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Reduced Number, G Protein Coupling, and Antinociceptive Efficacy of Spinal Mu-Opioid Receptors in Diabetic Rats Are Reversed by Nerve Growth Factor

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Abstract: **This study investigated putative mechanisms of impaired spinal opioid antinociception such as a downregulation of mu-opioid receptor (MOR) number, coupling, and efficacy in rats with advanced (12 weeks) streptozotocin (STZ)-induced diabetes. Intravenous injection of STZ (45 mg/kg) in Wistar rats led to selective degeneration of insulin-producing pancreatic ß-cells, elevated blood glucose, and mechanical hyperalgesia. In these animals, dose-dependent and naloxone-reversible intrathecal fentanyl antinociception was significantly impaired and associated with a loss in MOR immunoreactivity of calcitonin gene-related peptide–immunoreactive (CGRP-IR) sensory nerve terminals, membrane-bound MOR binding sites, and MOR-stimulated G protein coupling within the dorsal horn of the spinal cord. Intrathecal delivery of nerve growth factor (NGF) in diabetic animals normalized spinal MOR number and G protein coupling and rescued spinal fentanyl-induced antinociception. These findings identify for the first time a loss in functional MOR on central terminals of sensory neurons as a contributing factor for the impaired spinal opioid responsiveness during advanced STZ-induced diabetes that can be reversed by NGF. Moreover, they support growing evidence of a distinct regulation of opioid responsiveness during various painful states of disease (eg, arthritis, cancer, neuropathy) and may give novel therapeutic incentives.**

Perspective: ***In diabetic neuropathy a loss in sensory neuron mu-opioid receptor number and coupling contributes to impaired spinal opioid antinociception that can be reversed by NGF. These findings support growing evidence of a distinct regulation of opioid responsiveness during various painful diseases and may give novel therapeutic incentives.***

Key words: *Diabetic neuropathy, pain, opioids, sensory neuron, intrathecal, nerve growth factor.*

In addition to their well-known systemic effects, opioids elicit potent analgesia when administered to the spinal cord. This concept was first established in animals[37] before it was adopted in clinical practice to effectively treat both acute postoperative pain[14] and chronic pain.[18] Spinally delivered opioids target pre- and postsynaptic opioid receptors in the dorsal horn of the spinal cord, of which 50 to 70% are presynaptically located.[1,15] Consistently, electrophysiological experiments performed in spinal cord slice preparations revealed that mu-opioid receptor (MOR) agonists reduced nociceptive signal transmission on central terminals of Aδ- and C-fibers mainly by inhibition of presynaptic voltage-dependent Ca^{2+} channels (VDCCs).[19,21]

Under pathological conditions such as neuropathic pain, there is a loss in spinal opioid responsiveness.[7,24] Indeed, peripheral nerve injury results in diminished MOR agonist-induced pre- and postsynaptic inhibition of excitatory postsynaptic currents.[24] At the same time the density of MOR immunoreactivity in the dorsal horn of the spinal cord is significantly decreased,[24,41] suggesting a possible link to the reduced opioid

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responsiveness. However, in diabetic neuropathic pain the mechanisms of a diminished spinal opioid responsiveness are less clear. While some studies reported alterations in the opioid distribution and clearance in diabetic rats,[12,43] others observed a reduced spinal MOR G protein coupling without changes in the number of MOR or G protein subunits,[9,10] a reduction in distinct G protein subunits,[16] or an increase in the concentrations of the pronociceptive peptides pronociceptin, nociceptin/orphanin FQ, and nocistatin.[25] The majority of these investigations were performed after 4 and only few of them after 8 weeks of streptozotocin (STZ)-induced diabetes. Therefore, we set out to systematically investigate the number, G protein coupling, and antinociceptive efficacy of spinal MOR in rats during a more advanced stage (12 weeks) of STZ-induced diabetes.

Interestingly, it was shown in the model of sciatic nerve constriction injury that intrathecal delivery of nerve growth factor (NGF) over 7 days could restore the antinociceptive efficacy of intrathecal opioids.[7] Consistently, NGF application in naïve animals[28] or overexpression of the *NGF* gene in transgenic mice[27,44] resulted in significantly increased MOR expression. Since NGF and other growth factors are known to be reduced in sensory neuron targets of experimental diabetic neuropathy,[5,11,13,23,31,40] we examined whether the intrathecal (i.t.) delivery of NGF could restore the number, functional coupling, and antinociceptive effects in rats with STZ-induced diabetes.

Methods

Reagents

The following substances were used: [^{3}H]DAMGO (DAMGO = [D-Ala2,N-MePhe4, Gly-ol]-enkephalin) (50 Ci/mmol); [^{35}S]GTPγS (1250 Ci/mmol) (Perkin Elmer, Rodgau, Germany); STZ, penicillin, naloxone hydrochloride (Sigma-Aldrich, Taufkirchen, Germany); and fentanyl citrate (Jansen, Neuss, Germany); scintillation fluid (Perkin Elmer Wallac, Turku, Finland); NGF beta-subunit of NGF (R & D Systems, Minneapolis, MN); rabbit polyclonal MOR antiserum (Gramsch Laboratories, Schwabhausen, Germany); mouse monoclonal antibody to rat GAD65 (Chemicon, Temecula, CA); polyclonal rabbit antibody against insulin (total insulin protein) (Cell Signaling Technology, Inc, Danvers, MA); and polyclonal guinea pig antibody against calcitonin gene-related peptide (CGRP) (Peninsula Laboratories, San Carlos, CA).

Animals and Streptozotocin-Induced Diabetes

Experiments were performed in male Wistar rats housed in cages lined with ground corn cob bedding. Rats with a mean starting body weight of 225 $\pm$ 10 g were kept in climate- and light-controlled rooms (22 $\pm$ .5°C; relative humidity, 60–65%; 12-hour light/dark cycle) with standard rodent food pellets and water ad libitum. Experiments were approved by the local animal care committee and done in accordance with the Declaration of the European Communities Council Directives (86/609/ECC). Rats were anesthetized with isoflurane in oxygen via nose cone and received an intravenous (i.v.) injection of STZ 45 mg/kg in .2 mL of citrate buffer (.1M, pH 4.7). The age-matched control animals received an equal volume of citrate buffer alone. Diabetes was verified by measuring hyperglycemia in the tail vein blood using a glucose strip Glucoflex (h&h Diabetes Care GmbH, Waiblingen, Germany). Body weights and blood glucose levels were monitored every week. Twelve weeks after the initial administration of STZ or buffer, in vivo and in vitro studies were carried out as described below. Animals were subdivided into 4 groups (n = 6–8 rats per group): nondiabetic control rats, STZ-treated diabetic rats, diabetic rats with i.t. NGF, or vehicle-treated rats.

Intrathecal Catheter and Osmotic Minipump Implantation

For continuous i.t. delivery of drugs, animals were implanted with chronic i.t. catheters, according to the method previously described.[28,39] Briefly, animals were anesthetized with isoflurane in oxygen via nose cone. A longitudinal skin incision was made in the lumbar region directly above the spinous processes of the L4–L6 vertebrae. The needle, through which the catheter was set up, was inserted at a 30° angle between the L5 and L6 vertebrae. The catheter (PE 10 tubing attached to PE 60 tubing for attachment to an osmotic pump; Portex Ltd, Hythe, Kent, United Kingdom) was carefully advanced while rotating it between the thumb and forefinger. The sign of dura penetration was observed by involuntary movements of the tail or hind limb. The catheter was then carefully pushed upward 1 cm into the intrathecal space. The needle was then carefully removed before the catheter was fixed with glue to the tissue and secured with 2 additional sutures. The correct location of the catheter was verified by 10 μL lidocaine 2%, which caused reversible bilateral hind limb paresis for 10 to 15 minutes. Diabetic rats received the following i.t. NGF treatment over the last 7 days of the 12-week STZ period: Alzet osmotic minipumps (200 μL; Alzet Corporation, Cupertino, CA) were filled with artificial cerebrospinal fluid (aCSF: 122 mM NaCl, 3.1 mM KCl, 5 mM $NaHCO_3$, .4 mM KH_2PO_4, 1.3 mM $CaCl_2$, 1.0 mM $MgSO_4$, 10 mM D-glucose) and rat serum albumin (1 mg/1 mL) with or without .125 μg/1 μL NGF and connected to the i.t. catheter to administer NGF or vehicle continuously at 1 μL/h.[7] Only animals exhibiting no motor deficits were used for further testing.

Nociceptive Testing

Mechanical pain thresholds and fentanyl-induced antinociception were tested by a mechanical paw pressure test. Animals were handled several days before the experiments to enable habituation to the experimental conditions. Paw pressure thresholds (PPTs) were assessed by a paw pressure algesiometer (Ugo Basile, Comerio, Italy) before (baseline) and