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Short communication

Decreased sensory neuropeptide release from trachea of rats with streptozotocin-induced diabetes

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Abstract

We studied the release of somatostatin, calcitonin gene-related peptide (CGRP) and substance P in response to electrical field stimulation from isolated tracheas of rats following 4 weeks of streptozotocin (50 mg/kg i.v.)-induced diabetes. Field stimulation (40 V, 0.1 ms, 10 Hz for 120 s) increased the release of somatostatin, CGRP and substance P from the baseline 0.18 ± 0.029, 0.17 ± 0.027, and 1.77 ± 0.086 to 0.51 ± 0.022, 0.69 ± 0.115, and 5.96 ± 0.377 in control preparations and 0.31 ± 0.081, 0.41 ± 0.142, and 3.14 ± 0.443 fmol/mg wet tissue weight in preparations from diabetic rats as measured by radioimmunoassay (control vs. diabetic $P < 0.01$ for each). The results show a simultaneous decrease in release of the three sensory neuropeptides and an enhanced plasma somatostatin level in rats with streptozotocin-induced diabetes.

Keywords: Diabetes; Somatostatin; CGRP (calcitonin gene-related peptide); Substance P; Field stimulation; Trachea; Rat

1. Introduction

Sensory neural dysfunction commonly occurs in patients with peripheral neuropathy, a major complication of diabetes mellitus. Sensory nerves not only transmit sensory information, but also act upon the local environment by releasing several biologically active mediators (see for reviews, Holzer, 1992; Szolcsanyi, 1996). Therefore, a deficiency in axon reflex vasodilation influences ongoing inflammatory disorders, wound healing processes, local control of vascular proliferative mechanisms, and sensory-effector nerve function in cardio-pulmonary diseases (Holzer, 1992; Tosaki et al., 1996; Brain, 1996; Lundberg, 1996). Moreover, we have recently found that somatostatin released from primary sensory neurons mediates a systemic anti-inflammatory effect as well (Szolcsanyi et al., 1998). Thus, beyond their sensory function, the functional integrity of these nerves is a prerequisite for widespread effector regulatory mechanisms (Szolcsanyi, 1996; Ferdinandy et al., 1997). Regarding the neurotransmitters involved, most evidence favours a role for calcitonin gene-related peptide (CRGP), substance P, somatostatin and nitric oxide (NO) (Szolcsanyi, 1996, Moncada and Higgs, 1995).

Depletion of CGRP and substance P has been shown to occur in the sciatic nerve of rats with streptozotocin-induced diabetes mellitus (Diemel et al., 1992). The decreased amount of these sensory neuropeptides has been suspected to underlie a weak neurogenic inflammatory response in both experimental animals and clinical patients with diabetes mellitus (Gyorfi et al., 1996; Walmsley and Wiles, 1991). Nevertheless, to the best of our knowledge, no studies have been conducted to study the effect of diabetes on simultaneous release of CGRP, substance P and somatostatin in response to nerve stimulation. The present work was therefore concerned with the possibility that experimental diabetes induced by streptozotocin would influence the field stimulation-induced release of these neuropeptides from isolated trachea of the rat.

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2. Methods

2.1. Ethics

The experiments performed in the present work conformed to European Community guiding principles for the care and use of laboratory animals. The experimental protocol applied was approved by the local ethical committee of Medical University of Pecs, Hungary.

2.2. Experimental groups

The study was carried out with 36 male Wistar rats weighing 200-210 g. They were housed in an animal room (12-h light/dark periods a day, temperature of 22–25°C, humidity of 50–70%) with four animals per cage fed commercial laboratory chow and allowed tap water ad libitum. The animals were randomly divided into two experimental groups. Group 1 (control): animals treated with the solvent for streptozotocin; Group 2 (treatment group): animals treated with 50 mg/kg streptozotocin i.v. (Zanosar, Upjohn, Kalamazoo, MI).

2.3. Neurotransmitter release studies

These have been described in detail elsewhere (Helyes et al., 1997; Nemeth et al., 1998). In brief, following exsanguination, the whole trachea was removed, cleaned of fat and adhering connective tissues. Tracheas from two animals were perfused (1 ml/min) in an organ bath (1.8 ml) with a temperature- (37°C) and pH- (7.2) controlled oxygenized Krebs solution for 60 minutes (equilibration period). After discontinuation of flow, the solution was changed three times for eight minutes to produce pre-stimulated, stimulated, post-stimulated fractions. Electrical field stimulation (40 V, 0.1 ms, 10 Hz for 120 s) was applied to elicit neurotransmitter release in the second 8-min period. Calcitonin gene-related peptide (CGRP), substance P, and somatostatin concentrations were determined from 200-μl samples of organ fluid of the preparations by means of radioimmunoassay methods developed in our laboratories as described (Nemeth et al., 1996, 1998; Helyes et al., 1997). The whole experimental protocol was repeated with separate preparations (three preparations from 6 animals per group) preincubated with 1 μM tetrodotoxin.

2.4. Experimental protocol

Fig. 1 provides a schematic representation of the experimental protocol applied. Four weeks after treatment with streptozotocin/solvent, the animals from both groups were exsanguinated. Food had been withdrawn 12 h prior to blood sampling for glucose and plasma somatostatin measurements. Plasma somatostatin immunoreactivity was determined by radioimmunoassay as described earlier (Nemeth et al., 1996; Szolcsanyi et al., 1998). The rats were divided into two experimental groups: 18 animals of the first group (control) were treated with the solvent for streptozotocin, the other 18 rats were given streptozotocin (second group). Twelve animals from each experimental group were killed for neuropeptide release studies. Blood samples were taken from the remaining 6 rats for fasting blood glucose and plasma somatostatin determination. The tracheas of these animals were removed for supplementary experiments to confirm the neuroselectivity of the field stimulation protocol used, i.e., the sensitivity of neuropeptide release to tetrodotoxin.

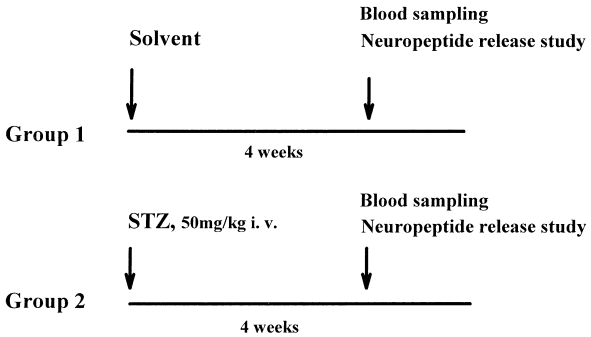

Fig. 1. Schematic representation of the experimental protocol. Group 1 indicates the controls, whereas Group 2 indicates the group of animals treated with streptozotocin (50 mg/kg i.v.). The horizontal lines represent the experimental process, the arrows indicate moments of particular interventions/examinations.

2.5. Statistical analysis

The results expressed as means ± standard deviation (S.D.) were analyzed with Student's *t*-test for paired data. Changes were considered significant at $P < 0.05$.

3. Results

3.1. Effects of diabetes on body weight, plasma glucose, and somatostatin levels

The animals in 'Group 1' grew steadily over the 4-week observation period, with an average weight gain of 42 ± 3.3 g, whereas the animals in 'Group 2' had a weight loss of 8 ± 1.3 g. Fasting blood glucose and plasma somatostatin levels were 4.4 ± 0.6 mmol/l and 6.36 ± 0.71 pmol/l in the controls (Group I) and 15.2 ± 3.9 mmol/l ($P < 0.01$) and 11.12 ± 3.36 pmol/l ($P < 0.05$) in the streptozotocin-treated animals (Group II), respectively.

3.2. Field stimulation-induced sensory neuropeptide release

Field stimulation induced a significant increase in levels of substance P, CGRP and somatostatin in the organ fluid of the tracheas from normal animals (Fig. 2). The release of these neuropeptides in response to the same field stimulation protocol was significantly attenuated when the tracheas were from diabetic animals. The resting neuropep-

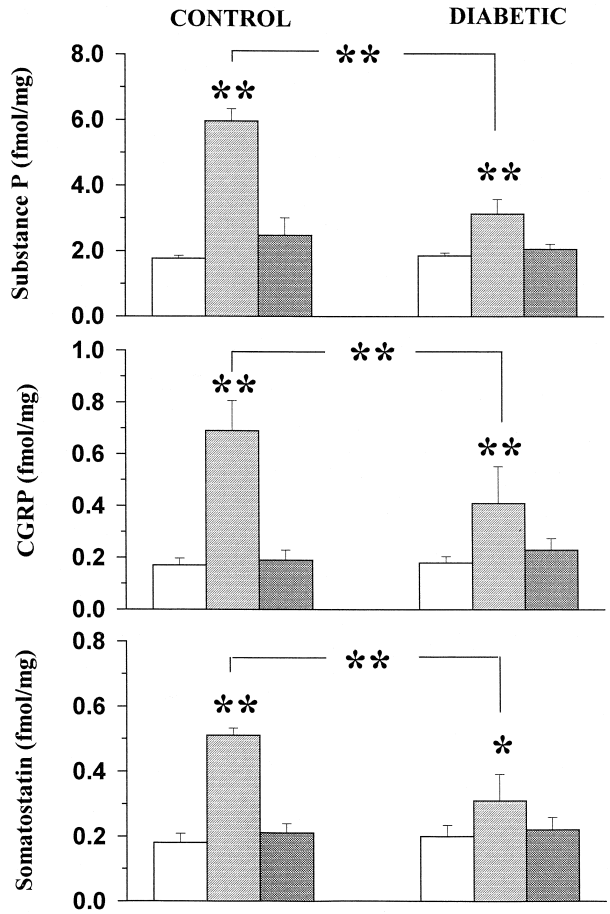

Fig. 2. Electrical field stimulation-induced sensory neuropeptide release from tracheas of normal and diabetic rats. The data are means ± S.D. obtained with 6 preparations in each group. Resting values are represented by blank columns (sampling 1; see Section 2). Hatched columns show neuropeptide concentrations in 'sample 2', i.e., after a 2-min period of field stimulation. Cross-hatched columns show corresponding 'sampling 3' data. * denotes a significant difference between values compared as indicated at $P < 0.05$; * * $p < 0.01$.

tide levels, however, were the same in both groups (Fig. 2). In normal animals, field stimulation failed to produce any significant increase in substance P, CGRP and somatostatin concentration in the presence of 1 μM tetrodotoxin; post-stimulation values of 2.23 ± 0.38, 0.15 ± 0.03 and 0.25 ± 0.03 vs. resting 2.32 ± 0.46, 0.13 ± 0.02 and 0.19 ± 0.03 fmol/mg wet wt tissue for substance P, CGRP and somatostatin, respectively. Similarly, tetrodotoxin completely blocked neuropeptide release in diabetic animals as well (data not shown).

4. Discussion

The results presented show that the release of substance P, CGRP and somatostatin from isolated tracheas of diabetic rats is substantially diminished in response to electrical field stimulation as compared to that of preparations from normal animals. Since the release of these peptides was completely blocked by tetrodotoxin, a fast Na^+ channel blocker, the neuropeptide release elicited by the field stimulation protocol applied can be considered to have been of neural origin. Fasting plasma somatostatin, however, was significantly increased in diabetic vs. normal animals.

Detrimental changes in autonomic, sensory and motor nerves are typically involved in diabetic neuropathy. Defective axonal transport, including that of neuropeptides, is believed to be a critical initiating factor in degenerative distal neuropathies leading to severe microcirculatory changes in diabetic patients. As the local effector function of peripheral sensory nerves is known to be supported by the ability of these nerves to release neuropeptides in response to various stimuli, direct measurement of the relevant neurotransmitters may serve as an indicator of the integrity of the effector function of sensory nerves. Depletion of CGRP and substance P content has been shown to occur in sensory nerves of streptozotocin-diabetic rats (Diemel et al., 1992); nevertheless, direct measurement of sensory neuropeptides released in response to a highly standardized challenge obviously provides a more accurate assessment of the functional effector capacity of sensory nerves in diseased states than does determination of tissue neurotransmitter contents. To the best of our knowledge, this report is the first to describe a deficiency in *simultaneous release* of the three neuropeptides, i.e., CGRP, substance P, and somatostatin in response to field stimulation in diabetic rats. Besides a significant decrease in neuropeptide release, the streptozotocin-treated rats had characteristic features of insulin-dependent (Type I) diabetes mellitus in that they failed to gain weight, and suffered from hyperglycaemia. It is therefore strongly suggested that the deficiency in neuropeptide release detected in the streptozotocin-treated group was caused by the ensuing diabetic state.

Beyond its local effector function, somatostatin has recently been shown to underlie a systemic anti-inflammatory effect (Szolcsanyi et al., 1998). The decrease in neural release of somatostatin in parallel with that of CGRP and substance P may participate in disturbed endogenous protective mechanisms against inflammation in insulin-deficient diabetes. The present data make it tempting to assume that the moderate increase in plasma somatostatin level recently described in streptozotocin-induced diabetes in rats (Fisher et al., 1998) may be somehow compensatory. Whatever the precise mechanism, the results provide a further description of the significant role diabetes-induced alterations in sensory effector neural mechanisms play in the development of late complications of diabetes mellitus.

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