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Pharmacological characterization of the TRPV1 receptor antagonist JYL1421 (SC0030) in vitro and in vivo in the rat

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Abstract

The TRPV1 capsaicin receptor is an integrator molecule on primary afferent neurones participating in inflammatory and nociceptive processes. The present paper characterizes the effects of JYL1421 (SC0030), a TRPV1 receptor antagonist, on capsaicin-evoked responses both in vitro and in vivo in the rat. JYL1421 concentration-dependently (0.1–2 μM) inhibited capsaicin-evoked substance P, calcitonin gene-related peptide and somatostatin release from isolated tracheae, while only 2 μM resulted in a significant inhibition of electrically induced neuropeptide release. Capsazepine (0.1–2 μM), as a reference compound, similarly diminished both capsaicin-evoked and electrically evoked peptide release. JYL1421 concentration-dependently decreased capsaicin-induced Ca^{2+} accumulation in cultured trigeminal ganglion cells, while capsazepine was much less effective. In vivo 2 mg/kg i.p. JYL1421, but not capsazepine, inhibited capsaicin-induced hypothermia, eye wiping movements and reflex hypotension (a component of the pulmonary chemoreflex or Bezold–Jarisch reflex). Based on these data JYL1421 is a more selective and in most models also a more potent TRPV1 receptor antagonist than capsazepine, therefore it may promote the assessment of the (patho)physiological roles of the TRPV1 receptor.

Keywords: TRPV1 receptor antagonist; JYL1421 (SC0030); Sensory neuropeptide release; Ca^{2+} accumulation; Wiping test; Hypothermia; Pulmonary chemoreflex

1. Introduction

Capsaicin (8-methyl-*N*-vanillyl-6-nonenamide), the pungent principle of hot chilli peppers, is a valuable pharmacological tool for the categorization and characterization of primary afferents. The existence of a capsaicin receptor in the cell membrane of a subgroup of nociceptive sensory neurones was hypothesized 30 years ago (Szolcsányi and Jancsó-Gábor, 1975), then it was cloned and identified in the rat (Caterina et al., 1997), guinea pig (McIntyre et al., 2001) and man (Hayes et al., 2000). This receptor was then called vanilloid receptor 1 (VR1), but now it is referred to as TRPV1 based on its phylogenetic relationship with the larger superfamily of transient receptor potential (TRP) channels (Clapham et al., 2001; Gunthorpe et al., 2002; Montell et al., 2002). This non-selective cation channel composed of 6 transmembrane domains has become a promising target for the development of a new generation of anti-inflammatory and analgesic agents with a target on sensory neurones (Szállási and Blumberg, 1999; Szolcsányi, 2002). Activation of the TRPV1 receptor induces Ca^{2+} entry and consequent release of sensory neuropeptides like calcitonin gene-related peptide (CGRP) and tachykinins (e.g. substance P), which mediate vasodilatation, plasma

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protein extravasation and immune cell accumulation in the innervated area (Maggi, 1995; Helyes et al., 2003). Furthermore, the released somatostatin exerts systemic anti-inflammatory and analgesic actions (Szolcsányi et al., 1998a,b; Helyes et al., 2000, 2004).

The TRPV1 channel can be considered a molecular integrator of noxious chemical and thermal stimuli in the peripheral terminals of primary sensory neurones involved in pain sensation (Garcia-Martinez et al., 2002). The classical pharmacological tools to study the role of this receptor are TRPV1 receptor antagonists, but the available compounds mostly show moderate potency, species difference and limited selectivity (Helyes et al., 2003). Of them, recent studies have shown that ruthenium red should be considered a non-selective inhibitor of all TRPV channels (Gunthorpe et al., 2002; Smith et al., 2002) and it also acts on many other ion channels and receptors (Amann and Maggi, 1991). Capsazepine is a relatively weak antagonist, which appears to show species- and modality-specific activity (McIntyre et al., 2001; Shin et al., 2002; Savidge et al., 2001, 2002; Seabrook et al., 2002; Walker et al., 2003). Furthermore, it also inhibits acetylcholine receptors (Liu and Simon, 1997), voltage-gated Ca^{2+} channels (Docherty et al., 1997), and hyperpolarisation-activated cyclic nucleotide gated channels (Gill et al., 2004) at similar concentrations at which it acts on TRPV1. Iodo-resiniferatoxin effectively inhibits capsaicin-evoked currents in Xenopus oocytes and nociceptive responses in the rat (Wahl et al., 2001; Seabrook et al., 2002). However, its deiodination to resiniferatoxin after systemic administration cannot be excluded, which raises the possibility of inducing desensitization. BCTC represents highly potent TRPV1 receptor antagonistic activity (Pomonis et al., 2003), but from a pharmaceutical development perspective, it has several possible shortcomings including poor metabolic stability, short half-life, poor aqueous solubility and moderate bioavailability (Tafesse et al., 2004). Therefore, there is still a great need for more potent and selective TRPV1 receptor antagonists to define the role of this receptor in physiological and pathophysiological conditions and reveal its significance as a therapeutic target. Recently, novel TRPV1 receptor antagonists with improved potency and/or selectivity compared to capsazepine have been synthesized. Among these, SB366791, a structurally novel inhibitor of rat and human TRPV1 receptor developed by GlaxoSmithKline (Rami et al., 2004) and AMG 9810, an Amgen compound (Gavva et al., 2005), have been reported to be highly effective.

A successful approach initiated by Uhtaek Oh in Seoul National University resulted in high affinity antagonists of the TRPV1 ion channels. [*N*-(4-*tert*-butylbenzyl)-*N*′[3-fluoro-4-(methylsulfonylamino)benzyl]-thiourea] called JYL1421 (SC0030; Wang et al., 2002; Kim et al., 2004) antagonizes capsaicin-induced Ca^{2+} uptake in rat TRPV1-expressing Chinese hamster ovary cells with an IC_{50} of 9.2 nM, having 60-fold greater potency than capsazepine (Wang et al., 2002).

The aim of the present series of experiments was to investigate the TRPV1 receptor antagonistic effect and selectivity of JYL1421 in vitro and in vivo using various rat models. Its effects were compared to capsazepine as a reference compound.

2. Materials and methods

2.1. Animals

Experiments were performed on male Wistar rats (Charles Rivers Hungary Ltd., Budapest, Hungary) weighing 180–220 g, which were kept in the Laboratory Animal Centre of the University of Pécs under standard pathogen free conditions at 24–25 °C and provided with standard chow and water ad libitum.

2.2. Drugs and chemicals

JYL1421 (SC0030) was synthesized and kindly donated by Uhtaek Oh at Seoul National University (Korea). Urethan, capsaicin and capsazepine were purchased from Sigma (St. Louis, MO, USA). The solvent of capsaicin, capsazepine and JYL1421 contained 10 v/v% ethanol, 5 v/v% Tween 80 and 85 v/v% saline. The concentration of the stock solution was 2 mg/ml and further dilutions were made with saline. Rats in the control group were treated with the vehicle.

2.3. Investigation of sensory neuropeptide release from isolated rat tracheae

After exsanguination 2–2 removed tracheae were perfused (1 ml/min) in an organ bath (1.8 ml) at 37 °C for 60 min with oxygenated (95% O_2 and 5% CO_2) Krebs solution of the following composition (in mM): NaCl 119; $NaHCO_3$ 25; KH_2PO_4 1.2; $MgSO_4$ 1.5; KCl 4.7; $CaCl_2$ 2.5; glucose 11. After stopping the flow, the solution was changed 3 times for 8 min (prestimulated–stimulated–poststimulated). Chemical stimulation with capsaicin (10^{-6} M) or electrical field stimulation (40 V, 0.1 ms, 10 Hz, 2 min) was performed to induce peptide release in the second fraction. JYL1421 or capsazepine (0.1–2 μM) as a reference compound was added into the incubation medium at the beginning of each 8 min period. In control experiments stimuli were applied in the absence of the antagonists. The fractions were collected in ice-cold tubes and the wet weight of each trachea was measured. Concentrations of substance P, CGRP and somatostatin were determined by specific and sensitive radioimmunoassay (RIA) methods developed in our laboratory (Nemeth et al., 1996, 1998, 1999), and peptide output was expressed as the released amount per tissue weight. Detection limits of the assays were 2 fmol/tube (substance P), 0.2 fmol/tube (CGRP), 2 fmol/tube (somatostatin) (Nemeth et al., 1996).

2.4. Investigation of Ca^{2+} accumulation in cells

2.4.1. Cell culture

Primary culture of trigeminal ganglion neurones were isolated enzymatically and mechanically as described elsewhere (Szoke et al., 2000). In brief, 12 neonatal Wistar rat pups were anaesthetized with ether, decapitated and bilateral trigeminal ganglia were

removed. They were washed in phosphate-buffered saline (PBS) containing penicillin and streptomycin, and incubated in PBS supplemented with collagenase for 35 min and deoxyribonuclease for 8 min at 37 °C in a humidified thermostat. Triturated trigeminal cells were plated on sterile 12 mm glass coverslips coated with poly-D-lysine (0.1 mg/ml) in a 24-well dish. Trigeminal suspension (100 μl) and nerve growth factor (7.5 ng/ml, 120 μl) were added to 1.5 ml medium in each well. Primary culture was grown for 1–7 days in a nutrient-supplemented medium (Dulbeccoś-Modified Eagle Medium, DMEM) at 37 °C in 5% CO_2 atmosphere.

2.4.2. Imaging intracellular Ca^{2+} levels

Trigeminal ganglion cell culture was incubated with 1 μM fura-2, AM (fluorescent Ca^{2+} indicator dye) for 15 min at 37 °C followed by at least 5 min wash in extracellular solution at room temperature on the stage of a epifluorescence microscope (Olympus BX50WI, Japan) in darkened room. Superfused extracellular solution was gravity fed to the cells using a single outlet tube from close vicinity. A single digital (12-bit) fluorescence image was taken with an Olympus LUMPLAN FI/×20 0.5 W water immersion objective and a digital camera (CCD, SensiCam PCO, Germany) connected to a Pentium II PC (Supertech, Hungary). Up to 12 dye-loaded small (<25 μm diameter) cells were selected in each plate to monitor their fluorescence individually. Fluorescence intensity ratios as a function of time were generated by the Axon Imaging Workbench 2.1 (AIW, Axon Instruments, CA) software and the obtained graphs provided data for analysis. Single cells were covered by small areas (regions of interest) representing approximately 800 to 1500 image pixels. Averaged light intensity from the selected areas, after background subtraction, was considered during automatic on line ratio calculations following conventional technique (Grynkiewicz et al., 1985). Cells were illuminated alternately at 340 and 380 nm light (for 80–400 ms each) generated by a monochromator (Polychrome II, Till Photonics, Germany) under the control of AIW 2.1. Emitted light >510 nm was measured. After subtraction of background fluorescence, the ratio of fluorescence intensity at the two wavelengths was calculated. The R=F340/F380 was monitored (rate 1 Hz) continuously for up to 120 s, while a few sample images were also recorded. JYL1421 (5 nM–1 μM) or capsazepine (0.5–10 μM) was administered for 5 min between two challenges with capsaicin (330 nM for 3 s), in control experiments extracellular solution was applied to the cells during this period. The second capsaicin-induced responses were compared to the first ones and expressed in percent.

2.5. Measurement of capsaicin-induced hypothermia

Capsaicin was injected s.c. (300 μg/kg), which produced a rapid reduction of core body temperature through hypothalamus-mediated autonomic responses such as cutaneous vasodilatation and hypersalivation (Jancsó-Gábor et al., 1970). Core body temperature was measured with a rectal thermometer every 10 min for 1 h. Pretreatment with JYL1421 (0.4, 1, 2, or 5 mg/kg) was performed i.p. 20 min before capsaicin administration. In control rats the solvent was injected in the same volume, in the reference group capsazepine (2 or 5 mg/kg) was administered i.p.

2.6. Measurement of capsaicin-induced eye wiping

The number of wiping movements in response to 50 μl capsaicin solution (10 μg/ml) dropped into the left eye of male Wistar rats (180–220 g) was determined during 60 s after instillation (Amann et al., 1995). Pretreatment with JYL1421 (0.4, 1, 2 or 5 mg/kg) or capsazepine (2 or 5 mg/kg) was performed i.p. 20 min before capsaicin administration. In the control group the solvent was injected i.p. in the same volume.

2.7. Examination of the pulmonary chemoreflex

Bolus intravenous capsaicin injection produces a triple response consisting of hypotension, bradycardia and apnoea known as the pulmonary chemoreflex or Bezold–Jarisch reflex (for rev. see Coleridge and Coleridge, 1984). Wistar rats (180–220 g) were anaesthetized with urethane (1.25 g/kg i.p.), the trachea was cannulated to facilitate spontaneous respiration and blood pressure was continuously recorded through a cannula inserted into the left carotid artery via a pressure transducer by the Haemosys software (Experimetria Ltd., Budapest, Hungary). The body temperature was maintained at 37 °C by a heating lamp. Drugs were administered through a cannula inserted into the right jugular vein. The pulmonary chemoreflex was evoked by rapid injections of capsaicin (1 and 2 μg/kg i.v.) separated by a 5 min interval. Thereafter JYL1421 (0.4 and 1.6 mg/kg i.v.) was administered and 5 min later capsaicin injections were repeated at increasing doses until the magnitude of the control responses could be achieved. For quantitative analysis the area under the curve (AUC) of the capsaicin-induced hypotension was determined with the help of the Haemosys software.

2.8. Ethics

All experimental procedures were carried out in accordance with the Declaration of Helsinki and the Animals (Scientific Procedures) Act 1998 (Hungary), complied with the recommendations of the International Association for the Study of Pain (Zimmermann, 1983). The studies were approved by the Ethics Committee on Animal Research of the University of Pécs.

2.9. Data analysis

The results are expressed as means±S.E.M. Statistical analysis was performed by Mann–Whitney U test in cases of the wiping and hypothermia tests and by analysis of variance followed by Bonferroni's modified t test in the pulmonary chemoreflex experiments. Student's t test for paired comparison in the peptide release studies or unpaired comparison in the Ca^{2+} imaging experiments were used. $P<0.05$ was regarded as significant.

3. Results

3.1. Release of substance P, CGRP and somatostatin from isolated rat tracheae

3.1.1. Effect of JYL1421 and capsazepine on capsaicin-evoked neuropeptide release

Capsaicin (10^{-6} M) evoked 2-, 13- and 3-fold increase of substance P, CGRP and somatostatin release, respectively (Fig. 1). The outflow of CGRP was the most sensitive to the stimulatory effect of capsaicin and enhanced release was observed even in the poststimulation period. The release of all the three peptides was

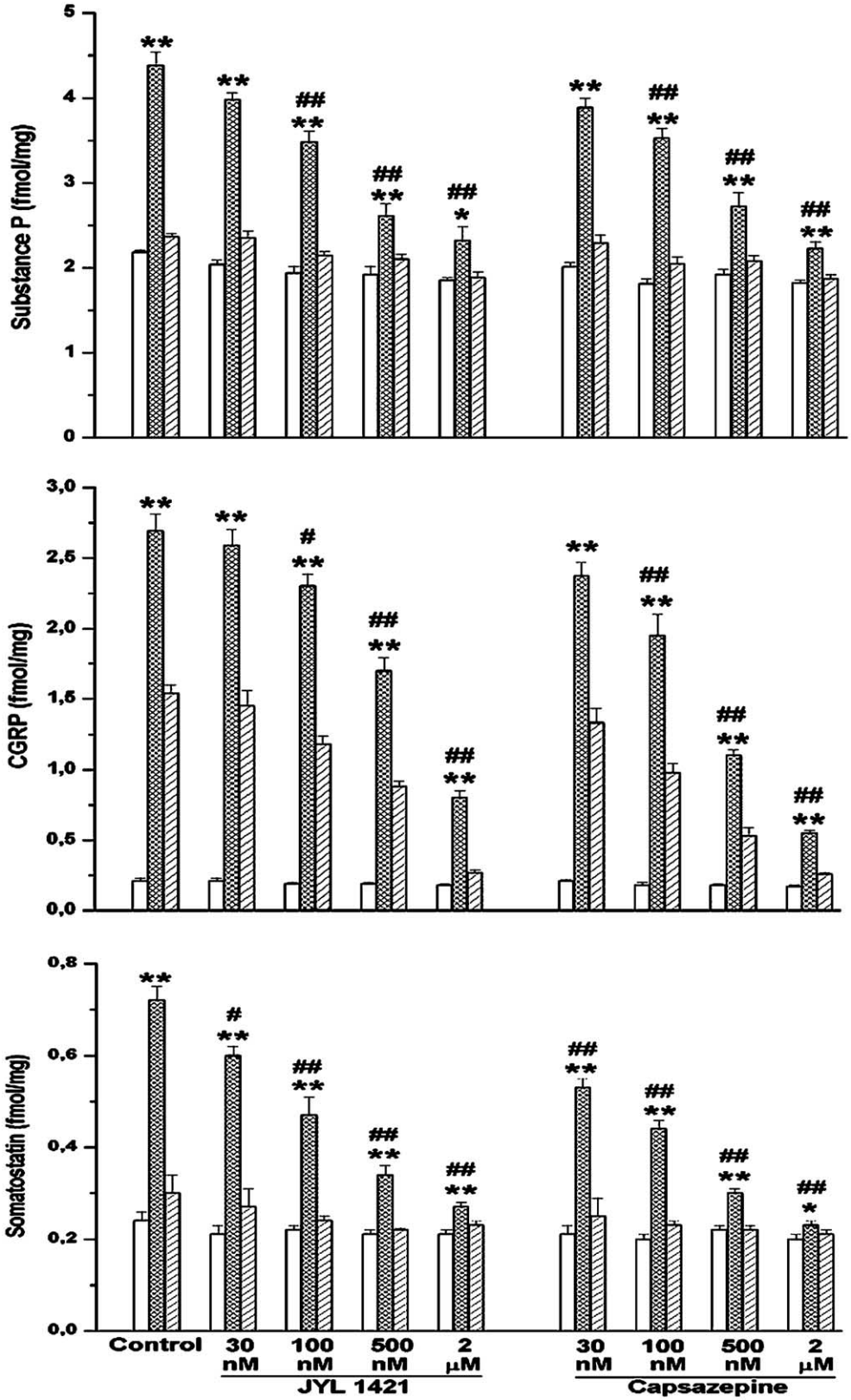

Fig. 1. Effect of JYL1421 and capsazepine on 10^{-6} M capsaicin-evoked release of sensory neuropeptides from isolated rat tracheae. Each column represents the mean ± S.E.M. concentration of the respective peptide measured in the incubation medium of the prestimulated (open columns), stimulated (cross-hatched columns) and poststimulated (hatched columns) fraction of $n = 6$ experiments; $^{*}P < 0.05$, $^{**}P < 0.01$ vs. prestimulated period; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ vs. control.

diminished in the presence of 0.1–2 μM JYL1421 in a concentration-dependent manner. The IC_{50} values for the inhibition of the release of substance P, CGRP and somatostatin were 331, 491 and 227 nM, respectively. The same applied concentrations of capsazepine produced similar inhibitory effects in the cases of all peptides, the corresponding IC_{50} values were 365, 229 and 85 nM. The correlation coefficients (R^2) were higher than 0.95 in cases of all curve fits (Fig. 1).

3.1.2. Effect of JYL1421 and capsazepine on electrically evoked neuropeptide release

Electrical field stimulation (1200 pulses) induced 3–4-fold increase of substance P, CGRP and somatostatin release from the isolated rat tracheae (Fig. 2). These responses were not influenced by 500 nM, but significantly diminished by 2 μM JYL1421 in the cases of CGRP and somatostatin. On the contrary, capsazepine in both the smaller (500 nM) and higher (2 μM) concentration

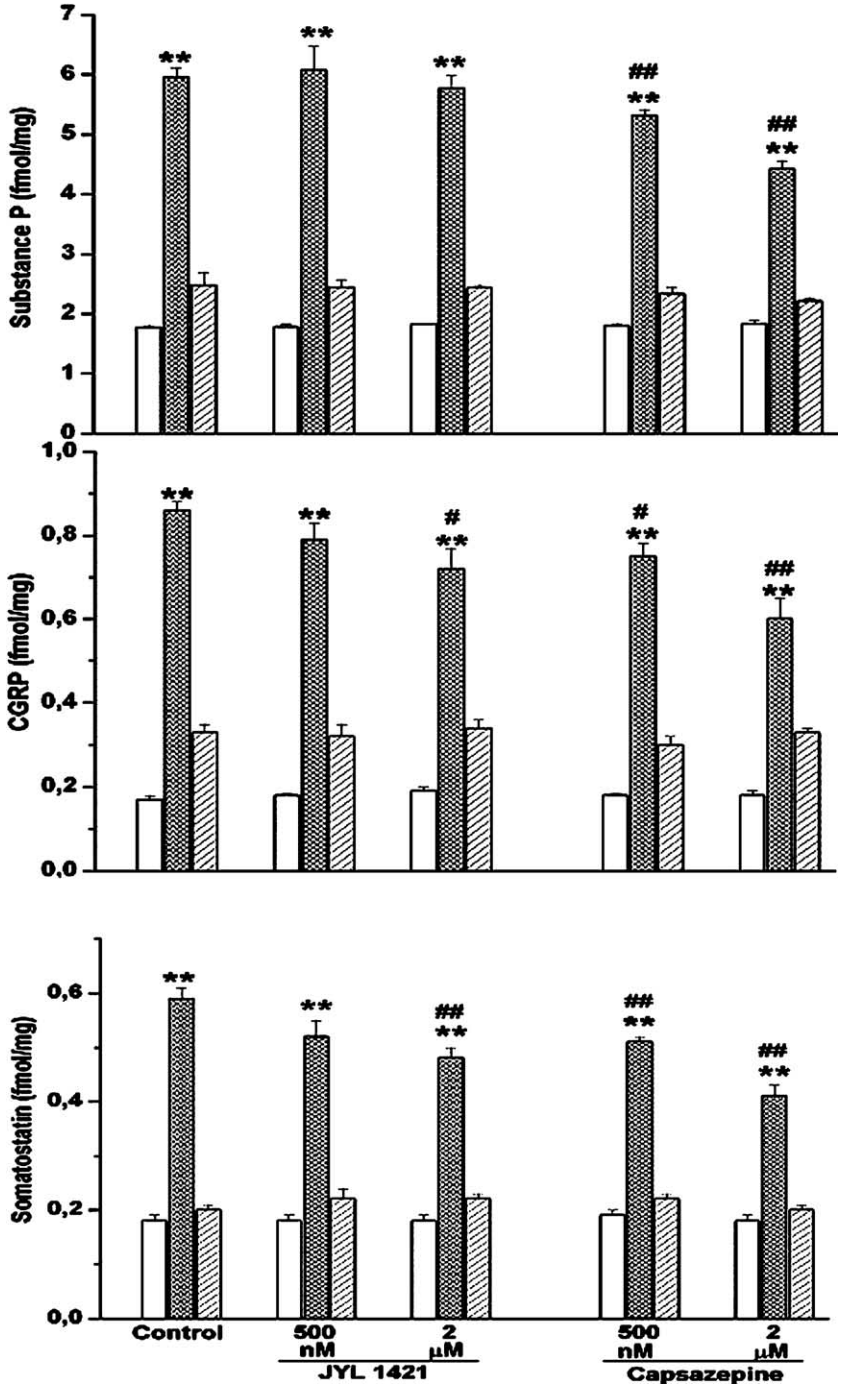

Fig. 2. Effect of JYL1421 and capsazepine on electrical field stimulation (40 V, 0.1 ms, 10 Hz, 2 min)-induced release of sensory neuropeptides from isolated rat tracheae. Each column represents the mean ± S.E.M. concentration of the respective peptide measured in the incubation medium of the prestimulated (open columns), stimulated (cross-hatched columns) and poststimulated (hatched columns) fraction of $n=6$ experiments; $^{*}P<0.05$; $^{**}P<0.01$ vs. prestimulated period; $^{\#}P<0.05$, $^{\#\#}P<0.01$ vs. control.

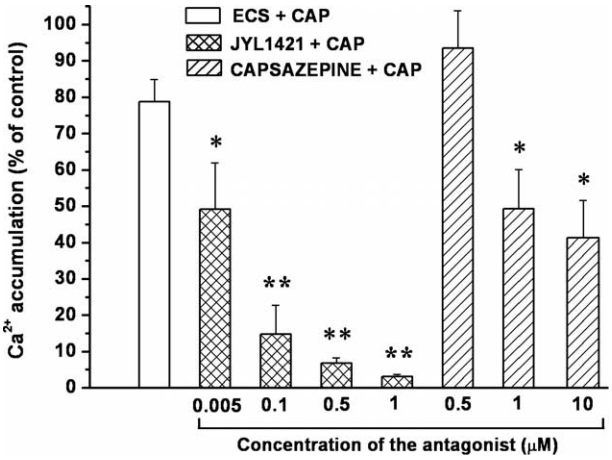

Fig. 3. Effect of JYL1421 and capsazepine on capsaicin (CAP)-evoked Ca^{2+} accumulation in cultured trigeminal ganglion neurones measured with microfluorimetric technique. The antagonists or in control experiments the extracellular solution (ECS) was administered for 5 min between two challenges with capsaicin (330 nM for 3 s). The second capsaicin-induced responses were compared to the first ones and expressed in percent. Results are means ± S.E.M. of $n = 9–17$ cells/group; $^*P < 0.05$, $^{**}P < 0.01$ vs. ECS-treated control.

induced significant inhibition on the electrically evoked release of all measured neuropeptides in a concentration-dependent manner. The effect of neither compounds reached the 50% inhibition, therefore IC_{50} values could not be determined (Fig. 2).

3.2. Effect of JYL1421 and capsazepine on capsaicin-induced Ca^{2+} accumulation in neurones

Incubation with JYL1421 for 5 min concentration-dependently inhibited the capsaicin (330 nM)-evoked Ca^{2+} accumulation measured with microfluorimetric technique in cultured trigeminal ganglion neurones. The inhibitory effect of the lowest concentration (5 nM) of this compound reached the level of significance and 1 μM almost abolished the response, only 3.1 ± 0.65% of the first capsaicin-induced Ca^{2+} influx could be observed (Fig. 3). The IC_{50} value was 8 nM for JYL1421. Meanwhile, the effect of capsazepine was markedly smaller, it induced 41.7% inhibition in 1 μM concentration, but this inhibitory effect did not increase further in 10 μM and did not reach 50% (Fig. 3). Administration of 30 μM capsazepine alone evoked marked Ca^{2+} influx resulting in obvious cell damage. Therefore, the effect of this high concentration was not included in the analysis of its antagonistic action.

3.3. Effect of JYL1421 and capsazepine on capsaicin-induced hypothermia

In the solvent-treated control group, the core body temperature decreased from 38.5 ± 0.2 to 36.3 ± 0.2 °C in response to s.c. administration of 300 μg/kg capsaicin. Results were calculated on the basis of maximal hypothermia data measured during the 60 min of the experiment. Pretreatment with JYL1421 (0.4–5 mg/kg) inhibited the capsaicin-induced drop of body temperature, although the effect of the two smaller doses was not significant. Capsazepine in 5 mg/kg dose evoked about 25% inhibition, but it did not reach the level of significance (Fig. 4).

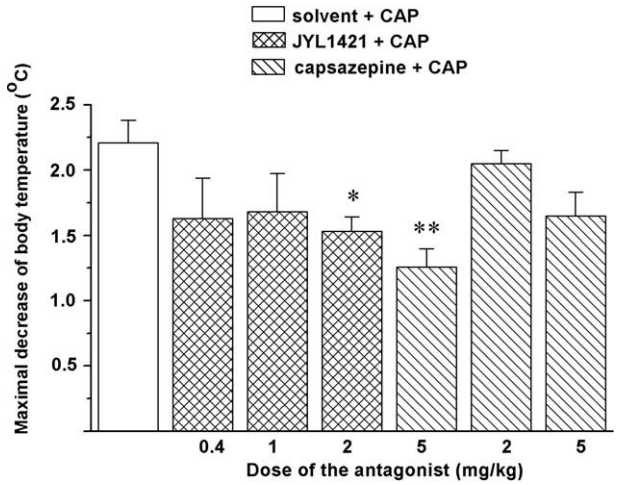

Fig. 4. Effect of i.p. pretreatment with JYL1421 or capsazepine on capsaicin (CAP; 300 μg/kg s.c.)-induced maximal decrease of core body temperature. In control experiments the solvent of the antagonists was administered i.p. Results are means ± S.E.M. of $n = 6–8$ rats/group, $^*P < 0.05$ vs. solvent-treated control.

3.4. Effect of JYL1421 and capsazepine on capsaicin-evoked wiping movements

Instillation of 50 μl capsaicin solution (10 μg/ml) into the left eye of the rat evoked 12.9 ± 1.3 wiping movements within 3 min. Pretreating the rats with 0.4 or 1 mg/kg i.p. JYL1421 did not influence significantly the wiping behaviour, but 2–5 mg/kg dose-dependently reduced the number of wiping movements. The ID_{50} value was 4.6 mg/kg. Capsazepine, as a reference compound, did not induce significant inhibition at the dose of 5 mg/kg (Fig. 5).

3.5. Effect of JYL1421 and capsazepine on capsaicin-evoked pulmonary chemoreflex

The mean arterial pressure of untreated rats was 109.9 ± 4.2 Hgmm ($n = 6$). One and 2 μg/kg capsaicin i.v. injection evoked a

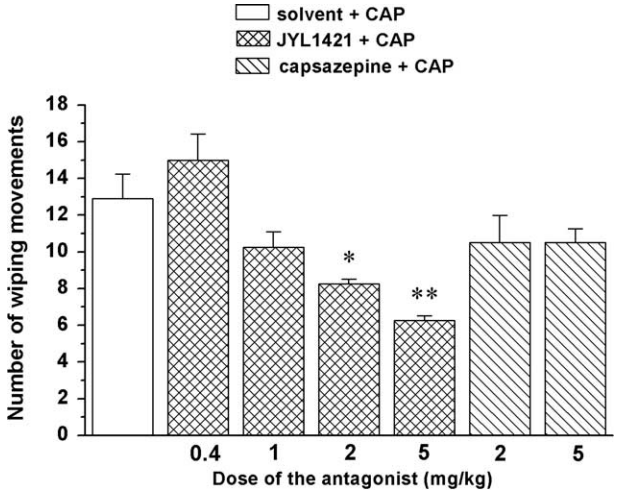

Fig. 5. Effect of i.p. pretreatment with JYL1421 or capsazepine on the number of wiping movements evoked by instillation of 50 μl capsaicin solution (CAP; 10 μg/ml) into one eye. In control experiments the solvent of the antagonists was administered i.p. Results are means ± S.E.M. of $n = 6–8$ rats/group, $^*P < 0.01$ vs. solvent-treated controls.

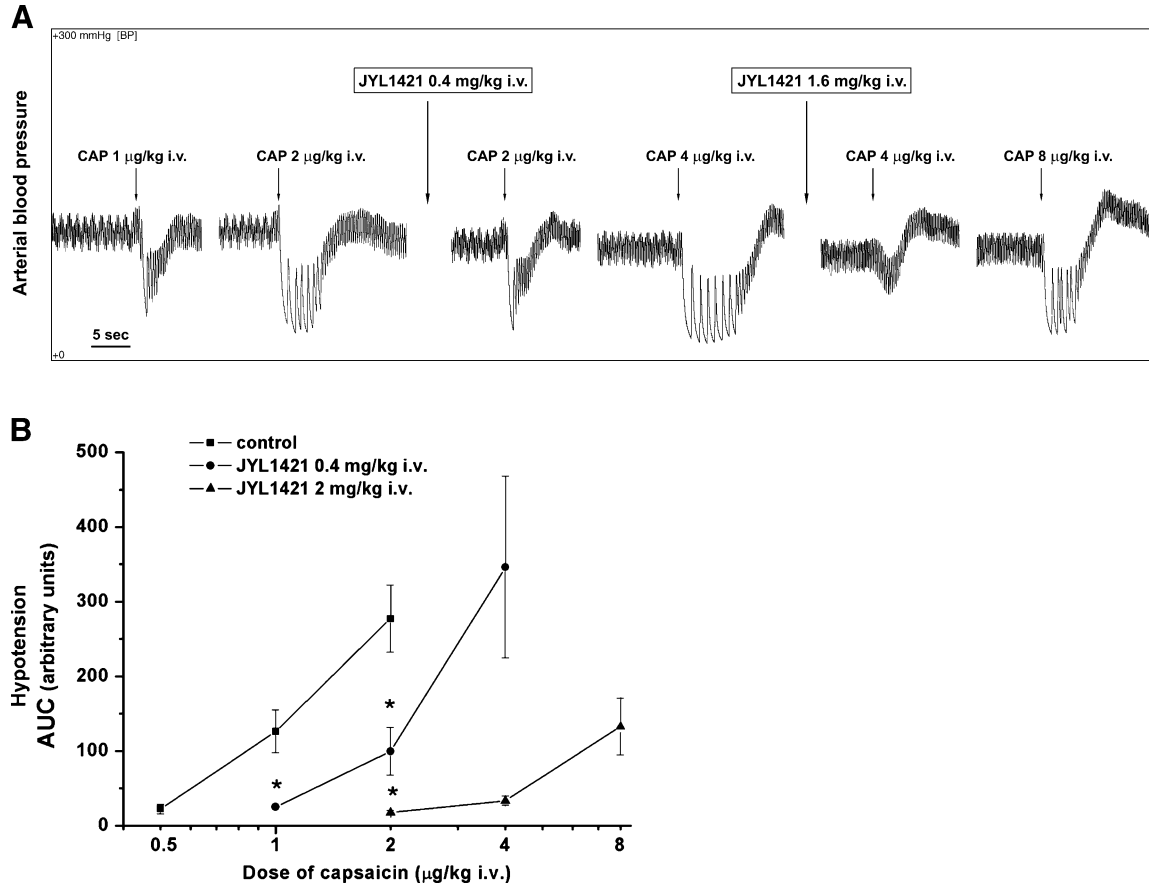

Fig. 6. Effect of JYL1421 on capsaicin (CAP)-evoked drop of mean arterial blood pressure. Panel A shows representative original recordings and panel B demonstrates the dose–response curves for the capsaicin-induced hypotension before and after JYL1421 administration constructed on the basis of the AUC of responses ($n=6$), *$P<0.01$ vs. control group. The 2 mg/kg dose is the sum of the two applied doses.

transient drop of blood pressure by 47.4±4.7 and 59.6±4.2 Hgmm ($n=6$), respectively (Fig. 6A). Both applied doses of JYL1421 (0.4 and 1.6 mg/kg) failed to evoke hypotension. JYL1421 dose-dependently inhibited the capsaicin-induced fall in blood pressure as shown by the need for higher capsaicin doses to elicit the reflex hypotension after JYL1421 administration than before treatment (Fig. 6A). Fig. 6B shows the dose–response curves for capsaicin before and after JYL1421 injection constructed on the basis of the AUC of the capsaicin-induced hypotensive responses. The dose ratio of JYL1421 was 2.1 and 8.0 for the 0.4 and 2 mg/kg cumulative dose, respectively. Capsazepine failed to induce a significant inhibition of the capsaicin-evoked hypotension up to a dose of 2 mg/kg.

4. Discussion and conclusions

A potential role for the TRPV1 receptor in nociception was evidenced long ago since injection of capsaicin induced nocifensive and hyperalgesic behaviours in rodents and pain in humans (Szolcsányi, 1977; Simone et al., 1987; Gilchrist et al., 1996). Results obtained in TRPV1 gene-deleted mice suggest that TRPV1 plays pivotal role in the peripheral sensitization of nociceptors in several inflammatory/nociceptive processes and tissue damage (Caterina et al., 2000; Davis et al., 2000). Therefore, it has been extensively investigated as a promising target for the development of novel type of analgesics. Its expression is predominantly restricted to primary nociceptive neurones and it is activated by pathological rather than physiological stimuli, including nociceptive factors like protons, pungent vanilloid compounds, noxious heat (>43 °C), and sensitized or activated by various endogenous pain producing substances (bradykinin, lipoxygenase products) through an intracellular signal transduction pathway (Cesare et al., 1999; Hwang et al., 2000; Liang et al., 2001). The TRPV1 receptor protein has attracted tremendous attention in the last few years because it can function as an integrator molecule of painful stimuli on primary sensory neurones. Based on these data, there is a great need for potent and selective TRPV1 receptor antagonists in both basic research and drug development. Capsazepine is the only TRPV1 antagonist that has been studied extensively so far (Bevan et al., 1992), but unfortunately it has only moderate potency and very limited selectivity (Docherty et al., 1997; Liu and Simon, 1997; Wardle et al., 1997).

JYL1421 (SC0030) is a novel TRPV1 receptor antagonist synthesized at Seoul National University and it has been reported to be 25- to 60-fold more potent than capsazepine on TRPV1 receptor-expressing CHO cells (Wang et al., 2002). Furthermore, in these cells the enhanced potency has been proved to be associated with enhanced selectivity since it did not block ATP-induced Ca^{2+} uptake while capsazepine did (Wang et al., 2002).

In the present series of experiments we characterized the antagonistic activity of JYL1421 on capsaicin-evoked TRPV1 activation, both in vitro and in vivo. It effectively and concentration-dependently inhibited capsaicin-evoked substance P, CGRP and somatostatin release from isolated rat tracheae, while only the highest concentration (2 μM) resulted in significant inhibition of electrical field stimulation-induced sensory neuropeptide release. Capsazepine caused a similar inhibition of the capsaicin-evoked release of all the three measured sensory neuropeptides but it markedly diminished the electrically induced peptide release as well, even in the lowest applied 500 nM concentration. Thus, in vitro, at the level of capsaicin-sensitive sensory nerve endings, JYL1421 proved to be as effective as capsazepine to inhibit sensory neuropeptide release, but the effect of JYL1421 was much more selective. In our other in vitro system it also concentration-dependently inhibited capsaicin-induced Ca^{2+} accumulation in trigeminal ganglion cells with an IC_{50} value of 8 nM. These results well correlate with previous experiments in which JYL1421 was found to antagonize capsaicin-induced Ca^{2+} uptake both in TRPV1-transfected and dorsal root ganglion cells with 60-fold higher potency than capsazepine (Wang et al., 2002). Several papers have provided evidence that capsazepine inhibited Ca^{2+} currents with an IC_{50} of 1.4–7.7 μM, but also inhibited the function of other ion channels, e.g. nicotinic receptors (Docherty et al., 1997; Liu and Simon, 1997; Szállási and Blumberg, 1999 for rev. Szolcsányi, 2002). On the contrary, a recent paper demonstrated that capsazepine by itself induced elevation of intracellular Ca^{2+} concentration in human osteosarcoma cells due to extracellular Ca^{2+} influx and mobilization from intracellular stores (Teng et al., 2004). In our experiments 1 and 10 μM of capsazepine caused significant inhibition of similar magnitude on capsaicin-induced Ca^{2+} accumulation in trigeminal ganglion neurones, but in 30 μM concentration Ca^{2+} accumulation response was observed suggesting partial agonism. The partial agonistic effect of capsazepine has previously been proposed (Pethő et al., 2004). This might be the reason why its inhibitory effect did not increase in 10 μM compared to 1 μM.

Furthermore, we provided evidence that JYL1421 is also effective in vivo in the rat. It is particularly important since several new TRPV1 receptor antagonists have been synthesized recently which proved to be very effective in vitro but many of them failed to act under in vivo conditions especially upon systemic administration. The in vivo tests used in this study belong to those that have been used previously to assess the agonistic or antagonistic activity of compounds acting at the TRPV1 capsaicin receptor. The capsaicin-induced hypothermia is mediated by stimulation of hypothalamic TRPV1 warm sensors and is the result of the coordinated heat loss mechanisms including cutaneous vasodilatation (Roberts et al., 2004; Szelényi et al., 2004). The finding that JYL1421 inhibited capsaicin-induced decrease of core body temperature suggests that the compound is able to cross the blood–brain barrier and gain access to hypothalamic warm sensors. Capsaicin-evoked eye wiping movements constitute a very simple, but reliable test for chemonociception. The pulmonary chemoreflex is initiated by activation of the vagal afferent fibers connected to pulmonary J receptors (Coleridge and Coleridge, 1984). Measurement of this reflex was used previously to describe the in vivo TRPV1 receptor agonistic and sensory desensitizing effects of resiniferatoxin and the antagonistic activity of ruthenium red (Szolcsányi et al., 1990, 1991). The inhibitory effect of JYL1421 on capsaicin-induced eye wiping and hypotension indicates that it blocks TRPV1 receptors both in exteroceptive and interoceptive areas. JYL1421 failed to evoke capsaicin-like actions in all of our experimental models suggesting that the compound is devoid of partial agonistic activity. Capsazepine even in a high dose (5 mg/kg; Perkins and Campbell, 1992) did not induce significant inhibition in the in vivo models. The failure of capsazepine in vivo might be due to its poor pharmacokinetic properties (Szállási and Appendino, 2004), as well as its partial agonistic activity suggested previously (Pethő et al., 2004) and also by our data.

Based on these data JYL1421 can be considered a more selective and in vivo also a more potent TRPV1 receptor antagonist than capsazepine, therefore it may promote the assessment of the (patho)physiological roles of the TRPV1 receptor.

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