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Original Contribution

EXPERIMENTAL PAINFUL DIABETIC NEUROPATHY: PHARMACOLOGICAL AND MORPHOLOGICAL APPROACH

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ABSTRACT
Development of streptozotocin-induced (STZ) diabetic neuropathy (behavioral and morphological) and effects of tramadol (30 mg/kg) and amitriptyline (10 mg/kg) on rats was investigated.
Changes in pain threshold were measured by dynamic plantar aesthesiometer, hot plate, analgesymeter. Transmission electron microscopy was applied. Tramadol and amitriptyline reduced the thermal hyperalgesia and mechanical allodynia. Tramadol showed similar efficacy on mechanical hyperalgesia. Partial separation of the axolemma from the myelin sheath, primary demyelination, hypertrophy of the basal lamina were found Our results suggest: i). STZ-induced diabetes as an animal model is useful for studying painful diabetic neuropathy; ii). tramadol is effective for this condition.
Key words: neuropathic pain, streptozotocin, diabetes, allodynia

INTRODUCTION

Neuropathic pain results from damage to the nervous system due to many diverse processes. Neuropathic pain is often reported as having a lancinating or continuous burning character and is often associated with the appearance of abnormal sensory signs, such as allodynia or hyperalgesia [1]. Rodent models of neuropathic pain are used to investigate the underlying mechanisms of pain associated with damage to peripheral nerves. The STZ-induced diabetic rat model demonstrates many of the abnormalities observed in humans [2]. Experimental models of diabetic neuropathy can spur development of understanding of pathogenesis and novel therapies approaches. Diabetic neuropathic pain is usually treated with antidepressants, anticonvulsants, nonsteroidal anti-inflammatory drugs, opioids. Admittedly neuropathic pain is resistant to opioid analgesia. Analgesic activity of tramadol was clearly established in nociceptive system in rats, but the effect of this atypical opioid on experimental neuropathic pain models is not fully investigated [3].

The aim of this study was to assess the development of diabetic neuropathy (behavioral and morphological) and effects of tramadol and amitriptiline in neuropathic pain syndrome in rat.

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MATERIALS AND METHODS

All experiments were approved by the Ethics Committee of the Medical University Sofia. Male rats (Wistar, 200-220g) were used. Animals were housed in groups of six under a 12 h light/dark cycle (lights on at 07.00 h) with food and water *ad libitum.* Each experimental group consisted of 6 - 8 animals. On day 0, diabetes was induced by the i.p. injection of STZ (75 mg/kg). Control animals received a similar administration of saline.

Behavioural testing: To assess tactile allodynia, paw withdrawal threshold to a non-noxious tactile stimulus was determined using von Frey filament application (Dynamic plantar aesthesiometer, Ugo Basile, Italy). Mechanical hyperalgesia was measured as the hind paw withdrawal, threshold to a noxious mechanical stimulus, and was determined using the paw pressure technique (Ugo Basile, Italy). Thermal

hyperalgesia was determined using hot plate test. Hind limb weight bearing was assessed using an incapacitance meter (2 Biolin, Italy).

Pharmacological treatment: Tramadol was applied in a dose of 30 mg/kg p.o., amitriptiline -10 mg/kg i.p. on day 15 from STZ injection.

Morphological investigation: Transmission electron microscopy was applied to study morphological changes. The rats were transcardially perfused with half-strength Karnovsky solution (2% paraformaldehyde and 2.5% glutaraldehyde) in 0,1M phosphate buffer. Tissue pieces from sciatic nerve were dehydrated, postfixed in $0s0_4$ and embedded in Durcupan. Ultrathin sections were stained with lead citrate and uranyl acetate and viewed in Hitachi H-500 electron microscope. Statistical analysis. The data are expressed as means ± SEM. The obtained data were evaluated by the one-way analysis of variance (ANOVA), followed by Dunnett's multiple comparison test; $p < 0.05$ was considered significant.

RESULTS AND DISSCUSION

Tactile allodynia was determined by measuring the paw withdrawal threshold. Animals with STZ-induced diabetes (hyperglycemia > or = 14 mM) displayed allodynia on day 15 in response to the mechanical stimulus with a mean withdrawal latency of the paw of 25.2 ± 3 g; the mean withdrawal latency of the paw of vehicle-treated diabetic rats was 35.4 ± 2 g. Treatment with tramadol 30 mg/kg and amitriptiline 10 mg/kg significantly increases the nociceptive threshold (Figure 1).

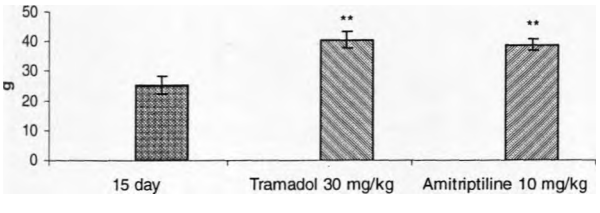


*Figure 1. STZ-induced diabetes. Effects of ramadol and amitriptiline on mechanical allodynia, von Frey withdrawal threshold (g); **p < 0.01* vs. *diabetic rats*

The paw pressure withdrawal threshold for diabetic rats reach significance about 20 day after induction of diabetes. Tramadol (30 mg/kg) showed analgesic activity on mechanical hyperalgesia, but amitriptiline (10 mg/kg) did not change pain threshold (Figure 2).

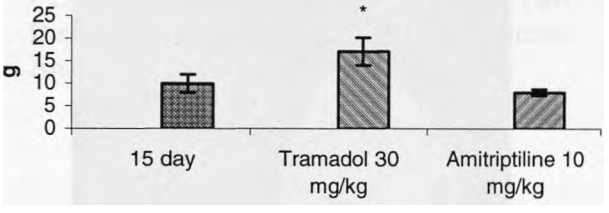
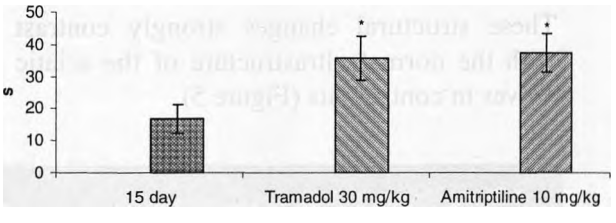


Figure 2. *STZ-induced diabetes. Effects of tramadol and amitriptiline on mechanical hyperalgesia on paw pressure test (gxlO): **p < 0.01* vs. *diabetic rats*

Thermal hyperalgesia was determined by measuring the withdrawal latency to a hot plate. Tramadol (36 ± 7 s) and amitriptiline (37.5 ± 6 s) increase withdrawal latency compared with the non treated diabetic group on day 15 (16.9 ±4.5 s) (Figure 3).

***Figure** 3. STZ-induced diabetes. Effects of*



*tramadol and amitriptiline on termal hyperalgesia, heat withdrawal threshold (s); * p < 0.05 vs. diabetic rats*

STZ-induced diabetic rats did not exhibit significant changes in the weight-distribution ratio (incapacitance test) compared with the non treated diabetic rats.

Under the electron microscope, the sciatic nerves of the diabetic rats expose several changes (Figure 4).

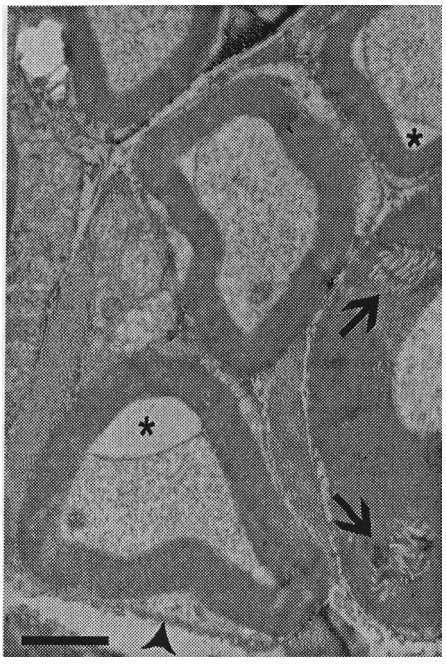


Figure 4. STZ diabetic rat. Sciatic nerve. Separation of the axolemma from the myelin (asterisks). Myelin defects (arrows). Schwann cell basal lamina is thickened (arrowhead). Scale bars = 1 μm

Axon and its axolemma are partially detached from the myelin sheath as a sign of an initial neurite injury. The myelin sheath profiles show focal destruction of the lamellar structure. The Schwann cell basal lamina is often thickened but also loosened. These structural changes strongly contrast with the normal ultrastructure of the sciatic nerves in control rats (Figure 5).

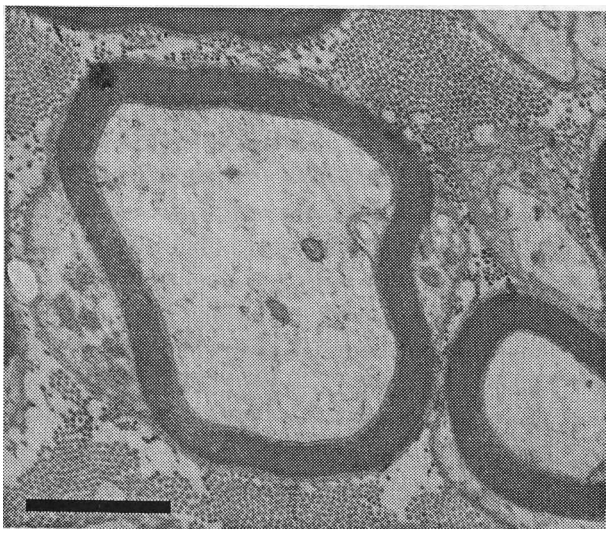


Figure 5. *Control rat. Sciatic nerve*

The majority of animal models of neuropathy have been based on a discrete peripheral nerve injury [4]. Streptozotocin model of peripheral diabetic neuropathy closely mimic individual disease [5]. Conflicting results have been obtained in experimentally diabetic animals subjected to pain stimuli [6]. In our experiment tactile allodynia develops early post STZ injection. Allodynia and thermal hyperalgesia are sensitive to treatment with amitriptyline, a non-selective serotonin and noradrenaline reuptake blocker, and tramadol, non typical opioid analgesic, inhibitor of norepinephrine and serotonin reuptake. We found a corelation between neuropathic pain and structural changes in peripheral nerve. It is possible that different mechanisms of neuropathic pain or mechanism of action of drugs explain our results.

CONCLUSION

Our results suggest that STZ-induced diabetes as an animal model is useful for studying painful diabetic neuropathy and that tramadol is an effective medication for this condition.

ACKNOWLEDGMENTS

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