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Short communication

Analgesic effects of the somatostatin sst_4 receptor selective agonist J-2156 in acute and chronic pain models

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Abstract

Somatostatin released from capsaicin-sensitive afferents exerts systemic anti-nociceptive actions, presumably via somatostatin receptor subtype 4 (sst_4). In the present study, the antinociceptive effects of a novel somatostatin sst_4 receptor selective peptidomimetic compound, J-2156 (1–100 μg/kg i.p.), were examined. J-2156 inhibited nocifensive behaviour of mice in the second phase of the formalin test. Adjuvant-evoked chronic inflammatory mechanical allodynia was decreased in rats treated with J-2156 for 21 days. Sciatic nerve ligation-induced neuropathic mechanical hyperalgesia was inhibited by J-2156 on the seventh postoperative day. Results obtained using this highly selective agonist suggest that somatostatin sst_4 receptors represent a promising target for new perspectives in analgesic therapy.

Keywords: Somatostatin; Anti-nociceptive effect; Formalin test; Adjuvant-induced inflammation; Traumatic mononeuropathy

1. Introduction

Somatostatin is synthesized and stored in capsaicin-sensitive, transient receptor potential vanilloid 1 receptor (TRPV1)-expressing nociceptive afferents as 14 and 28 amino acid peptides (Hökfelt et al., 1976; Carlton et al., 2001). Exogenously administered somatostatin has been shown to inhibit nociceptive processes in different experimental assessments (Lembeck et al., 1982; Chrubasik, 1991; Karalis et al., 1994; Fioravanti et al., 1995). Furthermore, we have recently provided several lines of evidence that, following somatostatin release from activated capsaicin-sensitive fibres, it reaches the circulation and exerts systemic anti-nociceptive and anti-inflammatory actions, presumably via somatostatin receptor subtype 4 (sst_4) (Szolcsanyi et al., 1998a,b; Helyes et al., 2004, 2000, 2001; Than et al., 2000). This endogenous counterregulatory mechanism of neurally derived somatostatin has been termed as its "sensocrine function" (Than et al., 2000; Szolcsanyi et al., 2004a).

The therapeutic value of native somatostatin is limited by its broad range of effects, mediated by five different receptor subtypes and its short (3 min) plasma half life (ten Bokum et al., 2000). However, potent and stable somatostatin receptor agonists acting selectively on sst_4 receptors on nociceptive nerve terminals (Hoyer et al., 1995) could be promising for analgesic drug development. This has been supported by the potent and broad spectrum antinociceptive effects of a stable heptapeptide agonist TT-232 (Helyes et al., 2000, 2004, 2005; Pinter et al., 2002). Progress has been made recently in the development of subtype-selective, nonpeptide somatostatin receptor agonists. A novel sulfonamido-peptidomimetic compound, J-2156 [(1′*S*,2*S*)-4-amino-*N*-(1′-carbamoyl-2′-phenylethyl)-2-(4″-methyl-1″-naphthalenesulfonamino)butanamide], synthesized at Juvantia Pharma (Turku, Finland), belongs to a chemically novel class of somatostatin receptor ligands. J-2156 possesses nanomolar affinity for the human somatostatin sst_4 receptor and is over 400-fold more selective for this receptor than for other human somatostatin receptor subtypes (Engstrom et al., 2005). In a cyclic AMP assay, the compound acted as a full agonist, similar to native somatostatin-14 or somatostatin-

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28. In a [^{35}S]guanosine-5′-*O*-(3-thio)triphosphate functional assay, J-2156 elicited a response two to three times larger than native somatostatin. In the same assay, increasing concentrations of somatostatin-14 caused a concentration-dependent rightward shift of the concentration–response curves of J-2156, without affecting its maximal effect. Since J-2156 exerted greater agonism on the sst_4 than its endogenous ligands, J-2156 has been defined as a "superagonist" (Engstrom et al., 2005).

The present study aimed at analysing the anti-nociceptive effects of J-2156 on formalin-induced acute chemonocifensive behaviour, chronic inflammatory hyperaesthesia and neuropathic hyperalgesia in mouse and rat experimental models.

2. Materials and methods

2.1. Animals

Experiments were performed on male Balb/c mice (20–25 g), Lewis rats (200–250 g) and Wistar rats (200–250 g) bred and kept in the Laboratory Animal Centre of the University of Pécs under standard pathogen free conditions at 24–25 °C and provided with standard rat chow and water ad libitum. Animals were randomized in all three experimental assessments. The examiner taking the measurements was blinded from the treatment the animals received.

2.2. Investigation of formalin-induced acute nocifensive behaviour

Formalin (20 μl, 2.5%) was injected intraplantarly into the left hindpaw of male Balb/c mice, which induced nocifensive reactions in two phases. Phase I (0–5 min) is thought to be due to a direct chemonociceptive effect of formalin, while phase II (20–45 min) is mainly mediated by inflammatory reactions (Tjolsen et al., 1992). Nocifensive behaviour was quantified by the duration of paw lickings. J-2156 was administered i.p. 20 min prior to formalin injection.

2.3. Measurement of mechanical touch sensitivity of the paw in adjuvant-induced chronic inflammation

Complete Freund's adjuvant (CFA; killed Mycobacteria suspended in paraffin oil; 0.1 ml, 1 mg/ml) was injected intraplantarly and into the root of the tail of male Lewis rats. In order to enhance systemic effects, an additional injection was given into the tail the following day. The mechanical touch sensitivity of the paws was measured by aesthesiometry (Ugo Basile Dynamic Plantar Aesthesiometer 37400, Comerio, Italy) before the experiment and 2, 5, 8, 12, 15, 18 and 21 days after CFA administration. This is an electronic von Frey device, in which the rats move about freely in one of the two compartments of the enclosure positioned on the metal mesh surface. Following acclimation and cessation of exploratory behaviour, the operator places the touch stimulator unit under the animal's paw, using the adjustable angled-mirror to position the filament below the target area of the paw. The paw withdrawal threshold is numerically shown in grams on the digital screen. Allodynia (hyperaesthesia) was expressed as percentage of initial control values. Previous studies have revealed that this method is the most appropriate to study mechanosensitivity when oedema develops on the dorsal surface of the paw during inflammatory models (Helyes et al., 2004). J-2156 (1 and 10 μg/kg) was injected i.p. three times daily throughout the whole experimental period.

2.4. Measurement of mechanonociceptive threshold of the paw in traumatic neuropathy

Male Wistar rats were anaesthetised with pentobarbital (Nembutal, 50 mg/kg i.p.). The common sciatic nerve was exposed unilaterally high in the thigh, then 1/3–1/2 of the nerve trunk was carefully separated and tightly ligated using a siliconised silk suture (Ethicone 8-0). The wound was closed and the animals were allowed to recover and survive for 8 days (Seltzer et al., 1990). During this period, signs of spontaneous pain (holding the legs in elevated position) were observed and a significant decrease of the mechanical threshold developed 7 days after the surgery. Mechanonociceptive threshold of the hindpaws was determined with the Randall-Selitto test (Ugo Basile Analgesimeter 7210, Comerio, Italy), which is a reliable technique for the measurement of mechanonociception in the partial sciatic nerve ligation model (Pinter et al., 2002). The analgesimeter contains a plastic cone-shaped pusher with a blunt end, under which the rat paw is placed. After pressing a pedal, an increasing force is applied on the paw and the weight to which the rat withdraws the paw is considered to be the mechanonociceptive threshold. This value can be read from the analog scale in grams. Hyperalgesia was expressed in % compared to the initial control values. J-2156 (1–100 μg/kg) was injected i.p. on the seventh day 20 min before the repeated measurement.

2.5. Ethics

All experimental procedures were carried out according to the 1998/XXVIII Act of the Hungarian Parliament on Animal Protection and Consideration Decree of Scientific Procedures of Animal Experiments (243/1988) and complied with the recommendations of the International Association for the Study of Pain and the Helsinki Declaration. The studies were approved by the Ethics Committee on Animal Research of Pécs University according to the Ethical Codex of Animal Experiments and licence was given (licence no.: BA 02/200-6-2001).

2.6. Statistical analysis

For determining statistically significant differences between the different groups, the non-parametric Mann–Whitney *U*-test was used, in all cases $p<0.05$ was considered significant.

2.7. Drugs and chemicals

Formalin (Formaldehydum solutum 37%; Ph.Hg. VII) was purchased from the Pharmacy of the University of Pécs.

Pentobarbital sodium (Nembutal) was bought from May and Baker (UK) and complete Freund's adjuvant from Sigma (St. Louis, Mo, USA). J-2156 was synthesized at Juvantia Pharma (Turku, Finland) and dissolved in isotonic saline.

3. Results

Signs of behavioural side-effects, such as sedation, motor disorders or coordination problems were not observed in rats treated with J-2156.

3.1. Effect of J-2156 on formalin-induced acute nocifensive behaviour

The anti-nociceptive effect of J-2156 (10–100 μg/kg i.p.) was examined on the two characteristic phases (Tjolsen et al., 1992) of the formalin test. In the early phase, nocifensive behaviour, expressed as the duration of paw licking and lifting, was not inhibited by any dose of the compound. In the second, acute inflammatory phase J-2156 induced a significant, dose-dependent anti-nociceptive effect (Fig. 1).

3.2. Effect of J-2156 on adjuvant-induced chronic inflammatory mechanical allodynia

Initial mechanonociceptive threshold of the paw was 48.8±0.2 g, which decreased to 26.8±0.3 g 5 days after CFA injection in control, saline-treated rats. J-2156 (1–10 μg/kg i.p., three times per day) diminished adjuvant-induced mechanical allodynia in a dose-dependent manner throughout the 21-day experimental period. The inhibition induced by 10 μg/kg J-2156 was around 40–50%, while the effect of the lower dose was not significant at any measuring points (Fig. 2A).

3.3. Effect of J-2156 on chronic neuropathic mechanical hyperalgesia

Partial ligation of the sciatic nerve resulted in a significant decrease of the mechanonociceptive threshold from 109±3.1 g to 72±2.3 g 7 days after surgery. J-2156 at doses of 10 and 100 μg/kg i.p. inhibited this neuropathic mechanical hyperalgesia by 62.5% and 50.6%, respectively, while the effect of the smallest, 1 μg/kg i.p. dose, was not statistically significant (Fig. 2B).

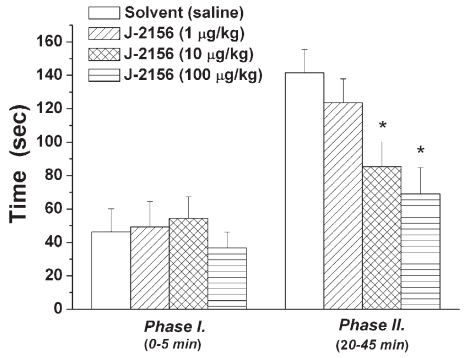

Fig. 1. Effect of J-2156 (i.p.) on formalin-induced acute nocifensive behaviour in mice. Results are shown as the mean duration of paw lickings and liftings (s) with S.E.M. of $n=9–10$ experiments/group. The Mann–Whitney U-test was used for statistical analysis (*$P<0.05$ compared to the solvent-treated control group).

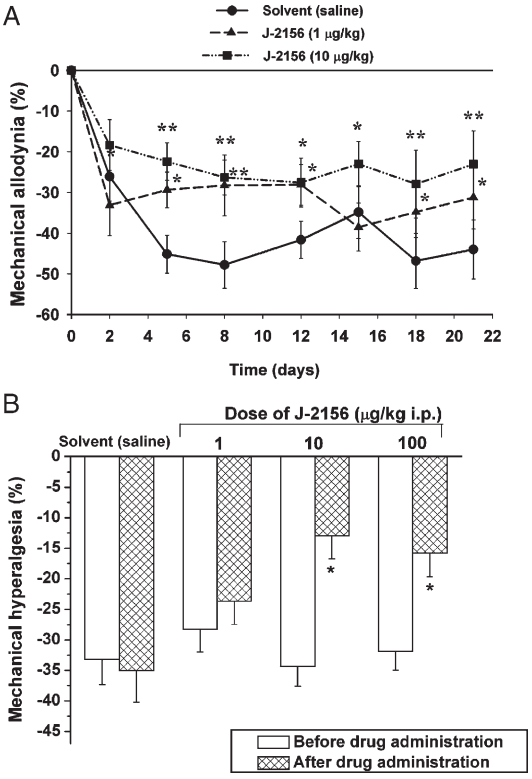

Fig. 2. (A) Effect of J-2156 (i.p.) on adjuvant-induced chronic inflammatory mechanical allodynia in rats. Data are expressed as percentage changes of the mechanonociceptive threshold of the hindpaws compared to the values measured before the induction of inflammation. Results are means with S.E.M. of $n=6–9$ experiments/group. The Mann–Whitney U-test was used for statistical analysis (*$P<0.05$, **$P<0.01$ compared to the solvent-treated control group). (B) Effect of J-2156 (i.p.) on mechanical hyperalgesia developed 7 days after partial ligation of the sciatic nerve in rats. Columns represent percentage changes of the mechanonociceptive threshold of the hindpaws compared to the pre-operation control values. Results are means with S.E.M. of $n=6–8$ experiments/group. The Mann–Whitney U-test was used for statistical analysis (*$P<0.01$ compared to the solvent-treated control group).

4. Discussion

The present results demonstrate that the peptidomimetic compound J-2156, a highly somatostatin sst_4 receptor selective agonist, was able to significantly inhibit nocifensive reactions in a conventional acute somatic chemonociceptive test in mice (formalin test) (Le Bars et al., 2001; Tjolsen et al., 1992), as well as in two chronic pain models in rats, thus indicating its effectiveness in both species.

At the cellular level, the activation of somatostatin receptors causes an inhibition in the accumulation of cyclic AMP, the opening of various K^+ channels and an inhibition of voltage-gated Ca^{2+} channels. The latter two effects lead to an inhibition of both spike generation and the release of neurotransmitters (Weckbecker et al., 2003). In addition, somatostatin sst_4 receptor agonists enhance signalling through mitogen-activated

protein kinase (MAPK), phospholipase C and phospholipase A_2, as well as activating/inhibiting phosphotyrosine phosphatases (Weckbecker et al., 2003). In other experiments, we have observed that J-2156 potently diminishes different types of inflammatory reactions in rodents (manuscript submitted), presumably as a consequence of inhibiting the release of substance P and calcitonin gene-related peptide from sensory neurones. Therefore, the inhibitory action of J-2156 on nocifensive behaviour in the second phase of the formalin test and on mechanical allodynia in the adjuvant-induced chronic inflammation model may be largely due to its anti-inflammatory effects.

Our results are of great practical significance, considering that, at present, the therapy of neuropathic and chronic inflammatory pain is still an unresolved problem. The analgesic effect of non-steroidal agents and opioids is almost absent in neuropathic conditions (Dellemijn, 1999) and high doses are required for the treatment of chronic inflammatory pain which are often not tolerated due to their unwanted actions. Patients treated with opioid analgesics often suffer severe side effects such as nausea, vomiting, drowsiness and constipation (Dellemijn, 1999). In our previous experiments, diclofenac (10 mg/kg i.p.) was ineffective in the rat neuropathy model, but the presynaptic $GABA_B$ receptor agonist baclofen (3 mg/kg i.p.) was able to abolish hyperalgesia (Pinter et al., 2002).

In earlier studies, the analgesic effects of TT-232, a peripherally acting heptapeptide somatostatin sst_4 and sst_1 receptor agonist (for review, Helyes et al., 2005), were analysed in detail (Helyes et al., 2000, 2004, 2005; Pinter et al., 2002; Szolcsanyi et al., 2004b). TT-232 diminished nocifensive behaviour in both phases of the formalin test, adjuvant-induced inflammatory allodynia and reversed development of mechanical hyperalgesia after partial sciatic nerve injury (Pinter et al., 2002; Szolcsanyi et al., 2004b; Helyes et al., 2004; for review, Helyes et al., 2005). Although penetration of J-2156 to the central nervous system cannot be ruled out, based on previous results that TT-232 exerts similar actions in the same models (Pinter et al., 2002; Szolcsanyi et al., 2004b), the observed anti-nociceptive actions might be, at least partially, due to a peripheral site of action. However, the major advantage of J-2156 over TT-232 is its much smaller size and peptidomimetic structure, which may make oral administration possible.

Our observations with the highly somatostatin sst_4 receptor-selective agonist J-2156 suggest that these receptors represent a promising target and opens interesting new perspectives for non-opioidergic pain control. Since J-2156 targets the somatostatin sst_4 receptor, it avoids the most common endocrine side effects of somatostatin agonists (namely the inhibition of growth hormone, glucagon and insulin release) as these effects are mainly ascribed to somatostatin sst_2 and sst_5 receptor (Weckbecker et al., 2003). The anti-nociceptive effect of J-2156 combined with its anti-inflammatory action makes this compound a promising lead molecule for further discovery and development work, particularly in the treatment of pain conditions, such as peripheral neuropathy, in which other analgesics are not effective (Dellemijn, 1999).

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