Coupling of rat somatostatin receptor subtypes to a G-protein gated inwardly rectifying potassium channel (GIRK1)

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Abstract The five different rat somatostatin receptor subtypes (SSTR1–SSTR5) were coexpressed with a subunit of G-protein gated inwardly rectifying potassium channel (GIRK1) in *Xenopus* oocytes. SSTR2–SSTR5, but not SSTR1 coupled efficiently to the activation of GIRK currents when stimulated by SST14 or SST28. A comparison of the dose-response curves and of the maximum currents obtained indicates that SSTR2 couples most efficiently to this effector, supporting the notion that SSTR2 is involved in activation of potassium conductances by SST in vivo.

Key words: Inwardly rectifying K^+-channel; G-protein; Somatostatin; Oocyte; TT232

1. Introduction

The neuropeptide somatostatin occurs in two isoforms, SST14 and SST28, both of which are abundant in brain and numerous peripheral tissues [1]. The actions of these two agonists are mediated by at least five different somatostatin receptor subtypes (SSTR1–SSTR5), which are all coupled to G-proteins of the pertussis toxin sensitive G_i/G_o class. In heterologous expression systems it has been shown that, dependent on the cellular environment, these receptors can be coupled to the inhibition of adenylyl cyclase [2] and voltage-dependent calcium channels [3], the activation of tyrosine phosphatases [4], phospholipase C [5] and of a phospholipase A_2 [6]. In addition, it has frequently been observed in neuronal and neuroendocrine cells that SST leads to the hyperpolarization of the cell membrane by activation of G-protein gated inwardly rectifying potassium (GIRK) channels [7]. The activation of GIRK channels by somatostatin in pituitary cells is mainly responsible for the inhibitory effect of SST on growth hormone release [8]. Whereas recently strong evidence was provided that in oligodendrocytes SSTR1 is involved in the inhibition of potassium currents [9], it has been unclear so far which of the known SSTR subtypes mediates the activation of GIRK currents. Here we describe the coexpression of SSTR1–SSTR5 together with the GIRK1 subunit in *Xenopus* oocytes. We observed strong activation of inward potassium currents by SST14 in cells coexpressing GIRK1 and either SSTR2, SSTR3, SSTR4, or SSTR5, but not SSTR1.

2. Materials and methods

cDNAs coding for the rat somatostatin receptors SSTR1–SSTR5 were cloned into the mammalian expression vector pcDNA3. A cDNA coding for the mouse GIRK1 subunit, also in pcDNA3, was obtained from Volker Höllt (Magdeburg). Plasmids were linearized with *Xba*I (SSTR1–SSTR5) or *Xho*I (GIRK1), and RNA was transcribed in vitro using T7 RNA polymerase (Fermentas, Vilnius, Lithuania).

Somatostatin receptor and GIRK1 cRNAs were mixed in a 1:1 ratio and injected at a concentration of 80 ng/μl total RNA into *Xenopus laevis* oocytes [10]. Oocytes were kept in Barth medium for 2 to 4 days at 20°C. For recordings, oocytes were superfused with ND-96 medium (96 mM NaCl, 2 mM KCl, 2.5 mM $CaCl_2$, 1 mM $MgSO_4$, 5 mM HEPES, pH 7.5) and clamped at −80 mV. For measurements of somatostatin agonists, the medium was changed to high K^+ medium (hK, ND-96 with 96 mM KCl and 2 mM NaCl); after the initial inward current reached a plateau, agonists were applied in the same medium. After washout of the agonists with hK, 300 μM $BaCl_2$ in hK was applied to determine the contribution of GIRK1-mediated currents to the total inward current observed.

3. Results and discussion

The individual rat somatostatin receptor subtypes SSTR1–SSTR5 were coexpressed together with GIRK1 in *Xenopus* oocytes. Stimulation of these oocytes with somatostatinergic agonists in hK medium under voltage-clamp conditions resulted in robust inward currents in cells expressing either rSSTR2, rSSTR3, rSSTR4 or rSSTR5 (Fig. 1). The peak current amplitude was usually several hundred nA at an agonist concentration of 100 nM SST14 or 100 nM SST28. The larg-

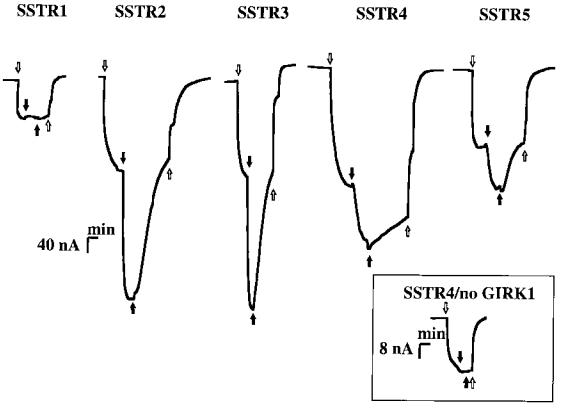

Fig. 1. Activation of inward potassium currents by SSTR subtypes. *Xenopus* oocytes injected with cRNA for the mouse GIRK1 subunit and rat somatostatin receptors were superfused with ND-96 medium and clamped at −80 mV. Open arrow, switch to hK medium; filled arrows, application of 100 nM SST14 (SSTR1–SSTR4) or 100 nM SST28 (SSTR5); upward filled arrows, washout with hK; upward open arrows, block of GIRK1 currents with 300 μM $BaCl_2$. Insert: Oocyte injected with SSTR4 only.

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Abbreviations: SST, somatostatin; SSTR, somatostatin receptor; hK, high potassium medium; ND-96, high sodium medium; GIRK, G-protein gated inwardly rectifying potassium channel

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est currents were reproducibly observed with SSTR2, with peak amplitudes often reaching values larger than 1 μA. SSTR2 was also efficiently activated by the stable somatostatin analog SMS 201–995, whereas none of the SSTRs could be activated by the newly discovered SST-like compound TT232 [11] at concentrations up to 1 μM (data not shown). In contrast to the other SSTRs, little or no inward current was observed upon stimulation of oocytes expressing SSTR1 and GIRK1 with SST14 or SST28.

Whereas the currents rapidly returned to baseline levels after washout of the agonist in cells expressing SSTR2, SSTR3 or SSTR5, currents elicited by activation of SSTR4 were relatively persistent and declined only slowly after washout of the agonist. This may be related to the observation that SSTR4 does not exhibit agonist-induced functional desensitization (Kreienkamp et al., manuscript in preparation).

No current was observed in oocytes expressing SSTR subtypes (e.g. SSTR4) in the absence of the GIRK1 subunit, suggesting that the current observed is due to the activation of the coexpressed GIRK1 (Fig. 1). This interpretation is supported by the observation that the currents were sensitive to block by 300 μM $BaCl_2$, a feature which is typical for GIRK-type channels [12]. As expected for an inward rectifier, agonist-induced currents were only observed in medium containing high external potassium concentrations, but not in ND-96 medium.

We investigated the dose-response relationship for activation of GIRK1 currents by the two naturally occurring somatostatin derivatives, i.e. SST14 and SST28. SSTR2, SSTR3 and SSTR4 showed a slight preference for SST14 over SST28, whereas the contrary was true for SSTR5, which was activated more efficiently by SST28 than by SST14 (Fig. 2, Table 1). In comparison with the other receptor subtypes, SSTR2 is by far the most efficient receptor in activating the GIRK-mediated current. The EC_{50} value for activation of this subtype by SST14 is about one order of magnitude lower than that observed for SSTR3 and SSTR4 (Fig. 2).

The data reported here clearly show that four out of the five somatostatin receptor subtypes identified so far are able to activate G-protein gated inwardly rectifying potassium currents. One remarkable exception is SSTR1 which did not couple to GIRK1 in our oocyte system. GIRK activation by G-proteins has been shown to occur via interaction of the free βγ-subunits with the potassium channel proteins [13]. As dissociation of βγ-subunits from the G-protein heterotrimer is a step common to the signal transduction by all G-protein coupled receptors, it remains unclear how coupling specificity as observed here might arise. One potential mechanism was recently elucidated by Schreibmayer et al. [14], who showed that several activated G_α-subunits inhibited GIRK

Table 1
EC_{50} values for activation of GIRK1 currents by SSTR2–SSTR5 using various SST agonists

Receptor subtype	SST14	SST28
SSTR2	4.19 nM	23.5 nM
SSTR3	54 nM	187 nM
SSTR4	56 nM	104 nM
SSTR5	290 nM	120 nM

The data were obtained from the non-linear regression analysis of the dose-response curves presented in Fig. 2.

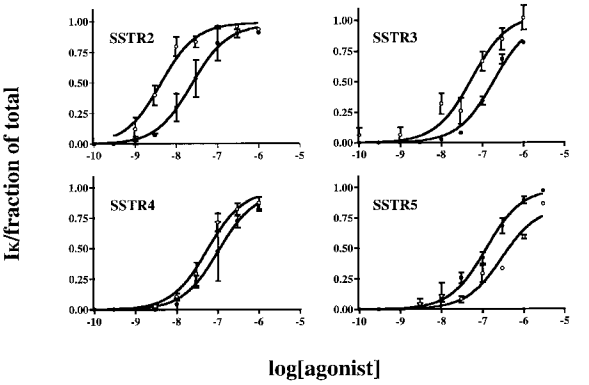

Fig. 2. Dose-response curves for activation of GIRK1 currents by SST14 and SST28. Peak currents were recorded from oocytes injected with the appropriate cRNAs and stimulated with various concentrations of agonist. Between applications a minimum of 15 min delay time was observed to avoid desensitization of SSTRs or GIRK1 channels. The data were normalized to the maximum response obtained from each oocyte and analyzed by non-linear regression using the GraphPad Prizm software (GraphPad, San Diego, CA). 3 to 6 oocytes were analyzed per receptor/ligand combination, and the data are presented as the mean +/− standard error. Open circles, SST14; filled circles, SST28.

channels activated by βγ. SSTR1 might therefore be coupled to one of these G-protein α-subunits in *Xenopus* oocytes. Alternatively, another GIRK subunit might be necessary for SSTR1 to be effective. In agreement with our results, it was recently observed that in oligodendrocytes SSTR1 inhibits inwardly rectifying potassium currents [9].

SSTR2 appears to be the receptor subtype that activates GIRK most efficiently. SSTR2 is coupled to $G_{\alpha i3}$- and $G_{\alpha o}$-subunits [15], and it was shown recently that $G_{\alpha i3}$ mediates the somatostatin-induced activation of GIRK currents in growth hormone secreting adenoma cells [16]. Thus it becomes very likely that SSTR2 is responsible for this effect in these cells. On the other hand, SSTR3 is likely to be the receptor subtype that mediates activation of GIRK channels by somatostatin observed in cerebellar granule cells [17], as SSTR3 is prominently expressed in this brain region [18]. The lower EC_{50} value for activation of SSTR5 by SST28 is in agreement with the higher affinity of this receptor subtype for the long SST isoform as determined in competition binding experiments [19].

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References

[1] Epelbaum, J., Dournaud, P., Fodor, M. and Viollet, C. (1994) Crit. Rev. Neurobiol. 8, 25–44.
[2] Garcia, P.D. and Myers, R.M. (1994) Mol. Pharmacol. 45, 402–409.
[3] Fujii, Y., Gonoi, T., Yamada, Y., Chihara, K., Inagaki, N. and Seino, S. (1994) FEBS Lett. 355, 117–120.
[4] Reardon, D.B., Dent, P., Wood, S.L., Kong, T. and Sturgill, T.W. (1997) Mol. Endocrinol. 11, 1062–1069.
[5] Akbar, M., Okajima, F., Tomura, H., Majid, M.A., Yamada, Y., Seino, S. and Kondo, Y. (1994) FEBS Lett. 348, 192–196.
[6] Bito, H., Mori, M., Sakanaka, C., Takano, T., Honda, Z.-i.,

Gotoh, Y., Nishida, E. and Shimizu, T. (1994) J. Biol. Chem. 269, 12722–12730.
[7] Shen, K.-Z., North, R.A. and Surprenant, A. (1992) J. Physiol. 455, 581–599.
[8] Yamashita, N., Shibuya, N. and Ogata, E. (1986) Proc. Natl. Acad. Sci. USA 83, 6198–6202.
[9] Karschin, A. (1995) Neuroreport 7, 121–124.
[10] Darlison, M.G., Greten, F.R., Harvey, R.J., Kreienkamp, H.-J., Stühmer, T., Zwiers, H., Lederis, K. and Richter, D. (1997) Proc. Natl. Acad. Sci. USA 94, 8214–8219.
[11] Kery, G., Erchegyi, J., Horvath, A., Mezo, I., Idei, M., Vantus, T., Balogh, A., Vadasz, Zs., Bökönyi, Gy., Seprodi, J., Teplan, I., Csuka, O., Tejeda, M., Gaal, D., Szegedi, Zs., Szende, B., Roze, C., Kalthoff, H. and Ullrich, A. (1996) Proc. Natl. Acad. Sci. USA 93, 12513–12518.
[12] Lesage, F., Guillemare, E., Fink, M., Duprat, F., Heurteaux, C., Fosset, M., Romey, G., Barhanin, J. and Lazdunski, M. (1995) J. Biol. Chem. 270, 28660–28667.
[13] Wickman, K.D., Iniguez-Lluhi, J.A., Davenport, P.A., Taussig, R., Krapivinsky, G.B., Linder, M.A., Gilman, A.G. and Clapham, D.E. (1994) Nature 368, 255–257.
[14] Schreibmayer, W., Dessauer, C.W., Vorobiov, D., Gilman, A.G., Lester, H.A., Davidson, N. and Dascal, N. (1996) Nature 380, 624–627.
[15] Law, S.F., Yasuda, K., Bell, G.I. and Reisine, T. (1993) J. Biol. Chem. 268, 10721–10727.
[16] Takano, K., Yasufuku-Takano, J., Teramoto, A. and Fujita, T. (1997) Endocrinology 138, 2405–2409.
[17] Surmeier, D.J., Mermelstein, P.G. and Goldowitz, D. (1996) Proc. Natl. Acad. Sci. USA 93, 11191–11195.
[18] Meyerhof, W., Wulfsen, I., Schönrock, C., Fehr, S. and Richter, D. (1992) Proc. Natl. Acad. Sci. USA 89, 10267–10271.
[19] O'Carroll, A.-M., Lolait, S.J., Konig, M. and Mahan, L. (1992) Mol. Pharmacol. 42, 939–946.