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Expression and localisation of somatostatin receptor subtypes sst1–sst5 in areas of the rat medulla oblongata involved in autonomic regulation

Emma J. Spary *, Azhar Maqbool, Trevor F.C. Batten

Academic Unit of Cardiovascular Medicine, Worsley Building, University of Leeds, Leeds LS2 9JT, UK

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Abstract

Somatostatin is known to modulate the activity of neurones of the medulla oblongata involved in autonomic regulation, mediated through five subtypes of G protein-coupled receptors, sst1–sst5. This study utilises reverse transcription polymerase chain reaction and immunohistochemistry to investigate the expression of sst1–sst5, including the $sst2_A$/$sst2_B$ isoforms, in the main autonomic centres of the rat medulla oblongata: nucleus of the solitary tract (NTS), dorsal motor vagal nucleus (DVN) and ventrolateral medulla (VLM). In tissue from the cerebral cortex, hippocampus and cerebellum all subtype mRNAs were detected, but sst5 signals were weak, and the distribution of sst1–sst5 immunoreactivities was consistent with previous reports. In the medulla, all sst mRNAs gave clear amplicons and subtype-specific antibodies produced characteristic patterns of immunolabelling, frequently in areas of somatostatinergic innervation. Anti-sst1 labelled beaded fibres, $sst2_A$, $sst2_B$, sst4 and sst5 gave somatodendritic labelling and sst3 labelled presumptive neuronal cilia. In NTS tissue, sst1, $sst2_A$, sst4 and sst5 mRNAs were strongly expressed, while in VLM tissue sst1, $sst2_A$, $sst2_B$ and sst4 predominated. In both areas of the medulla, neurones with intense somatodendritic $sst2_A$ immunoreactivity were principally catecholaminergic in phenotype, being double labelled for tyrosine hydroxylase (TH) and phenylethanolamine-*N*-methyl-transferase (PNMT). Some TH/PNMT positive neurones were also $sst2_B$ and sst4 immunoreactive. Cholinergic parasympathetic neurones in the DVN were immunoreactive for the $sst2_A$, $sst2_B$, sst4 and sst5 subtypes. These observations are consistent with the proposal that multiple somatostatin receptor subtypes, possibly combining as heterodimers, are involved in mediating the modulatory effects of somatostatin on autonomic function, including cardiovascular, respiratory and gastrointestinal reflex activity.

Keywords: Sst subtype; Polymerase chain reaction; Nucleus of the solitary tract; Ventrolateral medulla; Catecholamine

1. Introduction

Somatostatin (SOM) is widely distributed throughout the CNS and is an important neurotransmitter and modulator of neural activity (Patel, 1999), mainly due to its inhibitory effects on neuronal excitability and by its ability to modify the release of neurotransmitters (Meyer et al., 1989; Tallent and Siggins, 1999). The neuropeptide exists in two biological active forms, SOM-14 and the amino terminally extended form SOM-28 (Brazeau et al., 1973; Pradayrol et al., 1980). Both forms have diverse effects mediated via interaction with seven transmembrane spanning G-protein-coupled receptors (GPCRs) and inhibition of Ca^{2+} currents or augmentation of K^+ conductance (Jacquin et al., 1988; Dryer et al., 1991; Selmer et al., 2000). To date five receptor genes encoding distinct somatostatin receptor (sst) subtypes have been identified, termed sst1–sst5 (Bruno et al., 1992; Kluxen et al., 1992; Li et al., 1992; Meyerhof et al., 1992; O'Carroll et al., 1992). The genes for sst1, sst3, sst4 and sst5 are not interrupted by introns in their protein coding regions; however, the rat sst2 subtype can undergo alternate mRNA splicing at its 3′ end generating two separate isoforms, a long variant 2_A and a short variant 2_B. These splice variants are identical except for the differences in their carboxyl terminal sequences (Vanetti et al., 1992).

Somatostatin receptor subtypes have been shown to form both functional hetero- and homodimers, with dimerisation modifying their functional properties (Rocheville et al., 2000a,b). The sst subtypes can also form heterodimers with

* Corresponding author. Tel.: +44 113 343 4172; fax: +44 113 343 4803.
E-mail address: E.J.Spary@leeds.ac.uk (E.J. Spary).

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other GPCRs, as demonstrated by the oligomerisation of sst5 with the human dopamine receptor 2 in Chinese hamster ovary cells (Rocheville et al., 2000a). In addition, there is also evidence to suggest that certain receptor subtypes can modulate the actions of other subtypes. For example sst5 has been shown to modulate the trafficking and cell surface regulation of $sst2_A$ when expressed in the same cells (Sharif et al., 2007).

The distribution of somatostatin receptor binding and somatostatin-immunoreactive (IR) fibres in the brainstem suggests that the neuropeptide may be involved in a number of central regulatory functions. These include the processing of somatosensory, proprioceptive and nociceptive information, arousal and the sleep-waking cycle and autonomic functions, including cardiovascular, respiratory and gastric reflexes (Carpentier et al., 1997). Within the medulla oblongata, transient blood pressure changes are controlled by the sympathetic reflex pathway, which is subserved by a series of three nuclei: the nucleus of the solitary tract (NTS), the caudal, and the rostral ventrolateral medulla (VLM) (Dampney, 1994; Aicher et al., 2000). Studies on the NTS, the primary region for the co-ordination and integration of sensory afferent inputs derived from the cardiovascular, respiratory and gastrointestinal systems (Loewy, 1990; Van Giersbergen et al., 1992; Spyer, 1994), have shown that a substantial proportion of the GABAergic terminals contain large dense core vesicles (Maqbool et al., 1991). This suggests that they may be capable of releasing additional peptide transmitters involved in modulation of autonomic reflexes.

The co-existence of somatostatin with GABA has been reported in many areas of the brain (Hendry et al., 1984; Somogyi et al., 1984). It is frequently co-released with GABA from hippocampal neurones and axonal terminals of the basolateral or central nucleus of the amygdala (Vezzani and Hoyer, 1999; McDonald and Mascagni, 2002; Saha et al., 2002). Thus, it is possible that GABA immunoreactive terminals in the NTS involved in cardiovascular regulation may also contain and release somatostatin. Indeed, microinjections of somatostatin in the NTS of anaesthetised rats have been shown to have modulatory actions on cardiovascular reflexes, producing hypotension and bradycardia (Koda et al., 1985).

This raises the question of which somatostatin receptor subtypes are present in the medullary nuclei and whether the activity of the NTS and VLM neurones involved in cardiovascular regulation may be modulated by somatostatin, released from GABAergic or other axon terminals, acting upon these receptor subtypes. Previous investigations on the distribution of the sst subtypes, using either immunohistochemistry or *in situ* hybridisation have not examined the medulla oblongata in any detail. In the present study, we analysed the expression of sst subtype mRNAs in the medulla oblongata of the adult rat using reverse transcription polymerase chain reaction (RT-PCR) and examined the distribution of sst receptor proteins using immunohistochemistry with well characterised, subtype-specific antibodies. Sst subtype expression in the cortex, hippocampus and cerebellum was also studied as a positive control or baseline comparison for our analyses of the medulla oblongata.

2. Materials and methods

2.1. Tissue isolation

Adult, 10–12-week-old male Wistar rats (160–220 g, $n = 6$) were killed by decapitation under anaesthesia (5% halothane in O_2) in accordance with the regulations of the UK Animals (Scientific Procedures) Act, 1986. Brains were removed and rapidly frozen on dry ice. Coronal slices of 0.5–1 mm thickness were cut from the brainstem, and tissue punches collected from the NTS and the rostral area of the VLM (RVLM) with a 0.69 mm corer under a ×5 dissecting microscope. Placement of the NTS punches was confirmed as previously described (Saha et al., 2004). Tissue samples for analysis were also dissected from the cerebral cortex, hippocampus, cerebellum and the remaining area of the medulla oblongata.

2.2. RNA extraction and reverse transcription polymerase chain reaction

Total RNA was isolated using the SV total RNA extraction system (Promega, Southampton, UK). Reverse transcription was initiated by adding 1 μg of RNA to 1 μl oligo (dT) primer (500 μg/ml) and heating at 70 °C for 5 min. Then 50 mM Tris–HCl pH 8.3, 1 mM deoxynucleotide triphosphates, RNase inhibitor (2U) and M-MLV reverse transcriptase (200U) were added to the mixture and the reaction incubated at 37 °C for 1 h and terminated by heating at 95 °C for 5 min.

Polymerase chain reaction (PCR) (50 mM Tris–HCl, pH 8.5, 1.5 mM $MgCl_2$, 200 μM dNTPs, 1.5 U Taq DNA polymerase) was performed in a 25 μl reaction using 2 μl of first strand product as a template, in a Perkin-Elmer GeneAmp 9700 (Applied Biosystems), with 0.4 μM subunit-specific primers (Table 1). Amplification was initiated by a 5 min pre-incubation at 95 °C, followed by 35 cycles of 95 °C (30 s), 60 °C (30 s) and 72 °C (1 min). A final extension step was performed at 72 °C for 7 min. Negative controls included amplification of RNA and water. Aliquots were separated by electrophoresis on 2% agarose gels containing ethidium bromide and visualised under UV light. The veracity of the PCR products was confirmed by DNA sequencing on an ABI 3100 genetic analyser using BigDye terminator cycle sequencing version 3.1.

2.3. Perfusion fixation

Adult Wistar rats (150–200 g, $n = 6$) were perfused transcardially with 4% paraformaldehyde and 0.2% picric acid in 0.1 M phosphate buffer as previously described (Saha et al., 2001). Brains were post-fixed for 2 h in 4% parafor-

Table 1
Forward (For) and reverse (Rev) primers used for detection of sst receptor subtypes by PCR

Subtype	Primer sequence 5′–3′	Amplicon size (bp)
Sst1	For—GCTGTCACACACAAGTCACA Rev—TTCAACAGTGCATTCGACCA	519
$Sst2_A$/$sst2_B$	For—GGTGACCCGAATGGTATCCA Rev—TGCCGGGTAGCTGCTTTCCA	619 (A) 305 (B)
Sst3	For—AGCAGCAACGGCCTTGCACA Rev—GTGGCTGAGGCCACAGAGCA	669
Sst4	For—GTCTCCTGGAAACAACTGGA Rev—CCCTATGCTACCACACAGCA	569
Sst5	For—CCCTCTCTCTGGCCTCCACA Rev—CGCGCTGGCCATCTTGGCTA	469

Primers are written 5′–3′ with their predicted size.