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α_1- and α_2-Adrenoreceptor antagonists in streptozotocin- and vincristine-induced hyperalgesia

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Abstract:
The effect of α_1- and α_2-adrenoreceptor antagonists (prazosin and yohimbine, respectively) on streptozotocin (STZ)- and vincristine (VIN)-induced hyperalgesia in rats was studied. In two experimental models, yohimbine (1.0 mg/kg *ip*) completely abolished STZ- and VIN-induced hyperalgesia. This effect was markedly prolonged in diabetic rats. Prazosin (0.3 mg/kg *ip*) attenuated and delayed development of STZ-induced hyperalgesia. In VIN-elicited neuropathy, the administration of prazosin not only delayed hyperalgesia but also produced antinociception. After cessation of drug administration, a significant decrease in nociceptive threshold was observed. The obtained results seem to indicate that both α_1- and α_2-adrenoreceptors are engaged in diabetic (STZ) and toxic (VIN) neuropathy.

Key words:
adrenergic system, hyperalgesia, prazosin, rats, streptozotocin, vincristine, yohimbine

Abbreviations: NE – norepinephrine, NO – nitric oxide, PGs – prostaglandins, PGSNs – postganglionic sympathetic neurons, PRA – prazosin, STZ – streptozotocin, VIN – vincristine, YOH – yohimbine

Introduction

Neuropathy still represents a significant clinical problem. This kind of pain results from peripheral nervous system damage caused by metabolic disorders, toxic chemicals or a direct mechanical injury. Neuropathy-related complications resulting from chronic diabetes and neuropathies occurring in the course of anticancer chemotherapy are typically accompanied by neuropathic pain. The experimental model of neuropathic pain caused by administration of streptozotocin (STZ), also known as a model of painful diabetic neuropathy/hyperalgesia, and model of pain caused by administration of vincristine (VIN), known as a model of VIN-induced neuropathy/hyperalgesia, are commonly used to investigate this type of pain in animal studies.

Mechanisms underlying neuropathic pain are still poorly understood. Not only structural damage of neurons, but also concurrent inflammatory process contributes to onset and development of neuropathic pain. This process includes the release of bradykinin, serotonin, prostaglandins (PGs), nitric oxide (NO), nerve growth factor, cytokines and free radicals at

the site of nerve injury [10]. Results of our previous studies [8] suggest that both B_1 and B_2 receptors are involved in transmission of nociceptive stimuli in VIN- and STZ-induced neuropathy. We also showed that cyclooxygenase-1, cyclooxygenase-2, as well as inducible NO synthase contributed to transmission of pain stimuli in the model of STZ-induced diabetic neuropathy [7].

It is well known that peripheral neuropathy has also been associated with abnormal function of sympathetic nervous system [35]. Both ascending and descending adrenergic systems seemed to be involved in the modulation of nociceptive transmission. α_2-Adrenoreceptors are widely distributed presynaptically on noradrenergic and nonadrenergic neurons and their stimulation resulted in inhibition of appropriate neurotransmitters [24, 26]. Stimulation of structures belonging to the bulbospinal monoaminergic descending inhibitory pathways i.e. parabrachial nuclei, locus coeruleus promote release of norepinephrine (NE) with consequent blockade of spinal nociceptive reflexes. This antinociceptive action is mediated by NE-releated activation of dorsal horn α_2-receptors [30]. Epidural and intrathecal administration of an α_2-agonist clonidine is known to abolish the sharp intra- or postoperative pain as well as cancer pain unresponsive to opioids. However, the possible role of α_1-receptors in the modulation of pain perception cannot be completely excluded. It was shown that nociceptors become overresponsive to NE if they are sensitized or their peripheral nerves are damaged. Chemical or surgical sympathectomy attenuated hyperalgesia in models of neuropathic pain after partial transection of the sciatic nerve [37].

It was, therefore, of interest to check the effect of α_1- and α_2-adrenoreceptor antagonists (prazosin – PRA and yohimbine – YOH, respectively) on STZ- and VIN-induced hyperalgesia.

Materials and Methods

Animals

Study was conducted according to the guidelines of the Ethical Committee for Experiments on Small Animals, Medical University of Warsaw. The aforementioned committee approved the experimental protocols.

Male Wistar rats (250–300 g) were housed in a room maintained at a temperature of 20 ± 2°C, under 12–12 h light/dark cycle. The animals had a free access to food and water. Experimental groups consisted of at least six rats. Food was removed 16 h before STZ administration. Each animal was used in one experiment only (i.e. for administration of STZ plus one agent).

Chemicals

Prazosin, yohimbine, streptozotocin (N-[methylnitrosocarbamoyl]-α-D-glucosamine) and vincristine sulfate were purchased from Sigma Chemical Co., USA.

Equipment

Experiments were conducted using analgesimeter to exert progressively increased pressure stimulus (type 7200, manufactured by Ugo-Basile Biological Research Apparatus, 21025 Comerio – Varese, Italy), and blood glucometer Accu-Check Active, manufactured by Roche Diagnostics Corporation.

Streptozotocin-induced diabetes

Diabetes was induced by intramuscular administration of STZ at a dose of 40 mg/kg, as described by Nakhoda and Wong [27]. STZ was dissolved in citrate buffer at pH 4.5 and administered at only one dose on the first day of the study into the thigh muscles of rat's leg. Before the induction of diabetes, the animals were fasted over 16 h. Following the injection, the food and water were available *ad libitum* during the remaining 30 days of experiment. Control rats received an equal volume of buffer. Starting on day 3 (72 h after STZ administration), levels of glucose were determined using a blood glucometer. Blood samples for the glucose determinations were drawn from tail vein. Permanent severe hyperglycemia was observed (> 400 mg/dl) in all rats.

In STZ-untreated animals, the glucose levels amounted to about 120 mg/dl and remained stable during 30 days of the observation period.

The STZ induced hyperglycemia was accompanied by a gradual decrease in rats' body mass, increase in food consumption, as well as considerable increase in water intake.

Chemotherapy (vincristine)-induced painful neuropathy

VIN neuropathy was induced as described by Aley et al. [1, 2]. VIN sulfate was dissolved in redistilled water to a stock concentration of 1 mg/ml and then stored at 4°C. Immediately before administration, the stock was diluted in distilled water to a concentration of 70 μg/ml. The solution was administered into the tail vein at a dose of 70 μg/kg. Administrations of VIN were performed daily, Monday through Friday, for 10 days (experiment lasted 12 days, no doses of drug was given on Saturdays or Sundays). The dosage calculations were based on daily body weight. Weight-matched control rats received injections of redistilled water [1].

No weight gain was observed in rats receiving intravenously (*iv*) VIN at a dose of 70 μg/kg.

Drug administration

STZ and VIN were administered as described above. PRA and YOH were dissolved in redistilled water immediately before injection. PRA (0.3 mg/kg) and YOH (1.0 mg/kg) were applied intraperitoneally (*ip*). Doses of both drugs were based on previous observations as well as on literature data [4, 6, 19].

Time schedule

In the model of diabetic neuropathy, PRA and YOH were administered on day one 15 min before STZ. All drugs (except for STZ given only on the first day) were administered once a day at the same time for 21 days.

In the chemotherapy-induced neuropathy model, PRA and YOH were administered daily 15 min before VIN for 10 days (2 × 5 – see above).

Control animals were injected according to the same time schedule: *ip* with redistilled water (control to PRA and YOH).

Increased locomotor activity and aggressive behavior were observed in animals receiving YOH. No marked changes in blood pressure were noted during 24 h after either YOH or PRA administration, except for small and insignificant decrease observed 1 h after PRA application.

Measurement of the nociceptive threshold

Changes in pain thresholds were determined using mechanical stimuli, according to the modification of the classic paw withdrawal test described by Randall and Selitto [29]. In order to measure mechanical stimulation, a progressively increased pressure was applied to the dorsal surface of the rat's paw using an analgesimeter. The used instrument increased the force on the paw at a rate of 32 grams per second. The nociceptive threshold was defined as force in grams, at which the rat attempted to withdraw its right hind paw, and values of pressure were recorded at this very moment. Nociceptive threshold was measured in triplicate and the mean was used for further calculations. At least two observers recorded the response.

Nociceptive thresholds (average of three trials) measured for each animal immediately before STZ (effect of STZ alone) or before drug and STZ administration on the first day of the study constituted the baseline pain threshold (A). Next measurements of withdrawal threshold to mechanical stimuli were performed daily before drug administration (until day 21) and after cessation of drugs until the end of experiment (B).

Similarly, nociceptive threshold measured on the first day immediately before VIN or drug + VIN administration was considered as baseline (A). Next measurements of withdrawal threshold to mechanical stimuli were performed daily (from day 2 to 5 and from day 8 to 12 of experiment) before VIN or drug + VIN administration. After cessation of VIN + drug nociceptive thresholds were measured daily until day 32 of experiment (B).

Values of thresholds obtained (B) in all experimental sessions (i.e., every day, investigated every drug), were compared to baseline (A).

Changes in pain threshold were calculated as percent of baseline value according to the following formula:

$$\text{Pressure in grams} = B - A$$

A – baseline pressure (in g), measurements on the first day of the study before drugs administration (as mentioned above);
B – pressure (in g), measurements performed daily (except for the first day) before drugs administration.

Pressures in grams calculated as described above were subsequently used for statistical analyses.

Statistical analysis

The results are expressed as the mean values ± standard error of the mean (± SEM). The statistical significance of differences between groups was evaluated by the two-way ANOVA test; $p \leq 0.05$ was accepted as statistically significant. All statistical calculations were performed using the computer software described by Tallarida and Murray [33].

Results

Effect of STZ on nociceptive threshold to mechanical stimuli

As shown in Figure 1, starting from the day 5 a statistically significant gradual decrease in the nociceptive threshold was observed in STZ-treated animals. The decrease reached its nadir on day 20 and remained at similar level until the end of experiment.

Effect of PRA and YOH on STZ-induced hyperalgesia

As shown in Figure 1 and 2, PRA and YOH significantly modified the development of diabetic hyperalgesia. However, marked differences between both drugs were observed. PRA delayed the development of hyperalgesia. Until day 8 nociceptive thresholds remained at the baseline level. Starting from the day 8, this effect gradually disappeared, and after drug withdrawal, the threshold returned to baseline values observed in STZ-treated animals. PRA administration did not modify nociceptive threshold in the naive rats (Fig. 1).

YOH completely prevented the development of hyperalgesia and during the entire period of its administration (i.e. until day 21) the nociceptive threshold remained at the baseline level. After cessation of YOH administration a small decrease in nociceptive threshold occurred. It is of interest to note that in contrast to PRA-pretreated animals, YOH significantly decreased the nociceptive threshold in naive rats. After cessation of drug administration nociceptive threshold returned to baseline values (Fig. 2).

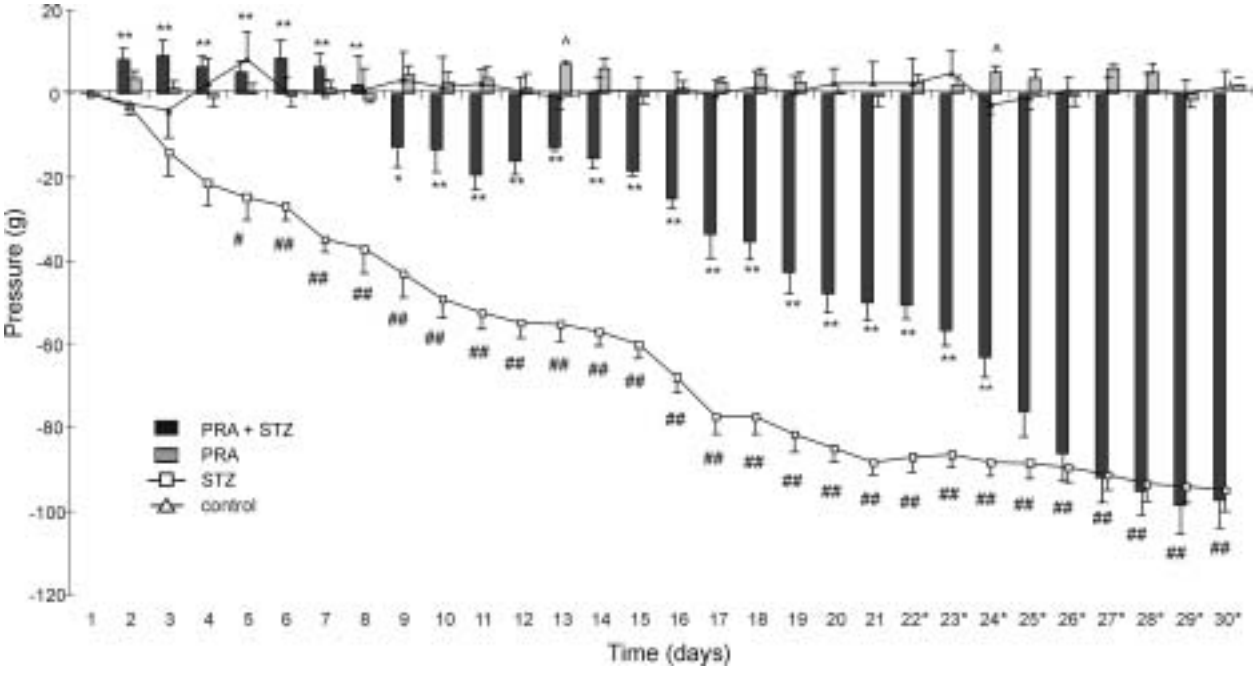

Fig. 1. Effect of prazosin (PRA) at a dose of 0.3 mg/kg *ip* administered to streptozotocin (STZ)-treated or naive rats. Days 1–21 – measurements of prolonged activity of the investigated drug. Days 22*–30* – after discontinuation of PRA administration. Values are the means ± SEM. PRA + STZ *vs.* STZ ** $p \leq 0.01$ * $p \leq 0.05$; PRA *vs.* control ^ $p \leq 0.05$; STZ *vs.* control ## $p \leq 0.01$ # $p \leq 0.05$

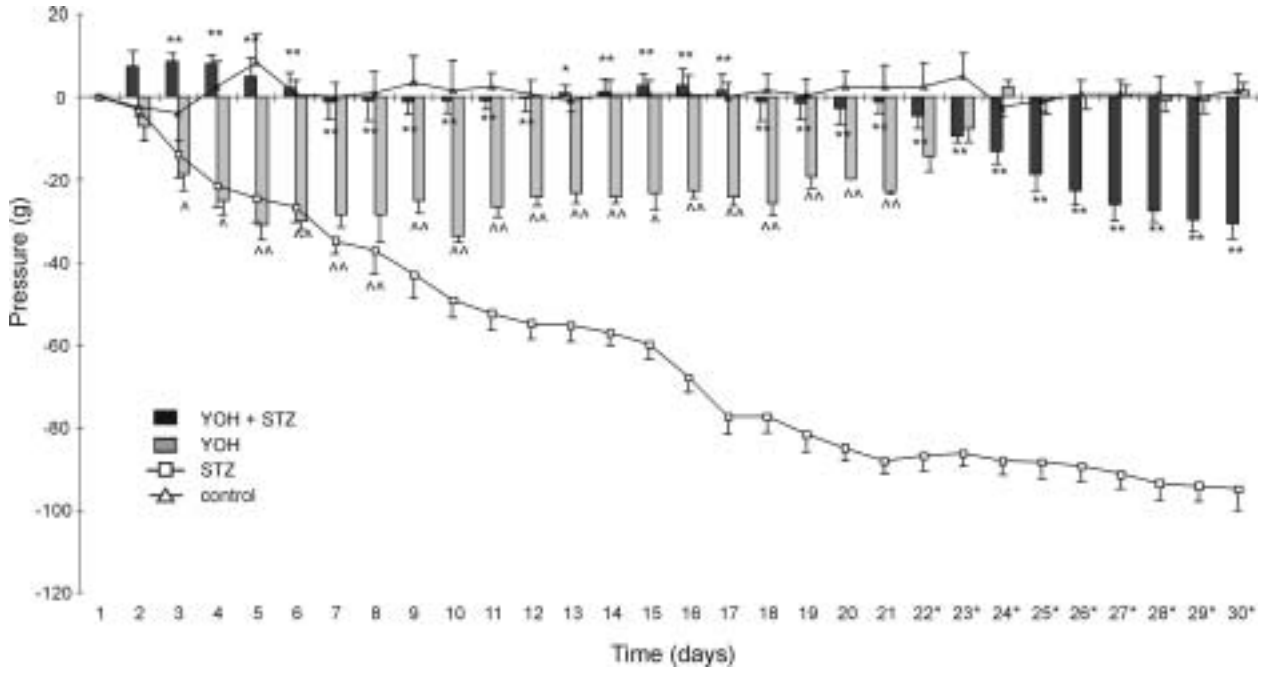

Fig. 2. Effect of yohimbine (YOH) at a dose of 1.0 mg/kg *ip* administered to streptozotocin (STZ)-treated or naive rats. Days 1–21, measurements of prolonged activity of the investigated drug. Days 22*–30*, after discontinuation of administration of YOH. Values are the means ± SEM. YOH + STZ *vs.* STZ ** $p \leq 0.01$ * $p \leq 0.05$; YOH *vs.* control ^^ $p \leq 0.01$ ^ $p \leq 0.05$

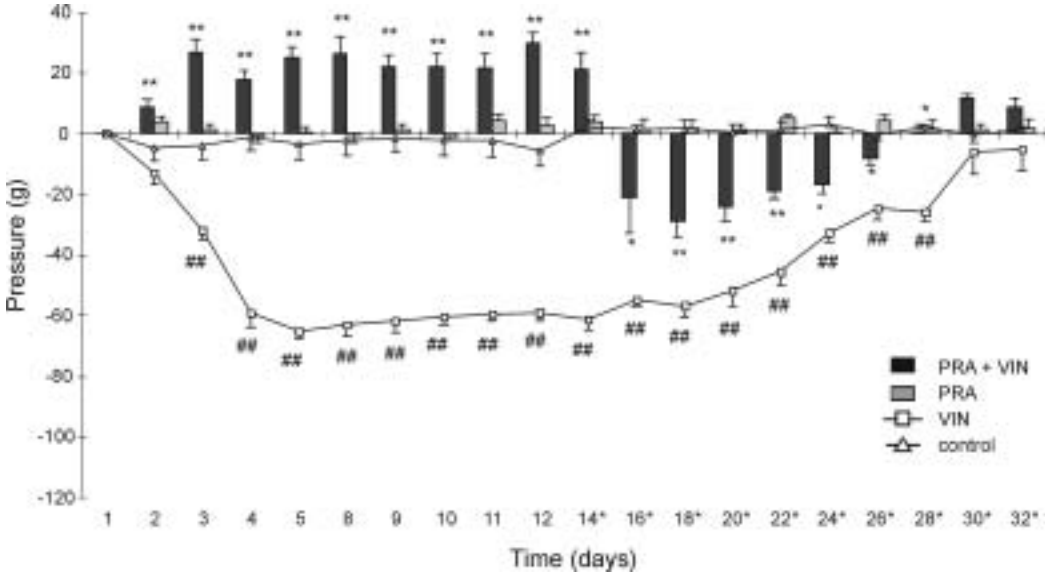

Fig. 3. Effect of prazosin (PRA) at a dose of 0.3 mg/kg *ip* administered to vincristine (VIN)-treated or naive rats. Days 1–12, measurements of prolonged activity of the investigated drug. Days 14*–32*, after discontinuation of administration of PRA or VIN + PRA. Values are the means ± SEM. PRA + VIN *vs.* VIN ** $p \leq 0.01$ * $p \leq 0.05$; VIN *vs.* control ## $p \leq 0.01$

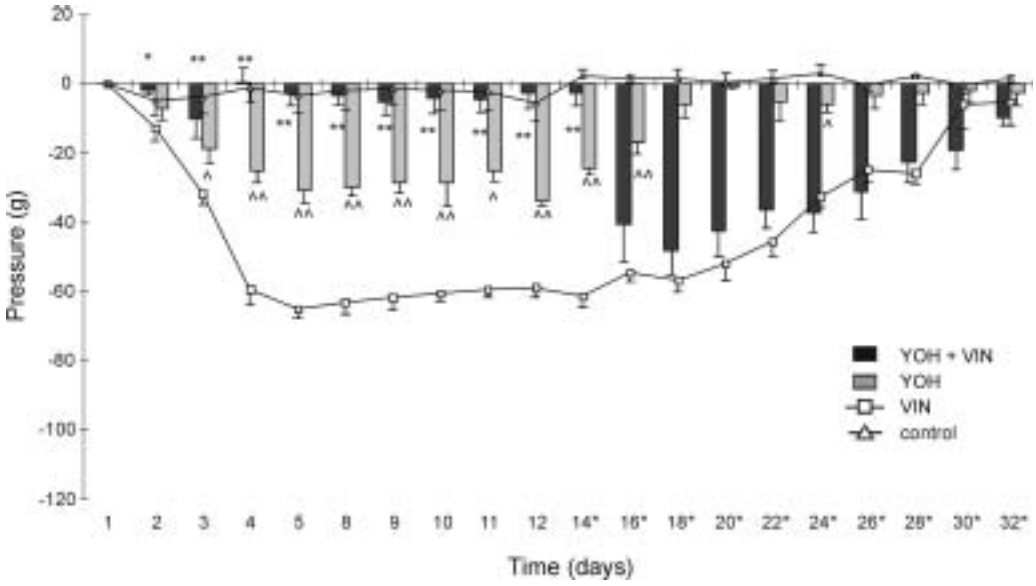

Fig. 4. Effect of yohimbine (YOH) at a dose of 1.0 mg/kg *ip* administered to vincristine (VIN)-treated or naive rats. Days 1–12, measurements of prolonged activity of the investigated drug. Days 14*–32*, after discontinuation of administration of YOH or VIN + YOH. Values are the means ± SEM. YOH + VIN *vs.* VIN ** $p \leq 0.01$ * $p \leq 0.05$; YOH *vs.* control ^^ $p \leq 0.01$ ^ $p \leq 0.05$

Effect of VIN on nociceptive thresholds to mechanical stimuli

Starting from day 3, a statistically significant gradual decrease in the nociceptive threshold was observed in VIN-treated animals. The decrease reached its nadir on day 5 of experiment and remained approximately stable until day 12. After discontinuation of VIN administration from the day 14, nociceptive thresholds to mechanical stimuli gradually increased and on day 32 returned to baseline values (Fig. 3).

Effect of PRA and YOH on the development of VIN-induced hyperalgesia

Daily administration of PRA not only prevented the VIN-induced hyperalgesia but also produced antinociception. After cessation of drug administration (from day 14 of experiment), a significant increase in nociceptive threshold in relation to the VIN-treated group was observed. When administered to the naive rats, PRA was without effect on nociceptive threshold. Daily administered YOH almost completely abolished VIN hyperalgesia. However, this effect was not prolonged. After cessation of drug administration, the values of hyperalgesia were comparable to those obtained in VIN alone-treated group. YOH administered daily markedly lowered the pain threshold in naive rats. After withdrawal of the drug, nociceptive threshold returned to normal values (Fig. 4).

Discussion

Diabetic neuropathy is typically accompanied by neuropathic pain and hyperalgesia. Furthermore, as shown both in human and animal studies, hyperalgesia precedes the development of neuropathy [14, 15, 17, 18, 28, 32]. In rats, diabetes produced by STZ administration seemed to represent an appropriate model for investigation of diabetes-induced hyperalgesia and neuropathy [14, 31].

In our investigations, a stable hyperglycemia was observed 72 h after STZ administration. Elevated blood glucose concentrations are maintained over the remaining period of experiment (30 days). An increase in water intake with accompanying rise of excreted urine volume, increase in food intake, as well as gradual decrease in body mass were also observed. Diabetogenic action of STZ was accompanied by development of persistent hyperalgesia. The considerable lowering of the withdrawal threshold to mechanical stimuli occurred on day 5 of investigations and

threshold values gradually decreased until day 20. From 21 to 30-day hyperalgesia remained at similar level.

These results are similar to those reported by Aley and Levine [1], who demonstrated the development of hyperalgesia in response to mechanical stimulus after administration of a single *iv* dose (50 mg/kg) of STZ.

The VIN model of chemotherapeutic-induced painful toxic neuropathy provides an opportunity to investigate mechanisms involved in this form of neuropathic pain [11, 23]. Tanner et al. [34] suggested that in rats VIN caused disorganization of the axonal microtubule cytoskeleton as well as an increase in the caliber of unmyelinated sensory axons. Topp et al. [36] drew similar conclusions. These authors observed that VIN-induced hyperalgesia was accompanied by a decrease in microtubule density (probably due to swelling of axons and not to decrease in the number of microtubules per axons) and an increase in tangentially oriented microtubules per axon compared to controls.

In this study, daily administration of VIN (70 μg/kg) resulted in progressive decrease in pain threshold. Diminishing of pain threshold was significant on day 3. Thereafter, a nociceptive threshold progressively decreased until day 5 of VIN administration and then remained approximately stable until withdrawal of the drug. In contrast to STZ which produced long-lasting persistent hyperalgesia, the hyperalgesia due to VIN was reversible and after cessation of drug administration (after 12 days) nociceptive threshold gradually returned to initial values.

The results presented here are similar to those reported by Aley and coworkers, who demonstrated the appearance of hyperalgesia in response to mechanical stimulus after administration of VIN at the doses of 100 μg/kg and 200 μg/kg. No significant differences in intensity of hyperalgetic effects between doses were observed in this study. However, unlike animals receiving VIN at 100 μg/kg dose, rats given 200 μg/kg lost on the average 12.5% of body weight during the experiment but regained weight when the drug administration was stopped [2].

In a previous study [8] administration of VIN at a dose of 100 μg/kg produced very intensive hyperalgesia, which persisted after discontinuation of drug administration and was connected with marked toxicity. Since a dose of 70 μg/kg also markedly decreased nociceptive threshold (without concomitant loss of body weight) but hyperalgesia disappeared after drug withdrawal, this dose was used in the present study.

Several evidences indicate that alterations in monoamine systems are implicated in the mechanism of neuropathic pain [16]. Whether these abnormalities are involved in diabetic and toxic neuropathy is unclear. The data from the experiments investigating the implication of adrenergic system in neuropathic diabetic pain are controversial and contradictory. In literature, there are no available data concerning the influence of adrenergic system on VIN hyperalgesia.

Lee et al. [21] showed that α_1-adrenoreceptor agonists, phenylephrine (3 mg/kg *sc*), aggravated, while α_1-adrenoreceptor antagonists, prazosin (3 mg/kg *sc*), alleviated mechanical and cold allodynia in the diabetic rats. Phenylephrine injected *sc* at a dose of 15 μg into the affected paw produced exacerbation of mechanical hyperalgesia in the experimental model in which the sciatic nerve is partially transected [37]. It is known that reduced peripheral nerve conduction velocity in diabetic rats is associated with decreased blood flow, which leads to endoneurial hypoxia. α_1-Adrenoreceptor antagonists improve conduction velocity in diabetic rats. Cotter and Cameron [12] showed that α_1-adrenoceptor blockade by doxazosin substantially reversed diabetic deficit in sciatic nutritive endoneurial blood flow (61.0%). Liu et al. [22] observed that nociceptive paw-withdrawal threshold in diabetic rats was significantly decreased by noradrenaline and increased by phentolamine or YOH, but not by PRA. However, after sympathetic postganglionic neuron damage by 6-hydroxydopamine, nociceptive pain threshold was not changed by the above-mentioned drugs. Courteix et al. [13] showed that α_2-receptor agonist, clonidine, administered at doses of 50, 100 and 150 μg/kg *sc*, alleviated STZ-hyperalgesia in paw pressure test. Bitar et al. [3, 4] showed that in comparison with normal animals, spinal release of NE was markedly suppressed in STZ-treated diabetic male and female rats 30 days after diabetes induction while clonidine-induced elevation in nociceptive threshold was attenuated. It was also shown that systemic administration of clonidine and YOH produced dose-dependent analgesic and hyperalgesic effects, respectively, in naive animals. On the other hand, diminishing ability of these compounds to alter nociceptive threshold was evident in chronically diabetic rats. In humans, only a weak effect of clonidine on diabetic neuropathy was reported [9, 25]. The above-mentioned discrepancies in the results of studies evaluating the effects of adrenoreceptor agonists and antagonists on the neuropathic pain may depend

on several factors: kind of tests and experimental procedures used, various strains of animal and different drugs and routes of its administration [20]. In addition, results obtained in this study are difficult to interpret.

In the present study, daily administration of prazosin attenuated and delayed development of STZ hyperalgesia. In fact, until day 8 of experiment, no hyperalgesia in STZ-treated animals was noted and then a small gradual decrease occurred. After cessation of drug administration pain threshold quickly returned to values observed in STZ-treated animals.

Also in the model of VIN neuropathy, daily administration of PRA not only delayed VIN hyperalgesia but produced antinociception. After cessation of drug administration, a significant increase in nociceptive threshold in relation to the VIN-treated group was observed. In two experimental models, YOH completely abolished STZ and VIN hyperalgesia. However, it is interesting to note that contrary to the VIN model, in diabetic rats this effect was markedly prolonged. The significant reduction of hyperalgesia was still observed 14 days after cessation of drug administration. It is worthy of notice that, in contrast to PRA, which was without effect on nociceptive threshold in naive animals, YOH significantly decreased the pain threshold. It is commonly known that NE released from endings of descending noradrenergic systems originating supraspinally-attenuated transmission of pain stimuli. This antinociceptive action is mediated by dorsal horn α_2-receptors [5, 30]. Thus, we can speculate that YOH significantly decreases the nociceptive threshold in naive rats by blocking α_2-receptors in the spinal cord. In contrast to the naive rats, in animals with chronic neuropathic pain, NE sensitizes nociceptors indirectly by acting on α_2-receptors located probably on postganglionic sympathetic neurons (PGSNs). This increases production and release of PGs from the PGSNs terminals. PGs directly act on the primary afferents producing hyperalgesia [37]. Thus, administration of an α_2-receptor agonist under conditions of increased expression of these receptors can intensify hyperalgesia, while blockade of α_2-receptors by an antagonist should possess an opposite action.

The observed similarities in the action of PRA and YOH on diabetic and VIN hyperalgesia are difficult to explain. However, other authors in different experimental models also showed that PRA alleviated allodynia in the diabetic rats [21] whereas injection of the non-specific α-adrenergic blocker, phentolamine, and YOH significantly relieved the hyperalgesia in the model in which the sciatic nerve was partially transected [30]. It cannot be excluded that these two drugs, at least at the doses used, act preferentially on two separate descending adrenergic pathways in the central nervous system i.e. tract originating in A5, A6 (locus coeruleus) and those whose cells are localized in pontine A7 region [24] , in other words they attenuated the activity of two different systems.

To confirm these hypothesis further studies are in progress, however, it seems that to resolve this problem, involvement of more complex and precise methods will be necessary.

Concluding, the results of this study for the first time indicate that adrenergic system is involved in VIN-induced hyperalgesia. This paper also suggests a participation of α_1- and α_2-receptors in STZ-hyperalgesia.

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