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Somatostatin 4 receptor activation modulates G-protein coupled inward rectifying potassium channels and voltage stimulated calcium signals in dorsal root ganglion neurons

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ABSTRACT

Somatostatin has a wide biological profile resulting from its actions on the five receptor subtypes (sst_{1-5}). Recently somatostatin was shown to exert analgesic effects via activation of the sst_4 receptor. Although the analgesia in pain models is established, the precise molecular mechanism has yet to be fully elucidated. This research aimed to identify possible anti-nociceptive mechanisms, showing functional links of the sst_4 receptor to G-protein coupled inward rectifying potassium (GIRK) channels and reduction of voltage stimulated calcium influx within the pain processing pathway. Whole cell voltage clamp experiments and calcium imaging experiments were conducted on DRG neurons prepared from adult rats. Application of an sst_4 receptor selective agonist, J-2156, on DRG neurons induced a GIRK modulated potassium current, and inhibited voltage sensitive calcium current. Both mechanisms are thought to contribute to the analgesic properties of sst_4 receptor agonists.

1. Introduction

Somatostain (sst), also known as somatostatin releasing-inhibiting factor (SRIF), is a cyclic tetradecapeptide (Schulz et al., 2000), first discovered just over 40 years ago in hypothalamic extracts. It was quickly characterized as a regulatory peptide (Krulich et al., 1968), and has been ascribed important roles both in the endocrine system and in the central nervous system (CNS) (Schulz et al., 2000). Somatostatin has the ability to exert such a broad range of biological effects by associating with the five receptor subtypes, sst_{1-5}, which are widely distributed throughout the body (Pinter et al., 2006). The receptors all belong to the family of G-protein coupled receptors (GPCRs) (Patel et al., 1996).

Several recent studies have shown that somatostatin and its receptors have roles in analgesia, where the specific receptor involved is the sst_4 receptor. This was concluded on the basis of data using a highly selective sst_4 receptor agonist, J-2156 (Engstrom et al., 2005), which significantly reduced mechanical allodynia in arthritic and neuropathic pain models (Sandor et al., 2006) and exhibits multiple anti-inflammatory effects in rodents (Helyes et al., 2006). In addition mice lacking the sst_4 receptor have increased inflammatory and nociceptive responses suggesting impaired defense mechanisms (Helyes et al., 2009). Somatostatin receptors are expressed in peripheral pain regulatory pathways, particularly DRG neurons, where 45% of cells showed sst_4 receptor-like immunoreactivity (Bar et al., 2004).

Although the analgesic effect of sst_4 receptor agonists is established, the precise molecular mechanism behind this has yet to be fully elucidated. Potential mechanisms could be unfolded via links to various ion channels involved in nerve transmission.

Pain transmission involves activation of multiple potassium channels, including GRIK. These belong to the superfamily of G-protein coupled inwardly-rectifying potassium channels which influence a wide spectrum of physiological processes (Luscher and Slesinger, 2010). Functionally the GIRK channels are responsible for maintaining the resting membrane potential close to that of potassium equilibrium (Walsh, 2011). Activation of GIRK channels results in hyperpolarization of the cell membrane (Luscher and Slesinger, 2010), thus reducing spontaneous action potential formation (Walsh, 2011). Such effects present the GIRK channel as a good target for excessive cell excitability, including pain (Bhave et al., 2010).

Voltage gated calcium channels (Ca_v) belong to a family of large multi-protein complexes (Catterall, 2000). These channels are expressed in all excitable cells and are able to transduce electrical activity into biochemical signals (Catterall, 2000). Ca_v can be

Abbreviations: ATP, adenosine triphosphate; CNS, central nervous system; DMEM, Dulbecco's Modified Eagle Medium; DRG, dorsal root ganglion neurons; GIRK, G-protein inward rectifying potassium channels; GPCR, G protein coupled receptor; GTP, guanosine triphosphate; HEPES, hydroxyethyl piperazineethanesulfonic; nM, nanomolar; sst, somatostatin; SRIF, somatotropin release-inhibiting factor; Ca_v, voltage gated calcium channels

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influenced by GPCRs by either direct binding or influence of phosphorylation and channel trafficking (Dolphin, 2003). Inhibition results in reduced cell excitability and release of neurotransmitters and peptides. Such effects present calcium channels, so reduce intracellular calcium ion content, as good targets for treatment of chronic pain (Gribkoff, 2006).

The objective of this study is to investigate the actions of the sst_4 receptor signaling on membrane excitability in rat DRG neurons. In the present report we show that the sst_4 receptor is functionally coupled to GIRK and is able to inhibit voltage stimulated calcium influx in DRG neurons.

2. Methods

2.1. Culture of DRG neurons

Adult DRG neuron cultures were prepared from 6 to 8 week old Crl:WI(Han) (Charles River, Germany) male rats. DRGs were removed and placed into DMEM (c.c.pro., Germany, FM-13-L) containing 1% penicillin–steptomycin (Sigma, Germany, P4333-100). These were digested in 4 mg/mL collagenase (Gibco, UK, 17104-019) and 2 mg/mL papain (Sigma, Germany, P4762) for 75 min in a water bath heated to 37 °C. This reaction was terminated by aspiration, and addition of DMEM (c.c.pro., Germany, FM-13-L) containing 1% penicillin–steptomycin (Sigma, Germany, P4333-100) and 10% FCS (Gibco, UK, 10500-064). Using fire-polished pasture pipettes the cells were mechanically dispersed into a homogenized cell suspension. Cells were plated on to 0.1 mg/mL poly-L-lysine (Sigma, Germany, P-7886) and 2 μg/mL laminin (Sigma, Germany, C2020-IMG) coated 35 mm tissue culture cover slips (Thermo scientific, Germany). The cells were stored at 37 °C, 10% carbon dioxide and with a humidity of 95%. Recordings were made within 48 h of plating. For the calcium imaging experiments, plated cells were left for 2 h at 37 °C, 5% CO_2 before being topped up to 1 mL of culture medium.

2.2. Whole cell patch clamp

Transmembrane currents were recorded by whole-cell voltage-clamp using an EPC 10 amplifier, with the TIDA 5.2 software (HEKA electronics, Germany) and a PCI-1600 interface (HEKA electronics, Germany) at room temperature. The average cell size of the neurons was approx. 35 μm. Data were low-pass filtered at 2.9 KHz and sampled at 20 KHz. Patch pipettes were pulled from thick-walled borosilicate glass capillaries (1.5 mm outer diameter) on a Sutter Instruments P-97 puller (Sutter instruments, USA), and had a resistance of 3–5 MΩ. The pipette solution consisted of: 20 mmol/l NaCl; 120 mmol/l KCl; 10 mmol/l glucose; 10 mmol/l HEPES; 1 mmol/l EGTA; 3 mmol/l $MgCl_2$, with the addition of 3 mmol/l Na_2ATP and 0.3 mmol/l NaGTP on the day of testing. The cells were bathed in normal Ringer's solution containing: 140 mmol/l NaCl; 5 mmol/l KCl; 10 mmol/l glucose; 10 mmol/l HEPES; 1.8 mmol/l $CaCl_2$; 0.8 mmol/l $MgCl_2$. The pH was adjusted to 7.4 using NaOH and the osmolarity remained between 300 and 320 mOsmol/l. Activation of the GIRK channels was achieved with a high potassium extracellular solution containing 100 mmol/l NaCl and 45 mmol/l KCl. The pipette potential was zeroed before seal formation; care was taken to maintain membrane access resistance as low as possible, between 3 and 7 MΩ. Command voltage protocols and data acquisition were performed by TIDA 5.2 (HEKA electronics, Germany). Cells were analyzed when J-2156 induced a potassium current of more than 0.5 nA, which was then reduced by more than 0.5 nA, Statistical analysis was carried out using the prism5 software. Data are expressed as mean ± S.E.M.. To test for normal distribution the Kolmogorov–Smirnov test was used. *t*-Test measurements allowed for significance to be determined.

2.3. Calcium imaging

The DRG neurons were pre-loaded with the Ca^{2+}-sensitive fluorophore, Fluo-4-AM, 2 μM (Invitrogen, CA) and 1 mM probenecid (Sigma, Germany) for one hour prior to imaging. The cells were then washed in Ringer buffer (140 mmol/l NaCl; 5 mmol/l KCl; 10 mmol/l glucose; 10 mmol/l HEPES; 1.8 mmol/l $CaCl_2$; 0.8 mmol/l $MgCl_2$. The pH was adjusted to 7.4 using NaOH) until use. The cells were used within one hour of pre-loading. Cells were imaged through a plan neofluar 20 × /0,5 objective, using an Axiovert 200 M microscope with inbuilt camera (Zeiss). Excitation was achieved at 488 nm with an argon laser, and images were collected, at an emission of 510 nm, through a long pass filter, using the computer software Laser Scanning Microscope 510 META version 3,2 SP2 (Zeiss). The fluorescent signal was optimized by the detector gain and amplifier offset. The cells were mounted on a custom made perfusion chamber. Depolarization of the cells was achieved via electrical stimulation of voltages between15–21 V, using an amplifier (HSE, Germany). The voltage was tested by a train of 40 pulses as 10 Hz, for 3 s. Cells were identified as responders to J-2156 if a greater effect was seen than the mean plus 2 times the S.E.M. of the control result. Statistical analysis was carried out using the prism5 software. Data are expressed as mean ± S.E.M., normalized to the maximal effect, of all voltage gated calcium channel blockers. To test for normal distribution the Kolmogorov–Smirnov test was used. *t*-Test measurements allowed for significance to be determined.

3. Results

In order to determine if the sst_4 receptor is functionally coupled to GIRK channels in DRG neurons, voltage dependent whole-cell currents from isolated DRG neurons were recorded, utilizing the sst_4 receptor selective agonist J-2156 (Engstrom et al., 2005).

Within each protocol 2 different concentrations of compound were applied, followed by potassium channel blockers. Around 30% of the cells tested responded to the sst_4 receptor agonist. These cells had an average capacitance of 50.75 ± 8.11 pF. Control experiments were run (Fig. 1A), the first was a standard control, applying only vehicle control ringer solution, showing stable readings. The second was a compound control, applying only the sst_4 receptor agonist J-2156, without the blocker application, confirming the effects of the blocker. A recording from one experiment shows the increase in current after J-2156 perfusion, then the blockage after barium chloride ($BaCl_2$) perfusion (Fig. 1C).

After 3 min of compound perfusion, a significant increase in the magnitude of the inward potassium current was seen at both 30 nM and 100 nM ($P < 0.001$). An increase of 1.22 ± 0.5 nA and 1.84 ± 0.5 nA ($n = 11$; Fig. 1B) in the current obtained at −80 mV, in 45 mmol/l potassium-containing ringer solution, was recorded respectively. When J-2156 was applied at a lower concentration of 10 nM, there was no significant increase in potassium current (Fig. 1B).

The current induced by J-2156 was significantly suppressed not only by the addition of 300 μM $BaCl_2$ by 1.59 ± 0.7 nA ($P < 0.01$, $n = 11$; Fig. 1D); but also by 500 nM tertiapin Q by 1.30 ± 0.7 nA ($P < 0.05$, $n = 11$; Fig. 1D). These results confirm that the potassium induced current is as a result of GIRK channel activation, indicating that the sst_4 receptor is functionally coupled to GIRK channels within DRG neurons.

In order to determine if the sst_4 receptor is able to influence voltage induced calcium influx in DRG neurons, calcium imaging