available at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/biochempharm

Intrathecal substance P (1–7) prevents morphine-evoked spontaneous pain behavior via spinal NMDA-NO cascade

Tsukasa Sakurada [a,*], Takaaki Komatsu [a], Hikari Kuwahata [a], Chizuko Watanabe [b], Toru Orito [b], Chikai Sakurada [c], Minoru Tsuzuki [c], Shinobu Sakurada [b]

[a] First Department of Pharmacology, Daiichi College of Pharmaceutical Sciences, 22-1 Tamagawa-cho, Minami-ku, Fukuoka 815-8511, Japan
[b] Department of Physiology and Anatomy, Tohoku Pharmaceutical University, 4-4-1 Komatsushima, Aoba-ku, Sendai 981-8558, Japan
[c] Department of Biochemistry, Nihon Pharmaceutical University, 10281, Komuro Ina-Machi Kitaadachi-gun, Saitama 362-0806, Japan

ARTICLE INFO

Article history:
Received 6 April 2007
Accepted 29 May 2007

Keywords:
High-dose morphine
Substance P (1–7)
Intrathecal injection
Vocalization and agitation
Glutamate
Nitric oxide
Spinal microdialysis

ABSTRACT

Previous research has shown that injection of high-dose of morphine into the spinal lumbar intrathecal (i.t.) space of rats elicits an excitatory behavioral syndrome indicative of severe vocalization and agitation. Substance P N-terminal fragments are known to inhibit nociceptive responses when injected i.t. into animals. In this study, we investigated the effect of i.t. substance P (1–7) on both the nociceptive response and the extracellular concentrations of glutamate and nitric oxide (NO) metabolites (nitrite/nitrate) evoked by high-dose i.t. morphine (500 nmol). The induced behavioral responses were attenuated dose-dependently by i.t. pretreatment with the substance P N-terminal fragment substance P (1–7) (100–400 pmol). The inhibitory effect of substance P (1–7) was reversed significantly by pretreatment with [D-Pro^2, D-Phe^7]substance P (1–7) (20 and 40 nmol), a D-isomer and antagonist of substance P (1–7). In vivo microdialysis analysis showed a significant elevation of extracellular glutamate and NO metabolites in the spinal cord after i.t. injection of high-dose morphine (500 nmol). Pretreatment with substance P (1–7) (400 pmol) produced a significant reduction on the elevated concentrations of glutamate and NO metabolites evoked by i.t. morphine. The reduced levels of glutamate and NO metabolites were significantly reversed by the substance P (1–7) antagonist (40 nmol). The present results suggest that i.t. substance P (1–7) may attenuate the excitatory behavior (vocalization and agitation) of high-dose i.t. morphine by inhibiting the presynaptic release of glutamate, and reducing NO production in the dorsal spinal cord.

1. Introduction

Morphine is widely used as a strong opioid-analgesic compound in the clinical management of moderate to severe pain. Despite widespread use of morphine, its treatment is often accompanied with the development of tolerance to and dependence on opioid analgesics. Analgesic tolerance has led to the higher opioid requirements and increases non-analgesic side effects such as respiratory depession, urinary retention, pruritis and myoclonic seizures. Hyperalgesic responses in animals occur during a precipitated opioid withdrawal. Previous studies have demonstrated that spinal administration of morphine at far higher doses than that required for analgesia produces spontaneous pain-related behavior (nociception) and hyperalgesia, which are naloxone-insensitive in mice and rats [1–3]. This observation suggests that spontaneous nociceptive behaviors and sensory hypersensitivity evoked by high-dose intrathecal (i.t.) morphine are not an opioid-mediated event.

* *Corresponding author*. Tel.: +81 92 541 0161; fax: +81 92 553 5698.
E-mail address: tsukasa@daiichi-cps.ac.jp (T. Sakurada).
0006-2952/$ – see front matter
doi:10.1016/j.bcp.2007.05.025

Clinical reports have also implicated that similar changes in pain sensitivity may also occur in pain patients after spinal administration of high-dose of morphine [4–7].

Substance P has been implicated as a major neurotransmitter/neuromodulator of pain in the spinal cord [8]. The i.t. administration of substance P causes a behavioral series of spontaneous scratching, biting and licking, and a hyperalgesic response to noxious stimuli. Furthermore, antagonists to neurokinin (NK_1) receptors were reported as having antinociceptive effect in mice [9–11], which provides additional strong support for the involvement of substance P in pain transmission and modulation in the dorsal horn of spinal cord. In contrast to a pivotal role of substance P in pain transmission, a major metabolite of substance P appears to be the N-terminal heptapeptide substance P (1–7) [12–14], which is known to have the opposite effect to substance P or to bioactive C-terminal substance P fragments. A membrane-bound protease capable of degrading substance P in synaptic region releases N-terminal fragments, substance P (1–7) and (1–8) from the parent peptide [15]. It is interesting to note that substance P specific endopeptidase present in human cerebrospinal fluid (CSF) and rat spinal cord is able to release the N-terminal fragments, substance P (1–7) and (1–8), from the parent peptide [12,16]. Substance P (1–7), the predominant N-terminal metabolite of substance P in the dorsal part of spinal cord [13,14], has been found to inhibit nociceptive behaviors in several nociceptive assays when injected i.t. into mice [17–20].

A role of nitric oxide (NO) in spinal nociceptive processing has been postulated in models of neuropathic pain [21]. NO synthase (NOS) inhibitors such as L-NAME prevent or reduce thermal hyperalgesia following chronic constriction injury. Recently, iNOS has become of significant interest in the phathophysiology of inflammatory and neuropathic pain [22–24]. NO has been shown to be involved in thermal hyperalgesia induced by endogenous and exogenous substance P in rats [25] and in substance P-induced itch-associated responses in mice [26]. In addition, it is of interest to note that an accumulation of substance P (1–7) down-regulates neuronal NOSmRNA and decreases constitutive NOS activity in the spinal cord and dorsal root ganglia [27].

The present study was to determine the effect of i.t. substance P (1–7) on the spontaneous vocalization and agitation responses evoked by high-dose i.t. morphine in rats. In addition, we measured released concentrations of glutamate and nitrite/nitrate by microdialysis in the spinal cord after high-dose i.t. morphine in the presence and absence of substance P (1–7) to determine whether the *N*-methyl-D-aspartate (NMDA)-NO cascade was influenced.

2. Materials and methods

2.1. Animals

Male Sprague–Dawley (SD) rats were obtained from Shizuoka Laboratory (Japan). All behavioral testing occurred when the rats were between 250 and 260 g. The rats were individually housed in a colony maintained in a controlled environment (12-h light:12-h dark cycle, room temperature 23 °C, 50–60% relative humidity). The animals had unlimited access to food and water. All behavioral experiments took place during the light period between 10:00 and 17:00 h in a quiet room. The animals belonging to the various treatment groups ($n = 7$ each group) were tested in randomized order. All experiments followed the Guidelines on Ethical Standards for Investigation of Experimental Pain in Animals [28]. Additionally, the study was approved by the Committees of Animal Care and Use of Daiichi College of Pharmaceutical Sciences and Tohoku Pharmaceutical University.

2.2. Intrathecal administration

The i.t. administration of compounds to the lumbar region of the spinal cord of rats was performed through a polyethylene catheter 10 μl in a volume. This involved inserting a length of polyethylene tubing (PE-10) following laminectomy between L3 and L4 and carefully placing the catheter tip in the subarachnoid space of L5 and L6 through a slit in the atlanta-occipital membrane [15]. The animals were allowed to recover 7 days following implantation of the catheter. The catheter was filled with sterile artificial cerebrospinal fluid (CSF), containing in (g/L) NaCl 7.4, KCl 0.19, $MgCl_2$ 0.19 and $CaCl_2$ 0.14. Drugs were administered in volumes of 10 μl followed by a flush of 15 μl of artificial CSF to ensure that each compound reached the spinal cord. Substance P (1–7) and [D-Pro^2, D-Phe^7]substance P (1–7) were administered i.t. 2 and 4 min prior to i.t. morphine (500 nmol), respectively. Rats showing motor weakness and signs of hindlimb paralysis upon recovery from anesthesia were immediately sacrificed.

2.3. Measurement of spontaneous behavior

Prior to testing, all rats were handled and habituated to an open Plexiglass chamber (34 cm × 30 cm × 17 cm) for 1 h before actual experimental sessions, which also served as an observation chamber after injection. The handling and habituation protocol were designed to limit stress-induced analgesia that has been reported to be influenced by rat strain [29]. Immediately after morphine (500 nmol) was injected i.t. using a microsyringe with a 26-gauge needle, behavioral observation was immediately started. The i.t. administration of 500 nmol morphine produced a striking behavioral syndrome consisting of spontaneous vocalization and agitation. The magnitudes of two behavioral responses were quantified; vocalization and agitation. Spontaneous vocalization was recorded by the stop-watch during each 5-min interval for a 40-min period after i.t. administration of morphine (500 nmol) in combination with artificial CSF (control), or substance P (1–7) and/or [D-Pro^2, D-Phe^7]substance P (1–7). The latency to induce the first vocalization after i.t. administration of morphine was also recorded. The second parameter studied was the appearance of spontaneous agitation. Spontaneous agitation was ranked visually every 1 min after i.t. administration of 500 nmol morphine in combination with artificial CSF (control), or substance P (1–7) and/or [D-Pro^2, D-Phe^7]substance P (1–7) as 0, no sign of excitation; 1, restlessness, scratching and biting at the flank or tail; 2, mild vocalization with restlessness, scratching and biting at the flank or tail; 3, vocalization with spontaneous running and circling; 4, vigorous vocalization with running, circling, rolling and