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EFFECT OF SELOL ON THE OPIOIDS ACTIVITY IN STREPTOZOTOCIN INDUCED HYPERALGESIA

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Abstract: We examined the effect of the opioid receptor agonists and the effect of an antioxidant selol, which is an organoselenium compound on antinociceptive action of opioid agonists in diabetic neuropathic pain model. Streptozotocin (STZ) induced hyperglycemia accompanied by a prolonged decrease in nociceptive threshold is considered a useful model of experimental hyperalgesia. The changes in pain thresholds were determined using mechanical stimuli – the modification of the classic paw withdrawal Randall-Selitto test. Neither morphine, fentanyl nor buprenorphine administered alone in 7 consecutives days modified the STZ induced hyperalgesia, whereas selol slightly increased the nociceptive threshold. Pretreatment with selol markedly enhanced the analgesic activity of all three investigated opioids. Concomitant administration of selol and opioids in alleviation of neoplastic pains seems to be justified.

Keywords: hyperalgesia, streptozotocin, selol, opioids, rats

Reactive oxygen species (ROS) are free radicals produced under physiological conditions in enzyme reactions. Under normal conditions, the level of intracellular ROS is regulated by removing excessive ROS through enzymatic reactions, which convert ROS to harmless non-radicals. The primary purpose of ROS is to protect against invading pathogens. Under pathological conditions, elevation of intracellular ROS levels is caused by increasing production or impaired removal, which leads to the cell damage (1).

The removal of excessive ROS is important for restoring normal conditions (1). This function meets endogenous antioxidants i.e. superoxide dismutase, glutathione peroxidase, glutathione reductase and catalase (2). Unfortunately, low level of endogenous antioxidants was observed in nerve fibres. Additionally, depletion of antioxidants by increased amount of ROS exposes the nerves to a risk of ROS damage (3).

Diabetic neuropathy is one of the most frequent complications of diabetes mellitus. It is typically accompanied by neuropathic pain and hyperalgesia. Furthermore, as shown both in human and animal studies, hyperalgesia precedes the development of neuropathy (4-8). In rats, diabetes produced by STZ administration seemed to represent an appropriate model for investigation of diabetes-induced hyperalgesia and neuropathy (8, 9).

It is known, that radical activity is elevated in diabetes mellitus and it has been implicated in the etiology of neuronal and vascular abnormalities. Treatment with free radical scavengers prevents development of peripheral nerve conduction abnormalities in diabetic rats. Cameron et al. (10) demonstrated that hydroxyl radical scavenger (dimethylthiourea) had significant effects on the deficits in small and large nerve fibre function and neural tissue perfusion in diabetic rats. In other study, Cotter et al. (11) showed that naturally available scavengers (vitamins A, E, beta-carotene) prevented nerve conduction and nutrive blood flow deficits in streptozotocin treated rats.

The role of selenium as an antioxidant is well known. Selenium (IV) competes with sulfur and is incorporated into the sulfur-containing amino acids, cystine and methionine and into the selenium-dependent enzymes i.e. glutathione peroxidase (12) or thioredoxin reductase families (13).

Unfortunately, neuropathic pain is difficult to treat. Classical analgetics, for example opioid receptor agonists, possess low activity in this kind of pain. However, opioids activity can be enhanced e.g. by Mg^{2+} ions (in press). Since free radical scavengers seemed also to increase opioid analgesia, it was of interest to investigate the influence of selol, which is organoselenium compound on antinociceptive action of opioids in streptozotocin-induced hyperalgesia.

EXPERIMENTAL

Laboratory animals

The study was conducted according to the guidelines of the Ethical Committee for Experiments on Small Animals, Medical University of Warsaw.

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Aforementioned Committee approved the experimental protocols. Male Wistar rats (250 – 320 g) were housed in a room maintained at a temperature of 20 ± 2°C, under 12-12 h light – dark cycle. Experimental groups consist of at least six rats. The animals had free access to food and water. Food was removed 16 h before streptozotocin administration. The individual animals were used in only one experiment (i.e. for administration of streptozotocin plus one agent).

Chemicals

Streptozotocin (N-[methylnitrosocarbamoyl] -α-D-glucosamine) was purchased from Sigma Chemical Co., USA; morphine sulfate [(7,8-didehydro-4,5-epoxy-17-methylmorphina-3,6-diol)sulfate], fentanyl [N-(1-phenethyl-4-piperidyl)-N-phenyl-propanamide], buprenorphine hydrochloride [21-cyclopropyl-7α-((S)-1-hydroxy-1,2,2-trimethylpropyl)-6,14-endo-ethano-6,7,8,14-tetrahydrooripavine hydrochloride] were from "Polfa" Warsaw S.A., Poland. Selenitetriglycerid, named selol [(9E,11E,13E)-octadeca-9,11,13-trienoic acid 2-hexadecanoyloxy-3-{7-[5-((1E, 3E)-nona-1,3-dienyl)-2-oxy-2λ⁴-[1,3,2]dioxaselenolan-4-yl]-heptanoyloxy}-propyl ester was synthesized at the Department of Drug Analysis at Medical University of Warsaw, Poland (14-17).

Equipment

1) Analgesimeter exerted progressively increased pressure stimulus (type 7200, manufactured by Ugo-Basile Biological Research Apparatus, 21025 Comerio – Varese, Italy).

2) Blood glucometer Accu-Check Active, manufactured by Roche Diagnostics Corporation.

Streptozotocin induced diabetes

Diabetes was induced by intramuscular (*im*) administration of streptozotocin (STZ) at a dose of 40 mg/kg of body weight, as described by Nakhoda and Wong (18). Streptozotocin was dissolved in citrate buffer at pH 4.5 and administered in only one dose on a first day of study into the thigh muscles of rat leg. Before the induction of diabetes, the animals were fasted over 16 h. Following injection, food and water were available *ad libitum* during the remaining 43 days of experiment. Control rats received an equal volume of buffer. Starting on day 3 (72 h) after streptozotocin administration, levels of glucose were determined using a blood glucometer. Blood samples for the glucose determinations were drawn from tail vein. In all rats, permanent hyperglycemia was observed (> 400 mg/dL).

In control animals, the glucose levels amounted about 120 mg/dL and remained stable during 43 days of the observation period.

The streptozotocin-induced hyperglycemia was accompanied by the gradual decrease of body mass, increase in food consumption, as well as considerable increase in water intake.

In the groups of animals receiving investigated compounds and STZ, food consumption, body mass or water intake did not differ statistically in comparison with animals treated with streptozotocin only.

Drugs administration

Streptozotocin (STZ) was administered as described above. Morphine (MRF), fentanyl (FEN) and buprenorphine (BPR) were dissolved in 0.9% saline immediately before injection and applied intraperitoneally (*ip*). MRF was applied in a dose of 2.5 mg/kg, FEN and BPR were administered in the doses, which corresponded to morphine's dose (FEN – 0.03125 mg/kg, BPR – 0.0375 mg/kg). Selol was diluted in vegetable oil and applied perorally (*po*) via gastric tube in a dose of 3 mg selenium (SE)/kg. Control animals were injected *ip* with 0.9% saline (control to MRF, FEN, and BPR) and/or were administered *po* with vegetable oil (control to selol) according to the same time schedule.

Time schedule

All the drugs (except STZ given only on day one) were applied in 7 consecutive days of experiment (from 18 to 24 day after STZ administration). Selol was administered thirty minutes before MRF, FEN, and BPR.

Measurement of the nociceptive threshold

The changes in pain thresholds were determined using mechanical stimuli – the modification of the classic paw withdrawal test described by Randall and Selitto (19). In order to perform mechanical stimulation, a progressively increased pressure was applied to the dorsal surface of the rat's paw using an analgesimeter. The instrument used increased 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 hind paw, and values of pressure were recorded at this very moment. The nociceptive threshold was measured in duplicates and mean was drawn for further calculations.

Nociceptive thresholds (average of two trials) measured for each animal immediately before streptozotocin or streptozotocin with investigated drugs constituted the value (A).

Measurements of prolonged activity of investigated drugs were performed on 7 consecutive days (for example, measurement on following days after administration of drugs and before consecutive drugs administration) from 19 to 25 days after STZ

administration and then, after cessation of drugs administration, to 43 day (B).

Measurements of withdrawal threshold to mechanical stimuli for STZ group of animals were performed each day of experiment (B) or from 2 to 43 day of experiment in particular period according to the same schedule.

In all experimental sessions, obtained thresholds (B) were compared to the baseline (A). Changes in pain threshold were calculated as percentage of baseline value according to the following formula:

$$\% \text{ of hyperalgesia} = (\frac{A}{B} \times 100\%) - 100\%$$

A – pressure (in g), baseline pain threshold; B – pressure (in g) in consecutive measurements.

Percents of hyperalgesia values calculated as above for individual animals were subsequently used to calculate average values in particular experimental groups and for statistical analysis.

Statistical analysis

The results are expressed as the mean values ± standard error of the mean (S.E.M.). The statistical significance of differences between groups was evaluated by Student t-test and the Newman-Keuls multiple-range test; $p \leq 0.05$ was accepted as statistically significant. All statistical calculations were performed using the computer software described by Tallarida and Murray (20).

RESULTS

Effect of STZ on nociceptive threshold to mechanical stimuli

As shown in Figure 1, starting from the day 3 a statistically significant gradual decrease of nociceptive threshold was observed in streptozotocin treated animals. The decrease reached its nadir on day 21 and remained on similar level until the end of experiment.

Influence of selol on antinociceptive activity of morphine, fentanyl, and buprenorphine in model of STZ-induced hyperalgesia

Morphine (MRF), fentanyl (FEN) or buprenorphine (BPR) administered alone in above mentioned doses on 7 consecutives days did not modify the STZ induced hyperalgesia (Figures 2-4), whereas selol slightly increased the nociceptive threshold. The pretreatment with selol resulted in progressive increase in the analgesic action of all three opioids (Figures 2-4). It is also of interest to note that an increase in nociceptive threshold persisted for two weeks after cessation of drugs administration, and then a gradual return of hyperalgesia was observed.

DISCUSSION AND CONCLUSIONS

The neuropathies are among the most common long-term complications of diabetes. In our investigations, 72 h after STZ administration, a stable hyperglycemia occurred. Elevated blood glucose concentrations were maintained over the remaining period of experiment (43 days). An increase in water intake with accompanying rise of excreted urine volume, an increase in food intake, as well as a gradual decrease of body mass was 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 3 of investigation and threshold values gradually decreased until day 19. From 21 to 43-day hyperalgesia remained on similar level.

These results are similar to those reported by Aley and Levine (21), who demonstrated the appearance of hyperalgesia in response to mechanical stimulus after administration of single intravenous dose (50 mg/kg) of STZ.

It was suggested that ROS, which include superoxide, hydrogen peroxide, hydroxyl as well as peroxynitrite, play an important role in development of neuropathic pain (2). In diabetes, autoxidation reactions of glucose could be additional important sources of reactive oxygen species. In patients with diabetic vascular complications, an increase in lipid superoxides was observed and it was suggested that lipid peroxidation represents mainstay of diabetic vasculitis. It was also documented that insulin attenuated lipid peroxidation, whereas hipoinsulinemia contributed to elevation of oxidative stress (22).

An information about the influence of free radicals and antioxidants in the central nervous system (CNS) during pain is almost completely lacking. Oxidative stress seems to be an important determinant in degenerative and sometimes painful peripheral nerve conditions (23). It is known that astrocytes with a high intracellular concentration of antioxidants are more resistant to free radicals than oligodendrocytes and neurons (24). It was suggested that ROS activate spinal glial cells, which in turn play an important role in chronic pain (25). Thus, when properly used, antioxidants may not only protect CNS against damage by free radicals, but they are also able to decrease the sensation of pain (1, 23).

In the literature, the role of selenium as an antioxidant and anticancer agent is documented. Selenium is a structural component of the active centre of many

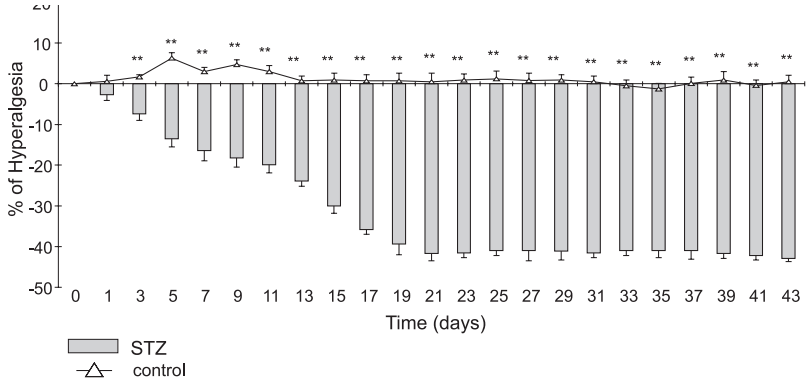

Figure 1. Influence of streptozotocin (STZ) at a dose of 40 mg/kg *im* on threshold to mechanical stimuli (days 1-43 of experiment). Values are the means ± S.E.M. STZ vs. control; **p = 0.01.

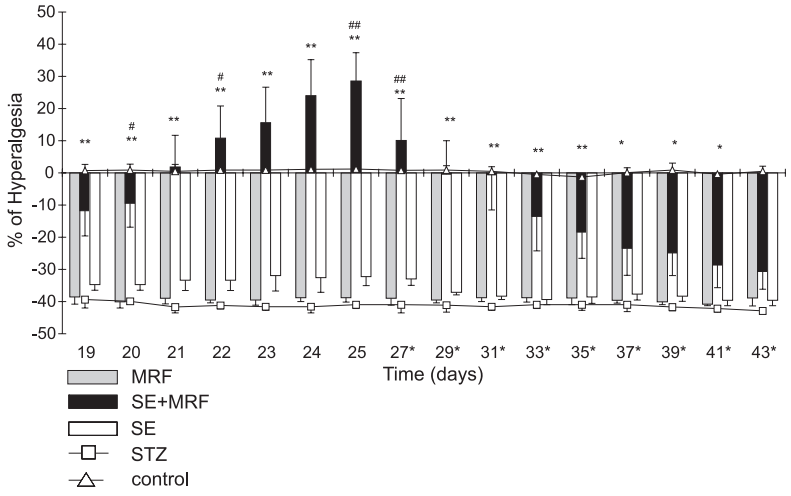

Figure 2. Effect of selol (SE) at a dose of 3 mg/kg *po* on analgesic activity of morphine (MRF) at a dose of 2.5 mg/kg *ip* in STZ treated rats. Days 19-25 – measurements of prolonged activity of investigated drugs; days 27*-43* – after discontinuation of administration. Values are the means ± S.E.M. MRF vs. SE+MRF: **p = 0.01; *p = 0.05, SE vs. STZ: $^{\#\#}$p = 0.01; $^{\#}$p = 0.05.

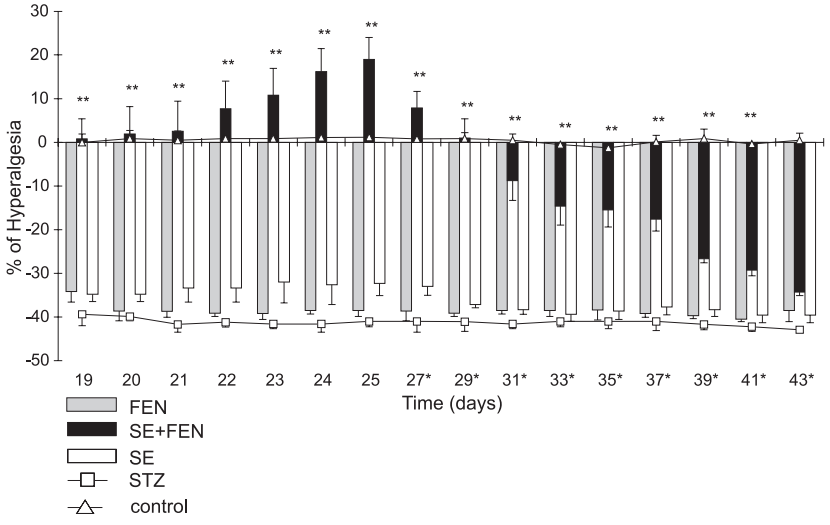

Figure 3. Enhancement of fentanyl – (FEN, 0.031 mg/kg *ip*) induced analgesia after selol (SE) pretreatment in STZ treated rats. Days 19-25 – measurements of prolonged activity of investigated drugs; days 27*-43* – after discontinuation of administration.
Values are the means ± S.E.M. FEN vs SE+FEN: **p = 0.01

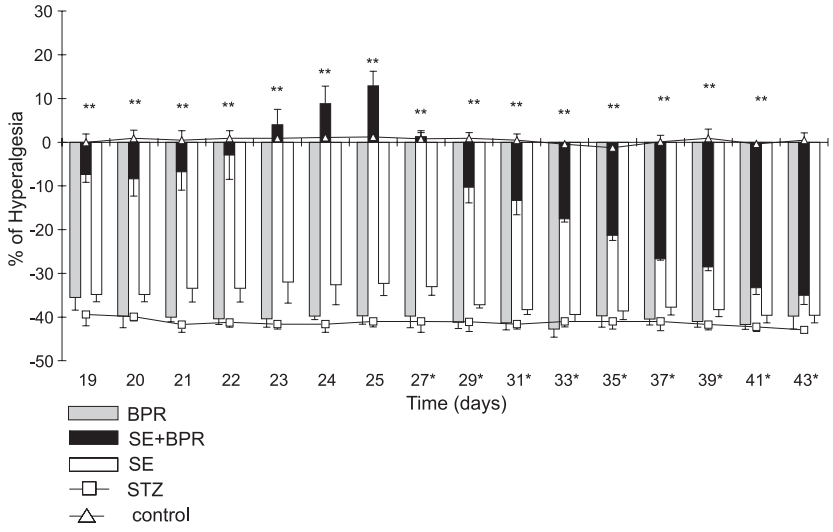

Figure 4. Enhancement of buprenorphine – (BPR, 0.038 mg/kg *ip*) induced analgesia after selol (SE) pretreatment in STZ treated rats. Days 19-25 – measurements of prolonged activity of investigated drugs; days 27*-43* – after discontinuation of administration.Values are the means ± S.E.M. BPR vs SE+BPR: **p = 0.01;

enzymes i.e. glutathione peroxidase, which is important for protection from oxidative stress. Organoselenium compounds have become attractive synthetic targets because of their low toxicity in comparison with inorganic compounds and their useful biological activity. In this study, we decided to use organoselenium compound – selenitetriglyceride (14, 16, 17).

The influence of selenium on nociceptive stimuli transmission was not satisfactorily examined. Savegnago et al. (26) showed that bis-selenide alkene administered subcutaneously (5-50 mg/kg) significantly reduced pain induced by acetic acid or capsaicin and increased the tail flick response latency time. Nogueira et al. (27) also reported that diphenyl diselenide produced dose-dependent antinociceptive and anti-inflammatory activity in acetic acid-induced abdominal constriction, tail-flick test or capsaicin and formalin test in mice.

In the present study selol administered in 7 consecutive days at a dose of 3 mg selenium/kg *po* slightly increased the nociceptive threshold in STZ treated animals.

Alleviation of neuropathic pain, considering its complex mechanism, makes an important problem for medicine. Data available in the literature, as well as the results of our previous study [in press] showed a limited efficacy of opioids in relieving of neuropathic pain. It was shown that in model of STZ-induced diabetic neuropathy morphine induced a dose-dependent antinociception but at doses twice as high as those used in normal rats (28). Zurek et al. (29) showed that fentanyl (10-100 µg/kg *ip*) produced a dose-related antinociceptive effect in both neuropathic and non-neuropathic rats to electrical stimuli, as well as in paw pressure and tail flick nociceptive tests. However, higher doses of fentanyl were needed in neuropathic rats. In the present study morphine, fentanyl and buprenorphine administered in low doses did not modify STZ induced hyperalgesia.

Insensitivity of neuropathic pain to opioid analgesic is difficult to explain. Raz et al. (30) suggested that this phenomenon could be connected with hyperglycemia accompanying diabetes. It was shown that glucose at high concentration might interfere with the interaction of morphine on the opioid receptor. The loss of opioid receptors expressed on C-fibre afferents, the excessive activation of NMDA receptors, an increase in the levels of cholecystokinin and accumulation of morphine-3-glucuronide may lead to reduced sensitivity to morphine in neuropathic pain states (31). Some authors explain limited efficacy of opioids in neuropathic pain relieve by the NO synthesis increase (32, 33).

In the literature, there are no data concerning the influence of selenium on antinociceptive activity of opioids in neuropathic pain. Rokyta et al. (24) showed that among other antioxidant (vitamins C, E, A) administration of selenium also led to a decrease the antinociceptive dose of morphine.

In this study, pretreatment with selol resulted in progressive increase in the analgesic action of three administered opioids. The increase in nociceptive threshold persisted for about two weeks after cessation of drugs administration. It is of interest to note that selol intensified and prolonged the analgesic activity of opioids in vincristine model of chemotherapeutic-induced painful toxic neuropathy (own observation). One of the possible explanations of the observed

potentiation of antinociceptive opioids activity in streptozotocin hyperalgesia by selol are antioxidant properties of this compound but other mechanism(s), for example inhibition of iNOS expression must also be taken into consideration (13, 34, 35).

Nevertheless, results of this study indicate that selol significantly increases analgesic activity of opioids in streptozotocin model of diabetic neuropathy. This observation can be clinically relevant since selol possesses anticancer activity (36). Therefore, concomitant administration of selenium and opioids may be beneficial in terminal neoplastic states.

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