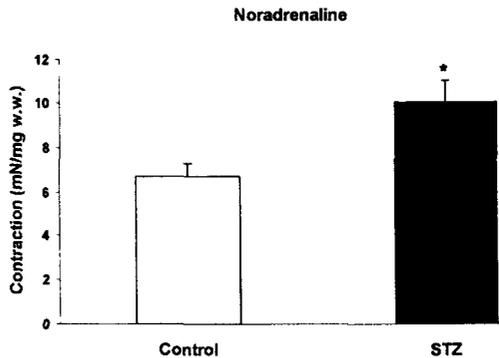

Figure 2. Responses of rat thoracic aorta from control (open column) and STZ (60 mg/kg)-diabetic (full column) animals to noradrenaline 10^{-6} mol/l. Values are means $\pm$ S.E.M. of 12 experiments. $*p < 0.05$ versus control group.

(i.e. in that which was allowed to "relax" for approximately 2 hours). Both results are shown in Table 1. Conduction velocity in the nerves from the STZ-treated animals (60 mg/kg, $n = 8$) was significantly lower compared to that in nerves from the control animals ($n = 6$). A similar difference occurred in both series of the preparations (Table 1).

Reduced glutathione and gastric damage

Pronounced hyperaemia was found in almost all stomachs from rats with STZ-induced diabetes. The gastric mucosa was fragile, spontaneous lesions were however rare. In most cases only petechiae and small erosions were found. After the administration of STZ in a dose of 40 mg/kg, gastric lesions were observed only in a few stomachs, yielding rather heterogenous data in this group. There was no

Table 1. Conduction velocity in rat sciatic nerve

	The 1st nerve	The 2nd nerve
Controls	22.93 ± 0.75	27.21 ± 0.73
STZ-treated animals	20.03 ± 0.73*	22.63 ± 0.87**

Conduction velocity is indicated in m/s. Means ± SEM are shown, number of animals 6–8. Dose of STZ was 60 mg/kg. $*p < 0.01$ and $**p < 0.001$ compared to the control groups.
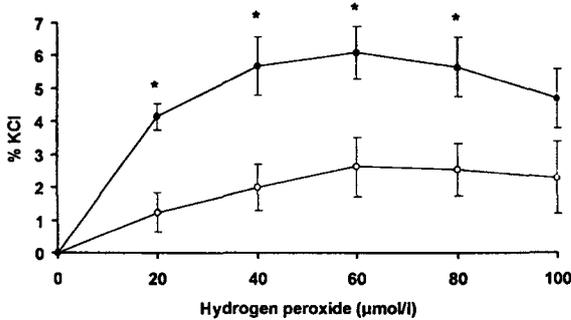

Figure 3. Responses of rat thoracic aorta from control (open circles) and STZ (60 mg/kg)-diabetic (full circles) animals to hydrogen peroxide expressed as % of KCl-induced contraction. Values are means ± S.E.M. of 12 experiments. $*p < 0.05$ versus control group.

Table 2. Length of gastric lesions in rats with diabetes induced by streptozotocin

Treatment dose	Lesion length (mm) mean ± S.E.M
control (vehicle)	0
STZ 40 mg/kg i.v.	7 .67 ± 6.97
STZ 50 mg/kg i.v.	5.75 ± 1.85
STZ 60 mg/kg i.v.	6.25 ± 3.80

$n = 6$–8 animals per each dose

statistically significant dose-related difference in the effect of STZ on the mean gastric lesion length (Table 2). Compared to controls, significantly lower levels of GSH were found in stomachs from rats with STZ-induced diabetes (Fig. 4), but the difference between the STZ doses used (50 and 60 mg/kg i.v.) was not significant.

Discussion

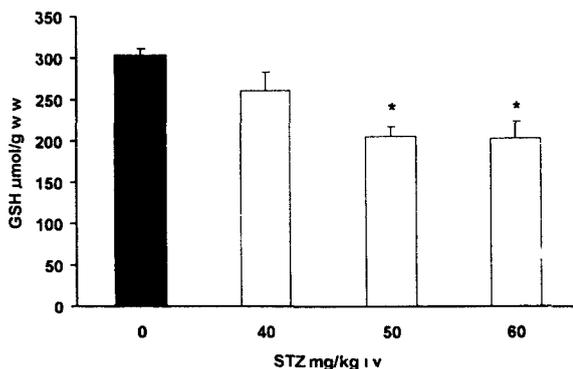Several studies demonstrated diabetes-associated endothelial dysfunction and re-

Figure 4. Gastrıc mucosal levels of reduced glutathıone (GSH) ın rats wıth streptozotocın (STZ) ınduced dıabetes Data are means ± S E M for at least 6 rats ın each group *$p < 0.05$, **$p < 0.01$ versus control

ductıon of endothelıum-dependent relaxatıon (Calver et al 1992, Johnstone et al 1993, Rodrıguez-Mañas et al 1998) In agreement wıth these findıngs, we observed dımınıshed relaxant response to AC ın rat aortas from dıabetıc rats Moreover, we found that aortas from STZ-treated rats responded to H_2O_2 wıth hıgher amplı tude of contractıon than aortas from control rats We reported prevıously (Sot- nıková 1998) that H_2O_2 (20–200 μmol/l) caused contractıon of rat ısolated aortıc rıngs Hıgher concentratıons of H_2O_2 ınduced aortıc relaxatıon The response of the rat aorta to H_2O_2 ınvolved two components   contractıle and relaxant, the latter beıng endothelıum-medıated Increased amplıtudes of contractıon ın the rat aorta wıth mechanıcally or functıonally damaged endothelıum resulted probably from the absence of the endothelıum-dependent relaxant component The onset of maxımal H_2O_2-ınduced contractıon at lower concentratıons could also reflect endothelıal ınjury A sımılar sıtuatıon may have occurred ın STZ-dıabetıc aortas where the ınjured endothelıum resulted ın enhanced H_2O_2-contractıon at lower concentratıons

    Several authors reported ıncreased NA-ınduced contractıon ın STZ-treated rats as a consequence of deficıent endothelıal actıvıty (Abebe et al 1990, Karasu et al 1997) One of the suppossed mechanısms seems to be the absence of a functıonal antagonıst of the tonıc phase of NA-ınduced contractıon, ı e spontaneously released NO These findıngs are ın agreement wıth our results of enhanced NA-contractıon ın STZ-treated aortas Increased contractıle responsıveness of the dıabetıc aorta does not appear to be a consequence of low responsıveness of vascular smooth cells to normal levels of NO as sodıum nıtroprussıde ınduced relaxatıon was not changed

    The demonstrated deficıency ın the mechanısms underlyıng the NO-medıated vascular responses ın dıabetıc rats mıght be ımportantly ınvolved also ın the dıs- turbance of gastrıc mucosal ıntegrıty observed ın our experıments Accordıng to

Tashima et al. (1998), a decreased release and/or production of endogenous NO seems to be partly responsible for the impaired gastric hyperaemic response in diabetic rats (in addition to dysfunction of capsaicin-sensitive sensory nerves). Compared to controls, the reduction of GSH content in the stomach of fasted diabetic rats was about 30 %. Since endogenous GSH plays a key role in maintaining the integrity of the gastric mucosa (Robert et al. 1984) and a 25 % decrease is considered critical, the observed degree of GSH depletion might also result in the development of gastric damage in diabetic rats. Increased GSH oxidation by enhanced oxidative stress may be a possible mechanism operative in GSH reduction.

The conduction velocity observed in the nerves studied was indicating that the dominant population of the axons belonged to the Aα type. The conduction velocity decreased by about 15% in the nerves isolated from the animals with STZ-induced hyperglycaemia. This is in fairly good agreement with the observation of Cameron and Cotter (1995), Cotter and Cameron (1995) and Cotter et al. (1995), who found a decrease of about 20%. The small difference between the two studies could be due to different experimental conditions used in the studies (*in vitro* versus *in vivo*) A contributing factor may have been also the use of different rat strains (Wistar versus Sprague-Dowley). The decrease in nerve conduction velocity in STZ-treated rats could be interpreted in terms of an impairment of the activation of the voltage-operated ion channels responsible for action potential generation in the relevant population of axons That could undoubtedly be ascribed to changes induced directly or indirectly by STZ pretreatment, probably to the impairment of nerve vasculature (Cameron and Cotter 1995) and/or to a long-term exposure of the peripheral nerves to increased levels of glucose with resulting glycosylation of some proteins in axonal membranes. The differences between the values observed in the nerves assessed immediately after dissection (the "first" nerves) and those stabilized for a period of approximately 2 hours (the "second" nerves) might be pointing to a recovery of the preparations from the injury caused by dissection.

In conclusion, our experiments showed that STZ-induced diabetes was accompanied by endothelial injury, action potential conduction impairment in peripheral nerves, gastric hyperaemia, occasional gastric lesions, and by a significant depletion of GSH in the gastric mucosa. The current study demonstrated the suitability of Wistar rats for the model of STZ-induced diabetes.

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