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ANTINOCICEPTION INDUCED BY A NOVEL BENZODIAZEPINE RECEPTOR AGONIST AND BRADYKININ RECEPTOR ANTAGONIST IN RODENT ACUTE AND CHRONIC PAIN MODELS

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ABSTRACT
The mechanisms and antinociceptive effects of a novel benzodiazepine receptor agonist and bradykinin receptor antagonist, 7- bromo-5-(*o*-chlorophenyl)-3-propoxy-1,2-dihydro-3H-1,4-benzodiazepin-2-one (propoxazepam) were studied on animal models of acute and chronic pain and compared to the antinociceptive profiles of gabapentin and ketorolac. We also studied the possible role of GABAergic and bradykinin-ergic system on propoxazepam effects. The effects of propoxazepam on pain responses were examined using tail-flick test (TFT) in rats, Streptozotoci-induced diabetes rat model (SPZ) and sciatic nerve injury (SNI)-induced hyperalgesia in rats. Propoxazepam (3 mg/kg)) produced statistically significant analgesic effect compared to the control and ketorolac values after acute application in TFT and SNI-induced hyperalgesia in rats. Propoxazepam (2 mg/kg) in compare to gabapentin (5 mg/kg) in greater degree after both acute single and chronic administrations produced analgesic action in SPZ-diabetic rats. Propoxazepam administration reduced bradykinin-induced (0.01%) hyperalgesia. At low dose (1 mg/kg) flumazenil diminished propoxazepam antinociceptive effect while at higher dose (10 mg/kg) had nearly no influence, possibly due to GABA-receptor complex stabilization. These results suggest that propoxazepam causes both nociceptive and neuropathic analgesia in rats and $GABA_A$-receptor and bradykinin B-receptor are a key sites of the analgesic action of propoxazepam.

KEYWORDS: Propoxazepam, antinociception, neuropathic pain, streptozotocin-induced diabetes, benzodiazepine receptor agonist, bradykinin antagonist.

INTRODUCTION
Almost since their introduction, there has been interest to the therapeutic application of the benzodiazepines for the management of pain. As with many other drugs initially developed and studied for indications other than pain, conclusive data regarding the analgesic activity of the benzodiazepines are lacking.

A relevant aspect of neuroplastic changes in inflammatory and neuropathic conditions is the reduction in inhibitory glycinergic and GABAergic control of dorsal horn neurons: a reduction in the $GABA_A$-mediated endogenous inhibitory control within the central nervous system leads to exaggerated pain and hyperalgesia.[1] Potentiation of $GABA_A$ receptor-mediated synaptic inhibition by benzodiazepines reverses pathologically increased pain sensitivity in animal studies.[2]

Knabl et al. (2008) has shown[3] that intrathecal injection of diazepam reduced inflammatory heat hyperalgesia, as well as chronic constriction injury (CCI)-induced heat hyperalgesia, cold allodynia and mechanical sensitization in mice. Subtype-selective compounds targeting the alpha2 and/or alpha3 subunit of the $GABA_A$ receptor produce antihyperalgesia in mice and rats without sedation and without tolerance induction. These findings open new perspectives for a more selective targeting of pain pathways with GABAergic drugs. Furthermore, C3-substituent 1,4-benzodiazepines can mimic the β-turn that is important for their pharmacological activity as selective bradykinin B1 antagonists.[4,5] The β -turn is a structural motif that has been postulated in biologically active form of Arg-Pro-Pro-Gly-Phe-Ser-Pro-Leu.[6,7]

A number of novel 3-substituted 1,4-benzodiazepines have been synthesized at the Physico-Chemical Institute of the National Academy of Sciences of Ukraine and

their pharmacological activity was studied. These compounds demonstrated clearly pronounced anti-inflammatory and antinociceptive properties in the acetic acid-induced writhing test (induction of visceral pain) in mice, test with carrageenan-induced paw edema in rats, and formalin test in mice.[8] They hypothesized the mechanism underlying inhibition of bradykinin receptors. Compounds demonstrated the similar inhibition effect on bradykinin-induced contraction of smooth muscle like competitive inhibitor des-arg^9-bradykinin-acetate to bradykinin B2-receptors.[9,10]

Additionally, there was examined[9] their *in vitro* affinity for both the central benzodiazepine receptor (CBR) and the peripheral benzodiazepine receptor (PBR) of rat brain, with one of them, propoxazepam, 7- bromo-5-(*o*-chlorophenyl)- 3-propoxy-1,2-dihydro-3H-1,4-benzodiazepin-2-one, is considered as a promising drug which now undergoes preclinical trials phase.

The aim of this study was to evaluate the antinociceptive effects of propoxazepam: 7-bromo-5-(o-chlorophenyl)-3-propoxy-1,2-dihydro-3H-1,4-benzodiazepin-2-one in animal models of acute and neuropathic pain and to compare its antinociceptive profile with those of gabapentin and ketorolac. Additionally, we examined the possible mechanism of action of propoxazepam–mediated antinociceptive effects.

MATERIALS AND METHODS

Animals and injection procedures

Male Wistar rats (180-210 g), obtained from Institute of Pharmacology and toxicology NAMS of Ukraine housed at the local animal department, were used. The animals were exposed to a 12 h light-dark cycle and were provided with food and water *ad libitum*. All experiments were conducted during the light part of the day (9.00-14.00). The experiments were carried out according to the recommendations of the Committee for Research and Ethical Issues of the IASP (1983) and were approved by the regional ethical committee for animal research. All manipulations were made to minimize animal suffering and to reduce the number of animals used.

The test compounds were suspended in tween 80 (1%) emulsion, and the control animals received corresponding amount of vehicle (1% tween 80).

Drugs and chemicals

Propoxazepam was synthesized according to the method described in.[**Error! Bookmark not defined.**] The structure of the substance was determined and approved by a complex of physicochemical methods (IR and mass spectroscopy, as well as X-ray diffraction analysis). Chemical purity was confirmed by elemental analysis (99%). Streptozotocin (STZ), bradykinin, Gabapentin and ketorolac were obtained from Sigma, USA. Flumazenil was obtained from Toronto Research Chemicals (Canada).

Antinociceptive activity test

Tail-flick test (TFT)

The tail flick latency, defined by the time (in seconds) of withdrawal of the tail from a radiant heat source, according to[11] was measured via the usage of a semiautomatic device (tail flick unit, Ugo Basil, Italy). After the placement of the rat tail into the apparatus in accordance with the procedure and activation of the apparatus at 55% power, the period required for tail flicking was calculated as tail-flick period. Constant heat intensity was applied to the dorsum of the upper third of rat tail and when the rat flicked its tail in response to the noxious thermal stimulus, both the heat source and the timer stopped automatically. A cut off-time of 22 seconds (3-4 times more than the basal tail-flick period) was imposed to prevent any injury to the tail. All the tests were repeated for 3 times at intervals of 5 min between each application. The nociceptive threshold was observed before the study, and 1, 2, 4 and 6 hours after the drug administration.

Rat Model for Sciatic Nerve Injury (SNI)

Rats were anesthetized with sodium pentobarbital (65 mg/kg, i.p.) The common sciatic nerve was exposed and dissected from surrounding connective tissue near the trochanter, just distal to the branching point of the posterior biceps semitendinosus nerve. Four ligatures (4.0 chromic gut) were tied loosely around the nerve with a 1-1.5 mm interval between ligatures so that the circulation through the superficial epineuria vasculature was not totally blocked.Sham-operated rats underwent the same surgery, the left sciatic nerve was exposed but no ligation was made. Animals were housed individually in cages after the surgery. The development of the pathological process lasted 14 days. The degree of hyperalgesia was determined using a dolorimeter (Dolorimeter Baseline, USA) by determining the threshold of pain sensitivity - the minimum pressure on the lower surface of the rat's foot (g/mm^2), which caused pain in the animal (vocalization and/or withdrawal of the foot). Each animal was given 5 attempts; the threshold value was taken with such a pressure force, which caused a positive response in at least one attempt. The threshold of pain sensitivity was compared on intact and damaged limbs on day 14 after tying (pathology without treatment), as well as on the injured limb after 2 hours (peak of action) after the drugs administration. The test compound and the reference drug (ketorolac) were administered intragastrally (as gavage) once at doses of 0.5 and 3 mg/kg.

Induction and assessment of diabetes in rats and experimental groups

After two weeks of acclimatization, the diabetes was induced in rats with intraperitoneal administered STZ in a dose of 60 mg/kg dissolved in citrate buffer (0.1M, pH 6.5, *ex tempore*) and normal pellet diet and water *ad libitum*, respectively.

Control rats received intraperitoneally citrate buffer. Blood glucose concentrations were determined by measuring blood glucose concentrations in blood samples obtained from the rates tails using a blood glucose meter (GluNeo® Lite Infopia, Korea).

The animals divided randomly into 6 groups: 1. Control (non-diabetic) animals; Group 2. was the diabetic control (STZ). Group 3 and Group 4 were treated with equimolar doses of propoxazepam (2 mg/kg i.p.) and gabapentin (5 mg/kg i.p.) once a day from 1st week to 6th week after STZ administration; Group 5 and Group 6 received single propoxazepam and gabapentin (2 mg/kg and 5 mg/kg respectively) treatment in the end of 6th week after STZ injection and 120 min prior to the pain assessment. The control groups received the vehicle of propoxazepam and gabapentin (0.5% carboxymethyl cellulose).

The following diagnostic criteria for diabetes mellitus, such as changes in animal behavior, lethality, state of the limbs (changes in nails and joints), and glycemic levels, were determined for validation of the method (compliance of diabetes mellitus in animals). These indicators were studied dynamically every 7 days.

Analysis of the possible mechanism of action of propoxazepam

Involvement of GABA system

The possible involvement of the GABA system in the antinociceptive effect of propoxazepam was examined by injecting flumazenil (1 and 10 mg/kg, i.p.), a selective GABA- receptor antagonist, 30 min prior to the administration of propoxazepam (1,8 and 10 mg/kg, orally). Then, the tail immersion latencies were measured at 0 and 120 min.

Involvement of bradykinin receptors

The possible contribution of bradykinin receptors in the antinociceptive effect of propoxazepam was evaluated by using the method described by.[12]

Bradykinin (0.01% solution) was injected to the subplantar area of rat right hind paw 0.1 ml/animal. Propoxazepam (1.83 mg/kg) was administered 2 hours prior to bradykinin injection. The nociception degree was estimated using a dolorimeter (Dolorimeter Baseline, USA) by determining the threshold of pain sensitivity - the minimum pressure on the lower surface of the rat's foot (g/mm^2), which caused pain in the animal (vocalization and/or withdrawal of the foot). The threshold of pain sensitivity was compared on intact (before bradykinin injection) and damaged limbs at day 14 after tying (pathology without treatment), as well as on 1 min after bradykinin injection.

Statistical analysis

Data were first subjected to analysis of normal distribution. Statistically significant differences between mean values presenting Gaussian distribution were analyzed using paired and unpaired T Student's test. Non-Gaussian data were analyzed using Mann-Whitney's test. Differences of $P<0.05$ were regarded statistically significant.

RESULTS

Antinociception effect of propoxazepam and ketorolac in tail flick method in rats

For estimating the analgesic (antinociceptive) action of the studied compound in the conditions of acute pain syndrome the thermal tail flick test was used. Propoxazepam administered dose was 0.5 mg/kg (the mean effective dose for analgesic effect). ketorolac was a reference drug at the equimolar dose (0.5 mg/kg). Also the analgesic action degree for both compounds was estimated at dose 3 mg/kg (ketorolac mean effective dose). In order to estimate propoxazepam antinociceptive action at different routes of administration, both compound (0.5 mg/kg) and vehicle (solvent) were administered intraperitoneally.

Tail flick latency period regarded as nociception threshold (NTh). This model allowed estimate both analgesic effect intensity and duration since the it was measured in dynamics.

In the used model propoxazepam after both oral and parenteral administrations (latter more pronouncing) in the dose 0.5 mg/kg produced analgesic action. In all the routes of administration used the most pronounced effect was registered by 2th hour after administration and lasted at least 2 hours , but intraperitoneally administration led to high effect by 6th hour after administration, while oral administration produced rapid decrease (Fig. 1).

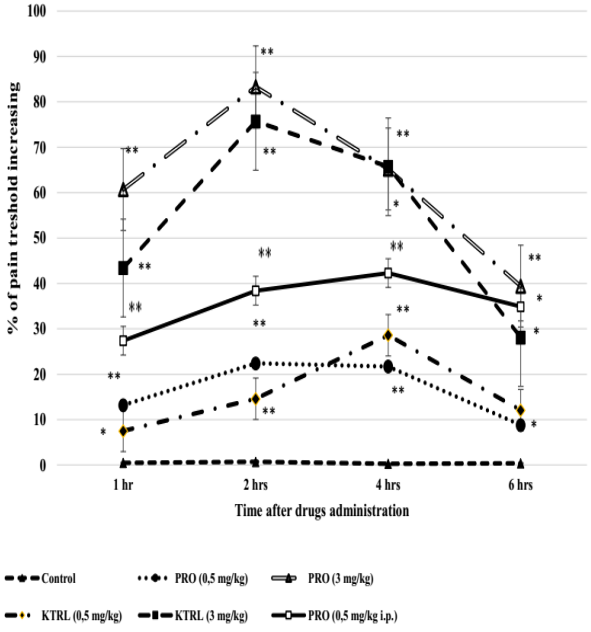

Figure 1: Effect of propoxazepam (PRO) and ketorolac (KTRL) on withdrawal threshold in tail-flick test after oral and parenteral administration. Control rats received equal volumes of vehicles. Data

are given as the mean ± S.D., n=10. * P<0.05 versus control; ** - P<0.01 versus control.

Analgesic action of ketorolac after oral administration at dose 0.5 mg/kg was similar to that of propoxazepam, registered at 4th and 6th hours. Propoxazepam or ketorolac administration at dose 3 mg/kg (ED_{50} according to the literature data) analgesic effect of propoxazepam prevailed the reference drug values both on intensity and duration (Fig. 1).

Effect of pharmacological interventions on neuropathic pain in rats

Effect propoxazepam interventions on SNI – induced pain in rats

Sciatic nerve ligation caused neuropathic pain syndrome development in rats, which manifested in behavioral reactions and decreasing of pain threshold approximately on 44-48% (Fig. 2). Under these conditions propoxazepam shared significant dose-dependent analgesic action, what was proved by the pain threshold increase 2 hours after oral administration. At dose 0.5 mg/kg (ED_{50} according to the previous studies results) antinociceptive effect was +23.1% in compare to non-injured hind limb. For this action the studied compound effect has no differences with effect of ketorolac (+24.6%) (Fig. 3).

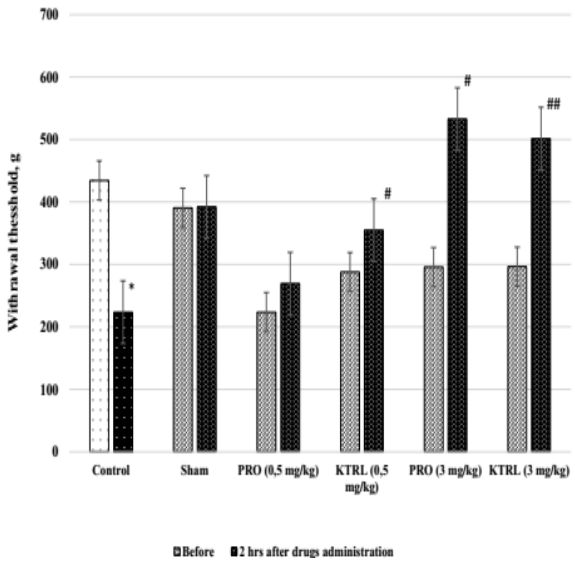

Figure 2: Therapeutic effects of propoxazepam (PRO) and ketorolac (KTRL) on neuropathic pain after sciatic nerve injury in rats. Control group shows changes in paw withdrawal threshold with contralateral hind paw on 7th day after sciatic nerve ligation. Other data show changes in paw withdrawal threshold on 7th day after sciatic nerve ligation before (baseline) and 2 hours after oral drugs administration or vehicles (sham). Data are given as the mean ± S.D., n=10. * P<0.01 versus uninjured paw group; # - P<0.05 versus injured paw before drugs administration; ## - P<0.01 versus injured paw before drugs administration.

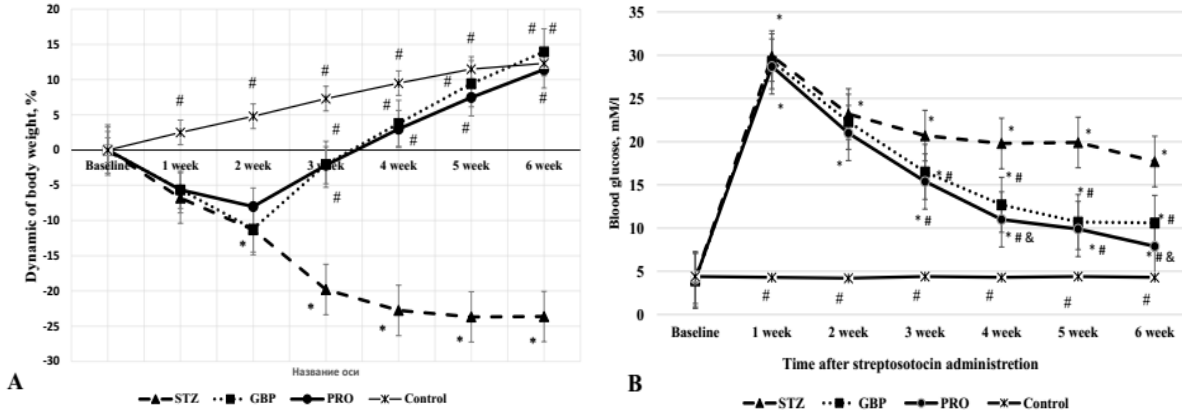

Figure 3: Effect of propoxazepam (2 mg/kg) and gabapentin (5 mg/kg) on body weight (A) and blood glucose level (B) in diabetic rats. The drugs were administered i.p. in equimolar doses for 5 weeks after the streptozotocin administration. Data are given as the mean ± S.D., n=10 for control, n=38 for diabetes (STZ), n=18 for propoxazepam (PRO) and gabapentin (GBP). * P<0.05 versus baseline; # - P<0.01 versus STZ; & - P<0.05 versus GBP.

Propoxazepam dose increase to 3 mg/kg led to significant increase of antinociceptive action: pain threshold increase after 2 hours was + 82.5% that was more than that of same ketorolac dose (+69.2%).

At dose 0.5 mg/kg propoxazepam-induced antinociceptive effect was on the average 20.5% in compare to non-injured limb. For this action propoxazepam was nearly equal to ketorolac at equimolar dose (+23.3%).

Propoxazepam dose increase to 3mg/kg was accompanied by significant increase in its antinociceptive action: pain threshold increase was

+80.2% after 2 hours exceeding ketorolac equal dose action (+69.2%).

The influence of propoxazepam on peripheral neuropathy and some characteristics of glucose in rat streptozotocin-induced diabetes model

Streptozotocin was used to induce the diabetic rat model. STZ rats were treated with propoxazepam (2 mg/kg daily, per os for 6 weeks) and gabapentin (5 mg/kg daily, per os), as a reference drug. The mechanism of action of the antiepileptic and antinociceptive drugs of the gabapentinoid family has remained poorly understood. It is now known that GBP binds to an exofacial epitope present in both the $\alpha_2\delta$-1 and $\alpha_2\delta$-2 subunits of voltage-gated calcium channels, but acute inhibition of calcium currents by GBP is either very minor or absent.[13]

Effect of pharmacological interventions on blood glucose level and body weight

The results of the experiments on diabetes mellitus models showed that during the 6 weeks of observation, animals receiving SZT in a dose of 60 mg/kg consumed significantly more water (polydipsia), urinary output (polyuria) significantly increased, they were sluggish, poorly fed food, were loosing the weight. During the first two weeks, there were cases of death. In addition, the animals developed polyneuropathy and autotomies, which were manifested by changes in animal nails, interphalangeal joints, and tarsus bones. At the 6th week after diabetes development and treatment with gabapentin or propoxazepam, animals showed signs of lesion of joints and nails, but the degree of severity was reduced. There was no difference between the two drugs on this indicator.

One of the main diagnostic criteria for diabetes mellitus is the level of glycaemia. In our study we found that the glucose concentration in the blood of animals in the control group waved within 3.9-4.2 mmol/l. The peak of hyperglycemia occurred in the 1st week of the experiment (Fig.3) when the glucose concentration increased 7-7.5 times. Subsequently, a decrease in glucose level was observed, however, at the 6th week it was approximately 4 times higher than the output value. The indicated indices testified the diabetes mellitus in rats and occurrence of DPN. Course administration of investigational drugs, especially propoxazepam, inhibited the development of hyperglycemia (Fig.3).

Thus, at the 6th week of propoxazepam administration, glucose levels in the blood exceeded the output value by only 1,9 times, whereas for gabapentin it was 2,7 times. At the same time, the development of experimental diabetes caused changes in the threshold of pain sensitivity in rats. The statistically significant changes in TPS were reached already on the 7th day after the administration of SZT. It was from this period that the investigational drugs began to be administered, and this treatment lasted for 5 weeks. It has been established that in animals with diabetes without correction of drugs, TPS continued to decrease during the entire observation period, and at the 6th week decreased by 69,5% in compare to baseline.

At the same time experimental diabetes development caused changes in TPS of rats. Statistically significant TPS changes were determined already at 7th day after SZT administration. Since this very time the tested compounds were administered throughout 5 weeks.

Effect of pharmacological interventions on pain in diabetic rats

Both drugs showed an analgesic effect, as evidenced by a statistically smaller decrease in TPS in animal groups that were daily treated with gabapentin or propoxazepam. The results are presented in Fig.4. In all of the experiments, propoxazepam was more effective than the reference drug, but the statistically significant difference was achieved within 4-6 weeks of treatment. At the end of the experiment, TPS in the gabapentin group was lower than output level by only 8,0%, while rats treated with propoxazepam were higher at 9,5% at output value.

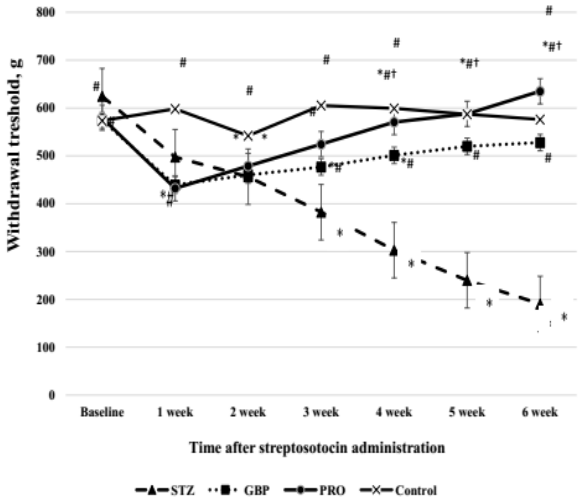

Figure 4: Effect of propoxazepam (2 mg/kg) and gabapentin (5 mg/kg) on withdrawal threshold in diabetic rats. The drugs were administered i.p. in equimolar doses for 5 weeks after the streptozotocin administration. Data are given as the mean ± S.D., n=10 for control, n=38 for diabetes (STZ), n=18 for propoxazepam (PRO) and gabapentin (GBP). * P<0.05 versus baseline; # - P<0.01 versus STZ; † - P<0.05 versus GBP.

Subsequently, the ability of gabapentin and propoxazepam to inhibit pain syndrome in animals with diabetic neuropathy after single intraperitoneal injection was investigated. For this purpose, animals with prevailing DM after measurements of the initial TPS index were injected with propoxazepam, gabapentin, or solvents (control). After 2 hours (peak of the analgesic activity of propoxazepam according to our previous studies), the changes in the studied index were evaluated.

The results are presented in Fig.5. According to the data obtained, at the 6th week of the experiment, on the background of the SZT administration, the pronounced neuropathy had been developed, which was confirmed by a significant decrease in TPS (68-71%), as well as other changes (autotomy, etc.). Administration of propoxazepam (2 mg/kg) showed a significant anti-nociceptive effect: the TPS increased up to 97,4% in compare to the pre-administration index, while gabapentin in the equimolar dose contributed to an increase of only 33,2% on the average.

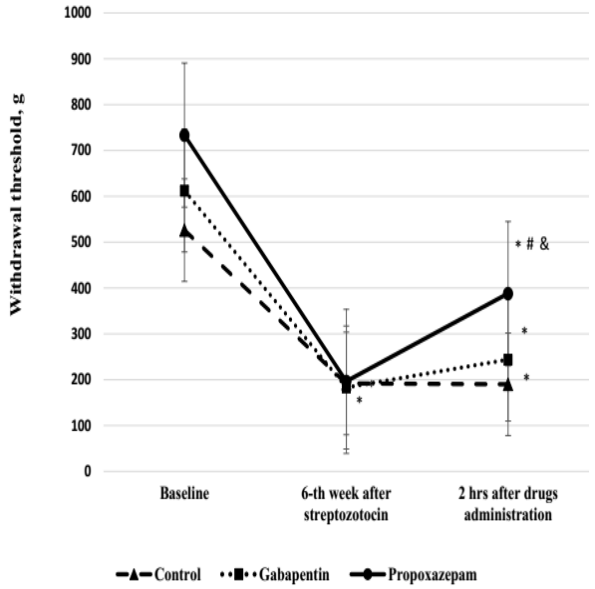

Figure 5: Effect of propoxazepam (2 mg/kg) and gabapentin (5 mg/kg) on withdrawal threshold in diabetic rats. Changes in pain threshold were registered on the 6th week after streptozotocin administration and then 2 hours after i.p, injection of drugs. Data are given as the mean ± S.D., n=11 for diabetes (control), n=13 for propoxazepam and n=14 for gabapentin. * P<0.05 versus baseline; # - P<0.01 versus 6-th week; & - P<0.05 versus gabapentin

Thus, the studies in this section suggest that propoxazepam slows down the course of pain in the experimental model of the DPN. propoxazepam (2 mg/kg), as well as gabapentin (5 mg/kg), under course of treatment for 5 weeks reduced hyperglycemia, clinical manifestations of polyneuropathy, as well as an analgesic effect, as evidenced by the increase in TPS. At the same time, throughout the study, propoxazepam was more effective than the referent drug, but the statistical significance of these differences were acquired for 4-6 weeks. At the end of the experiment, TPS in the gabapentin group was below the output level of 8,03%, whereas in rats treated with propoxazepam it was higher than the output value on 9,5%. Propoxazepam also showed the ability to reduce pain syndrome after single administration in animals with diabetic polyneuropathy, thus exceeding the action of gabapentin threefold. Under these experimental conditions, both drugs practically did not affect the level of glycaemia in animals.

Effect of flumazenil on propoxazepam antinociception in tail flick test in rats

At the dose 1 mg/kg flumazenil hadn`t changed the TPS of the experimental animals but the concomitant administration with propoxazepam totally abolished the antinociceptive action of the latter (tail flick latency period changes were -1.6% in compare to initial levels and +55.1 in compare to propoxazepam alone administration) (Fig. 6).

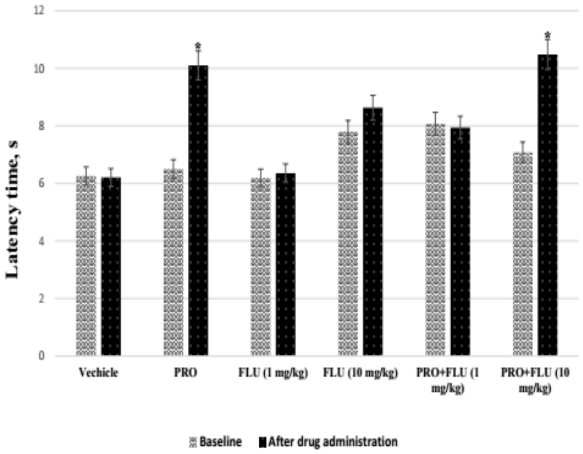

Figure 6: Effect of propoxazepam (2 mg/kg p.o.) on tail flick latency time in rats under flumazenil pretreatment. Tail flick latency time to heat influence were evaluated before (baseline) and 2 hours after propoxazepam administration. Inhibitor of benzodiazepine receptors (flumazenil) was injected p.o. 0.5 hour before propoxazepam administration. Flumazenil in low dose (1 mg/kg) inhibited analgesic effect of propoxazepam, whereas in high dose (10 mg/kg) it did not affect propoxazepam-induced analgesia. PRO – propoxazepam; FLU – flumazenil. Data are given as the mean ± S.D., n=7. * P<0.05 versus baseline.

In the contrast to this, flumazenil at the dose 10 mg/kg had nearly no influence on propoxazepam analgesic action (tail flick latency time increase after their concomitant administration was +48.0% in compare to initial values), but after its administration alone even shoved the tendency for own analgesic action, which however hadn`t reached the statistically significant level (latency period increased up to 10.6% on average).

Propoxazepam antinociceptive action on the bradykinin-induced hyperalgesia

Pathophysiological mechanisms of pain syndrome development involve nociceptors irritation with their further sensitization (increased sensitivity) to harmful stimuli. One of the causes for nociceptors sensitization development is inflammation mediators formation in the place of injury among which are bradykinin, arachidonic acid metabolites (prostaglandins, leukotrienes), biogenic amines, purines and other substances. They interact with corresponding receptors on the nociceptive afferent

nerves, increase their sensitivity to mechanical and thermal stimuli.[14]

Bradykinin injection to rats induced statistically significant TPS decrease on 71.7%. Under these conditions propoxazepam induced prominent antibradykinin effect, since on the background of its administration bradykinin-induced TPS decrease was threefold less that that of in control group (23.8% and 71.7% respectively) (Fig. 7).

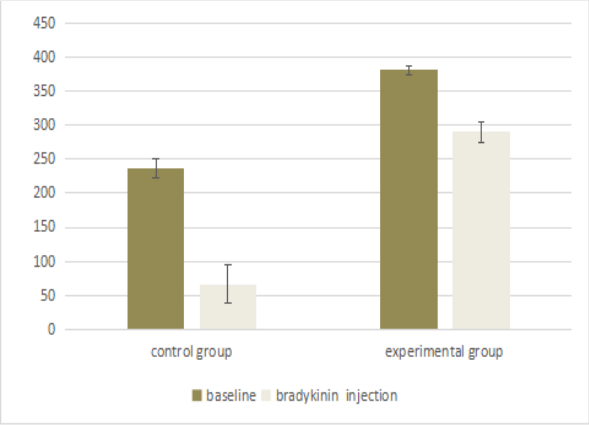

Figure 7: Effect of propoxazepam (2 mg/kg p.o.) on bradykinin-induced hyperalgesia in rats. Pressure-induced hind paw withdrawal were evaluated before (baseline), and 1 min after intraplantar bradykinin (0.01%) administration. Two hours before examination control group received vehicle, and experimental group received propoxazepam. Data are given as the mean ± S.D., n=7. * P<0.05 versus baseline, # - P<0.05 versus vehicle.

DISCUSSION

Pain experts have divided the physical causes of pain into two types: nociceptive and neuropathic pain. The differences are important for understanding the nature of the pain problem and especially for determining how to treat the pain. Among the medicines, which simultaneously inhibit pain of different genesis (both somatic and neuropathic) can be new 1.4-benzodiazepine derivatives provided less pronounced sedative effect or less dependence.

This study demonstrated that propoxazepam (3-alkoxy-1,2-dihydro-3H-1,4-benzodiazepin-2-one), a novel bradykinin receptor antagonist and benzodiazepines receptor agonist reduces responses indicative of acute thermal pain (tail-flick test) and neuropathic pain (SNI - and STZ – induced) in rats.

To study the spinal antinociceptive action, we performed the tail flick test. This model, like the hot plate test, measures animal nociceptive response latencies to thermal stimulus but tail flick is principally a spinal response and hot plate is predominantly supraspinal. In the tail flick test propoxazepam was equal to exceeded by both intensity and duration the effect of reference drug (ketorolac) under the similar doses and administration conditions.

Nonsteroidal anti-inflammatory drugs (NSAIDs) such as ketorolac, cause gastrointestinal complications such as ulcers and erosions. The pathophysiology of these complications has mostly been attributed to NSAID's action on the cyclooxygenase (COX) inhibition and the subsequent prostaglandin (PG) deficiency. PGs play a key role in gastric epithelial defense by enhancing the pre-epithelial, epithelial, post-epithelial defense mechanisms: PGs regulate the secretion of bicarbonate and mucous, inhibit gastric acid secretion, and are important in maintaining epithelial cells restitution and mucosal blood flow. NSAIDs can damage the gastrointestinal tract, causing widespread morbidity and mortality.[15]

Propoxazepam had no damaging influence on the stomach after acute, subacute and chronic administration, as well as didn`t change alimentary behavior and total animals activity, what differs it from ketorolac on the action mechanism and proves the harmlessness of this compound in particular for gastrointestinal tract.[16]

Unlike ketorolac, which acts through the prostaglandin synthesis inhibition, propoxazepam has a central mechanism of pain control through the GABA-ergic system, that was confirmed in experiments with flumazenil.

Flumazenil is a $GABA_A$-receptor benzodiazepine site neutral allosteric modulator, selective antagonist of α1-subunit and partial agonist of α4-subunit, that determines its anticonvulsive and analgesic action.[17] It is possible that flumazenil dose increase lead to prevailing of agonistic influence on this part of GABA-receptor, as well as in the case of propoxazepam, since for flumazenil there was shown both antinociceptive and anticonvulsive actions[5] through GABA-receptor complex stabilization.

Our radioreceptor studies[18] demonstrated that a value of the K_i in inhibition of specific binding of [^{3}H]flumazenil with synaptic membranes from the rat brain by propoxazepam is, on average, 3.5 ± 0.3 nM. As compared with the respective values for other benzodiazepine agents, it is a rather significant value. In particular, the analogous indices for diazepam, chlordiazepoxide, nitrazepam, and oxazepam are 6.3, 220, 6.4, and 14.0, respectively.[19]

Estimation of the internal activity of the compound is a rather important moment in our study. This index can be estimated according to the value of a GABA-shift in the graph of displacement of the radioligand by the examined ligand in the absence and presence of GABA (10^{-4} M). The respective calculations showed that the GABA shift for propoxazepam is equal to 1.9. This fact allows us to consider the examined compound as a full agonist of the GABA RCs.[20] The corresponding indices

for such full agonists of the above-mentioned receptor as diazepam and flunitrazepam are 2.89 and 2.73, respectively.

Within the framework of such approach, the functioning of the $GABA_A$-receptor in the CNS can be examined *in vivo* by estimation of the competitive effects of benzodiazepines and seizure-inducing agents.[21] Using effector analysis, we studied the mechanism of anti-seizure activity of the alcoxy-derived benzodiazepine (propoxazepam). In models of chemically induced seizures we determined the average molar and weight effective doses (ED_{50}) of propoxazepam as an antagonist of picrotoxin (1.67 ± 0.09 mg/kg), penthylenetetrazole (0.9 ± 0.04 mg/kg), and strychnine (14.24 ± 0.47 mg/kg), which reflect the high activity level of the substance. On the base of dose–effect curves, using comparative quantile analysis for chemoconvulsants with different mechanisms of action, we showed different stages of interaction of propoxazepam with GABA and glycine receptors under *in vivo* conditions. We evaluated the partial contribution of myoclonic and tonic components to the general structure of seizures induced by various chemoconvulsants. We believe that the results we obtained indicate that the anti-seizure action of propoxazepam is predominantly mediated by a GABAergic mechanism. Glycinergic components of the inhibition of strychnine-induced seizures by propoxazepam occur at doses that exceed the ED_{50} and seem to be an additional means of anticonvulsive action.

Chronic abnormal pain syndromes that follow peripheral nerve damage have been found to have a much reduced sensitivity to the two major classes of analgesics, opioids and non-steroidal anti-inflammatory drugs. In the search for alternative forms of treatment, anticonvulsants have emerged amongst the more commonly used pharmacologicals.

Gabapentin is anticonvulsant drug that is active in a variety of animal seizure models and prevents nociceptive responses from hyperalgesia in animal models.[22] Gabapentin has no activity at $GABA_A$ or $GABA_B$ receptors of GABA uptake carriers of brain. Gabapentin interacts with a high-affinity binding site in brain membranes, which has been identified as an α2δ-1 subunit of the voltage gated calcium channels on the DRG neurons.[23]

Propoxazepam (2 mg/kg i.p.) similar to gabapentin (5 mg/kg i.p.) reduced hyperglycemia, clinical signs of polyneuropathy with course of administration for 5 weeks, and also showed analgesic effect, as evidenced by an increase in the threshold of pain sensitivity (TPS). At the same time, propoxazepam was more effective than the reference drug in the study, but these differences reached a statistically significant level at 4-6 weeks. At the end of the experiment, the TPS in the gabapentin group was lower than the baseline value by 8,03%, and in rats treated with propoxazepam it was 9,5% higher than the baseline value. Propoxazepam also revealed the ability to reduce pain syndrome with a single dose in animals with experimental diabetes mellitus, threefold more efficient than reference drug. Under these experimental conditions, both drugs had no effect on the level of glycaemia in animals.

Bradykinin diversely influences on the pathophysiological processes accompanying pain and inflammation. Its biological actions are mediated by two known G-protein coupled receptors named B1 and B2. The bradykinin B2 receptor is constitutively expressed in most cell types and evokes acute pain responses following tissue injury, whereas the bradykinin B1 receptor is induced during inflammatory insults or painful stimuli.[24]

The obtained data show that propoxazepam in this experiment reduced hyperalgesia on the in the model of bradykinin-induced edema.

An additional argument for possible interaction of propoxazepam with bradykinin receptors is the study[25], dedicated to the investigation of compound influence on the maximal normalized speed of bradykinin-induced contraction of the rat stomach smooth muscles in the presence of gadolinium ions and verapamil. For propoxazepam the statistically significant changes if the noted indicator have been shown, as it is able to additionally inhibit the bradykinin-induced contraction in the presence of Gd^{3+} and verapamil on 19.3% and 32.0% respectively, and demonstrates the effects similar to those of des-Arg^9-bradykinin-acetate (B2-bradykinin receptors concurrent antagonist), which proves either interaction with receptor, or influence on signal transduction pathways.

CONCLUSIONS

Propoxazepam causes both nociceptive and neuropathic analgesia in rodents acute and chronic pain models. These results suggest that GABAF-receptor and bradykinin B-receptor are a key site of the analgesic action of propoxazepam.

Conflicts of interest

None of the authors have any conflicts of interest to declare.

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