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Relationship between noradrenaline release in the locus coeruleus and antiallodynic efficacy of analgesics in rats with painful diabetic neuropathy

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ABSTRACT

Aims: In animal models of neuropathic pain, the noradrenergic descending pain inhibitory pathways from the locus coeruleus (LC) may be suppressed. However, no study has investigated the correlation between noradrenaline (NA) release in the LC and efficacy of analgesics in rats with painful diabetic neuropathy. Using microdialysis and analysis of mechanical hypersensitivity, we investigated the correlation between NA release in the LC and efficacy of morphine, tramadol, and clomipramine in rats with diabetic mellitus (DM).
Main methods: In freely moving rats, basal NA concentrations in LC perfusate were quantitated 72 to 96 h after microdialysis probe implantation. Following intravenous administration of each drug, NA concentrations were expressed as a percentage of basal values. We concurrently measured the threshold to elicit a paw withdrawal response every 20 min for 80 min.
Key findings: NA concentrations in the LC perfusate were significantly higher in the tramadol and clomipramine groups compared to the morphine group. Naloxone administration did not significantly affect NA concentrations. In the morphine group, NA release in the LC was not significantly correlated with the pain threshold. In contrast, in the tramadol and clomipramine groups, NA release in the LC was significantly correlated with the pain threshold. The correlation coefficient was higher in the clomipramine group than in the tramadol group.
Significance: Our results suggest that the descending noradrenergic pathway can play an important role in analgesia for DM neuropathy and that there is a significant correlation between NA release in the LC and the efficacy of tramadol and clomipramine.

Introduction

Diabetic peripheral neuropathy is a common complication of diabetes mellitus (DM). The prevalence of diabetic peripheral neuropathy is 10 to 50% in patients with DM (Ko et al., 2010; Partanen et al., 1995; Veves et al., 2008), with 10 to 20% of these patients experiencing pain (Boulton et al., 2004; Clark and Lee, 1995; Raskin et al., 2005), expressed as a steady burning or aching pain, characterized by allodynia, hyperalgesia, and paresthesia (Calissi and Jaber, 1995; Courteix et al., 1993; Kim and Chung, 1992). Postherpetic neuralgia and painful diabetic neuropathy are among the most common types of neuropathic pain, often causing mood and sleep disturbances that can influence quality of life and life expectancy (Sator-Katzenschlager et al., 2003; Schmader, 2002). Clomipramine is a tricyclic antidepressant recommended as a first-line analgesic for patients with neuropathic pain (Saarto and Wiffen, 2007). The mechanism underlying its antinociceptive efficacy involves the descending inhibitory bulbospinal pathway (Ardid et al., 1995). Tramadol is often recommended as a second-line analgesic (Hollingshead et al., 2006), and its mechanism of action involves low-affinity binding to μ-opioid receptors and weak inhibition of noradrenaline and serotonin reuptake (Christoph et al., 2007). Serotonergic and noradrenergic neurons have been implicated in the mediation of endogenous pain inhibitory mechanisms via descending inhibitory pain pathways in the brain and spinal cord (Basbaum and Fields, 1984; Clark and Proundfit, 1993). Opioid administration shows the decreased in potency and efficacy in the neuropathic pain treatment (Sounvoravong et al., 2004). Its mechanism may involve the descending pathway from the rostral ventromedial medulla, and the spinal cholinergic system of the dorsal horn (Gilbert and Franklin, 2002; Chen et al., 2005). In pathological pain states, these endogenous pain inhibitory mechanisms may be dysfunctional, contributing to the central sensitization and hyperexcitability of spinal and supraspinal pain-transmitting pathways, resulting in persistent pain (Coderre and Katz, 1997).

The locus coeruleus (LC) is the main source of noradrenaline in the brain and is involved in physiological processes such as adaptation to antinociception, stress, and opioid withdrawal (Pudovkina et al., 2001). The locus coeruleus may modulate nociception through its descending pathways. In neuropathic animals, the descending pain inhibitory pathway from the LC may be suppressed, since rhizotomy attenuates the spinal antinociceptive effect induced by LC stimulation (Hodge et al., 1983). To study LC activity, extracellular noradrenaline concentrations were measured using the microdialysis method (Pudovkina et al., 2001). Several investigators have measured the

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release of noradrenaline in the LC of freely moving, awake rats by performing in vivo microdialysis (Fernández-Pastor et al., 2005; Pudovkina et al., 2001). Other studies have shown the efficacy of tramadol and morphine in animal models of neuropathic pain (Ardid et al., 1995; Gong et al., 2011). However, no studies have investigated the correlation between noradrenaline release in the LC and the efficacy of drugs such as tramadol and clomipramine for painful diabetic neuropathy in rats.

In the present study, the brain microdialysis technique was used in freely moving diabetic rats to quantitate extracellular noradrenaline release in the LC area. Additionally, we examined the correlation between noradrenaline release in the LC and antinociceptive efficacy of morphine, tramadol, and clomipramine by analyzing mechanical hypersensitivity in streptozotocin (STZ)-induced diabetic rats.

Materials and methods

Animals and drug treatment

Upon approval from the Institutional Animal Care and Use Committee of our institution, 6-week-old female Sprague–Dawley rats weighing 180 to 230 g (Keari Inc. Osaka, Japan) were acquired for this study. Since Calcutt (2004) reported that there were no sex differences in the effects of diabetes on nocifensive behavior, we used female rats. Rats had free access to food and water and were housed in plastic cages under a 12-h light/dark cycle. Following probe implantation and intravenous cannulation, rats were housed individually in a plastic cage. Experiments were carried out during the light cycle.

The following drugs were used: STZ (Sigma Aldrich Japan, Tokyo, Japan), morphine hydrochloride (Daiichi Sankyo, Tokyo, Japan), naloxone (Daiichi Sankyo, Tokyo, Japan), tramadol hydrochloride (Sigma Aldrich Japan, Tokyo, Japan), and clomipramine hydrochloride (Alfresa Pharma, Osaka, Japan). All drugs except STZ were dissolved in saline.

Induction of diabetic neuropathy

Diabetes was induced with a single intravenous injection of STZ at 60 mg/kg of body weight. STZ was dissolved in 0.1 M citrate buffer, adjusted to pH 4.5, and injected immediately into a tail vein under sevoflurane anesthesia. Diabetes was indicated by blood glucose concentrations greater than 250 mg/dL. All rats developed diabetes 7 d after treatment with STZ. Four weeks after STZ treatment, all rats developed painful diabetic neuropathy, and were used in experiments. Normoglycemic rats were injected with a vehicle (0.1 M citrate buffer) and used in experiments 4 weeks later.

Measurement of mechanical allodynia

We determined the threshold for avoidance of hind paw exposure to mechanical stimulation. These thresholds were determined by quantifying the mechanical sensitivity of the paw. Rats were individually placed in a clear plastic cage with a wire-mesh bottom, which allowed access to their hind paws. A series of calibrated von Frey filaments (North Coast Medical Inc., Morgan Hill, CA, USA) were positioned perpendicularly to the plantar surface of the paws, with 6 s of force applied to the filaments such that a response was elicited (von Frey test). Brisk paw withdrawal or paw flinching was considered a positive response. In the absence of avoidance behavior, the next greater force was applied, whereas in case of a response, the next lower force was applied. The tactile stimulus producing a 50% likelihood of withdrawal was determined using the "up-down" method, as described in a previous study by Chaplan et al. (1994). Filament forces ranged from 1 g to 26 g, with a predetermined cut-off value of 26 g. Blood glucose concentrations and mechanical hypersensitivity tests were performed on the same day (0, 7, 14, 21, and 28 d after STZ administration). We measured blood glucose levels under general anesthesia using sevoflurane. In addition we had an adequate time between the measurement of blood glycemia and mechanical threshold. Therefore, the measurement of blood glycemia was not a stressful event for the rats and did not influence the mechanical threshold values.

Surgical procedures

Guide cannulas (7 mm in length and 0.5 mm in OD, AG-7; Eicom, Kyoto, Japan) were inserted into the brain 7 d before experiments by using a stereotaxic instrument (Narishige, Tokyo, Japan) while the rat was under general anesthesia (5% sevoflurane via nose cone). The tips of the guide cannulas were placed into the LC (A/P, −9.8 mm; L/M, 1.4 mm; and V/D, 6.5 mm from the bregma) based on the atlas of Paxinos and Watson (2006). To facilitate drug infusion, the cervical vein was cannulated under general anesthesia (3–5% sevoflurane via nose cone) 3 or 4 d before experiments. An intramuscular injection of cephalosporin 25 mg/kg was administered into the triceps muscle of rats that were subjected to a surgical procedure.

In vivo brain microdialysis experiments

Microdialysis experiments were carried out in freely moving rats 72 to 96 h after implantation of the probe. An on-line approach was used in which the probe was perfused with Ringer's solution at a flow rate of 2.0 μL/min (ESP-32; Eicom). The composition of Ringer's solution was in mM as follows: NaCl 147.0, KCl 4.0, and $CaCl_2$ 2.3. After constant perfusion for 120 min, the perfusate was collected to determine basal noradrenaline concentrations. Infusion of drugs was concurrent with collection of the dialysate, which was collected every 20 min and directly injected into an electrochemical detector (HTEC-500; Eicom) by using an autoinjector (EAS-20; Eicom). The mobile phase consisted of 0.1 M ammonium acetate buffer (pH 6.0) and methanol (7:3 v/v) containing 0.03 M sodium sulfonate and 50 mg/L EDTA-2Na. Analysis was conducted using an EICOMPAC CAX (2.0 mm × 200 mm; Eicom) column that was maintained at room temperature. Concentrations of noradrenaline in the dialysate were measured using a pure graphite working electrode (WE-PG; Eicom) and a salt bridge Ag–AgCl reference electrode. The working potential was set at 450 mV. The signal from the current-potential converter (the integrator output) was filtered with a low-pass in-line noise filter and integrated by a computerized data acquisition system using chromatography data software (PowerChrom; AD-Instruments Pty Ltd., Castle Hill, New South Wales, Australia). After completion of the microdialysis experiment, animals were euthanized with an overdose of intravenous pentobarbital (50 mg/kg), and brain specimens were prepared for histology to confirm the location of the microdialysis probe (Fig. 1A, B).

Statistical analysis

The mean values of 3 dialysate samples obtained before drug administration were considered as the 100% basal values. Extracellular concentrations of noradrenaline in dialysate samples collected during each experiment were normalized and expressed as a percentage of basal values. We performed microdialysis in normoglycemic and DM neuropathy rats and compared the extracellular noradrenaline concentrations between these 2 groups at each time point. We measured the threshold to elicit a paw withdrawal response 20, 40, 60, and 80 min after drug administration (morphine 0.44 mg/kg, naloxone 4 μg/kg plus morphine 0.44 mg/kg, tramadol 4.4 mg/kg, naloxone 4 μg/kg plus tramadol 4.4 mg/kg, and clomipramine 0.5 mg/kg). The doses of these drugs were selected based on data from previously published studies (morphine: Coudoré-Civiale et al., 2000; Plomp et al., 1981, tramadol: Wrzosek et al., 2009; Beier et al., 2008; Christoph et al., 2007, clomipramine: Ardid and Guilbaud, 1992). We also investigated