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Life Sciences 74 (2004) 3085–3092
Life Sciences
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Effect of melatonin on vascular reactivity in pancreatectomized rats

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Received 24 July 2003; accepted 23 October 2003

Abstract

The present study was undertaken to assess whether the improvement of contractile performance of aortic rings by melatonin described in streptozotocin diabetic rats also occurs in another model of type I diabetes, the pancreatectomized rats. Adult male Wistar rats submitted to a subtotal pancreatectomy and exhibiting altered levels of fasting glucose and an abnormal tolerance glucose test, were used. Sham-operated laparotomized rats were employed as controls. Dose–response curves for acetylcholine-induced, endothelium-related relaxation of aortic rings (after previous exposure to phenylephrine) and for phenylephrine-induced vasoconstriction were conducted. This protocol was repeated with rings pre-incubated in a high glucose solution (44 mmol/l). Pancreatectomy decreased significantly acetylcholine-induced relaxation of aortic rings, but not phenylephrine-induced vasoconstriction, the effect being amplified by preincubation in high glucose solution. The deleterious effect of a high glucose medium was more pronounced in pancreatectomized rats. Melatonin (10^{-5} M) did not modify acetylcholine-induced relaxation in normal glucose concentration but was effective to prevent the impairment of relaxation brought about by exposure to high glucose solution. The contractile response to phenylephrine of aortic rings obtained from pancreatectomized rats was not affected by melatonin. The results further support the improvement by melatonin of endothelial-mediated relaxation in blood vessels of diabetic rats.

Keywords: Vascular reactivity; Diabetes; Melatonin; Pancreatectomy

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doi:10.1016/j.lfs.2003.10.032

Introduction

Early type I (insulin-dependent diabetes) is associated with changes in vascular function leading to overperfusion of the vasculature (Pieper, 1998) whereas the later stages are associated with atherosclerotic and hypertensive changes promoting the late stage complications of the disease (Sowers and Epstein, 1995). While the mechanisms of these changes are not completely understood, it is possible that shifts in the actions of endothelial derived relaxing and contracting factors are mainly involved (Calles-Escandon and Cipolla, 2001).

Animal models for type I diabetes include pancreatectomy, chemicals (e.g. streptozotocin, alloxan or Zinc chelating compounds) and viruses. In a previous study performed in streptozotocin-diabetic rats we reported the impairment of vascular reactivity as a function of severity of hyperglycemia, both on the vasoconstrictor responses to phenylephrine or serotonin, as well as on the acetylcholine-evoked, endothelium-mediated relaxation (Reyes-Toso et al., 2002). A pharmacological concentration of melatonin curtailed the maximal tension of aortic contraction after serotonin and restored acetylcholine-induced relaxation after preincubation in high glucose medium. The present study was undertaken to assess whether the improvement of contractile performance by a pharmacological concentration of melatonin also occurred in another model of type I diabetes, the pancreatectomized rats. Development of diabetes after pancreatectomy depends on the animal species and on the surgical procedure employed. In rats it is difficult to achieve a total pancreatectomy because of the diffuse distribution of pancreatic tissue. Therefore, several surgical techniques were developed to obtain a subtotal pancreatectomy (Foglia, 1944). Wistar rats submitted to subtotal pancreatectomy have altered tolerance glucose test and variable levels of fasting glucose (Shinagawa and Suzuki, 1992).

Methods

Animals

Experiments were carried out in adult male Wistar rats (150–180 g body weight), kept under light between 08:00 and 20:00 h daily. Rats had access to food and water ad libitum. Subtotal pancreatectomy was performed under diethylether anesthesia (Foglia, 1944). By this procedure all pancreatic tissue was removed, except for the portion lying between the bile duct and the duodenum. Rats receiving a sham-operation (laparotomy) were used as controls. Adequate measures were taken to minimize pain or discomfort, in accordance with the principles and procedures outlined in European Communities Council Directives (86/609/EEC).

Blood glucose tests

Blood glucose was determined 90 min after food withdrawal in rats that had been pre-starved for 12 h and then fed for 1 h. Glucose tolerance was measured after an oral glucose load (10 ml/kg body weight of a glucose solution containing 0.32 g of glucose/ml H_2O) in rats pre-starved for 12 h. Blood was taken from the tip of the tail at 0, 30 and 60 min. Glucose was determined by a conventional enzymatic method (glucose-oxidase) using the Accutrend Glucose® test strips. Rats that had fasting blood glucose levels

>8.3 mmol/l or an oral glucose tolerance test = 11.1 mmol/l were considered to be diabetic and were used for the subsequent studies.

Drugs

All drugs and reagents employed were obtained from Sigma Chemical Co, St. Louis, MO, USA. Melatonin was first dissolved in ethanol and further diluted in bidistilled water; ethanol concentration in the organ bath was lower than 0.01%. The rest of drugs were prepared in bidistilled water and diluted with Krebs immediately before the experiment.

Preparation of tissue

Rats were killed by decapitation and the thoracic aorta was removed and placed in cold Krebs-bicarbonate solution (mmol/l): NaCl, 120; KCl, 4.8; $MgSO_4$, 1.3; $CaCl_2$, 1.6; $NaHCO_3$, 25; glucose, 10; disodium EDTA, 0.03. The segments were carefully cleaned of fat and loose connective tissue and were sectioned into 3-mm long rings. Two rings were cut from each rat aorta. Rings were mounted on stainless steel hooks and suspended in 5-ml tissue baths. Tension development was measured by isometric force transducers (GRASS FT03) connected to an amplifier. Baths were filled with Krebs solution at 37 ± 0.1 °C, pH 7.4, with 95% O_2, 5% CO_2 as a gas phase. Aortic rings were stretched to a 2-g initial tension and were equilibrated for 60 min. The Krebs buffer solution of tissue bath was replaced every 15 min, tension being readjusted each time. At the end of the equilibration period, the maximal force generated by adding a depolarizing solution of 60 mM KCl was determined. After washing, one ring was used as control and the other was incubated in the presence of melatonin (10^{-5} mol/l). Cumulative dose–response curves to phenylephrine or acetylcholine were obtained to evaluate acetylcholine-induced vasodilatation, rings were pre-constricted with phenylephrine (3.5×10^{-7} mol/l) to obtain a stable plateau, and then a cumulative dose–response curve was obtained. The whole protocol was repeated with rings pre-incubated for 1 hour in a 44 mmol/l glucose solution.

Data analysis

Statistical analysis was performed by using a one-way analysis of variance (ANOVA) followed by Newman-Keuls test, a factorial ANOVA or a Student's t test.

Results

Eight weeks after pancreatectomy fasting blood glucose levels (mmol/l, mean ± SEM; n = 10 animals/group) of pancreatectomized rats (5.88 ± 0.14) differed significantly from those of controls (5.29 ± 0.15) ($p < 0.01$, Student's t test). Blood glucose levels attained after an oral glucose load were much greater in pancreatectomized (11.93 ± 0.43) than in control rats (7.8 ± 0.23) ($p < 0.001$).

Fig. 1 depicts the contractile response of aortic rings derived from pancreatectomized and control rats after exposure to acetylcholine or phenylephrine. Only the observed changes in acetylcholine-induced relaxation were significant. Pancreatectomy decreased significantly relaxation of aortic rings ($F = 35.4$, $p = 0.006$, factorial ANOVA), the effect being amplified by preincubation in 44 mmol/l glucose solution.

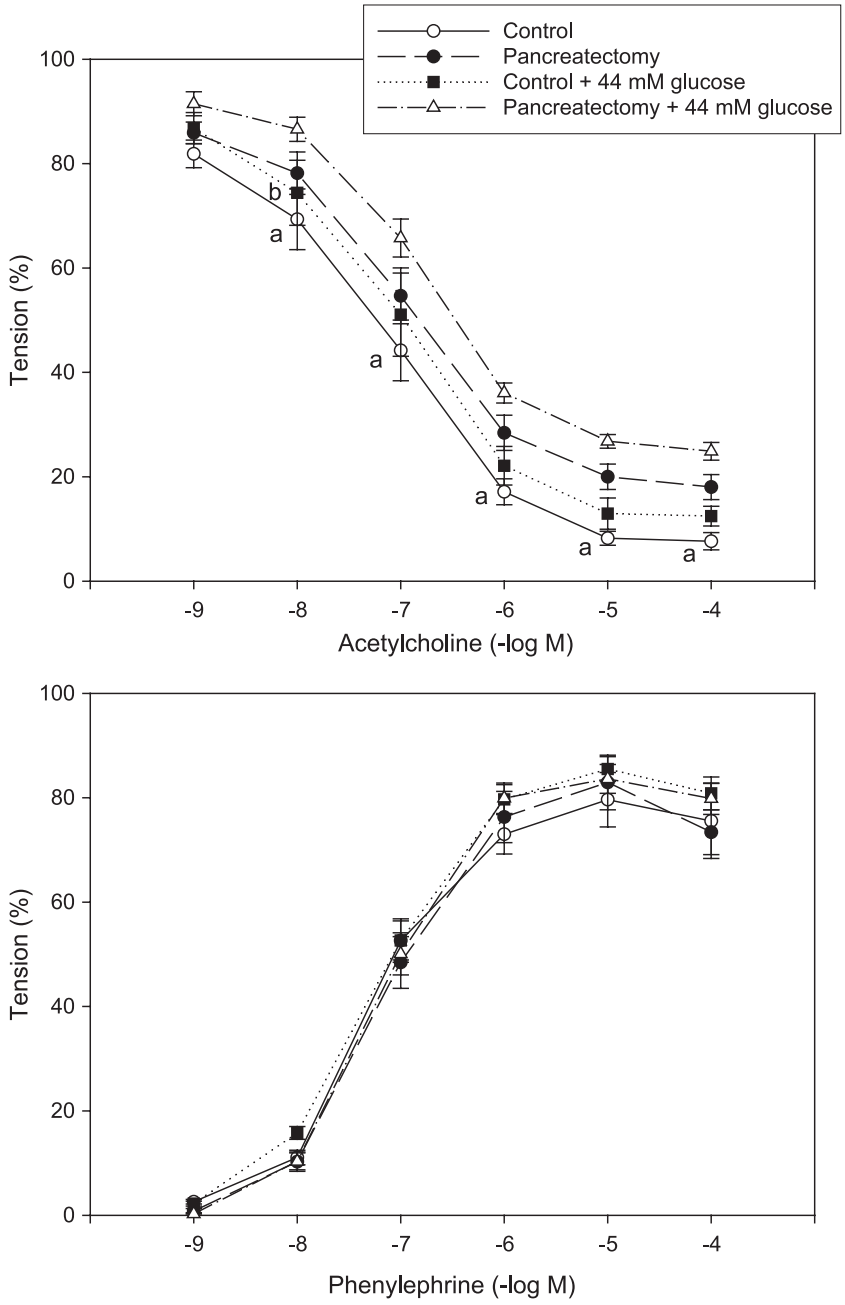

Fig. 1. Acetylcholine-induced relaxation (upper panel) and phenylephrine-induced contraction (lower panel) in aortic rings obtained from control and pancreatectomized rats and incubated in normal (10 mM) or high glucose (44 mM) medium. Shown are the means ± SEM (n = 9–10 rats/group). [a] $p < 0.01$ vs. pancreatectomy + high glucose, [b] $p < 0.05$ vs. pancreatectomy + 44 mM glucose, one-way ANOVA and Newman-Keuls test. For other statistical analysis, see text.

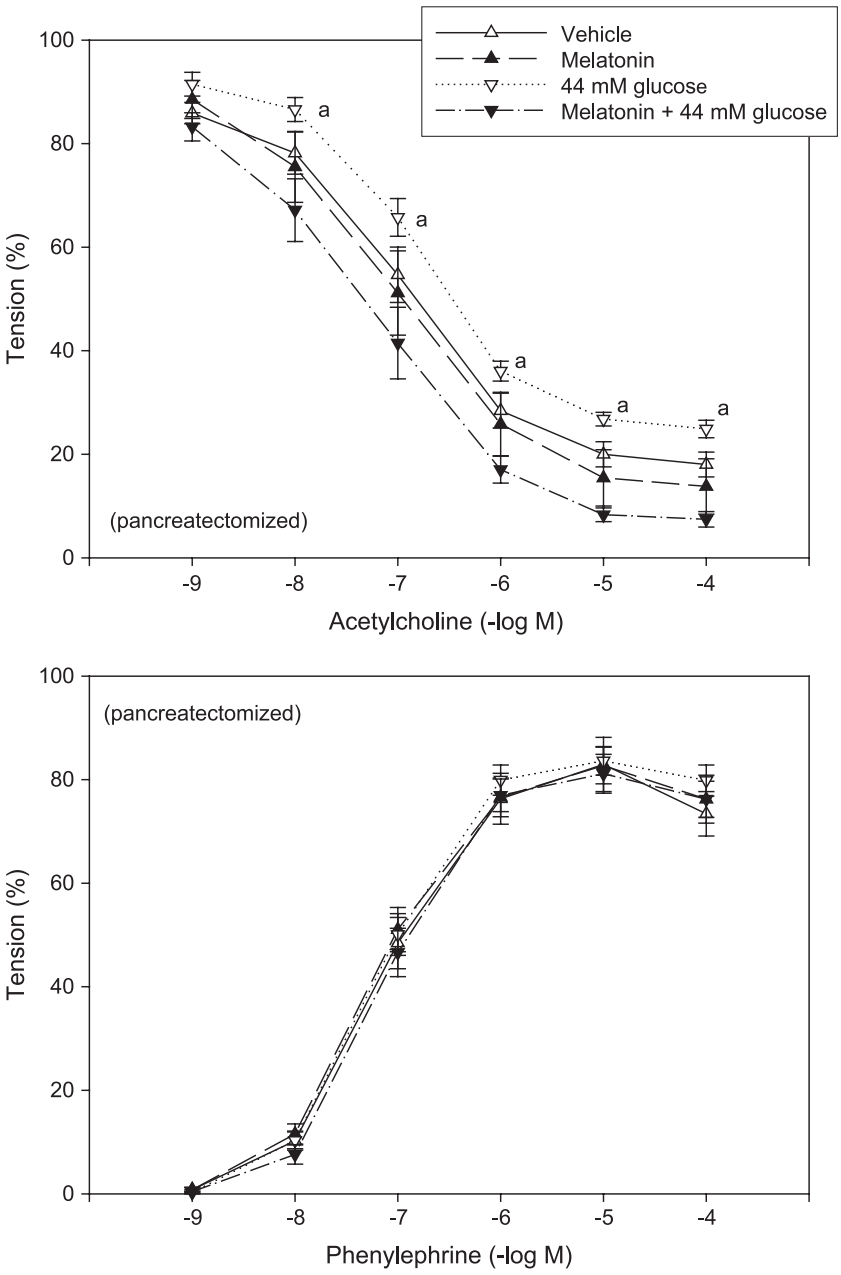

Fig. 2. Effect of melatonin (10^{-5} M) on acetylcholine-induced relaxation (upper panel) and phenylephrine-induced contraction (lower panel) of aortic rings obtained from pancreatectomized rats and incubated in normal (10 mM) or high glucose (44 mM) medium. Shown are the means ± SEM (n = 9–10 rats/group). [a] $p < 0.01$ vs. pancreatectomy + high glucose, [b] $p < 0.05$ vs. melatonin + 44 mM glucose, one-way ANOVA and Newman-Keuls test. For other statistical analysis, see text.

A significant interaction "surgery x high glucose medium" was found in the factorial ANOVA ($F = 24.3$, $p = 0.004$), i.e. the deleterious effect of the high glucose medium was more pronounced in pancreatectomized rats (Fig. 1).

Fig. 2 shows the effect of melatonin (10^{-5} M) on the contractile response of aortic rings of pancreatectomized rats towards acetylcholine or phenylephrine. Melatonin did not modify acetylcholine-induced relaxation in normal glucose concentration but was highly effective to prevent the impairment of relaxation brought about by exposure to a 44 mmol/l glucose solution. Indeed, aortic rings exposed to acetylcholine in the presence of melatonin and a high glucose medium showed the most effective relaxation response. This was reflected in the factorial ANOVA by a highly significant "melatonin x high glucose medium" interaction ($F = 119.21$, $p < 0.000001$) with non-significant effect of the main factors tested: high glucose ($F = 0.2$) or melatonin ($F = 1.70$). Melatonin did not modify the contractile response of aortic rings of pancreatectomized rats to phenylephrine (Fig. 2).

Discussion

Abnormal vascular function is one of the complicating features of human and experimental diabetes. Some of the vascular changes in diabetes are related to alterations in endothelial activity (Ruderman and Haudenschild, 1984; Calles-Escandon and Cipolla, 2001). Impaired endothelium-dependent relaxation has been demonstrated in blood vessels from streptozotocin-induced diabetic rats (Pieper and Gross, 1988; Kamata et al., 1989), spontaneously diabetic rats (Durante et al., 1988) and alloxan-induced diabetic rabbits (Abiru et al., 1990). Evidence suggests that the underlying defect may be a reduction in nitric oxide (NO) synthesis or, in some instances, an increase in the synthesis of constrictor prostanoids (Kamata and Hosokawa, 1997). However, not all investigators have reported an impaired endothelium-dependent response in diabetes. For example, some have reported that endothelium-dependent relaxation did not differ between control and streptozotocin-induced diabetic rats (Wakabayashi et al., 1987) while others have reported an increase in diabetic rats (Bhardwaj and Moore, 1988).

The foregoing results indicate that vascular reactivity in another experimental model of type I diabetes, i.e. the partially pancreatectomized rats, was altered as far as the endothelial-mediated relaxation response to acetylcholine. Pancreatectomy decreased significantly acetylcholine-induced relaxation of aortic rings, an effect which was more profound in the presence of a high glucose solution. Incubation in high glucose medium results in oxidative stress mainly through superoxide anion accumulation (Gryglewski et al., 1986).

Acetylcholine acts on the endothelium to release NO. In addition, a significant proportion of acetylcholine-induced endothelium-dependent vasodilatation is not mediated by NO and cGMP pathways (Adeagbo and Malik, 1990) but through the release of a factor that causes vascular smooth muscle hyperpolarization (endothelium-derived hyperpolarization factor) (Komori and Vanhoutte, 1990).

There is clear evidence that reactive oxygen species are increased in diabetes. The main sources are metabolic including autoxidation of glucose and its metabolites, advanced glycation, altered prostanoid production and abnormal or inefficient mitochondrial function (Baynes, 1991; Wolff, 1993). Superoxide neutralizes NO (Gryglewski et al., 1986) and the peroxynitrite formed is a source of hydroxyl radicals that can cause endothelial damage (Beckman et al., 1990). Oxidative stress therefore diminishes vessel endothelium-dependent relaxation, also demonstrable in type 1 and type 2 diabetic patients (McVeigh et al., 1992; Elliott et al., 1993; Johnstone et al., 1993; Morris et al., 1995).

We previously reported the occurrence of a significant protective effect of a pharmacological concentration of melatonin in aortic rings from streptozotocin-treated rats, particularly on the impairment of function caused by preincubation in a high glucose medium (Reyes-Toso et al., 2002).The present results further extent such a protective activity to the changes of endothelium-mediated relaxation in pancreatectomized rats, particularly on the impairment induced by a high glucose concentration.

Increasing attention has recently turned to the antioxidant properties of melatonin, which is a powerful scavenger of oxygen-free radicals, including hydroxyl radical, singlet molecular oxygen, peroxynitrite ion (Tan et al., 2003) and possibly also peroxyl radicals (Pieri et al., 1994). Melatonin also stimulates several antioxidant enzymes including superoxide dismutase, glutathione peroxidase and glutathione reductase (Tan et al., 2003). Indeed, there have been several reports indicating an inhibitory action of melatonin on oxidative stress in diabetic rats (Sailaja Devi et al., 2000; Andersson and Sandler, 2001; Baydas et al., 2002). Present and previous results (Reyes-Toso et al., 2002) point out to prevention of oxyradical damage as the way melatonin improves endothelial-mediated relaxation in blood vessels of diabetic rats.

Conclusions

The results support the improvement by melatonin of endothelial-mediated relaxation in blood vessels of rats turned diabetic by a partial pancreatectomy.

Acknowledgements

This work was supported by grants from the Agencia Nacional de Promoción Científica y Tecnológica, Argentina (PICT 6153), Fundación Antorchas, Argentina, University of Buenos Aires (PID M003) and Fundación Bunge y Born, Argentina.

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