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ORIGINAL ARTICLE

Somatostatin increases rat locomotor activity by activating sst_2 and sst_4 receptors in the striatum and via glutamatergic involvement

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Abstract The involvement of striatal somatostatin receptors (sst_1, sst_2 and sst_4) in locomotor activity was investigated. Male Sprague–Dawley rats, 280–350 g, received in the striatum bilateral infusions of saline, somatostatin, and selective sst_1, sst_2, and sst_4 ligands. Spontaneous locomotor activity was recorded for 60 min. The involvement of excitatory amino acid receptors (AMPA and NMDA) on somatostatin's actions was also examined. Western blot analysis was employed for the identification of *somatostatin receptors* in striatal membranes. Somatostatin, sst_2 and sst_4, but not sst_1, selective ligands increased rat locomotor activity in a dose-dependent manner. Blockade of AMPA and NMDA receptors reversed somatostatin's actions. In conclusion, striatal *somatostatin receptor* activation differentially influence rat locomotor activity, while glutamatergic actions underlie the behavioral actions of somatostatin.

Keywords Basal ganglia · Behavior · Somatostatin receptor subtypes

Introduction

The neuropeptide somatostatin [somatotropin release inhibitory factor (SRIF)] is a cyclic tetradecapeptide, which is widely distributed in the peripheral and central nervous system (Brazeau et al. 1973; Epelbaum 1986). SRIF mediates its actions by interacting with specific receptors, which belong to the G-protein-coupled receptor (GPCR) family. *Five* receptor subtypes for SRIF have been cloned, namely, $sst_{1\text{-}5}$ (for classification and nomenclature see Hoyer et al. 1995). In the brain, a differential expression of the six receptor mRNAs has been reported (Kong et al. 1994; Raulf et al. 1994; Fehlmann et al. 2000), while immunohistochemistry studies support the presence primarily of subtypes sst_1, sst_2, sst_3, and sst_4 (Schulz et al. 2000; Olias et al. 2004).

In the basal ganglia, SRIF is synthesized in medium-sized aspiny neurons of the caudate-putamen and the accumbens (NAc), where it is co-localized with nitric oxide and neuropeptide-Y (Vincent and Jonansson 1983; Kawaguchi et al. 1995). Functionally, SRIF has been reported to increase dopamine levels in the striatum and the NAc, and to influence dopamine-mediated behaviors such as locomotor activity (Chesselet and Reisine 1983; Thermos et al. 1996; Pallis et al. 2001; Raynor et al. 1993). In the striatum, the sst_2 receptor has been reported to be responsible for the regulation of dopamine release (Hathway et al. 1999).

Immunohistochemical studies have reported the presence of the sst_1, sst_2, and sst_4 receptors in nuclei of the basal ganglia, including the striatum (Schulz et al. 2000; Schreff et al. 2000; Selmer et al. 2000). Behavioral studies showed that activation of the sst_2 subtype in the NAc increased the locomotor activity of the rat (Raynor et al. 1993), while mice deficient in the sst_2 subtype showed impaired locomotor activity (Viollet et al. 2000; Hathway et al. 2003; Allen et al. 2003). Activation of specific ssts in other basal ganglia nuclei also resulted in the regulation of locomotor activity. Infusion of sst_1 and sst_2 selective agonists in the rat ventral pallidum (VP) led to the

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attenuation of locomotor activity (Marazioti et al. 2005), showing for the first time the involvement of the sst_1 subtype in this dopamine-mediated behavior. More recently, activation of the sst_1, sst_2, and sst_4 receptor subtypes in rat globus pallidus was shown to increase rat locomotor activity. These latter findings depicted for the first time a functional role for the sst_4 receptor in the basal ganglia (Marazioti et al., unpublished results).

As presented above, there are a number of investigations that have focused on the effect of SRIF and its receptors in the basal ganglia, at the neurochemical or behavioral level. While neurochemical studies involving SRIF–dopamine interactions focused mostly in the striatum, there are a few reports addressing the functional role of *ssts* in this nucleus and their involvement in locomotor activity. Tashev et al. (2001, 2004) examined the effect of the bilateral infusion of SRIF in the striatum of Wistar rats and reported a biphasic profile, while the same group reported a dose-dependent decrease in exploratory activity and horizontal and vertical movements after unilateral SRIF injections (Tashev et al. 2001).

The aim of the present study was to investigate (a) the functional presence of the subtypes sst_1, sst_2, and sst_4 in the striatum by examining their involvement in rat locomotor behavior and (b) to ascertain the involvement of the glutamatergic system in SRIF's behavioral actions.

Materials and methods

Animals

Male Sprague–Dawley rats weighting 280–350 g were used for all experiments. Animals were housed three per cage before surgery and one per cage after surgery in a room maintained at 22°C with an alternating 12-h light–dark cycle. Food and water were available ad libitum. All animal experiments were carried out in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH publication No. 86-23, revised 1996). Efforts were made in order to minimize the number of animals used and reduce their suffering.

Surgery for cannulae transplantation

Each rat was anesthetized with 55 mg (i.m.) of ketamine HCL plus xylazine (4 mg, i.m.) and atropine (2 mg, i.m.) and secured in the stereotaxic frame (David Kopf, Tujunga, CA, USA). Guide cannulae made from 23-gauge stainless-steel tube (Plastics One) were positioned bilaterally toward the dorsal striatum. The coordinates, taken from the atlas of Paxinos and Watson (2005), were as follows: AP +0.2, ML ±3, VD −6 from bregma. The two guides were secured to the skull by three stainless-steel anchor screws and rapid-setting acrylic dental cement, which surrounded the entire assembly. The skin was sutured around the assembly, and a 7-day postoperative interval was allowed.

Drug administration

A cannula for injecting drug solutions into the brain through the guides was made with a 30-gauge needle (Plastics One) connected by polyethylene tube (PE 10) to a 1-μl Hamilton syringe. The injection sites were reached by the cannula tip extending beyond the implanted guides by 1.0 mm. All drugs were dissolved in sterile normal saline (sodium chloride, 0.9%, B. Braun, Melsungen, Germany). Also, sterile normal saline (0.5 μl/side) was infused as vehicle.

Each animal received up to three doses of drugs as well as vehicle (four injections in total). The sequence of injection was counterbalanced with respect to order at a rate of one injection every 2 days. The test session was initiated immediately after the injection, time zero (T0) and lasted for 60 min. After the termination of behavioral testing, animals were perfused, brains were removed and cut into sections for verification of the site of injection.

Locomotor activity measurements

Spontaneous locomotor activity was quantified using an automated activity recording system (Model 7420, Ugo Basile). This system is a plastic activity chamber equipped with two series of photocells, which provide an estimation of horizontal and vertical movements. On the day of the behavioral testing, rats were habituated to the room for 60 min in the locomotor activity cage before drug administration. Drug solutions were administered as stated above at T0, and the spontaneous locomotor activity was recorded every 5 min for a total of 60 min through the activity recording system. Only the horizontal movements were employed for the analysis of the somatostatin effects on locomotor activity.

Effect of somatostatin and somatostatin receptor (sst_1, sst_2, sst_3, sst_4) ligands on locomotor activity

Somatostatin [0 (n=16), 30 (n=7), 100 (n=16), and 300 (n=6) ng/0.5 μl/side] was delivered bilaterally. CYN-154806 [(sst_2 antagonist) 100 ng/0.5 μl/side (n=12)] was co-infused with somatostatin [100 ng/0.5 μl/side, (n=12)] in order to examine whether the somatostatin-mediated effect on locomotor activity is mediated by the sst_2 receptor. The effect of CYN-154806 [100 ng/0.5 μl/side (n=6)] alone on locomotor activity was also examined. The sst_2 selective agonist MK678 [0 (n=15), 10 (n=5), 30 (n=10),

and 100 (n=7) ng/0.5 μl/side] was delivered bilaterally. CYN-154806 [100 ng/0.5 μl/side (n=6)] was also co-infused with MK678 [100 ng/0.5 μl/side (n=7)]. L-797,591 [(sst_1 selective agonist), 0 (n=8), 10 (n=5), 30 (n=7), 100 (n=7), and 300 (n=4) ng/0.5 μl/side], L-796,778 [(sst_3 selective agonist), 0 (n=9), 100 (n=3), and 300 (n=4) ng/0.5 μl/side], and L-803,087 [(sst_4 selective agonist), 0 (n=10), 10 (n=5), 30 (n=7), and 100 (n=8) ng/0.5 μl/side] were delivered bilaterally in the striatum. CYN-154806 [100 ng/0.5 μl/side] was also co-infused with L-803,087 [100 ng/0.5 μl/side] (n=5).

Effect of DNQX and AP5 on the somatostatin-mediated locomotor activity

The AMPA/kainate receptor antagonist DNQX [0 (n=12), 5 (n=6), and 50 (n=5) nmol/0.5 μl/side] and the NMDA receptor antagonist AP5 [0 (n=7), 5 (n=6), and 50 (n=5) nmol/0.5 μl/side) were delivered bilaterally 5 min prior to somatostatin (100 ng/0.5 μl/side, n=12) administration. The effect of the excitatory amino acid antagonists alone on the locomotor activity of the rat was also examined [50 nmol DNQX (n=6) and 50 nmol AP5 (n=6)].

Histology

After the termination of the behavioral testing, animals were deeply anesthetized with an overdose of sodium pentobarbital and perfused intracardially with 10% buffered formalin. Brains were removed from the skulls and stored in 10% buffered formalin. They were then frozen and cut at 60 μm using a Leica cryostat (model CM1850) and examined with the naked eye for verification of the site of injection. Only animals with bilateral cannula placements in the striatum were used for statistical analysis.

Statistical analysis

Locomotor activity counts were analyzed by repeated measures analysis of variance (ANOVA) followed by Student's t test comparisons and one-way analysis of variance (one-way ANOVA), followed Dunnett or Neuman–Keuls post hoc analysis.

Western blot analysis using striatal membrane preparations

Sprague–Dawley rats were killed by decapitation, and the striatum was removed and homogenized using an Ultra-Turrax homogeniser in Tris pH 7.4 ice-cold buffer. The homogenate was centrifuged at 1,000×g for 10 min; the supernatant was aspirated and stored. The pellet was resuspended, rehomogenized, and centrifuged as above. The two supernatants were combined and centrifuged at 11,000×g for 20 min. The pellet was resuspended in 3 ml of Tris ice-cold buffer and centrifuged at 27,000×g for 10 min. Finally, the pellet was resuspended in 1 ml lysis buffer (10 mM Tris–HCl pH 7.4, 250 mM sucrose, 1 mM EDTA, 1 mM PMSF, 1% Triton, 2 μg/ml leupeptin, 2 μg/ml aprotinin). All steps of the above procedure were performed at 4°C. The protein content was determined according to Bradford. Striatal membranes (20–100 μg) were analyzed by sodium dodecyl sulfate/polyacrylamide gel electrophoresis (SDS/PAGE; 10%), and the proteins were transferred from the gel to nitrocellulose overnight at 4°C. The residual binding sites were blocked by incubating the membrane for 2 h in 5% (w/v) milk and 0.1% (v/v) Tween-20.

The blots were subsequently incubated with primary antibodies for sst_1 (1:5000) and sst_{2A} (1:2500), overnight at 4°C, followed by incubation with peroxidase-conjugated anti-mouse IgG. Immunoreactive proteins were visualized by chemiluminescence (Chemicon International, Temecula, CA, USA). These experiments were performed four times from the same or different preparations of striatum. For adsorption controls, sst_{2A} (2 μg/ml) antigen was incubated with the antisera for 2 h at room temperature. Antigens and anti-sst antibodies were kindly provided by Prof S. Schulz.

Results

Effect of somatostatin on locomotor activity

Somatostatin was infused in the striatum according to the coordinates of Paxinos and Watson (2005). Locomotor activity was measured over 5-min intervals for the total 60-min observation period. As shown in Fig. 1a, SRIF increased locomotor activity when compared to the vehicle group. This effect was more pronounced in the time period 0–30 min. Two-way ANOVA with repeated measures over time yielded a significant time effect [$F_{(11,30)}$=68.84, P<0.0001], treatment effect [$F_{(1,30)}$= 21.75, P<0.0001] and a significant interaction effect [$F_{(11,30)}$=4.771, P<0.0001]. Student's t test analysis revealed statistically significant increases in locomotor activity at time points 5 min (P=0.01), 15 min (P= 0.0020), and 20 min (P=0.0003). The locomotor activity was increased in a dose-dependent manner (Fig. 1b). One-way ANOVA and Dunnet post hoc analysis of the data revealed that the increase was statistically significant at the concentrations of 30, 100, and 300 ng/0.5/ μl/side. The increase produced by SRIF (100 ng/0.5/μl/side) was reversed by the same dose of the sst_2 selective antagonist, CYN-154806 (Fig. 1c). CYN-154806 (100 ng/0.5/μl/side) alone had no effect (P>0.05) on rat locomotor activity (Fig. 1d).

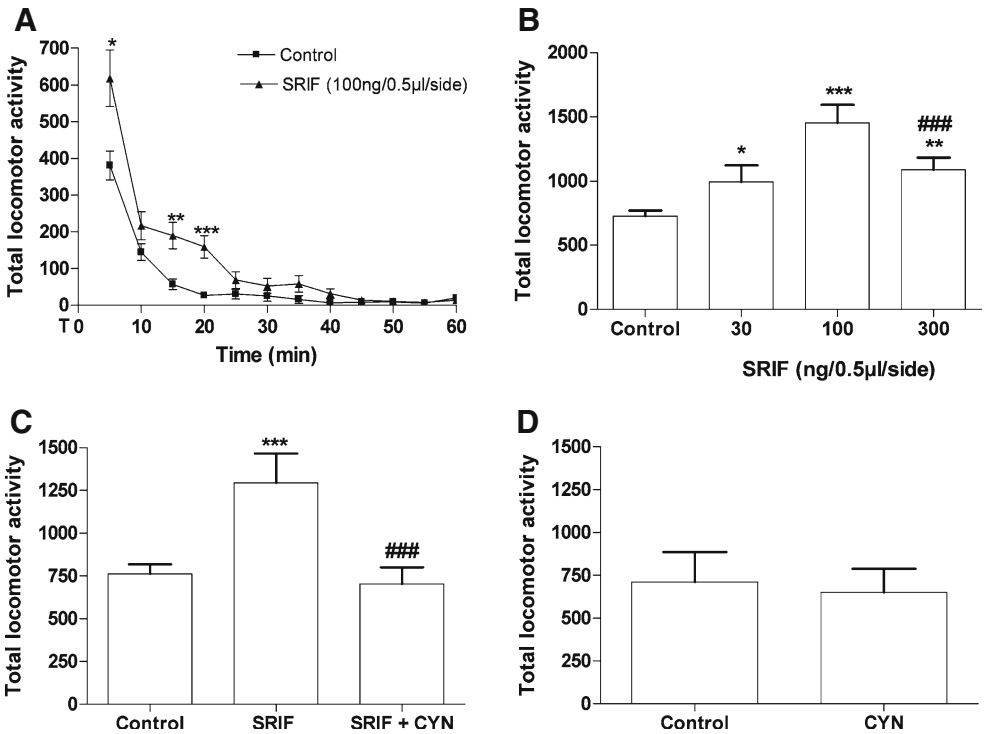

Fig. 1 SRIF increased locomotor activity when injected into the striatum of the rat. **a** Time course of locomotor activity produced by vehicle (Control, n=16) and somatostatin (100 ng/0.5 µl/side, n=16). Values shown are the mean number±SEM of line crosses in each 5-min time block during the 60-min time period. *P<0.05, **P<0.01, ***P<0.001 compared to vehicle. **b** Animals were administered either saline (Control, n=16) or somatostatin in the doses of 30 (n=7), 100 (n=16), 300 (n=6) ng/0.5 µl/side into the striatum and tested for locomotor activity. Values shown are the mean number±SEM of line crosses in the 60-min test period. *P<0.05, **P<0.01, ***P<0.001 compared to Control, ###P<0.001 compared to the SRIF (100 ng/0.5 µl/side). **c** Effect of CYN154806 [100 ng/0.5 µl/side, (n=12)] on somatostatin action [100 ng/0.5 µl/side, (n=12)]. **P<0.01 compared to Control, ##P<0.01 compared to somatostatin group. P>0.05 CYN154806 + SRIF versus Control. **d** Effect of CYN154806 on rat locomotor activity. P>0.05 CYN154806 (n=6) versus Control (n=6)

Effect of sst_1, sst_2, sst_3, and sst_4 ligands on locomotor activity

To assess further the role of the sst_2 subtype on SRIF's actions, the sst_2 agonist MK678 was administered bilaterally in the striatum. MK678 increased locomotor activity when compared to the vehicle group (Fig. 2a). Two-way ANOVA with repeated measures over time yielded a significant time effect [$F_{(11,20)}$=18.46, P<0.0001, treatment effect [$F_{(1,20)}$=13.87, P<0.0013, but no significant interaction effect [$F_{(11,20)}$=1.120, P<0.3464. Student's t test analysis revealed statistically significant increases in locomotor activity at time points 10 min (P=0.0298), 15 min (P=0.0002), 20 min (P=0.0220), 25 min (P=0.0031), 30 min (P=0.0024), 35 min (P=0.0171), 40 min (P=0.0082), 45 min (P=0.0364), 50 min (P=0.0070), and 60 min (P=0.0129). Locomotor activity was increased in a in a dose-dependent manner (Fig. 2b). Post hoc analysis of the data showed a statistically significant increase at the concentrations of 30 and 100 ng/0.5/µl/side (Fig. 2b). One-way ANOVA and post hoc analysis of the data revealed that the increase produced by MK678 100 ng/0.5 µl/side was reversed by the same dose of the selective sst_2 antagonist (Fig. 2c).

To examine further the involvement of the other somatostatin receptor subtypes, selective *ssts* agonists were employed. Sst_1 activation with the selective agonist L-797-591 did not influence locomotor activity even at the highest concentration used (300 ng/0.5 µl/side, Fig. 3a). No effect was observed when the sst_3 selective agonist L-796,778 (100 and 300 ng/0.5 µl/side) was employed (data not shown). However, activation of the sst_4 receptor subtype by L-803,087 increased locomotor activity in a statistically significant manner at the highest dose tested, that of 100 ng/0.5 µl/side (p<0.01 when compared to control; Fig. 3b). To examine whether this effect was partially due to a nonselective activation of the sst_2 receptor by the L-803,087 analog, CYN-154806 (100 ng/0.5/µl/side) was coinfused with L-803,087 (100 ng/0.5 µl/side) and the locomotor activity measured. CYN-154806 had no effect on the L-803,087-dependent increase of locomotor activity (Fig. 3c).

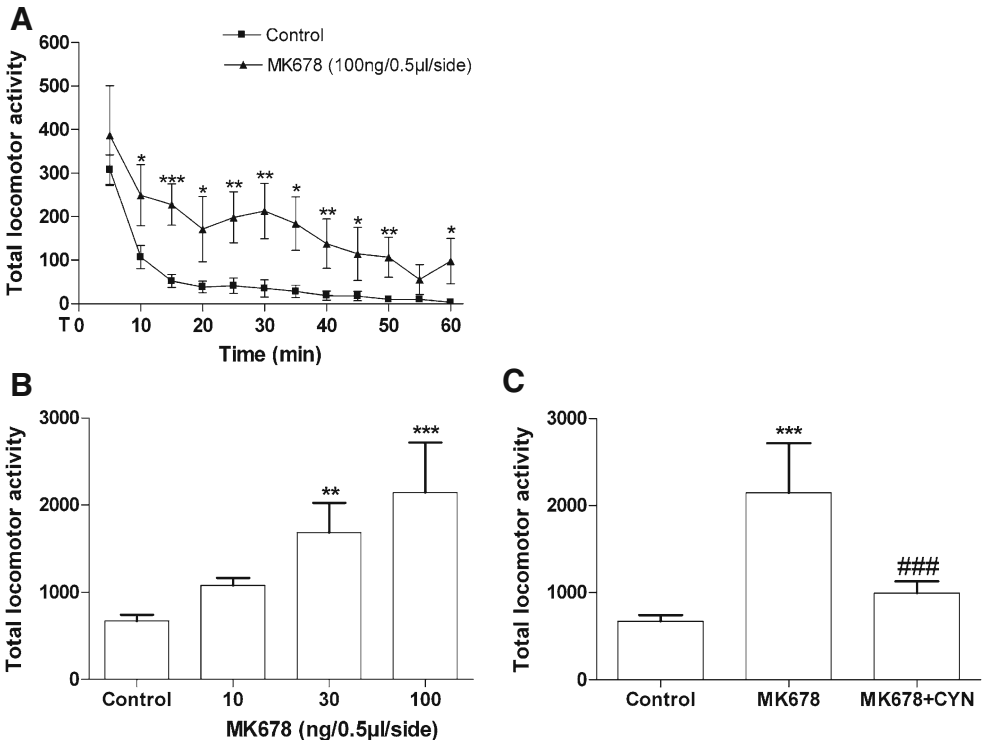

Fig. 2 MK678 increased locomotor activity when injected into the striatum of the rat. **a** Time course of locomotor activity produced by vehicle (Control, $n=15$) and MK678 (100 ng/0.5 µl/side, $n=7$). Values shown are the mean number±SEM of line crosses in each 5-min time block during the 60-min time period.*$P<0.05$, **$P<0.01$, ***$P<0.001$ compared to vehicle. **b** Animals were administered either saline (Control, $n=15$) or MK678 in doses of 10 ($n=5$), 30 ($n=10$), and 100 ng/0.5 µl/side ($n=7$) into the striatum and tested for locomotor activity. Values shown are the mean number±SEM of line crosses in the 60-min test period. *$P<0.05$, ***$P<0.001$ compared to Control. **c** Effect of CYN154806 [100 ng/0.5 µl/side, ($n=6$)] on MK678 action [100 ng/0.5 µl/side, ($n=7$)]. ***$P<0.001$ compared to Control ($n=15$), ###$P<0.001$ compared to MK678 group. $P>0.05$ CYN154806+MK678 versus Control

Effect of the AMPA and NMDA receptor antagonists on somatostatin-mediated locomotor activity

To examine whether the glutamatergic system is involved in SRIF's actions on locomotor activity, antagonists of the NMDA and AMPA receptors were employed. Infusion of AP5 (Fig. 4a) and DNQX (Fig. 5a), respectively, at the concentrations of 5 and 50 nmol prior to the administration of SRIF (100 ng/0.5 µl/side) reversed SRIF's action on locomotor activity. Infusion of the antagonists alone (50 nmol) had no statistically significant effect on the locomotor activity of the rat (Figs. 4b and 5b).

Localization of SRIF receptors in striatal membranes using Western blotting

Peptide antisera for sst_1 and sst_{2A} were employed to assess the presence of receptor protein in striatal membranes by Western blot methodology. As shown in Fig. 6, the sst_1 antiserum reacted specifically with a band migrating at a molecular mass of approximately 73 kDa. The higher and lower molecular weight bands present in Fig. 6a are also visible in the sst_{2A} blot and considered nonspecific. The sst_{2A} antiserum reacted with a number of bands. In order to examine whether one of these bands was specific for the receptor, the antiserum was first pretreated with antigen. A band migrating at a molecular mass of approximately 81 kDa was not detected in the presence of antigen and therefore specific for the sst_2 receptor.

Discussion

The present study supports the presence of the sst_1, sst_2, and sst_4 receptors in the striatum and the functional implication of sst_2 and sst_4 in locomotor activity. Furthermore, it is shown that the SRIF-mediated behavioral actions are influenced by glutamatergic neurotransmission.

Somatostatin is synthesized in the nuclei of the basal ganglia involved in dopamine-mediated behaviors, such as the striatum, and the NAc, and is implicated in the pathophysiology of neurological and affective disorders (Gerner and Yamada 1982; Lu and Stoessl 2002). In the early eighties, SRIF was shown to increase dopamine levels

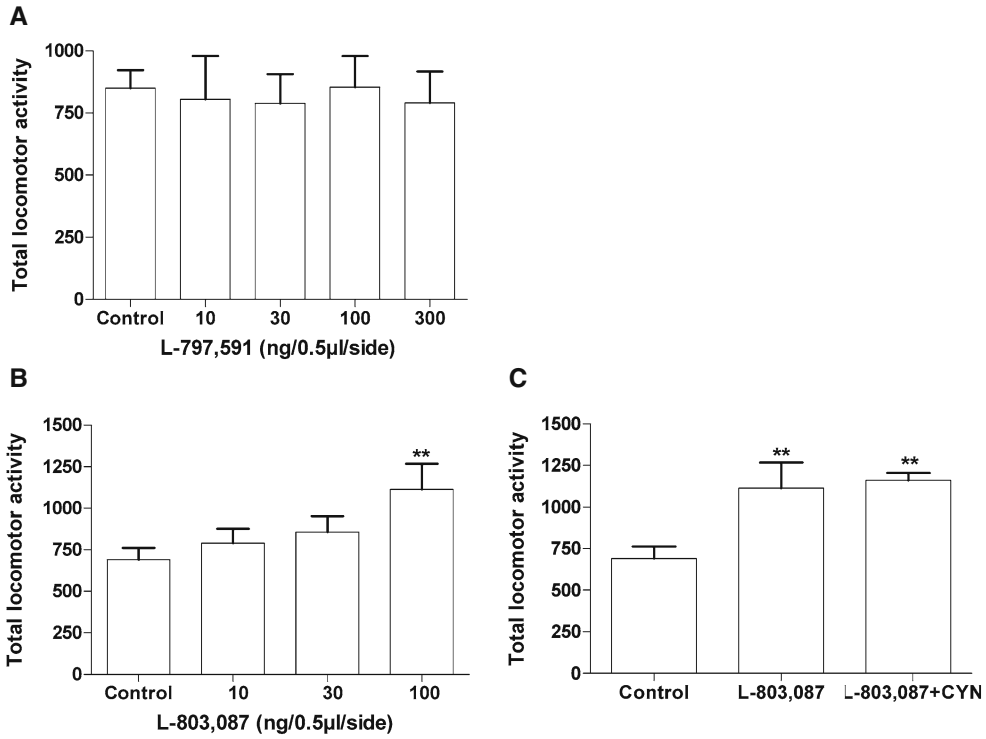

Fig. 3 Effect of sst1 and sst4 activation on locomotor activity. **a** L-797–591 [(sst1 agonist), 0 (Control, n=8), 10 (n=5), 30 (n=7), 100 (n=7), 300 ng/0.5 µl/side (n=4)]. **b** L-803,087 [(sst4 agonist), 0 (n=10), 10 (n=5), 30 (n=7), 100 ng/0.5 µl/side, (n=8)] increased locomotor activity in a statistically significant manner only at the highest dose used (100 ng/0.5 µl/side) **P<0.01 when compared to Control. **c** CYN154806 (100 ng/0.5 µl/side) coinfused with L-803,087 (100 ng/0.5 µl/side; n=5) had no effect on the L-803,087-induced increase (n=8) in locomotor activity. **P<0.01 versus Control (n=10)

in striatal slices (Chesselet and Reisine 1983). Subsequent in vivo microdialysis studies showed SRIF to increase dopamine release when infused bilaterally in the rat striatum (Thermos et al. 1996) and the NAc (Pallis et al. 2001).

Corticostriatal glutamatergic innervation influences striatal neurochemistry. Activation of glutamate receptors has led to increases in DA release (Imperato et al. 1990; Smolders et al. 1996; Kendrick et al. 1996), and further evidence supports that the SRIF-induced dopamine release involves a glutamatergic mechanism. SRIF increased glutamate release from corticostriatal terminals (Hathway et al. 1998). Glutamate's subsequent activation of AMPA/kainate or NMDA receptors possibly located on DA terminals is believed to be responsible for the SRIF-mediated increases in DA levels (Imperato et al. 1990; Wheeler et al. 1995; Kendrick et al. 1996).

In the striatum, SRIF was shown to increases DA levels by activating the sst_2 subtype (Hathway et al. 1999). Immunohistochemical studies have shown the presence of the sst_2 and sst_4 in this nucleus (Schulz et al. 2000; Schreff et al. 2000; Selmer et al. 2000). The sst_1 has been functionally observed in the NAc (Schulz et al. 2000) where it acts as an autoreceptor (Vasilaki et al. 2004; Thermos et al. 2006). In the present study, Western blot analysis showed the presence of the sst_1 and sst_2 in striatal membranes. The molecular masses obtained for the sst_1 and

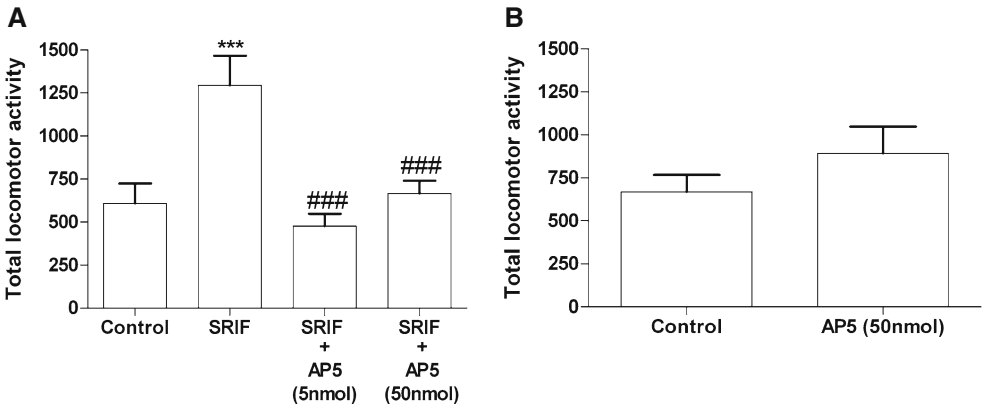

Fig. 4 Effect of NMDA receptor blockade on the somatostatin-induced increase of locomotor activity. **a** Effect of AP5 [5 (n=6) and 50 (n=5) nmol/0.5 µl/side] on somatostatin action (100 ng/0.5 µl/side). ***P<0.001 compared to Control (n=7), ###P<0.001 compared to SRIF group (n=12). P>0.05 AP5 5 nmol/0.5 µl/side + SRIF versus AP5 50 nmol/0.5 µl/side + SRIF. **b** Effect of AP5 [50 (n=6) nmol/0.5 µl/side] on locomotor activity, P>0.05 versus Control (n=9)

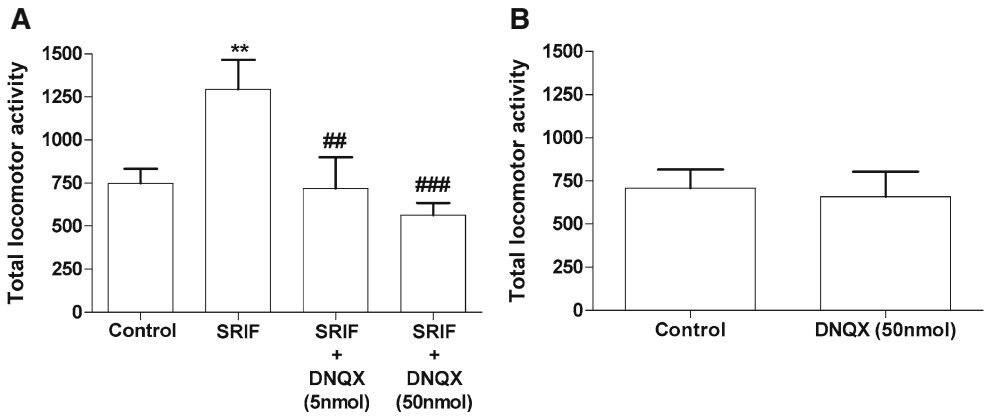

Fig. 5 Effect of AMPA receptor blockade on the somatostatin-induced increase of locomotor activity. **a** Effect of DNQX [5 (n=6)and 50 (n=5) nmol/0.5 μl/side] on somatostatin action (100 ng/0.5 μl/side, n=12). **P<0.01 compared to Control (n=12), ##P<0.01, ###P<0.001 compared to SRIF group (n=12). P>0.05 DNQX 5 nmol/0.5 μl/side + SRIF versus DNQX 50 nmol/0.5 μl/side + SRIF. **b** Effect of DNQX [50 (n=6) nmol/0.5 μl/side] on locomotor activity. P>0.05 versus Control (n=10)

sst_2 subtypes are in agreement with previous published results (Helboe et al. 1997; Hofland et al. 1999). In the sst_1 blot (Fig. 6a), only the 71-kDa band was specific, since the other two bands observed were also present in the sst_{2A} blot. The *ssts* usually provide diffuse and not sharp bands, as shown in the present study. However, a recent publication also depicted similar results (Aguado-Llera et al. 2008). This may be a characteristic of the antibodies employed. Western blot analysis for the presence of the sst_4 subtype was also attempted in the present study, but a number of bands were observed with questionable results regarding the specificity (data not shown). However, the work by Schreff et al. (2000) leaves no doubt that this subtype is present in the striatum. Therefore, we did not consider it essential to pursue the use of a different antibody or other technical aspects to substantiate the presence of the sst_4 subtype in our preparation. Its presence, as will be discussed below, is substantiated functionally in this study in agreement to the work by Schreff et al. (2000).

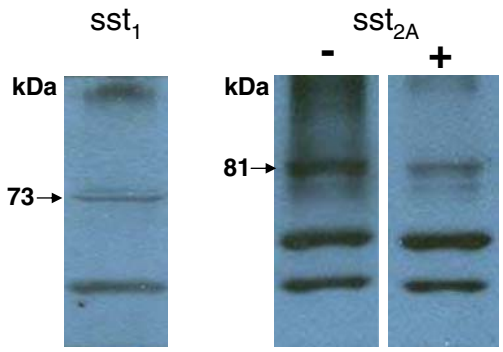

Fig. 6 Western blotting of somatostatin receptors in the striatum. Striatal membranes (20–100 μg) were separated on 10% SDS-polyacrylamide gels and blotted onto nitrocellulose membranes. Membranes were then incubated with either anti-sst1 or anti-sst2A. Blots were developed using enhanced chemiluminescence. (−) in the absence or (+) presence of antigen

The present behavioral data support that the sst_2 receptor subtype is important in the mediation of SRIF's effects on locomotor activity. This was shown by the inhibition of SRIF's actions by the $sst_{2/5}$ selective antagonist CYN-154806, as well as the increase in locomotor activity produced by the direct infusion of the $sst_{2/5}$ agonist MK678. The sst_5 subtype has not been found in the striatum (Schulz et al. 2000), and thus, it can be presumed that the MK678 effect is due to its activation of the sst_2 receptor. CYN-154806 had no effect on locomotor activity when administered alone. These results complement the neurochemical findings of Hathway et al (1999), who showed that SRIF increases dopamine levels in rat striatum by activating the sst_2 receptor. The same group has also shown that unilateral infusion of SRIF in the mouse striatum elicited ipsilateral turning associated with increased dopamine (Hathway et al. 2003).

The present data agree with the older work by Raynor et al. (1993) in the NAc. The locomotor activity was increased in a statistically significant manner during the first 30 min after the bilateral infusion of SRIF. In the Raynor study, SRIF was found to induce a statistically significant increase in locomotor activity starting at the doses of 10 and 30 ng/side). The data depicted an inverted U-shaped dose–response curve. The higher dose of SRIF 100 ng/side did not produce any effect. In the present study, the dose of 100 ng/side gave the highest increase in locomotor activity. The higher dose of 300 ng/side produced a statistically significant increase in locomotor activity compared to the control, but it was statistically lower than that produced by the dose of 100 ng/side (Fig. 1b).

In addition, in the NAc, the $sst_{2/5}$ agonist MK678 increased locomotor activity in a dose-dependent manner with optimum responses at the dose of 100 ng/side (Raynor et al. 1993), in agreement with the results of the present study. Also, there is agreement with the time course data,

namely, a statistically significant increase in locomotor activity was observed for up to 60 min after the MK678 infusion in the striatum and the NAc. The differences observed in the duration of action of SRIF and MK678, as depicted in the time course graphs, may *be* due to SRIF's reduced stability and higher degradation.

Another investigation examined the behavioral responses of Wistar rats to bilateral infusions of SRIF (20, 50, 100 ng/side) into the caudate putamen (Tashev et al. 2001). These investigators reported that SRIF exerts an inhibitory and a facilitatory effect on locomotor activity, but the identity of the receptors possibly involved in these actions was not examined.

The involvement of the sst_1 receptor in the mediation of locomotor behavior was also examined in the present study. The sst_1 selective agonist used had no effect on the locomotor activity even at the highest dose employed (300 ng/side). This *dose* was effective in the SRIF experiments. Activation of the sst_1 receptor was shown to attenuate SRIF levels in the NAc and thus act as SRIF's autoreceptor (Vasilaki et al. 2004; Thermos et al. 2006). While such a function has not been established in the dorsal striatum, one can question whether the decrease of SRIF levels as a result of sst_1 activation would be sufficient to influence locomotor activity in a negative manner.

Activation of the sst_4 receptor subtype, like the sst_2, influenced locomotor activity in a positive manner at the dose of 100 ng/side. Due to the paucity of the selective ligand used, we could not examine its effect at the higher dose (300 ng/side). To examine whether the increase in locomotor activity was due to a sst_2 nonselective effect of the ligand, we coinfused the sst_2 antagonist with the L-803,087. The data support that the sst_2 receptors do not mediate the locomotor activity actions of the sst_4 ligand. Recently, data from our group showed the functional presence of the sst_4 subtype in the globus pallidus and its role in the positive regulation of the locomotor activity of the rat (Marazioti et al. unpublished results). While further investigations are essential in order to examine whether a causal relationship exists between SRIF's locomotor inducing effects in the GP and dopamine release in the striatum, it appears that the sst_4 receptors in the basal ganglia are involved in the mediation of SRIF's actions on locomotor activity. In addition, the identity of the neurons hosting the sst_4 receptors in the striatum, as well as the effect of sst_4 agonists on dopamine levels in the same nucleus need to be elucidated. Infusion of a sst_3-selective agent in the striatum did not affect locomotor activity (data not shown). This receptor has not been reported to be present in striatal neurons (Schulz et al. 2000), and thus, these experiments were performed as control studies. Therefore, the behavioral and pharmacological results presented so far suggest the functional importance of the sst_2 and sst_4 receptors in rat locomotor activity.

Data in the literature suggested that SRIF's actions on DA release are mediated by increases in excitatory amino acid release, thus via a glutamatergic-dependent mechanism. An increase in glutamate levels was observed concomitant to the dopamine increase induced by SRIF in the anesthetized rat (Hathway et al. 1998). In addition, the SRIF-induced DA release was reduced in the presence of NMDA and AMPA/kainate receptor antagonists. In the present study, we also examined the effect of AMPA and NMDA receptor antagonists on SRIF's effects on locomotor activity. Antagonist administration prior to the SRIF infusion in the striatum reversed SRIF's mediated increase in locomotor behavior. Therefore, the present behavioral data complement the above cited neurochemical findings. The actions of the antagonists were shown to be specific to the actions of the *ssts*, since the infusion of the AMPA and NMDA receptor antagonists alone in the striatum had no effect on the animals' locomotor activity.

In conclusion, these results provide behavioral evidence to support the functional significance of the sst_2 and sst_4 receptor subtypes in the striatum, a nucleus with an important role in locomotor activity. While more information is accumulating on the role of SRIF in the basal ganglia, further studies are essential to pinpoint the physiological role of the specific receptors in the complicated neurochemical circuitry of the basal ganglia nuclei and subsequently in behavior.

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