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NEUROMIDIN ATTENUATES NEUROPATHIC PAIN IN THE STREPTOZOCIN-INDUCED DIABETES MODEL IN RATS

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Diabetic neuropathy, which affects all peripheral nerves and may cause dramatic pain, is one of the most severe pathologies associated with hyperglycaemia, damage in the blood vessels, and inflammation in nerves. Anticonvulsants and antidepressants are still the most commonly used options to manage diabetic neuropathy. However, to improve clinical benefit in the treatment of diabetic neuropathies, as well as to minimize side effects, search for a new type of drugs to protect/treat neuropathic pain is still important. The aim of this study was to investigate neuromidin (ipidacrine, amiridin, NIK-247), an anticholinesterase drug of tetrahydroaminoacridine series, in the streptozocin (STZ)-induced diabetic neuropathic pain model in rats. Neuromidin was administered per os at daily doses 0.3, 1.0 and 3.0 mg/kg for ten days. The dynamics in the development of hyperalgesia (pain threshold) was measured by algesimeter for five weeks. The data obtained show that neuromidin considerably protects the development of peripheral neuropathic pain caused by STZ. The most active dose was the lowest—0.3 mg/kg. Neuromidin did not affect STZ-hyperglycemia, nor the weight gain in animal groups. Neuromidin per se at the doses 0.3 and 1.0 mg/kg showed a short-term analgesic activity. The cholinergic mechanism of neuromidin may be considered as essential in attenuating of diabetic neuropathic pain; other mechanisms remain to be elucidated.

Key words: *neuromidin, streptozocin, diabetic neuropathy model, hyperalgesia.*

INTRODUCTION

Diabetic neuropathy is a heterogenous peripheral nerve disorder caused by diabetes (for review see Wong *et al*., 2007; Ziegler, 2008). About 60 to 70 per cent of people with diabetes have some form of neuropathy. Diabetic neuropathy affects all peripheral nerves: pain fibres, motor neurons, autonomic nerves. In this syndrome, a glove-stocking distribution of numbness, sensory loss, dysesthesia and nighttime pain occur. The main risk factor for diabetic neuropathy is hyperglycaemia (Tomlinson and Gardiner, 2008), but nerve damage is likely due to a combination of this factor with others, such as abnormal blood fat levels, and low levels of insulin; neurovascular factors, leading to damage to the blood vessels; autoimmune factors that cause inflammation in nerves; lifestyle factors etc. In the development of diabetic neuropathy at least four factors are involved: 1) microvascular vasoconstriction and hence, neuronal ischemia; 2) advanced glycation end products that can be produced by forming of non-enzymatic covalent binding of glucose with proteins, which alters protein structure and destroys their function; 3) protein kinase C which is implicated in the pathology of diabetic neuropathy; 4) polyol pathway or sorbitol/aldose reductase pathway, which means a much higher level of sorbitol accumulated, producing osmotic stress and formation of reactive oxygen molecules.

Despite advances in the understanding of the metabolic causes of neuropathy, nowadays treatments aimed at interrupting these pathological processes are focused on different cell processes. Therefore, drugs of different chemical classes and action mechanisms have been used. The most commonly used drugs to control pain are the well-known tricyclic antidepressants (TCA, e.g. imipramine, amitriptyline, desipramine and nortriptyline.), antiepileptic drugs of the sodium channel blocking class (e.g. carbamazepine, oxcarbazepine), and the ligands of $\alpha 2\delta$ subunit of the voltage-dependent calcium channel in the central nervous system (gabapentin, pregabalin). However, their use is described as limited due to insufficient efficacy and side effects (Cruccu, 2007; Ziegler, 2008). Therefore, the need for novel types of drugs capable of protecting from and treating diabetic neuropathic pain remains important. Recently, a cholinergic mechanism has been raised as essential in the attenuating neuropathic pain (Lynch *et al*., 2005; Liu, 2007). In this context, the aim of the present study was to test for the first time neuromidin (ipidacrine, amiridin, NIK-247, Fig. 1), an anticholinesterase drug of tetrahydroaminoacridine (THA) series, in the neuropathic pain model in rats. The drug neuromidin is manufactured in the Joint Stock Company „OlainFarm", Latvia, and the study was performed in the framework of the contract between the

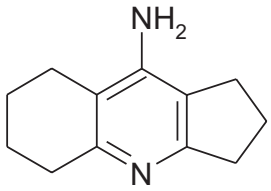

Fig. 1. Structure of neuromidin (9-amino-2, 3, 5, 6, 7, 8-hexahydro-1H-cyclopenta [b] quinoline monohydrochloride monohydrate).

Joint Stock Company „OlainFarm" and the University of Latvia). Neuromidin is well-known anti-dementia/anti-Alzheimer drug (Yoshida and Suzuki, 1993). The mechanism of its action is based on inhibition of both acetylcholinesterase, which is expressed mainly in the central nervous system, and also butyrylcholinesterase, which is formed in periphery (blood plasma, liver, pancreas, gut mucosa etc). Therefore, neuromidin causes considerable accumulation of acetylcholine in neural synapses and potentiates synaptic transmission (Kojima and Onodera, 1998). At present, we could not find any literature on the influence of neuromidin and other anticholinesterase drugs on the development of neuropathic pain in diabetic pain model systems.

In the present study, the influence of neuromidin on the development of streptozocin (STZ)-induced diabetic neuropathic pain in rats was examined. STZ is a cytotoxic agent which is particularly toxic to the insulin-producing beta cells of the pancreas in mammals (Like and Rossini, 1976). The dynamics of the development of hyperalgesia (pain threshold) was measured.

MATERIALS AND METHODS

Drugs. Neuromidin (9-amino-2, 3, 5, 6, 7, 8-hexahydro-1H-cyclopenta [b] quinoline monohydrochloride monohydrate) was obtained from the Joint Stock Company „OlainFarm", Latvia; streptozocin (STZ, 1-methyl-1-nitroso-3-[2,4,5-trihydroxy-6-(hydroxymethyl)oxan-3-yl]-urea) from „Sigma", Germany.

Animals. The experiments were performed on male Wistar rats (from Rīga Stradiņš University, Rīga, Latvia) weighing 180–220g; number of animals per group n = 8.

Animal ethics. All experimental procedures were carried out in accordance with guidelines of the Directive 86/609/EEC "European Convention for the Protection of Vertebrate Animals Used for Experimental and other Scientific Purposes" (1986) and were approved by the Animal Ethics Committee of the Food and Veterinary Service (Rīga, Latvia).

Experimental design. Solvents used were saline for streptozocin (STZ), and distilled water for neuromidin. Streptozocin was injected intraperitoneally at a dose 60 mg/kg, daily for two days (total dose 120 mg/kg). This dosage regimen was chosen as appropriate by modifying that of used in other studies (Runge-Morris and Vento, 1995). The first STZ administration day was taken as the first experimental day and the data taken—as initial (basic) ones.

Neuromidin doses 0.3, 1.0 and 3.0 mg/kg were chosen from literature data that showed the most expressed activity in memory tests (Yoshida and Suzuki, 1993).

Neuromidin was administered daily *per os* for ten days, beginning from the first STZ administration day. The development of hyperalgesia (pain threshold) was measured by algesimeter for five weeks. One week before starting of the experiment, rats were housed and handled.

Rats were divided into eight groups (n = 8):

Group I: Saline i.p., two days + distilled water, *per os*, ten days;

Group II: STZ. 60 mg/kg i.p., two days + distilled water, *per os*, ten days;

Group III: Saline i.p. two days + neuromidin 0.3 mg/kg *per os*, ten days;

Group IV: Saline i.p. two days + neuromidin 1.0 mg/kg *per os*, ten days;

Group V: Saline i.p. two days + neuromidin 3.0 mg/kg *per os*, ten days;

Group VI: STZ 60 mg/kg i.p., two days + neuromidin 0.3 mg/kg *per os*, ten days;

Group VII: STZ 60 mg/kg i.p., two days + neuromidin 1.0 mg/kg *per os*, ten days;

Group VIII: STZ 60 mg/kg i.p., two days + neuromidin 3.0 mg/kg *per os*, ten days.

Assessment of pain (mechanociceptive threshold). Three consecutive days before the experiment, the pain threshold was estimated by algesimeter (Ugo Basile, Italy, Model. 17181), by measuring paw withdrawal responses to mechanical pressure (in g). The mean value from these three measurements was taken as the initial (basic) nociceptive response. Further, a pain threshold was estimated weekly.

Assessment of glucose level. Before the experiment, a blood drop was taken from the tail vein, and basic glucose level was estimated by use of a glucometer (Accu-Check Active, Roche). Eighteen hours before this manipulation, rats did not receive food. Rats with a mean glucose levels of 4.5–5.8 mmol/L were used for the experiment. Glucose level was assessed once per week.

Weight gain. Rats were weighed before the experiment; and this weight for each rat was taken as the initial weight. Weighing was repeated once per week until the end of the experiment.

Statistics. For statistical analysis GraphPad Prism 4 software was used. Statistical analysis was made by ANOVA followed by Bonferroni test (significance level $P < 0.05$).

RESULTS

Influence of neuromidin on the development of neuropathic pain. Figure 2 shows that STZ caused a significant lowering of pain threshold (on average by about 30%) beginning from the second week after its administration. A neuromidin dose of 0.3 mg/kg completely eliminated the STZ effect beginning from the second week, until the end of the experiment. A dose of 1.0 mg/kg showed a protecting effect from the third week after neuromidin administration, until the end of the experiment similarly to that of the lowest dose (Fig. 3). At a dose of 3.0 mg/kg neuromidin also protected from the development of STZ-induced hyperalgesia, but only on the third and fourth weeks of the experiment. (Fig. 4).

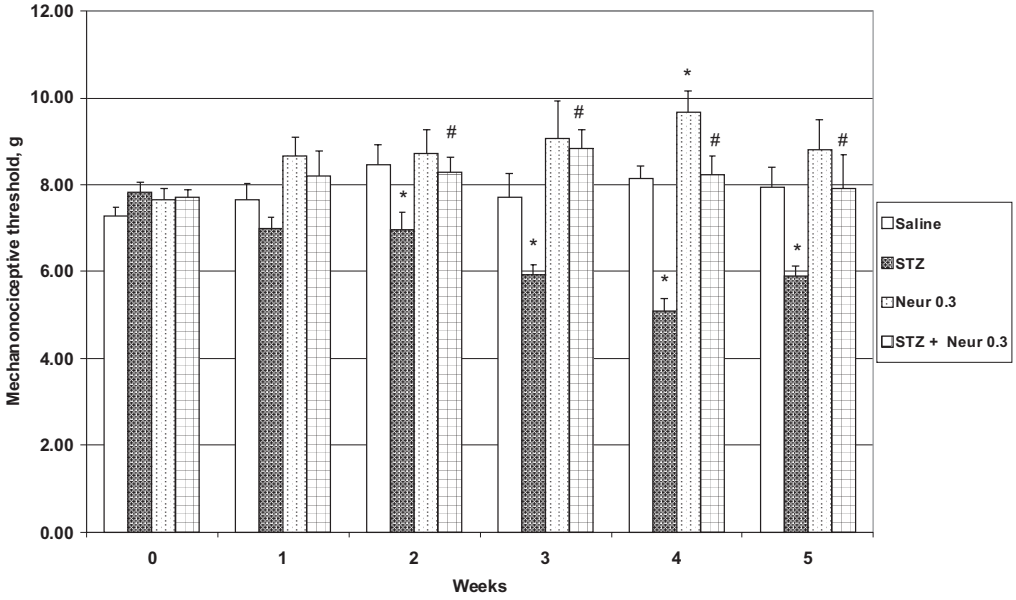

Fig. 2. Influence of neuromidin on the development of neuropathic pain in rats (algesimetric data in g) during five weeks. Streptozocin (STZ) administered intraperitoneally at the dose 120 mg/kg (daily 60 mg/kg for two days); neuromidin (Neur) was administered *per os* for ten days at the dose of 0.3 mg/kg *per se* or in combination with STZ (STZ + Neur).

$P < 0.05$ vs. Saline control; # $P < 0.05$ vs. STZ.

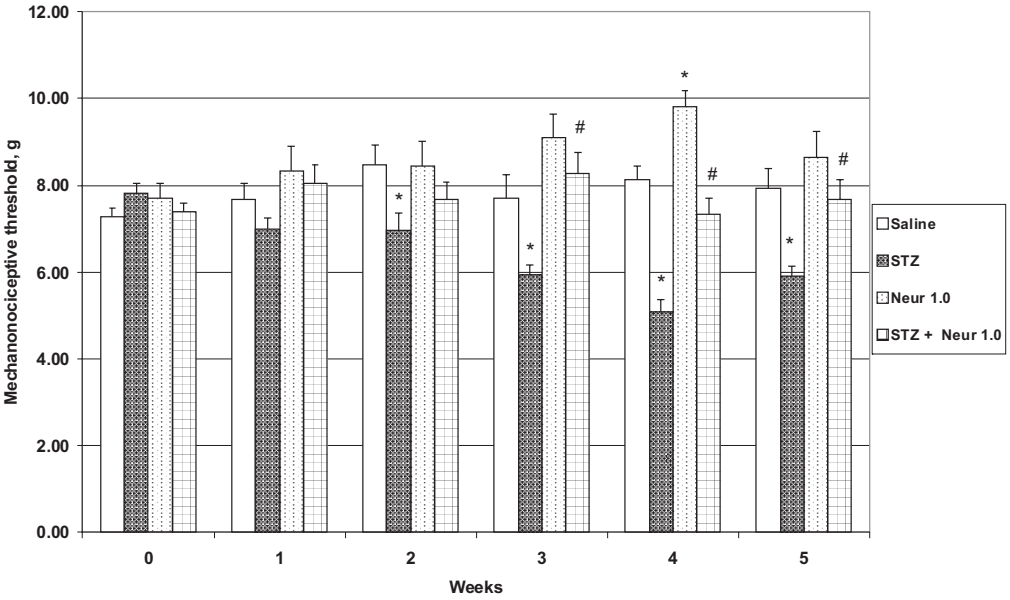

Fig. 3. Influence of neuromidin on the development of neuropathic pain in rats (algesimetric data in g) during five weeks. Streptozocin (STZ) administered intraperitoneally at the dose 120 mg/kg (daily 60 mg/kg for two days); neuromidin (Neur) was administered *per os* for ten days at the dose of 1.0 mg/kg *per se* or in combination with STZ (STZ + Neur).

$P < 0.05$ vs. Saline control; # $P < 0.05$ vs. STZ.

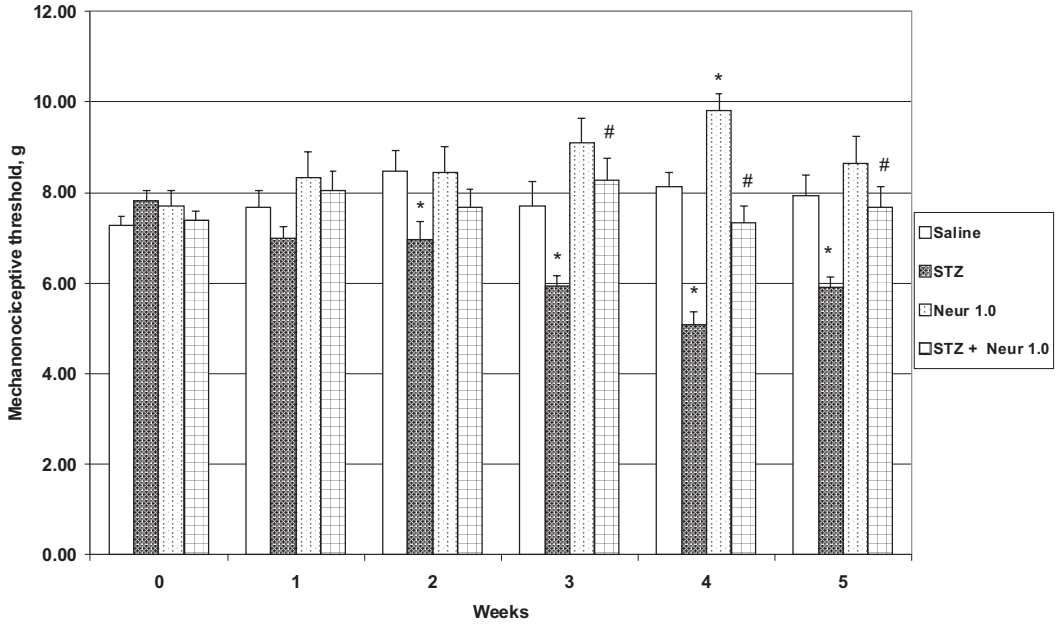

Fig. 4. Influence of neuromidin on the development of neuropathic pain in rats (algesimetric data in g during five weeks. Streptozocin (STZ) administered intraperitoneally at the dose 120 mg/kg (daily 60 mg/kg for two days); neuromidin (Neur) was administered *per os* for ten days at the dose of 3.0 mg/kg *per se* or in combination with STZ (STZ + Neur). .

$P < 0.05$ vs. Saline control; # $P < 0.05$ vs. STZ.

Neuromidin *per se* ar the doses 0.3 and 1.0 mg/kg showed a short-term analgesic activity manifested as the elongation of pain threshold on the third week (Figs. 2 and 3).

Influence of neuromidin on weight gain. In this experiment, weight gain for all experimental groups was comparable (data not shown).

Influence of neuromidin on blood glucose level. Before starting the experiment, the glucose level for all rat groups was 4.5–5.8 mmol/L (basic level). On the second week it reached 15.8–16.9 mmol/L for STZ-treated rats, and this level was maintained during the experimental period. Neuromidin control group rats (without STZ) had about the same glucose level as the initial level, but the STZ + neuromidin groups showed the same glucose level as in STZ rats.

DISCUSSION

Over the past few years, the precise causes of the neuropathic pain were not known and treatment was insufficient. However, now we have a better knowledge of the physiopathological aspects and there is a wider diffusion of the research for target-aimed therapies (Aurilio *et al*., 2008).

Here we have shown for the first time that neuromidin, an anti-cholinesterase drug of the tetrahydroaminoacridine series, in the diabetic neuropathy model in rats considerably protects from the development of peripheral neuropathic pain caused by streptozocin (STZ), a neurotoxin that destroys pancreatic beta-cells. The lowest dose (0.3 mg/kg) of neuromidin demonstrates its activity already from the second week, others from the third week. The highest dose of 3.0 mg/kg showed a shorter effect, as the protecting activity was not demonstrated in the last (fifth) experimental week. Therefore, the obtained data indicate that the highest activity can be reached by lower neuromidin doses, and those exceeding 1.0 mg/kg may even decrease the efficacy of the drug. Other data from literature (Yoshida and Suzuki, 1993) show that in memory tests, very high neuromidin doses about 30 mg/kg caused toxic effects, such as hypersalivation and tremor. Our data demonstrate that neuromidin *per se* at doses 0.3 and 1.0 mg/kg showed a short-term analgesic effect (only on the fourth week).

Neuromidin did not influence STZ-induced hyperglycemia, hence the anti-hyperalgesic effect of neuromidin cannot be explained by its influence on hyperglycemia caused by STZ. At present, activation of the sorbitol/aldose reductase pathway caused by hyperglycemia is considered as the main pathologic cause for the development of cell damage in diabetic neuropathy (Tomlinson and Gardiner, 2008). However, other hyperglycemia-induced factors may be involved. For instance, it is known that hyperglycemia increases production of reactive oxygen species (ROS) in mitochondria and decreases levels of nitric oxide and glutathione, leading to increased osmotic stress on the cell membrane. Growing evidence indicates that oxidative stress is increased in diabetes due to both overproduction of ROS and decreased efficiency of antioxidant defences. ROS formation is a process that starts very early and worsens over the course of the disease (Amaral *et al*., 2008). Recently the anti-cholinesterase drug tacrine (the same chemical class as neuromidin) was found to decrease ROS production and TNF alpha level without preventing loss of mitochondrial activity (Ezoulin *et al*., 2007).

Another explanation of the efficacy of neuromidin in the diabetic neuropathy model can be associated with its eventual influence on sodium ion channels. Neuropathy generates a local accumulation of sodium channels and seems to be the basis of neurohyperexcitability (Aurilio *et al*., 2008). Interesting results have been shown for tacrine: in addition to blocking other voltage-gated ion channels, tacrine blocked Na(+) channels in guinea-pig ventricular myocytes (Wang *et al.,* 2004).

In analogy with tacrine, we can suggest also the central anti-neuroinflammatory mechanisms of neuromidin. Recently, anti-cholinesterase drugs, such as tacrine, rivastigmine and donepezil in mice significantly attenuated the lipopolysaccharide (LPS)-induced increase in levels of cytokine IL-2, indicating that anti-dementia drugs of cholinesterase inhibitor class are effective against LPS-induced neuroinflammation, which may be linked to enhanced cholinergic activity (Tyagi *et al*., 2007). A possible anti-inflammatory action of neuromidin remains to be clarified.

And, finally, the cholinergic mechanism as essential in the attenuating of neuropathic pain is demonstrated recently. It has been shown that N-cholinoreceptor agonists (Lynch *et al.,* 2005), toxins affecting cholinergic transmission (Liu *et al*., 2007), and cholinergic channel-modulating substances (Bannon *et al*., 1998) acted beneficially in the treatment of different neuropathic pain, including diabetic neuropathies. Besides, the analgesic effect in neuropathic pain produced by clonidine, an alpha2-adrenoreceptor agonist, is believed to provide its effect at least in part through stimulation of cholinergic interneurons in the spinal cord (Obata *et al*., 2005). Some patent data (WO/2005/027975) show a good anti-allodinic effect from the combinations of gabapentin and acetylcholinesterase inhibitors, such as donepezil or tacrine. Therefore, the anti-hyperalgesic action of neuromidin can be put forward as more relevant mechanism for the explanation of its efficacy.

The data obtained from the present study in the STZ-model in rats, which showed for the first time an ability of neuromidin (administered only for ten days) to alleviate neuropathic pain in the STZ-diabetic model rats, may broaden neuromidin's beneficial application in neurological diseases, particularly in peripheral polyneuropathies caused by diabetes mellitus. Particularly the lowest doses can be suggested as more beneficial. The efficacy of neuromidin in other neuropathies, as well its possible anti-inflammatory action remain to be examined. Moreover, comparative studies of the efficacy of neuromidin and other anticholinesterase drugs should be done in the future, since to the best of our knowledge their influence on diabetic pain production has never been assessed.

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REFERENCES

Amaral, S., Oliveira, P.J., Ramalho-Santos, J. (2008). Diabetes and the impairment of reproductive function: Possible role of mitochondria and reactive oxygen species. *Curr. Diabetes Rev*., **4** (1), 46–54.

Aurilio, C., Pota, V., Pace, M.C., Passavanti, M.B., Barbarisi, M. (2008). Ionic channels and neuropathic pain: Physiopathology and applications. *J. Cell Physiol.*, **215**(1), 8–14.

Bannon, A.W., Decker, M.W., Kim, D.J., Campbell, J.E., Arneric, S.P. (1998). ABT-594, a novel cholinergic channel modulator, is efficacious in nerve ligation and diabetic neuropathy models of neuropathic pain. *Brain Res.,* **801**(1–2), 158–163.

Calcutt, N.A., Backonja, M.M. (2007). Pathogenesis of pain in peripheral diabetic neuropathy. *Curr. Diab. Rep*., **7**(6), 429–434.

Cruccu, G. (2007). Treatment of painful neuropathy. *Curr. Opin. Neurol.*, **20**(5), 531–535.

Ezoulin, M.J., Liu, Z., Dutertre-Catella, H., Wu, G., Dong, C.Z., Heymans, F,. Ombetta, J.E., Rat, P., Massicot, F. (2007). A new acetylcholinesterase inhibitor with anti-PAF activity modulates oxidative stress and pro-inflammatory mediators release in stimulated RAW 264.7 macrophage cells. Comparison with tacrine. *Int. Immunopharmacol.*, **7**(13), 1685–1694.

Kojima, J., Onodera, K. (1998). NIK-247 induces long-term potentiation of synaptic transmission in the CA1 region of rat hippocampal slices through M2 muscarinic receptors. *Gen. Pharmacol*., **31**(2), 297–300.

Like, A.A., Rossini, A.A. (1976). Streptozocin-induced pancreatic insulitis: New model of diabetes mellitus. *Science,* **193**, 415–417.

Liu, H.C. (2007). Botulinum toxin type a as a prophylactic treatment for chronic daily headache: Time to conduct a large clinical trial in Taiwan? *J. Chin. Med. Assoc*., **70**(12), 519–520.

Lynch, J.J., 3rd., Wade, C.L., Mikusa, J.P., Decker, M.W., Honore, P. (2005). ABT-594 (a nicotinic acetylcholine agonist): Anti-allodynia in a rat chemotherapy-induced pain model. *Eur. J. Pharmacol.*, **509**(1), 43–48.

Obata, H., Li X., Eisenach, J.C. (2005). alpha2-Adrenoceptor activation by clonidine enhances stimulation-evoked acetylcholine release from spinal cord tissue after nerve ligation in rats. *Anesthesiology,* **102**(3), 657–662.

Runge-Morris, M., Vento, C. (1995). Effects of streptozotocin-induced diabetes on rat liver sulfotransferase gene expression. *Drug Metabol. Dispos.,* **23**(4), 455–459.

Tomlinson, D.R., Gardiner, N.J. (2008). Glucose neurotoxicity. *Nat. Rev. Neurosci.*, **9**(1), 36–45.

Tracy, J.A., Dyck, P.J. (2008). The spectrum of diabetic neuropathies. *Phys. Med. Rehabil. Clin. N. Amer*., **19**(1), 1–26.

Tyagi, E., Agrawal, R., Nath, C., Shukla, R. (2007). Effect of anti-dementia drugs on LPS induced neuroinflammation in mice. *Life Sci.*, **80**(21), 1977–1983.

Wang, W., Wang, Y.P., Hu, G.Y. (2004). Tetrahydroacridine inhibits voltage-dependent Na(+) current in guinea-pig ventricular myocytes. *Acta Pharmacol. Sin*., **25**(9), 1138–1144.

Wong, M.C., Chung, J.W., Wong, T.K. (2007). Effects of treatments for symptoms of painful diabetic neuropathy: Systematic review. *BMJ*, **335**(7610), 87.

Yoshida, S., Suzuki, N. (1993). Antiamnestic and cholinomimetic side-effects of the cholinesterase inhibitors, physostigmine, tacrine and NIK-247 in rats. *Eur. J. Pharmacol.*, **250**(1), 117–124

Ziegler, D. (2008). Treatment of diabetic neuropathy and neuropathic pain: How far have we come? *Diabetes Care,* **31** Suppl 2, S255–261

Ziegler, D., Ametov, A., Barinov, A., Dyck, P.J., Gurieva, I., Low, P.A., Munzel, U., Yakhno, N., Raz, I., Novosadova, M., Maus, J., Samigullin, R. (2006). Oral treatment with alpha-lipoic acid improves symptomatic diabetic polyneuropathy: The SYDNEY 2 trial. *Diabetes Care* **29**(11), 2365–2370.

NEIROMIDĪNS SAMAZINA NEIROPĀTISKĀS SĀPES STREPTOZOCĪNA IZRAISĪTĀ DIABĒTA MODELĪ ŽURKĀM

Šajā pētījumā pirmo reizi parādīta antiholīnesterāzes vielas neiromidīna (sinonīmi: ipidakrīns, amiridīns, NIK-247) spēja samazināt neiropātiskās sāpes žurkām streptozocīna izraisītā diabēta modelī. Neiromidīns ievadīts *per os* desmit dienas 0,3, 1,0 un 3,0 mg/kg devās. Mehāniskās hiperalgēzijas (sāpju sliekšņa) dinamika noteikta piecu nedēļu laikā. Mērīts arī žurku asins glikozes līmenis un dzīvnieku masas (svara) pieaugums. Visas pētītās devas protektēja STZ hiperalgēzijas attīstību, taču visaktīvākā izrādījās deva 0,3 mg/kg. Neiromidīns neietekmēja STZ izraisīto hiperglikēmiju. Svara pieaugums visās grupās bija salīdzināms. Neiromidīns *per se* uzrādīja arī īslaicīgu analgētisku aktivitāti. Iegūtie dati liecina par holīnerģiskā mehānisma lomu neiromidīna anti-hiperalgētiskajā darbībā, kas paplašina šī preparāta lietošanas spektru diabētisko neiropātiju (iespējams, arī citu neiropātiju) ārstēšanā.