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ORIGINAL ARTICLE

Bradykinin receptors antagonists and nitric oxide synthase inhibitors in vincristine and streptozotocin induced hyperalgesia in chemotherapy and diabetic neuropathy rat model

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Abstract

PURPOSE: The influence of an irreversible inhibitor of constitutive NO synthase (L-NOArg; 1.0 mg/kg ip), a relatively selective inhibitor of inducible NO synthase (L-NIL; 1.0 mg/kg ip) and a relatively specific inhibitor of neuronal NO synthase (7-NI; 0.1 mg/kg ip), on antihyperalgesic action of selective antagonists of B_2 and B_1 receptors: D-Arg-[Hyp^3,Thi^5,D-Tic^7,Oic^8] bradykinin (HOE 140; 70 nmol/kg ip) or des-Arg^{10}-HOE 140 (70 nmol/kg ip) respectively, in model of diabetic (streptozotocin-induced) and toxic (vincristine-induced) neuropathy was investigated.

METHODS: The changes in pain thresholds were determined using mechanical stimuli – the modification of the classic paw withdrawal test described by Randall-Selitto.

RESULTS: The results of this paper confirm that inhibition of bradykinin receptors and inducible NO synthase but not neuronal NO synthase activity reduces diabetic hyperalgesia. Pretreatment with L-NOArg and L-NIL but not 7-NI, significantly increases antihyperalgesic activity both HOE 140 and des-Arg^{10}-HOE 140.

It was also shown that both products of inducible NO synthase and neuronal NO synthase activation as well as bradykinin are involved in hyperalgesia produced by vincristine. Moreover, L-NOArg and 7-NI but not L-NIL intensify antihyperalgesic activity of HOE 140 or des-Arg^{10}HOE 140 in toxic neuropathy.

CONCLUSIONS: Results of these studies suggest that B_1 and B_2 receptors are engaged in transmission of nociceptive stimuli in both diabetic and toxic neuropathy. In streptozotocin-induced hyperalgesia, inducible NO synthase participates in pronociceptive activity of bradykinin, whereas in vincristine-induced hyperalgesia bradykinin seemed to activate neuronal NO synthase pathway.

Therefore, concomitant administration of small doses of bradykinin receptor antagonists and NO synthase inhibitors can be effective in alleviation of neuropathic pain, even in hospital care.

Abbreviations:

AGEs – advanced glycation end-products,
BK – bradykinin,
EDRF – endothelium-derived factor,
HOE 140 – D-Arg-[Hyp^3,Thi^5,D-Tic^7,Oic^8],
7-NI – 7-nitroindazole,
L-NIL – L-N6-(1-iminoethyl)lysine,
L-NOArg – N^G-nitro-L-arginine,
NO – nitric oxide,
NOS – nitric oxide synthases,
cNOS – constitutive nitric oxide synthase,
eNOS – endothelial nitric oxide synthase,
iNOS – inducible nitric oxide synthase,
nNOS – neuronal nitric oxide synthase,
PGs – prostaglandins,
STZ – streptozotocin,
VIN – vincristine

INTRODUCTION

Bradykinin (BK) is nonapeptide formed at site of tissue trauma. It is an important mediator, which participates in the development of inflammatory responses (Wang *et al*. 2006). Moreover, BK is known to activate nitric oxide synthases (NOS) systems to produce nitric oxide (NO). The important role of BK and NO in an induction and transmission of pain is commonly known (Calixto *et al*. 2000, Millan 1999).

As previously reported, both nitric oxide (NO) and bradykinin (BK) are involved in persistent hyperalgesia produced by either streptozotocin (STZ) or vincristine (VIN) hyperalgesia (Bujalska *et al*. 2008, Bujalska *et al*. 2008a).

Therefore, it was of interest to investigate the influence of inhibitors of various NOS isoforms: N^G-nitro-L-arginine (L-NOArg), an irreversible inhibitor of constitutive nitric oxide synthase (cNOS), 7-nitroindazole (7-NI), a relatively specific inhibitor of neuronal nitric oxide synthase (nNOS), as well as L-N6-(1-iminoethyl)lysine (L-NIL), a relatively selective inhibitor of inducible nitric oxide synthase (iNOS), on antihyperalgesic action of bradykinin receptors. D-Arg-[Hyp^3,Thi^5,D-Tic^7,Oic^8] bradykinin (icatibant, HOE 140) and des-Arg^{10}-HOE 140 were used as selective B_2 and B_1 receptor antagonists.

MATERIAL AND METHODS

Laboratory animals

The study was conducted according to the guidelines of the Ethical Committee for Experiments on Small Animals, Medical University of Warsaw. 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. Experimental groups consisted of six rats. Animals had a free access to food and water, except 16-hour period before first experimental session (streptozotocin administration) in diabetic neuropathy model. The individual animals were used in one experiment only.

Chemicals

HOE 140 and des-Arg^{10} – HOE 140 were purchased from Research Biochemical's International; N^G-nitro-L-arginine and 7-nitroindazole were purchased from Research Biochemicals International; L-N6-(1-iminoethyl) lysine from Bachem Switzerland, streptozotocin (N-[methylnitrosocarbamoyl]-α-D-glucosamine) and vincristine sulphate from Sigma Chemical Co., USA.

Equipment

Equipment included an analgesimeter (Type 7200, Ugo-Basile Biological Research Apparatus, Comerio, Italy) which progressively exerted increased pressure stimulus, and a blood glucometer (Accu-Check Active, Roche Diagnostics Corp.).

ANIMAL MODELS OF NEUROPATHIC PAIN

Streptozotocin-induced (diabetic) painful neuropathy

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 (1979). STZ was dissolved in citrate buffer at pH 4.5 and administered in a single 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 hours. Following the injection, food and water were available ad libitum during the remaining 30 days of experiment. Effects of STZ given alone or with investigated drugs on withdrawal threshold to mechanical stimuli were investigated. Control rats received an equal volume of buffer. Starting on day 3 (72 hours after STZ administration), glucose levels were determined using a blood glucometer. Blood samples for the glucose determinations were drawn from tail vein. Permanent hyperglycemia was detected (>400 mg/dl) in all rats and it remained stable during 30 days of observation period. In vehicle-treated animals, the glucose levels amounted about 80 mg/dl.

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.

Chemotherapy (vincristine) – induced painful neuropathy

Vincristine (VIN) neuropathy was induced as described by Aley *et al*. (1996). Vincristine sulphate was dissolved in distilled 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 100 μg/ml. This solution was administered into the tail vein at a dose of 70 μg/kg. Because high doses of VIN (100 μg/kg) resulted in toxic events (marked decrease of body mass) leading even to cachexia, therefore in the present study lower doses (70 μg/kg) of the drug was used. Administrations of vincristine were performed daily – Monday through Friday – for 10 days (this phase of the experiment lasted 12 days, no drug doses were given on Saturdays or Sundays). The dosage

calculations were based on daily body weight. Weight-matched control rats received injections of distilled water.

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

Drug administration

STZ and VIN were administered as described above.

Preparation of Drugs. HOE 140 and des-Arg^{10}HOE 140 were dissolved in distilled water whereas N^{G}-nitro-L-arginine (L-NOArg) and 7-nitroindazole (7-NI) were suspended in 0.1% solution of methylcellulose immediately before injection. L-N6-(1-iminoethyl) lysine (L-NIL) was dissolved in 0.9% saline.

Aplication of Drugs. HOE 140 and des-Arg^{10}HOE 140 were applied intraperitoneally (ip) at 70 nmol/kg dose. N^{G}-nitro-L-arginine and L-N6-(1-iminoethyl) lysine were applied intraperitoneally (ip) in a dose of 1.0 mg/kg, 7-nitroindazole in a dose of 0.1 mg/kg (ip).

Time Schedule. In a diabetic neuropathy model, all the drugs (except of STZ given only on day 1) were applied for six consecutive days of experiment (from day 18 to 23 after STZ administration). L-NOArg, 7-NI and L-NIL were administered 10 min before HOE 140 or des-Arg^{10}HOE 140.

In chemotherapy (VIN) – induced neuropathy model, HOE 140 and des-Arg^{10}HOE 140 were administered daily 10 minutes before vincristine for 10 days (2 × 5 – see above). L-NOArg, 7-NI and L-NIL were administered 10 min before.

Controls. Control animals were injected according to the same time schedule: (1) intraperitoneally with equal volume of distilled water (control to HOE 140 and des-Arg^{10}–HOE 140), with 0.1% solution of methylcellulose (control to L-NOArg and 7-NI), with 0.9% saline (control to L-NIL).

Measurement of the nociceptive threshold

The changes in nociceptive thresholds were determined using mechanical stimuli; the modification of the classic paw withdrawal test described by Randall and Selitto (1957). In order to perform a mechanical stimulation, a progressively increasing pressure was applied to the dorsal surface of the rat's paw using an analgesymeter. The instrument increased the force on the rat's paw at a rate of 32 grams per second. The nociceptive threshold was defined as the force in grams, at which the rat attempted to withdraw its right hind paw. The values of that pressure were recorded at this moment. Three threshold measurements were performed daily per each rat. Nociceptive threshold was measured in triplicate, and the mean was drawn for further calculations.

In streptozotocin model (study lasting 30 days) nociceptive thresholds (average of tree trials) measured for each animal immediately before STZ (on day 1 of study) constituted the baseline pain threshold (A).

Measurements of prolonged activity of the investigated drugs were performed for six consecutive days (for example measurement following administration of drugs and before consecutive drugs administration) from day 19 to 24 after STZ administration and then, after cessation of drugs administration, to day 30 (B).

Measurements of withdrawal threshold to mechanical stimuli for STZ group of animals were performed daily from day 2 to 30 during particular period according to the same schedule (B).

In all experimental sessions, values of thresholds obtained (B) were compared to baseline (A).

In vincristine neuropathy model, a mean of nociceptive thresholds to mechanical stimuli of 18 days lasting study were measured on a first day immediately before administration of vincristine alone or vincristine with investigated drugs and constituted the baseline pain threshold (A). Consecutive measurements of nociceptive thresholds to mechanical stimuli (B) were conducted daily before administration of investigated compounds (from day 2 of experiment to day 5 and from day 8 to day 12) and then after drugs discontinuation (from day 14 to 18 of experiment). In all experimental sessions until end of the study, values of thresholds obtained (B) were compared to the baseline (A) defined above.

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

$$\% \text{ of Hyperalgesia} = \left(\frac{B}{A} \bullet 100\%\right) - 100\%$$

where A indicates pressure (in g) at baseline and B indicates pressure (in g) in consecutive measurements performed daily (excluding day 1) before drug administration.

Percentage of hyperalgesia values calculated as above for individual animals were subsequently used to calculate averages in particular experimental groups and for statistical analyses.

Statistical analysis

The results are expressed as mean values ± standard error of the mean (± S.E.M.). The statistical significance of differences between groups was evaluated by Student-test and the Newman-Keuls multiple-range test. $p \leq 0.05$ was accepted as statistically significant. All statistical calculations were performed using a software described by Tallarida and Murray (1986).

RESULTS

Effect of STZ on threshold to mechanical stimuli

As shown in Fig. 1, starting from the day 2, a statistically significant gradual decrease of the nociceptive threshold was observed in STZ-treated animals. The decrease reached its plateau phase on day 17 and remained approximately stable in the next 13 days i.e. until end of experiment.

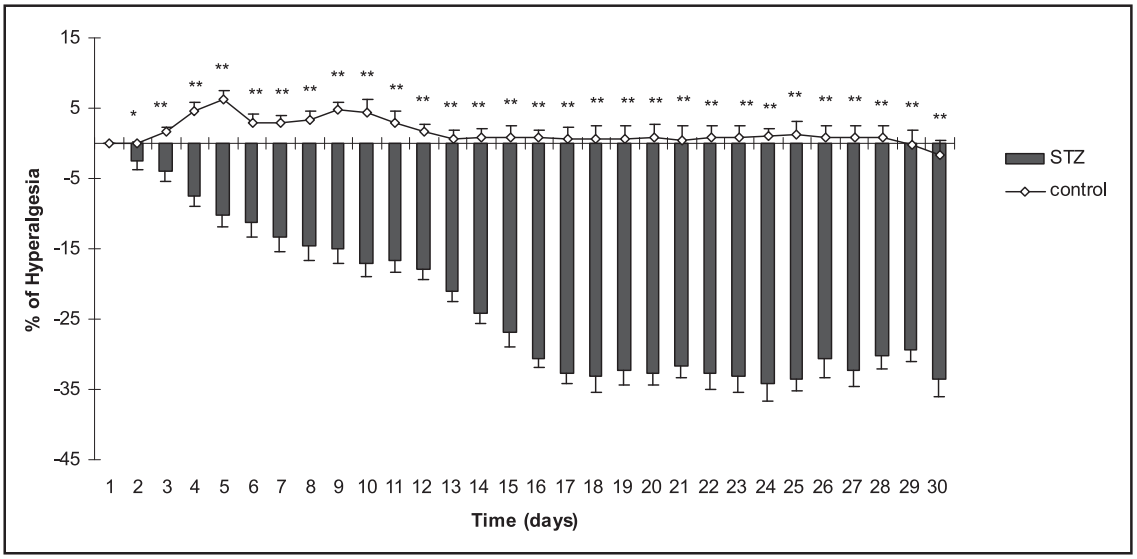

Fig. 1 Influence of streptozotocin (STZ) at a dose of 40 mg/kg (im) on threshold to mechanical stimuli (days 1-30 of experiment). Values are means ± S.E.M. STZ vs. control; ** p ≤ 0.01 * p ≤ 0.05. Darc column = STZ; ◊ = control.

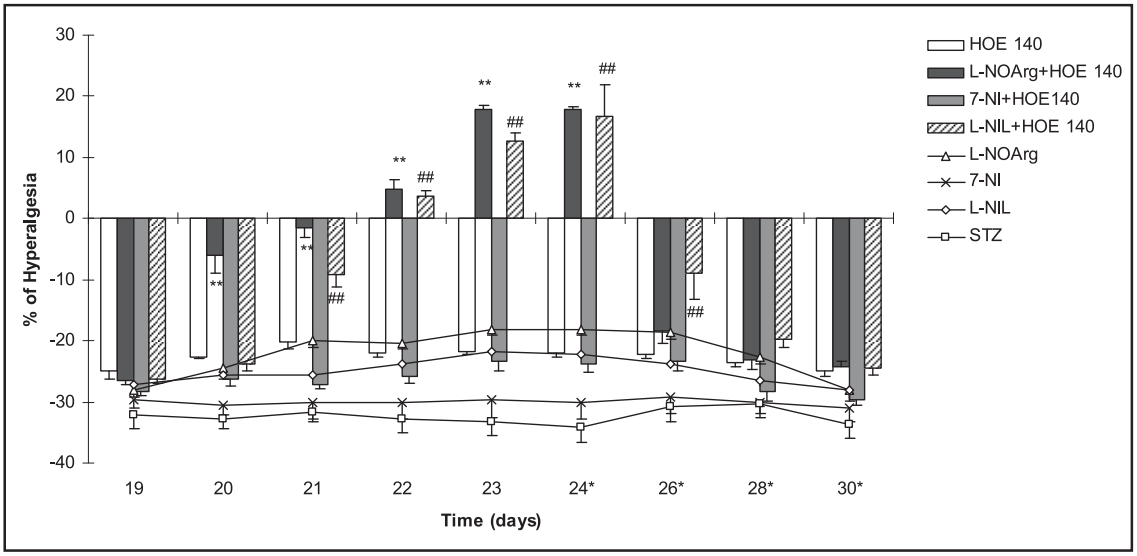

Fig. 2 Effect of L-NOArg and L-NIL at a dose of 1.0 mg/kg (ip) or 7-NI at a dose of 0.1 mg/kg (ip) on the antihyperalgesic activity of HOE 140 at a dose of 70 nmol/kg (ip) in STZ-treated rats. Days 19-24* – measurements of prolonged activity of investigated drugs; days 26*-30* - after discontinuation of administration.
Values are means ± S.E.M. HOE 140 vs. L-NOArg + HOE 140;
**p ≤ 0.01, HOE 140 vs. L-NIL + HOE 140 ##p≤ 0.01.
White column = HOE 140; Dark column = L-NOArg+HOE 140; Grey column =7-NI+HOE 140;
White striped column = L-NIL+HOE140; △ = L-NOArg; X = 7-NI; ◊ = L-NIL; □ = STZ.

Influence of L-NOArg, L-NIL and 7-NI on activity of HOE 140 or des Arg10HOE 140 administered in low doses in a STZ-induced hyperalgesia model

Both HOE 140 and des-Arg^{10}HOE 140 administered at relatively low daily doses (70 nmol/kg) slightly but significantly, decreased diabetic hyperalgesia i.e. increasing the nociceptive threshold. Antihyperalgesic activity of L-NOArg (1.0 mg/kg) and L-NIL (1.0 mg/kg) was also emphasized. However, 7-NI (0.1 mg/kg) not changed STZ hyperalgesia.

Pretreatment with L-NOArg progressively increased the antihyperalgesic activity of HOE 140 or des-Arg^{10}-HOE 140. On days 22–24, even significant antinociception was observed. Pre-medication of L-NIL before HOE 140 or des-Arg^{10}HOE 140 not only progressively reduced STZ hyperalgesia but in the last 3 or 2 days respectively, antinociceptive effect appeared. After cessation of drugs administration, hyperalgesia quickly returned to the baseline threshold. In contrast, 7-NI did not modify antihyperalgesic effect of either HOE 140 or des-Arg^{10}HOE 140 (Fig. 2, 3).

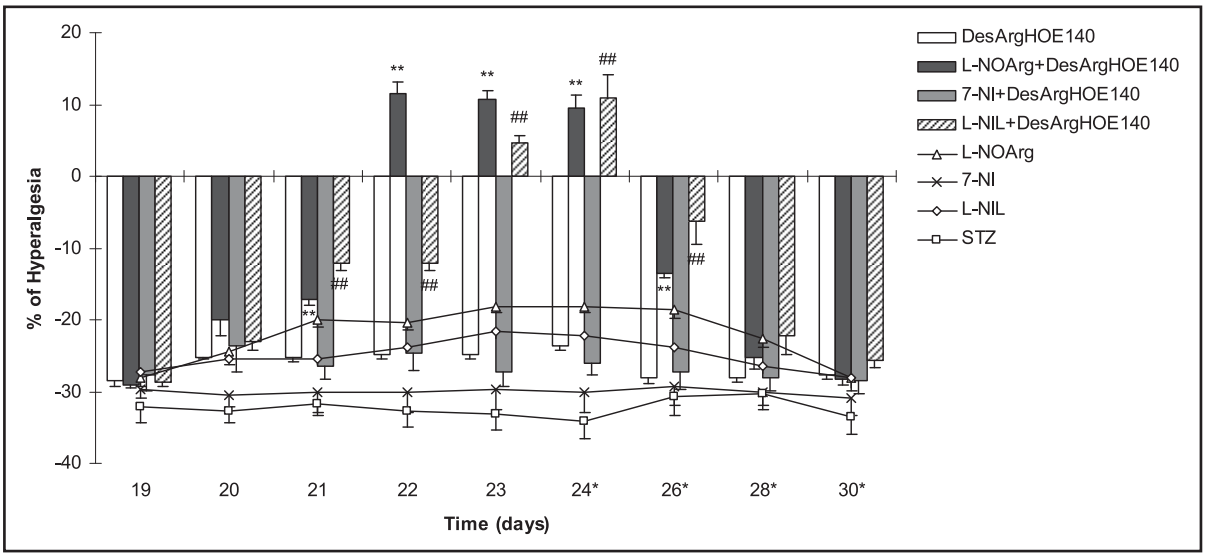

Fig. 3 Effect of L-NOArg and L-NIL at a dose of 1.0 mg/kg (ip) or 7-NI at a dose of 0.1 mg/kg (ip) on the antihyperalgesic activity of des-Arg^{10}-HOE 140 at a dose of 70 nmol/kg (ip) in STZ-treated rats. Days 19-24* – measurements of prolonged activity of investigated drugs; days 26*-30* - after discontinuation of administration.
Values are means ± S.E.M. des-Arg^{10}-HOE 140 vs. L-NOArg+ des-Arg^{10}-HOE 140 **p ≤ 0.01; des-Arg^{10}-HOE 140 vs. L-NIL+ des-Arg^{10}-HOE 140 ##p ≤ 0.01.
White column = des-Arg^{10}-HOE 140; Dark column = L-NOArg+ des-Arg^{10}-HOE 140; Grey column =7-NI+ des-Arg^{10}-HOE 140; White striped column = L-NIL+ des-Arg^{10}-HOE 140 △ = L-NOArg; X = 7-NI; ◊ = L-NIL; □ = STZ.

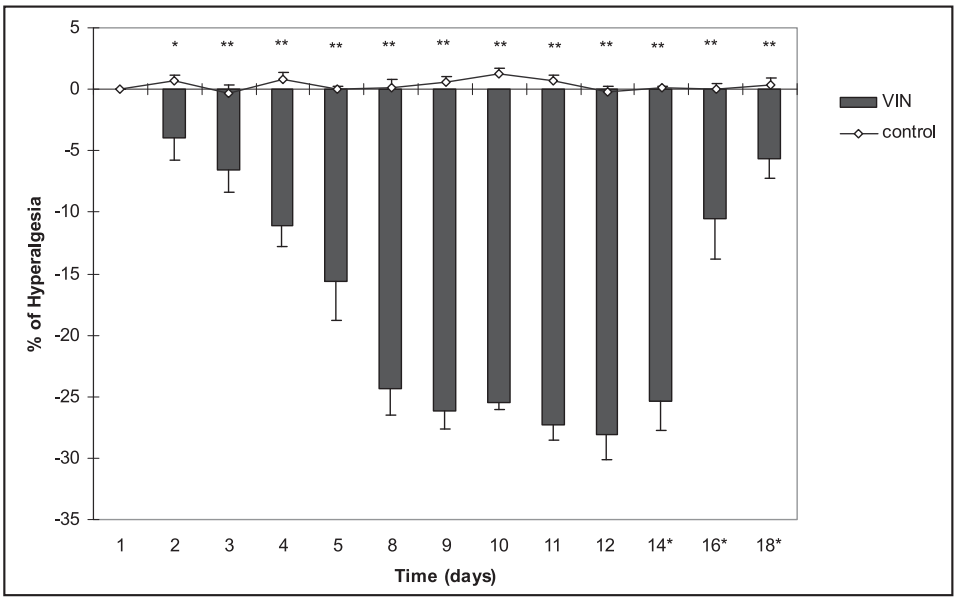

Fig. 4 Influence of vincristine (VIN) at a dose of 70 µg/kg (iv) on threshold to mechanical stimuli (days 1-12 administration of VIN; days 14*-28* after discontinuation of administration).
Values are means ± S.E.M. VIN vs. control
**p≤ 0.01, *p≤ 0.05. Dark column = VIN; ◊ = control.

Effect of VIN on nociceptive thresholds to mechanical stimuli

Starting from day 2, a statistically significant gradual decrease of the nociceptive threshold was observed in VIN-treated animals. The decrease reached its peak on day 8 of experiment and remained approximately stable until day 12. After discontinuation of VIN administration nociceptive thresholds to mechanical stimuli gradually increased, and on day 18 amounted only to 5% (Fig. 4).

Influence of L-NOArg, L-NIL and 7-NI on activity of HOE 140 or des-Arg^{10}HOE 140 administered in low doses in VIN-induced hyperalgesia model

Daily administration of HOE 140 (70 nmol/kg) markedly attenuated VIN hyperalgesia, although from day 5 to 14 only a small decrease occurred (Fig. 5). In contrast, des-Arg^{10}HOE 140 practically prevented the development of VIN hyperalgesia (Fig. 6). L-NOArg (1.0 mg/kg) and L-NIL (1.0 mg/kg) significantly dimin-

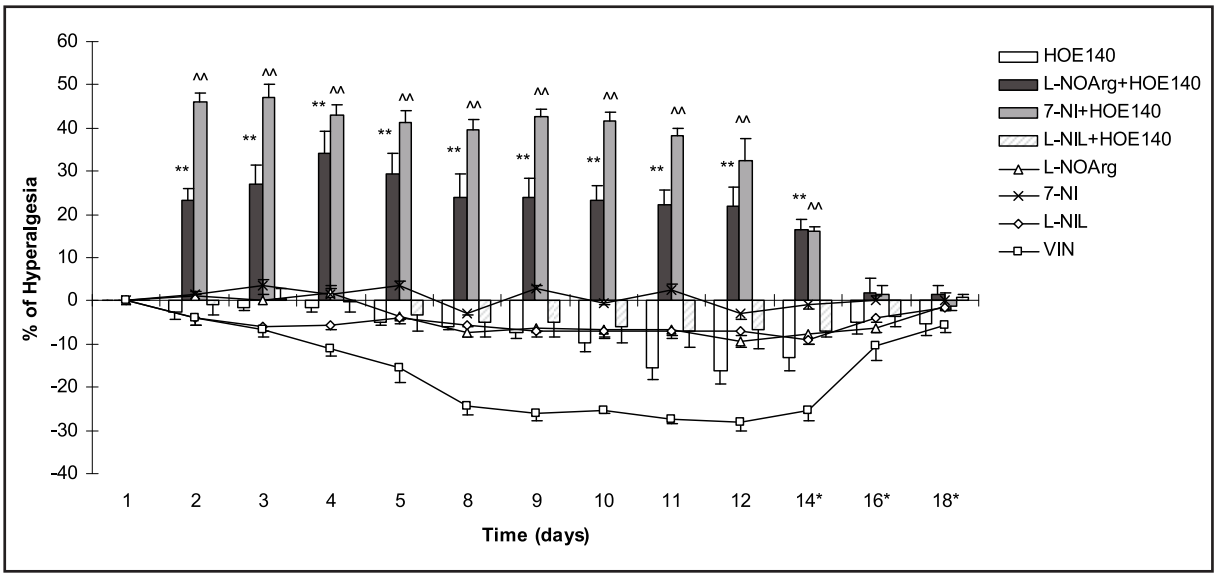

Fig.5 Effect of L-NOArg and L-NIL at a dose of 1.0 mg/kg (ip) or 7-NI at a dose of 0.1 mg/kg (ip) on the antihyperalgesic activity of HOE 140 at a dose of 70 nmol/kg (ip) in VIN treated rats. Days 1-12 – measurements of prolonged activity of investigated drugs; days 14*-18* - after discontinuation of administration.
Values are means ± S.E.M. HOE 140 vs. L-NOArg + HOE 140
**p ≤ 0.01; HOE 140 vs 7-NI+HOE 140 ^^p≤ 0.01.
White column = HOE 140; Dark column = L-NOArg+ HOE 140; Grey column =7-NI+ HOE 140;
White striped column = L-NIL+ HOE 140; △ = L-NOArg; x = 7-NI; ◊ = L-NIL; □ = VIN.

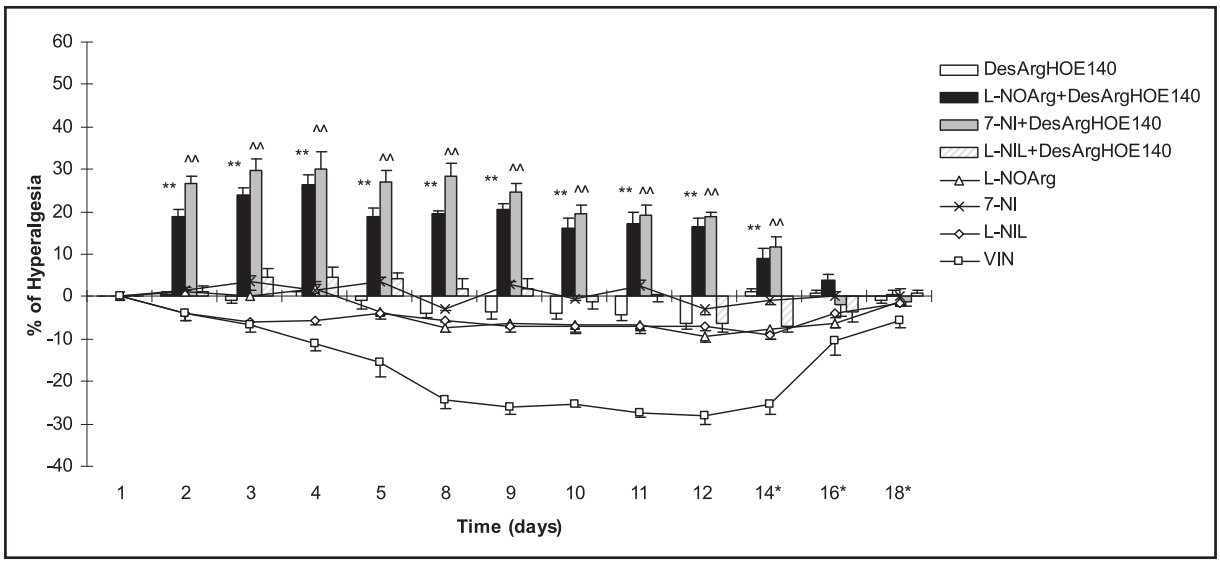

Fig.6 Effect of L-NOArg and L-NIL at a dose of 1.0 mg/kg (ip) or 7-NI at a dose of 0.1 mg/kg (ip) on the antihyperalgesic activity of des-Arg^{10}-HOE 140 at a dose of 70 nmol/kg (ip) in VIN treated rats. Days 1-12 – measurements of prolonged activity of investigated drugs; days 14*-18* - after discontinuation of administration.
Values are means ± S.E.M. des-Arg^{10}-HOE 140 vs. L-NOArg + des-Arg^{10}-HOE 140
**p ≤ 0.01; des-Arg^{10}-HOE 140 vs. 7-NI+ des-Arg^{10}-HOE 140 ^^p ≤ 0.01.
White column = des-Arg^{10}-HOE 140; Dark column = L-NOArg+ des-Arg^{10}-HOE 140;
Grey column =7-NI+ des-Arg^{10}-HOE 140; White striped column = L-NIL+ des-Arg^{10}-HOE 140;
△ = L-NOArg; X = 7-NI; ◊ = L-NIL; □ = VIN.

ished and 7-NI completely abolished the hyperalgesia produced by VIN.

Furthermore, pretreatment with L-NOArg or 7-NI before HOE 140 or des-Arg^{10}HOE 140 resulted in a significant antinociception from day 2 of experiment. This analgesic effect was especially emphasized for concomitant administration of 7-NI and HOE 140. After cessation of drug administration, the nociceptive threshold returned to control values. However, L-NIL did not modify antihyperalgesic activity of HOE 140 and des-Arg^{10}HOE 140 (Fig. 5, 6).

DISCUSSION

Neuropathic pain is difficult to treat. Classical analgetics, e.g. opioid receptor agonists, posses low activity; therefore, other agents which often have many adverse effects are used.

The experimental model of neuropathic pain caused by administration of streptozotocin (STZ), also known as painful diabetic neuropathy model, and model of pain caused by administration of vincristine (known as a vincristine-induced toxic neuropathy model), respectively, are commonly used in animal studies.

As reported previously (Bujalska *et al*. 2008, Bujalska *et al*. 2008a), in painful diabetic neuropathy model, administration of STZ at a dose of 40 mg/kg induces a development of stable hyperglycemia with an increase in water intake, an increase in food intake, and a gradual decrease of body mass. Diabetogenic action of STZ was accompanied by development of persistent hyperalgesia, which reached its plateau phase on day 17 and remained approximately stable during the next 13 days i.e. until the end of the experiment.

According to the previous study, an appearance of hyperalgesia after vincristine administration (70 μg/kg) was also observed. However, in contrast to STZ, which produced long-lasting and persistent hyperalgesia, hyperalgesia due to vincristine was reversible and nociceptive threshold gradually returned to the initial values after drug withdrawal (Bujalska and Gumułka 2008b).

Studies performed in the recent years have emphasized the important contribution of inflammatory process in development of neuropathic pain. Infiltration of inflammatory cells in response to nervous system damage, leads to subsequent production and secretion of various cytokins, growth factors and inflammatory mediators such as BK, NO, prostaglandins (PGs), serotonin. BK subsequently releases a large number of inflammatory mediators, such as PGs and NO. These inflammatory mediators (BK, NO, PGs) are known to sensitize nociceptors and elicit hyperalgesia as well as they participate in a pathophysiology of diseases accompanied by inflammatory reactions (Moalem and Tracey 2006, Maes *et al*. 2007).

BK not only causes excitation and sensitization of primary afferent nociceptors but also elicits vasodilatation and enhances vascular permeability, which contributes to inflammatory processes (Dray and Perkins 1993). BK-induced vasodilatation demonstrated to be mediated via endothelium-derived factor (EDRF), identified as NO (Palmer *et al*. 1987). It was also shown, that NOS is involved in the formation of inflammatory oedema induced by BK in peripheral tissues (Teixeira *et al*. 1993).

There are no literature reports about the role of bradykinin in vincristine neuropathy mechanism, and the data concerning bradykinin involvement in diabetic neuropathy is limited. Results of a few studies indicate that B_1 receptors are engaged in pain mechanism(s) of diabetic neuropathy (Campos *et al*. 2005, Gabra *et al*. 2006, 2005, Gabra and Sirois 2005, 2003, Pesquero *et al*. 2000). Campos *et al*. (2005) indeed suggested a very discrete and temporal increase of B_2 receptor density (without affinity changes) in spinal cord and brain of STZ-diabetic rats but data concerning involvement of B_2 receptors in this kind of neuropathic pain are not available.

As demonstrated in the previous study, a treatment with a specific antagonist of B_2 receptors (HOE 140) or a selective B_1 receptors antagonist (des-Arg^{10}-HOE 140) administered chronically at 200 nmol/kg doses prevented the development of both STZ and vincristine hyperalgesia (Bujalska *et al*. 2008). However, it was suspected that lower doses could also be effective. In fact, as shown in this paper, an administration of HOE 140 or des-Arg^{10}-HOE 140 at markedly lower doses (70 nmol/kg) significantly reduced or even abolished VIN hyperalgesia but only slightly attenuated STZ hyperalgesia.

There is limited data pertaining to the influence of NOS pathways in STZ- and VIN-induced hyperalgesia and results of these studies are controversial.

It was shown, that a decrease NO production by endothelial nitric oxide synthase (eNOS) system resulted in decreased vasodilatation and perfusion, which in turn lead to impairment of the peripheral nervous system(s) function (Timar-Peregrin and Guy 2001, Cameron *et al*. 2001).

Defect in axonal transport probably contributes to lose of axonal NOS content without diminishing nNOS level in the cell bodies. Because nNOS cannot be transported down to the axons, it accumulates in the cell body. Increased nNOS protein and NO production coincide with accumulation of advanced glycation end – products (AGEs) in blood and tissues. Synergistic action of AGEs and endogenous NO lead to increased oxidative stress of cell bodies resulting in their apoptosis (Cellek *et al*. 2005, 2004, Cellek 2004).

Thus, disturbances in NOS pathways (nNOS and eNOS) function may contribute to a development of diabetic neuropathy.

Results of the studies describing an influence of non-selective inhibitors of NOS on STZ-hyperalgesia are also incoherent. Fox *et al*. (1999) showed that administration of unselective nitric oxide synthase inhibitor L-NAME in streptozotocin-diabetic rats was without significant effect on hyperalgesia to mechanical stimuli. On the other hand, Joseph and Levine (2003) found that a nonspecific inhibitor of the constitutive and inducible nitric oxide synthase N-methyl-L-arginine (L-NMA) attenuate hyperalgesia to mechanical stimuli in diabetic neuropathy.

A role of NO pathway in VIN toxic neuropathy model is not satisfactorily explained. Aley and Levine (2002) showed that a nitric oxide synthase inhibitor (L-NMA) administered intraplantarly significantly increased paw

withdrawal threshold in vincristine neuropathy model. These Authors suggested that NO, acting peripherally, markedly contributes to the vincristine hyperalgesia. In contrast, Kamei *et al*. (2005) showed that sc pretreatment with a substrate of NO synthase, L-arginine or cGMP analog dose-dependently increased the tail-flick latencies in vincristine-treated mice. This action was reversed by intrathecal pretreatment with nonselective inhibitor NO synthase (L-NAME). Furthermore, the protein level of nNOS, but not iNOS was decreased in the spinal cord of vincristine treated mice as compared to that in naïve mice. Authors suggested that dysfunction of the L-arginine/NO/cGMP cascade in the spinal cord may trigger vincristine induced hyperalgesia to thermal stimuli in mice.

In the previous study, a non-selective inhibitor of NOS, L-NOArg, and a selective inhibitor of iNOS, L-NIL, at a dose of 10.0 mg/kg (ip) not only suppressed STZ- and VIN-induced hyperalgesia but also evoked antinociception. In contrast, the selective inhibitor of nNOS, 7-NI administered at a dose of 1.0 mg/kg (ip) prevented the VIN-hyperalgesia. However, it did not change nociceptive threshold in STZ neuropathy model (Bujalska *et al*. 2008a).

In the current paper, L-NOArg and L-NIL administered at a lower dose (1.0 mg/kg) diminished STZ, as well as VIN hyperalgesia. In accordance to the previous study, a lower dose of 7-NI (0.1 mg/kg) did not modify STZ-hyperalgesia but reduced VIN-hyperalgesia.

Thus, the results of this paper seem to confirm a participation of inducible nitric oxide synthase (iNOS) and neuronal nitric oxide synthase (nNOS) in VIN-induced model of chemotherapeutic neuropathy and iNOS but not nNOS in STZ-induced diabetic neuropathy.

BK is known to activate NO-cyclic GMP pathway. Moreover, in literature, the data supporting an involvement of NO-cGMP in pronociceptive effect of BK exists. Bauer *et al*. (1993) demonstrated that BK stimulates the production of cGMP by B_2 receptors in neurons cultured from embryonic rat dorsal root ganglion. This effect was inhibited by the specific guanylate cyclase inhibitor, methylene blue, and nonselective inhibitor of NOS, L-NMMA. Nakamura *et al*. (1996) showed that after administration of L-NAME or L-NMMA into the hind-paw, dose-related suppressed of BK-induced hyperalgesia in paw-pressure test occurred. L-Arginine reversed the suppressive effects of L-NAME and L-NMMA on BK-induced hyperalgesia. These Authors also demonstrated that BK activated the peripheral NO-cyclic GMP pathway via the B_2 receptor. An intact NO-synthesis pathway was also engaged in the generation of vein pains evoked by bradykinin in humans Kindgen-Milles and Arndt (Kindgen-Milles and Arndt 1996). Holthusen (1997) demonstrated that pretreatment with methylene blue inhibited bradykinin-evoked vein pain in a concentration-dependent manner. The author suggested that these results are consistent with the hypothesis that cyclic GMP plays a role in the transduction of NO-mediated noxious stimuli in vascular nociceptors in humans. In the contrary, in electrophysiological study Kelly *et al*. (2001) described that NO modulates particular C-fibre activity and reduces responsiveness to bradykinin. The authors showed that L-NAME enhanced responsiveness to bradykinin in arthritic but not normal rats, whereas L-arginine reduced the excitation by BK in both group.

There is only limited data concerning mutual interaction between bradykinin receptors activation and NOS pathways in the neuropathic pain models. Gabra and Sirois (2004) showed that bradykinin B_1 receptor – dependent activation of NO, SP and calcitinin gene-related peptide (CGRP) pathways contributed to development of diabetic hyperalgesia in STZ- induced diabetes in mice.

The results of this paper confirm that inhibition of bradykinin receptors as well as iNOS but not nNOS activity diminishes diabetic hyperalgesia (Bujalska *et al*. 2008, Bujalska *et al*. 2008a). Thus, it is not surprising that an irreversible inhibitor of cNOS, L-NOArg, and a relatively selective inhibitor of iNOS, L-NIL, but not a relatively specific inhibitor of nNOS, 7-NI, significantly increased antihyperalgesic activity of both a specific antagonist of B_2 receptors (HOE 140) and selective B_1 receptors antagonist (des-Arg^{10}-HOE 140).

It was also confirmed, that both products of iNOS and nNOS activation and bradykinin are involved in hyperalgesia produced by vincristine (Bujalska *et al*. 2008, in press). Moreover, in VIN induced toxic neuropathy L-NOArg or 7-NI administered before not only intensified antihyperalgesic activity of HOE 140 or des-Arg^{10}HOE 140 but also produced antinociception. In contrast, L-NIL did not change the nociceptive threshold increased after administration of HOE 140 or des-Arg^{10}HOE 140.

CONCLUDING REMARKS

Results of these studies suggest that B_1 and B_2 receptors are engaged in transmission of nociceptive stimuli in both diabetic and toxic neuropathy models.

Furthermore, in STZ-induced hyperalgesia in pronociceptive activity of bradykinin, iNOS is involved. Whereas in VIN-induced hyperalgesia, BK activates nNOS pathways.

Therefore, the results of this study may suggest that concomitant administration of small doses of bradykinin receptor antagonists and NOS inhibitors can be effective in alleviation of neuropathic pain, even in hospital care.

REFERENCES

1 Aley KO, Levine JD (2002). Different peripheral mechanisms mediate enhanced nociception in metabolic/toxic and traumatic painful peripheral neuropathies in the rat. Neuroscience. **111**: 389–397.
2 Aley KO, Reichling DB, Levine JD (1996). Vincristine hyperalgesia in the rat: a model of painful vincristine neuropathy in humans. Neuroscience. **73**: 259–265.
3 Bauer MB, Simmons ML, Murphy S, Gebhart GF (1993). Bradykinin and capsaicin stimulate cyclic GMP production in cultured rat dorsal root ganglion neurons via a nitrosyl intermediate. J Neurosci Res. **36**: 280–289.
4 Bujalska M, Tatarkiewicz J, de Cordé A, Gumułka SW (2008a). Effect of cyclooxygenase and nitric oxide synthase inhibitors on streptozotocin-induced hyperalgesia in rats. Pharmacology. **81**: 151–157.
5 Bujalska M, Gumułka SW (2008b). Effect of selenium compound (selol) on the opioid activity in vincristine induced hyperalgesia. Neuro Endocrinol Lett. **29**: 552-557.
6 Bujalska M, Tatarkiewicz J, Gumułka SW (2008). Effect of bradykinin receptor antagonists on vincristine- and streptozotocin-induced hyperalgesia in a rat model of chemotherapy-induced and diabetic neuropathy. Pharmacology. **81**: 158–163.
7 Calixto JB, Cabrini DA, Ferreira J, Campos MM (2000). Kinins in pain and inflammation. Pain. **87**: 1–5.
8 Cameron NE, Jack AM, Cotter MA (2001). Effect of alpha-lipoic acid on vascular responses and nociception in diabetic rats. Free Radic Biol Med. **31**: 125–135.
9 Campos MM, Ongali B, Thibault G, Neugebauer W, Couture R (2005). Autoradiographic distribution and alterations of kinin B(2) receptors in the brain and spinal cord of streptozotocin-diabetic rats. Synapse. **58**: 184–192.
10 Cellek S (2004). Point of NO return for nitrergic nerves in diabetes: a new insight into diabetic complications. Curr Pharm Des. **10**: 3683–3695.
11 Cellek S, Anderson PN, Foxwell NA (2005). Nitrergic neurodegeneration in cerebral arteries of streptozotocin-induced diabetic rats: a new insight into diabetic stroke. Diabetes. **54**: 212–219.
12 Cellek S, Qu, W, Schmidt AM, Moncada S (2004). Synergistic action of advanced glycation end products and endogenous nitric oxide leads to neuronal apoptosis in vitro: a new insight into selective nitrergic neuropathy in diabetes. Diabetologia. **47**: 331–339.
13 Dray A, Perkins M (1993). Bradykinin and inflammatory pain. Trends Neurosci. **16**: 99–104.
14 Fox A, Eastwood C, Gentry C, Manning D, Urban L (1999). Critical evaluation of the streptozotocin model of painful diabetic neuropathy in the rat. Pain. **81**: 307–316.
15 Gabra BH, Benrezzak O, Pheng LH, Duta D, Daull P, Sirois P, Nantel F, Battistini B (2005). Inhibition of type 1 diabetic hyperalgesia in streptozotocin-induced Wistar versus spontaneous gene-prone BB/Worchester rats: efficacy of a selective bradykinin B1 receptor antagonist. J Neuropathol Exp Neurol. **64**: 782–790.
16 Gabra BH, Berthiaume N, Sirois P, Nantel F, Battistini B (2006) The kinin system mediates hyperalgesia through the inducible bradykinin B_1 receptor subtype: evidence in various experimental animal models of type 1 and type 2 diabetic neuropathy. Biol Chem. **387**: 127–143.
17 Gabra BH, Merino VF, Bader M, Pesquero JB, Sirois P (2005). Absence of diabetic hyperalgesia in bradykinin B1 receptor-knockout mice. Regul Pept. **127**: 245–248.
18 Gabra BH, Sirois P (2003). Beneficial effect of chronic treatment with the selective bradykinin B1 receptor antagonists, R-715 and R-954, in attenuating streptozotocin-diabetic thermal hyperalgesia in mice. Peptides. **24**: 1131–1139.
19 Gabra BH, Sirois P (2004). Pathways for the bradykinin B1 receptor-mediated diabetic hyperalgesia in mice. Inflamm Res. **53**: 653–657.
20 Gabra BH, Sirois P (2005). Hyperalgesia in non-obese diabetic (NOD) mice: a role for the inducible bradykinin B1 receptor. Eur J Pharmacol. **514**: 61–67.
21 Holthusen H (1997). Involvement of the NO/cyclic GMP pathway in bradykinin-evoked pain from veins in humans. Pain. **69**: 87–92.
22 Joseph EK, Levine JD (2003). Sexual dimorphism in the contribution of protein kinase C isoforms to nociception in the streptozotocin diabetic rat. Neuroscience. **120**: 907–913.
23 Kamei J, Tamura N, Saitoh A (2005). Possible involvement of the spinal nitric oxide/cGMP pathway in vincristine-induced painful neuropathy in mice. Pain. **117**: 112-120.
24 Kelly DC, Asghar AU, Marr CG, McQueen DS (2001). Nitric oxide modulates articular sensory discharge and responsiveness to bradykinin in normal and arthritic rats in vivo. Neuroreport. **12**: 121–125.
25 Kindgen-Milles D, Arndt JO: Nitric oxide as a chemical link in the generation of pain from veins in humans. Pain 1996; **64**: 139–142.
26 Maes M, Mihaylova I, Kubera M, Bosmans E (2007). Not in the mind but in the cell: increased production of cyclo-oxygenase-2 and inducible NO synthase in chronic fatigue syndrome. Neuro Endocrinol Lett. **28**: 463–469.
27 Millan MJ: The induction of pain: an integrative review (1999). Prog Neurobiol. **57**: 1–164.
28 Moalem G, Tracey DJ (2006). Immune and inflammatory mechanisms in neuropathic pain. Brain Res Rev. **51**: 240–264.
29 Nakamura A, Fujita M, Shiomi H (1996). Involvement of endogenous nitric oxide in the mechanism of bradykinin-induced peripheral hyperalgesia. Br J Pharmacol. **117**: 407–412.
30 Nakhoda A, Wong HA (1979). The induction of diabetes in rats by intramuscular administration of streptozotocin. Experientia 35, Birkhäuser Verlag, Basel (Schweiz). **35**: 1679–1680.
31 Palmer RM, Ferrige AG, Moncada S (1987). Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. Nature. **327**: 524–526.
32 Pesquero JB, Araujo RC, Heppenstall PA Stucky CL, Silva JA, Walther T, Oliveira SM, Pesquero JL, Paiva AC, Calixto JB, Lewin GR, Bader M (2000). Hypoalgesia and altered inflammatory responses in mice lacking kinin B_1 receptors. Proc Natl Acad Sci U S A. **97**: 8140–8145.
33 Randall LO, Selitto JJ (1957). A method for measurement of analgesic activity on inflamed tissue. Arch Int Pharmacodyn Ther. **111**: 409–419.
34 Tallarida RJ, Murray RB: Manual of Pharmacologic Calculation with Computer Programs (1986), ed 2, New York: Springer, 113–117.
35 Teixeira MM, Williams TJ, Hellewell PG (1993). Role of prostaglandins and nitric oxide in acute inflammatory reactions in guinea-pig skin. Br J Pharmacol. **110**: 1515–1521.
36 Timar-Peregrin A, Guy RG (2001). Recovery of microvascular responses during streptozotocin-induced diabetes. Eur J Pharmacol. **414**: 63–70.
37 Wang H, Ehnert C, Brenner GJ, Woolf CJ (2006). Bradykinin and peripheral sensitization. Biol Chem. **387**: 11–14.