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$GABA_B$ receptor antagonist CGP-36742 enhances somatostatin release in the rat hippocampus in vivo and in vitro

Gabriella Nyitrai[a,*], Katalin A. Kékesi[b], Zsuzsa Emri[a], Éva Szárics[a], Gábor Juhász[c], Julianna Kardos[a]

[a] Department of Neurochemistry, Chemical Research Center, Hungarian Academy of Sciences, H-1025 Budapest, Pusztaszeri út 59-67, Hungary
[b] Department of Physiology and Neurobiology, Eötvös Loránd University, Budapest, Hungary
[c] Neurobiology Research Group, Eötvös Loránd University, Hungarian Academy of Sciences, Budapest, Hungary

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Abstract

Here, we show the modulation of somatostatin functions in the hippocampus by the orally active 'cognition enhancer' $GABA_B$ receptor antagonist, (3-aminopropyl)*n*-butylphosphinic acid (CGP-36742), both in vivo and in vitro. Using high-pressure liquid chromatography-coupled electrospray mass spectrometry, we measured a two-fold increase in the extracellular level of somatostatin to CGP-36742 application in the hippocampus of anaesthetised rats. The basal release of $[^{125}I]$somatostatin in the synaptosomal fraction was increased by CGP-36742 in concentrations lower than 1 μM. Simultaneous measurement of $[^{14}C]$Glu and $[^{3}H]$γ-aminobutyric-acid ($[^{3}H]$GABA) showed that CGP-36742 increased their basal release. However, prior $[^{125}I]$somatostatin application suppressed the increase in the basal release of $[^{14}C]$Glu and induced a net decrease in the basal release of $[^{3}H]$GABA. Somatostatin application had a similar effect. In slices, CGP-36742 increased the postsynaptic effect of somatostatin on CA1 pyramidal cells. These results suggest a pre- and postsynaptic functional 'cross-talk' between coexisting $GABA_B$ and somatostatin receptors in the rat hippocampus.
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Keywords: Somatostatin; $GABA_B$ receptors; GABA (γ-aminobutyric acid); Glutamate; Hippocampus; Brain, rat

1. Introduction

$GABA_B$ receptors are often located presynaptically on axon terminals and are commonly believed to modulate the release of different neurotransmitters (Bonanno and Raiteri, 1993; Bowery, 1993). Isolation of $GABA_{B1}$ and $GABA_{B2}$ receptor proteins (Kaupmann et al., 1997; White et al., 1998) revealed the heterodimeric structure of active $GABA_B$ receptors (Marshall et al., 1999; White et al., 1998; Bowery and Enna, 2000; Bowery et al., 2002). Although molecular biology has not revealed subtype-multiplicity of $GABA_B$ receptors as yet, several lines of evidence suggest that pharmacologically distinct $GABA_B$ receptor subtypes may exist (Bonanno and Raiteri, 1993; Kerr and Ong, 1996; Deisz et al., 1997; Bonanno et al., 1999; Ong et al., 2001).

The orally active $GABA_B$ receptor antagonist, (3-aminopropyl)*n*-butylphosphinic acid (CGP-36742), has been demonstrated to be active both at pre- and postsynaptic $GABA_B$ receptors, including autoreceptors regulating the release of γ-aminobutyric acid (GABA) in the cerebral cortex (Olpe et al., 1993; Froestl et al., 1995) and in the hippocampus as well (Pozza et al., 1999). CGP-36742-sensitive receptors have been suggested to mediate Glu release in other central areas in the central nervous system, also (Teoh et al., 1996; Nyitrai et al., 1999). Furthermore, in superfused rat and human cerebrocortical synaptosomes, CGP-36742 was shown to antagonise the baclofen-induced inhibition of the release of the cyclopeptide, and has been suggested to act selectively on a pharmacologically distinct presynaptic $GABA_B$ receptor subtype that regulates the release of somatostatin (Bonanno and Raiteri, 1993; Gemignani et al., 1994; Bonanno et al., 1999). CGP-36742 is reported to facilitate memory in a social recognition test in rats (Mondadori et al., 1996) and to improve cognitive performance in different species (Mondadori et al., 1993; Froestl

* Corresponding author. Tel.: +36-1-2090555x8110; fax: +36-1-325-7554.
E-mail address: nyitraig@chemres.hu (G. Nyitrai).

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et al., 1995), so it has been selected as a drug candidate for the treatment of cognition deficits (Bittiger et al., 1992; Froestl et al., 1995).

Somatostatin and GABA are co-transmitters of interneurons that synapse with pyramidal cells (Freund and Buzsáki, 1996), and each subtype of somatostatin receptor is present in the hippocampus of rats (for a review see Selmer et al., 2000). Somatostatin receptors are suggested to modulate GABA*ergic* synaptic transmission in the thalamus (Leresche et al., 2000) and both Glu*ergic* and GABA*ergic* transmission in the hippocampus (Boehm and Betz, 1997; Gardette et al., 1995; Scharfman and Schwartzkroin, 1989; Tallent and Siggins, 1997; Xie and Sastry, 1992).

Somatostatin is reported to have promnesic effect in animal models of dementia (Matsuoka et al., 1995) as well as a facilitatory role in long-term potentiation (Nakata et al., 1996; Kaneko et al., 1997). Acute stress and dexamethasone have been shown to induce the rapid synthesis and release of somatostatin from the dentate gyrus hilus (Arancibia et al., 2001). It may be conjectured that somatostatin levels are decreased in Alzheimer's disease patients (Davies et al., 1980; Grouselle et al., 1998), suggesting that increased levels of somatostatin may help to restore memory function. How somatostatin affects the neural mechanisms of memory formation, however, is far from being understood. Therefore, we studied the effect of $GABA_B$ receptor antagonist, CGP-36742, on somatostatin level and functions in the rat hippocampus since these changes might correlate with the 'cognition enhancer' property of the compound. To investigate this possibility, we used a strategy of comparing the ability of CGP-36742 (i) to modify hippocampal somatostatin levels in vivo and in vitro and (ii) to influence pyramidal cell excitability by affecting the postsynaptic effect of somatostatin. In addition, we measured the effect of CGP-36742 and somatostatin receptor activation on basal GABA and glutamate releases from nerve endings in vitro.

2. Materials and methods

2.1. Determination of somatostatin concentration in dialysate samples (Fig. 1)

Animal experiments were carried out as previously described (Kardos et al., 1996; Nyitrai et al., 1996) on the basis of local ethical rules in accordance with the European Council Directive of 24 November 1986 (86/609/EEC) and with the Hungarian Animal Act, 1998, and associated guidelines. Sprague–Dawley rats (350–400 g) were anaesthetised with 1% halothane in air and placed into a stereotaxic frame. To increase the amount of somatostatin in the dialysate samples, microdialysis probes (5 mm long and 0.5–1 mm wide) were implanted bilaterally into the hippocampus (A: −5, L: 5, V: −8 mm) according to the atlas of Paxinos and Watson (1997) (Fig. 1A). Since immunostaining for somtatostatin visualises a large number of neurons in all subfields of the hippocampus (Freund and Buzsáki, 1996), the extracellular fluid was collected from numerous subfields of the hippocampus (CA1, CA3, *stratum radiatum*, *stratum oriens*; Fig. 1A). To minimise tissue damage, the final position of the probes was reached slowly, in not less than 15 min. We started to collect samples 60 min after the implantation of the microdialysis probe. Owing to the complexity of solute diffusion into the microdialysis probe, the concentrations of somatostatin in the dialysate samples is not identical with its extracellular concentration. Therefore, the somatostatin concentration measured in dialysate samples collected during CGP-36742 application was related to the average somatostatin concentration of the samples collected before drug application (control) and is given as percent change from control. To determine the somatostatin concentration in dialysate samples, high-pressure liquid chromatography (HPLC) separation with electrospray mass spectrometric (MS) detection (Agilent 1100 Series HPLC) was used in combination with the HP ChemStation 6.1 software. Perfusion with non-pyrogenic, amino acid-free artificial cerebrospinal fluid (ACSF) containing 144 mM NaCl, 3 mM KCl, 1 mM $MgCl_2$ and 2 mM $CaCl_2$ (Department of Pharmacology, Heim Pál Hospital, Budapest, Hungary) was performed at a rate of 0.6 μl/min. The pH of ACSF was adjusted to 7.4 with NaOH (Merck) before the microdialysis experiment started. Each experiment started with the collection of a control sample (200 min—2 × 120 μl); the dialysate from the left and the right hippocampus was collected into the same glass vial. In the next 200 min, either 5 mM CGP-36742 (from W. Froestl, NOVARTIS, Basel) in ACSF or ACSF (control) was applied via the microdialysis probes bilaterally and the dialysate from both sides was collected as a single sample. Dialysate samples (240 μl) were freeze-dried and kept at −20 °C until analysed. Freeze-dried samples dissolved in 60 μl of 50% methanol (Merck) in distilled water were injected by the autosampler (55 μl). We used gradient elution for the separation of somatostatin and for the elimination of salts from samples with the following eluents: (A) 10 mM ammonium formiate (Merck) in distilled water pH = 3.5, (B) 10 mM ammonium formiate in 80% methanol pH = 3.5. Somatostatin was eluted at 10.4 min. Scanning over a wide mass range (500–2000 *m*/*z*) showed the highest abundance of standard somatostatin (Tocris) solutions (dissolved in water/methanol = 1:1) at 819.6 *m*/*z*, which represents the doubly charged somatostatin ion, thus providing a scanning window for dialysate samples with a detection limit of 0.5–1 ng/ml. Lastly, a Zorbax Poroshell 300SB C18 column was connected in close proximity to the electrospray needle, and the quadrupole was scanned over a narrow mass range (815–825 *m*/*z*; Fig. 1).

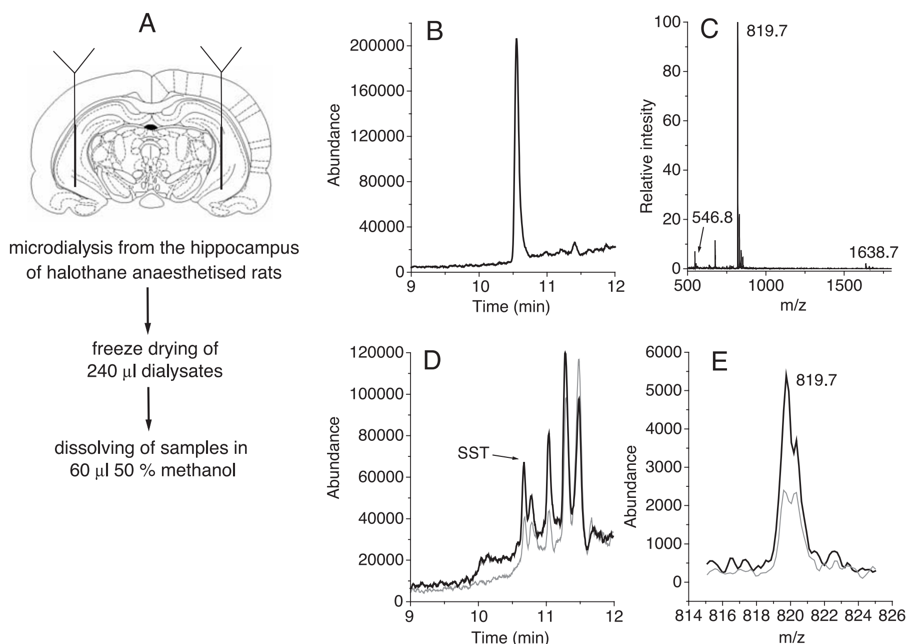

Fig. 1. Determination of somatostatin concentration in the hippocampus in vivo. (A) Position of the probes in the hippocampus of halothane-anaesthetised rats (A: − 5, L: 5, V: − 8 mm). Dialysis samples were collected for 200 min at a flow rate of 0.6 µl/min and then freeze-dried. (B) Total Ion Current chromatogram of 200 ng/50 µl standard solution of somatostatin. We used gradient elution for the separation of somatostatin on a Zorbax Poroshell 300SB C18 column (eluents: A: 10 mM ammonium formiate pH = 3.5, B: 10 mM ammonium formiate in 80% methanol pH = 3.5.); somatostatin was eluted at 10.4 min. (C) Mass spectrum of 200 ng/50 µl standard solution of somatostatin. Quadrupole was scanned over 500–2000 *m/z*. Three characteristic *m/z* values (at 546.8, 819.7, 1638.7 *m/z*) represent three differently charged forms of the peptide; the doubly charged ion (at 819.7 *m/z*) was most abundant. (D) Total Ion Current chromatogram of dialysates, dissolved in 60 µl 50% methanol, from the hippocampus of halothane-anaesthetised rats. The separation method was identical with that for standard somatostatin solutions. Grey line shows chromatogram of a control dialysate and the black line shows chromatogram of a dialysate collected after CGP-36742 application from the same animal. (E) Mass spectrum of dialysates shown in (D). Grey line shows spectrum of a control dialysate and the black line shows spectrum of a dialysate collected after CGP-36742 application from the same animal. Scanning window was 815–825 *m/z*. The chromatographic peak detected at the retention time of somatostatin standard had an *m/z* (819.7) identical to that of the somatostatin doubly charged ion.

2.2. Release of somatostatin, Glu and GABA from rat hippocampal synaptosomes

2.2.1. Preparation of purified synaptosomal fraction and membranes

Three- to six-week-old male Wistar rats were purchased from Toxicoop (Budapest, Hungary). Rats were housed at constant temperature (22 ± 1 °C) and relative humidity (50%) under a regular light–dark schedule (dark: 1900–0700 h). Food and water were freely available. Rats were killed by decapitation with a guillotine, and the brains were rapidly removed, washed in ice-cold physiological saline and the hippocampi were dissected on ice. Preparation of a purified synaptosomal fraction was performed as described previously (Hajós, 1975) with minor modifications. Briefly, hippocampi from seven rats were homogenised in 10 vol. ice-cold 0.32 M sucrose solution containing enzyme inhibitors (Sigma) such as bacitracin (0.2 mg/ml), antipain, leupeptin, pepstatin A (0.005 mg/ml each), aprotinin (0.01 mg/ml), phenyl-methyl-sulphonyl-fluoride (1 mM) and the antioxidant, 2,6-di-*t*-butyl-*p*-cresol (0.02 mM), with a motor-driven Potter–Elvehjem glass–Teflon homogeniser (Braun, Melsungen, Germany) at 1400 revolutions per minute. The homogenate was centrifuged at 1500 × *g* for 10 min at 4 °C and the supernatant was further centrifuged at 9500 × *g* for 20 min at 4 °C. The pellet was re-suspended in 10 ml 0.32 M ice-cold sucrose solution. Aliquots (5 ml) were layered on the top of 0.80 M ice-cold sucrose solution and centrifuged at 9500 × *g* for 25 min at 4 °C. Combined 0.80 M fractions were gradually diluted with 5 mM Tris–HCl buffer (pH 7.4 at 4 °C) to 0.40 M sucrose concentration and centrifuged at 20,000 × *g* for 15 min at 4 °C. One of the two pellets was re-suspended with 0.7 ml buffer A (5 mg protein/ml; Lowry et al., 1951) 145 mM NaCl, 5 mM KCl, 1.3 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM glucose, enzyme inhibitors (1 mM phenyl methyl sulphonyl fluoride, 0.01 mg/ml aprotinin, 0.02 µg/ml bacitracin), 10 mg/ml bovine serum albumin (Srikant and Patel, 1981) and 20 mM HEPES adjusted to pH 7.4 with NaOH, and kept on ice until used for the simultaneous measurement of [^{3}H]GABA, [^{14}C]Glu and [3-[^{125}I]iodotyrosyl^{11}]-somatostatin 14 ([^{125}I]somatostatin) release.

The other pellet was exposed to distilled water for 70 min at 4 °C. Thereafter, it was centrifuged at 20,000 × *g* for 15 min at 4 °C. The pellet obtained was re-homogenised in 5 mM Tris–HCl buffer, and the homogenate was centrifuged at 20,000 × *g* for 15 min at 4 °C. This washing procedure was repeated twice. The final pellet was re-suspended in 0.7

ml buffer A (3 mg protein/ml) and was kept on ice until used for the simultaneous measurement of [^{3}H]GABA, [^{14}C]Glu and [^{125}I]somatostatin binding.

2.2.2. Release measurements

[^{3}H]GABA (100 nM, 94 Ci/mmol, Amersham) and [^{14}C]Glu (8 μM, 238 mCi/mmol, Amersham) were incubated with the freshly isolated synaptosomal fraction in buffer A in the presence or absence of about 0.75 nM of 3-[^{125}I]iodotyrosyl^{11}somatostatin14 (~ 2000 Ci/mmol, Amersham; [^{125}I]somatostatin) at 37 °C for 45 min. Aliquots of the suspension (100 μl) were centrifuged at 10,000 × *g* for 3 min, the supernatants were aspirated under vacuum and the pellets were washed twice with 1 ml buffer A. Pellets were re-suspended in 100 μl buffer A in the absence and presence of CGP-36742 (10^{-9}–10^{-4} M) or somatostatin (10^{-10}–10^{-5} M), and were incubated at 37 °C for 10 min (Iversen et al., 1978) followed by centrifugation at 10,000 × *g* for 5 min. Thereafter, samples of supernatants (2 × 30 μl) were taken, and the pellets were washed twice with 1 ml buffer A and were re-suspended in 30 μl buffer A. Sodium dodecyl sulphate solution (10%, 150 μl) was added to the supernatant samples and the pellet suspensions. After vortexing, the samples were mixed with 900 μl HiSafe 3 LKB scintillation fluid and were left in the dark overnight. Radioactivity was counted for 5 min in a Wallac WinSpectral α/β 1414 liquid scintillation counter (Perkin Elmer Life Sciences).

Release and binding processes occurred simultaneously during the measurement with the synaptosomal fraction. To estimate the 'true' release component, the binding component was measured and subtracted from the amount of ^{3}H, ^{14}C and ^{125}I detected in the supernatants and the pellets of intact synaptosomes. The release and binding experiments were performed identically except for the use of different preparations, i.e. intact synaptosomes (release) vs. disrupted membranes isolated from the synaptosomes (binding).

Since the preparation containing intact synaptosomes was expected to metabolise somatostatin more actively than brain membrane homogenates (Srikant and Patel, 1981), we compared the stability of [^{125}I]somatostatin with and without (control) incubation with synaptosomes and synaptosomal membranes in buffer A, i.e. in the presence of enzyme inhibitors (1 mM phenyl-methyl-sulphonyl-fluoride, 0.01 mg/ml aprotinin, 0.02 μg/ml bacitracin). At the end of the incubation, the free radioligand was separated from bound radioligand by centrifugation (10,000 × *g* for 5 min) and subjected to thin layer chromatography to determine the percentage of [^{125}I]tyrosine formed during proteolytic degradation of [^{125}I]somatostatin. Lastly, the L-Tyr standard (10 mg/ml, mixed in 96% ethanol and dissolved by adding 1 N HCl) was eluted with samples of supernatants from incubations with synaptosomes and synaptosomal membranes, using a 16:4:4 mixture of ethylacetate, acetic acid and water as eluent. Under the conditions that were used in the experiments, [^{125}I]somatostatin was relatively less degraded in supernatant samples of synaptosomal membranes (25% of the control) whereas it was more susceptible to damage, with only 50% of the radioactivity remaining as intact ligand after incubation with synaptosomes.

2.3. Electrophysiological measurement from hippocampal CA1 pyramidal cells

Horizontal hippocampal slices (400 μm) from Wistar rats (100–150 g) were prepared using Vibroslice (Campden Instruments, UK) in ice-cold medium. Slices were left to

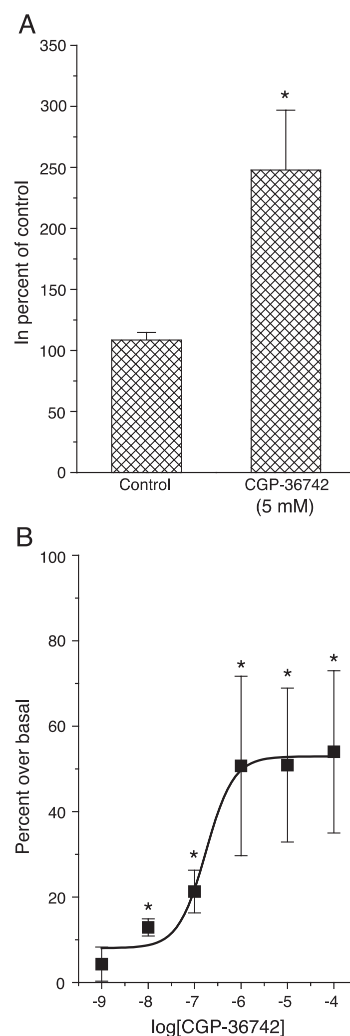

Fig. 2. Effect of CGP-36742 on hippocampal somatostatin level in vivo and in vitro. (A) Effect of CGP-36742 (5 mM applied locally via the microdialysis probe) on the extracellular somatostatin concentration in the ventral hippocampus of halothane anaesthetised rats in vivo. Data from ($n = 6$) experiments are expressed as mean percentage (± S.E.M.) of control samples collected before infusion of 5 mM CGP 36742 in ACSF. Student's *t*-test indicated significant increase in comparison with control rats at $^{*}P < 0.05$. (B) Effect of CGP-36742 on the basal outflux of [3-[^{125}I]iodotyrosyl^{11}]somatostatin 14 from hippocampal synaptosomes in vitro. Data from ($n = 4$) experiments are expressed as mean percent change (± S.E.M.) above basal release. The outflux was significantly enhanced (Student's *t*-test, $^{*}P < 0.05$) from 10^{-8} M to 10^{-4} CGP-36742.

recover at room temperature for about 1 h, and were thereafter transferred into an interface-type slice bath and perfused with 35 °C oxygenated (95% O_2, 5% CO_2) medium of composition: 134 mM NaCl, 2 mM KCl, 1.25 mM KH_2PO_4, 2 mM $CaCl_2$, 1 mM $MgSO_4$, 16 mM $NaHCO_3$ and 10 mM glucose. Intracellular glass microelectrodes were filled with 1 M potassium acetate. Current-clamp recordings were made using an Axoclamp 2A (Axon Instruments, USA) amplifier and stored on DAT Biologic tape recorder (Intracell, UK) for later analysis using Pclamp software (Axon Instruments). All drugs were bath applied. The results are presented as means ± S.D.

For statistical analysis of data we used Student's *t*-test and one-way analysis of variances (ANOVA) test. Percent change from the control value was considered significant at $P < 0.05$ levels.

3. Results

3.1. Effect of CGP-36742 on the extracellular concentration of somatostatin in the hippocampus in vivo

Local application of 5 mM CGP-36742 via the microdialysis probe increased the extracellular concentration of somatostatin by 149 ± 44% in the hippocampus 200 min after the start of drug application ($n = 6$, $P < 0.05$; Fig. 2A). The average somatostatin concentration of control samples was 1.8 ± 0.4 ng/ml in the hippocampus of halothane-anaesthetised rats.

3.2. Effect of CGP-36742 on the outflow of [^{125}I]somatostatin from hippocampal synaptosomes

After incubation of synaptosomes with 0.75 nM [^{125}I]somatostatin for 45 min at 37 °C (loading), about half of the radioligand was recovered from the pellet (45 ± 12%, $n = 4$, $P < 0.05$). After freezing, the loaded preparation released 40 ± 18% of the basal radioactivity ($n = 4$, $P < 0.05$), indicating that [^{125}I]somatostatin had been internalised in hippocampal synaptosomes. KCl (50 mM) evoked a 78 ± 35% ($n = 8$, $P < 0.05$) increase over basal outflow of [^{125}I]somatostatin in hippocampal synaptosomes. The effect was not dependent on the presence (KCl loading) or absence (basal loading) of 50 mM KCl during loading. CGP-36742 (10^{-8}–10^{-4} M) increased the basal outflow of [^{125}I]somatostatin from hippocampal synaptosomes with an EC_{50} value of 0.2 μM ($n = 4$, $P < 0.05$; Fig. 2B).

3.3. Effect of CGP-36742 and somatostatin on the release of [^{14}C]Glu and [^{3}H]GABA from hippocampal synaptosomes

CGP-36742 (10^{-8}–10^{-4} M) increased the basal release of [^{3}H]GABA and [^{14}C]Glu ($EC_{50} < 0.1$ μM, $P < 0.05$) from the hippocampal synaptosomal fraction (Fig. 3A). After preincubation with 0.75 nM [^{125}I]somatostatin, CGP-36742 (10^{-8}–10^{-4} M) reduced the basal release of [^{3}H]GABA from the hippocampal synaptosomal fraction ($EC_{50} < 0.1$ μM, $n = 5$, $P < 0.05$; Fig. 3C). Also, under the same condition, the increase in the basal release of [^{14}C]Glu in response to CGP-36742 was suppressed (Fig. 3C). Somatostatin alone (10^{-10}–10^{-5} M) reduced the basal release of [^{3}H]GABA and increased that of [^{14}C]Glu from the hippocampal synaptosomal fraction ($EC_{50} < 1$ nM, $n = 4$, $P < 0.05$; Fig. 3B).

3.4. Effect of CGP-36742 on passive membrane properties of CA1 pyramidal cells in hippocampal slices

Somatostatin (1 μm) caused hyperpolarisation (3.7 ± 1.0 mV, $n = 6$, $P < 0.05$) associated with a decrease in the input resistance (19.5 ± 6.6%, $n = 6$, $P < 0.05$). CGP-36742 (5 μM)

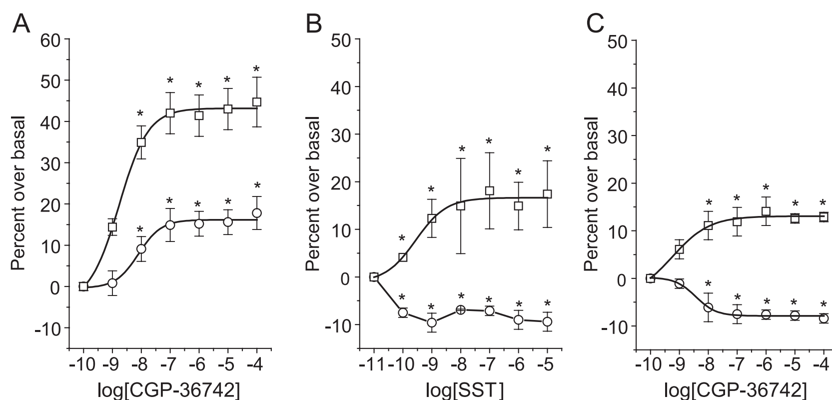

Fig. 3. Effect of CGP-36742 (10^{-9}–10^{-4} M) and somatostatin (10^{-10}–10^{-5} M) on the basal release of [^{14}C]Glu (squares) and [^{3}H]GABA (circles) from hippocampal synaptosomes. (A) In the absence of [^{125}I]somatostatin, CGP-36742 (10^{-8}–10^{-4} M) increased the basal release of [^{3}H]GABA and [^{14}C]Glu ($EC_{50} < 0.1$ μM, $n = 5$, $P < 0.05$) from hippocampal synaptosomes. Data from ($n = 5$) experiments are expressed as mean percent change (± S.E.M.) above basal release (*$P < 0.05$). (B) Somatostatin alone (10^{-10}–10^{-5} M) reduced the basal release of [^{3}H]GABA and increased that of [^{14}C]Glu from the hippocampal synaptosomal fraction ($EC_{50} < 1$ nM, $n = 4$, *$P < 0.05$). (C) After incubation with 0.75 nM [^{125}I]somatostatin, CGP-36742 (10^{-8}–10^{-4} M) reduced the basal release of [^{3}H]GABA from the hippocampal synaptosomal fraction ($EC_{50} < 0.1$ μM, $n = 5$, *$P < 0.05$). Under these conditions, the increase in the basal release of [^{14}C]Glu in response to CGP-36742 application was suppressed.

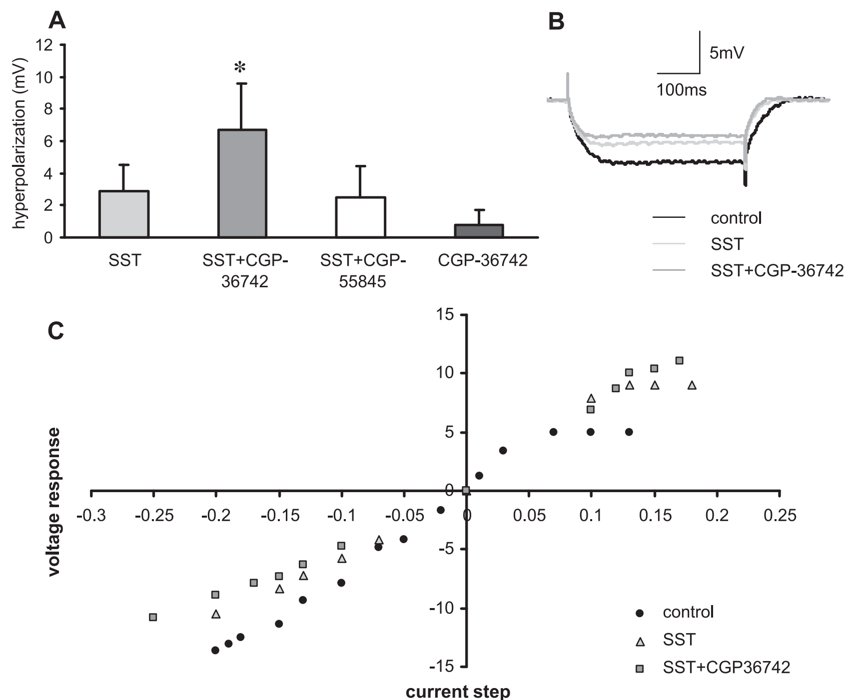

Fig. 4. Effect of somatostatin and $GABA_B$ receptor antagonist CGP-36742, CGP-55845 on the passive membrane properties of hippocampal CA1 pyramidal cells. (A) Hyperpolarisation evoked by the application of somatostatin and $GABA_B$ receptor antagonists. The somatostatin-induced membrane hyperpolarisation was elevated by CGP-36742 (*$P<0.05$) but not by CGP-55845. CGP-36742 alone had no effect on the resting membrane potential. (B) Superimposed traces show the decrease in input resistance after somatostatin application and the coapplication of somatostatin with CGP-36742. All traces are the average of 10 consecutive traces. (C) Voltage responses of a CA1 pyramidal cell to different current steps in control and somatostatin- or somatostatin and CGP-36742-containing solutions. The application of somatostatin or somatostatin and CGP-36742 decreased the voltage response to hyperpolarising current steps. The voltage changes are given in mV, the current steps in nA.

when applied in the continuing presence of somatostatin enhanced the hyperpolarising response (7.3 ± 3.4 mV compared to control, $n=5$, $P<0.05$), and further decreased the input resistance ($28.7 \pm 6.6\%$ compared to control, $n=5$, $P<0.05$). CGP-36742 alone (up to 10 μM) had no effect on the passive membrane properties (0.75 ± 0.96 mV hyperpolarisation, $n=3$). In contrast to the effect of CGP-36742, $GABA_B$ receptor antagonist without a known interaction with somatostatin release (Bonanno et al., 1999), [3-[[1-(*S*)-(3,4-dichlorophenyl)-ethyl]amino]-2-(*S*)-hydroxypropyl-phenylmethylphosphinic acid hydrochloride (CGP-55845A) (10 μM), alone or after somatostatin did not induce any change in the resting membrane potential or input resistance ($n=4$, Fig. 4).

4. Discussion

Here, we present evidence that the extracellular level of somatostatin can be regulated by $GABA_B$ receptors in vivo in the hippocampus, a brain area with a crucial role in memory formation. Our in vivo and in vitro results suggest an interaction between hippocampal $GABA_B$ and somatostatin receptor-mediated neurotransmission. First, the blockade of CGP-36742-sensitive $GABA_B$ receptors altered the somatostatin level and somatostatin release: CGP-36742 increased the level of extracellular somatostatin in vivo in the hippocampus of anaesthetised rats, and increased the outflow of [^{125}I]somatostatin from hippocampal nerve endings. Second, somatostatin affected basal GABA and Glu release: it decreased the basal release of GABA, and enhanced the basal release of Glu. Third, pre- and postsynaptic effects of CGP-36742 were altered in the presence of somatostatin: the enhancement of basal GABA release in response to CGP-36742 was inverted to a net decrease whereby basal Glu release remained elevated; and CGP-36742 increased a somatostatin-activated K^+ conductance in hippocampal slices. These data suggest pre- and postsynaptic 'cross-talks' (Nicholls, 1994) between coexisting $GABA_B$ and somatostatin receptors in the rat hippocampus. Inhibition of GABA release by a presynaptic negative feedback mechanism via activation of somatostatin receptors is shown in Fig. 5A. The facilitation of the hyperpolarising effect of somatostatin may occur via direct or indirect activation of postsynaptic somatostatin receptors (Fig. 5B). The increased excitation due to presynaptic decrease in GABA release together with the postsynaptic hyperpolarisation may selectively facilitate strong excitatory inputs pertinent to memory formation.

Receptor-mediated internalisation of somatostatin has been observed in rat cortical and hippocampal neurones (Stroh et al., 2000). The endocytosed receptor and bound

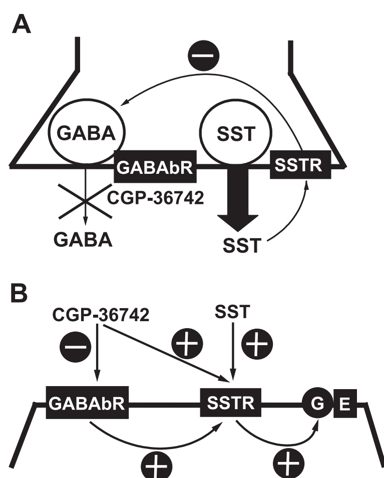

Fig. 5. A simplified model of the functional 'cross-talk' between coexisting $GABA_B$ and somatostatin receptors. (A) Presynaptic 'cross-talk' between somatostatin and GABA cotransmitters. In the rat hippocampus, blockade of $GABA_B$ receptors increased somatostatin release, while somatostatin inhibited GABA release via activation of somatostatin receptors. (B) Postsynaptic interactions of $GABA_B$ and somatostatin receptors. In the rat hippocampus, the $GABA_B$ receptor antagonist, CGP-36742 increased somatostatin receptor activation either directly or via $GABA_B$-somatostatin receptor 'cross-talk'. GABAbR-$GABA_B$ receptor, SSTR-somatostatin receptor.

somatostatin may be either metabolised or recycled back to the cell surface (Koenig et al., 1998). These findings suggest that stimulation of the [^{125}I]somatostatin outflow by CGP-36742 was mediated in part through modulation of the recycling rate of somatostatin in the hippocampal synaptosomal fraction; however, a direct presynaptic effect on somatostatin-containing nerve endings cannot be excluded. The concentration of CGP-36742 that enhanced [^{125}I]somatostatin outflow from purified hippocampal synaptosomes (this study, EC_{50} = 0.2 μM) and that antagonised the baclofen-induced inhibition of the evoked release of somatostatin-like immunoreactivity in cortical synaptosomes (Bonanno et al., 1999; IC_{50} = 0.14 μM) and hippocampal slices (Pittaluga et al., 2001, 1 μM) was similar. It is noteworthy that these in vitro effective concentrations of CGP-36742 on somatostatin release (0.1–1 μM) are similar to those low oral doses (0.03–10 mg/kg) affecting cognitive performance in vivo (Mondadori et al., 1996).

CGP-36742 increased the basal release of GABA and Glu in the same range, accompanied by the activation of basal somatostatin release. Regulation of the release of GABA and Glu was shown by using different $GABA_B$ receptor antagonists (Waldmeier et al., 1994; Froestl et al., 1995; Pozza et al., 1999) including CGP-36742 (Froestl et al., 1995; Teoh et al., 1996; Nyitrai et al., 1999; Pozza et al., 1999). In contrast to previous results (Bonanno et al., 1999), we did not find specific differences in the affinity of CGP-36742 in modulating the release of somatostatin or Glu or GABA. Our data suggest that CGP-36742-sensitive, high-affinity $GABA_B$ receptors in the hippocampus may control the release of GABA and Glu together with somatostatin.

Somatostatin receptor activation is suggested to decrease evoked GABA release from hippocampal slices (Meyer et al., 1989). In accordance, we found that somatostatin caused a net decrease in the basal release of GABA at 0.1 nM. Moreover, preincubation with somatostatin reversed the effect of CGP-36742 on the release of GABA. The effect of somatostatin on GABA release was comparable to that of CGP-36742 after preincubation with somatostatin at concentrations two orders of magnitude higher. The increase in basal Glu release observed after CGP-36742 application was reduced by somatostatin, but still remained elevated.

We investigated the postsynaptic action of somatostatin and the effect of CGP-36742 therein. To this end, the effect of CGP-36742 on postsynaptic somatostatin receptors was measured in hippocampal CA1 pyramidal cells. In hippocampal slices, CGP-36742 (5 μM) enhanced the postsynaptic effect of somatostatin, but was not effective when applied alone. Somatostatin was reported to hyperpolarise CA1 pyramidal neurons by activating various K^+ conductances (Moore et al., 1988; Sodickson and Bean, 1998), including the interaction with $GABA_B$ receptor-mediated K^+ channels (Sodickson and Bean, 1998). To explain the enhancement of the postsynaptic effect of somatostatin, indirect activation of somatostatin receptors by blockade of the $GABA_B$ receptors can be supposed. However, a direct postsynaptic interaction of CGP-36742 with activated somatostatin receptors cannot be excluded (Fig. 5B). Thus, direct or indirect disinhibitory mechanisms may underlie the enhancement of postsynaptic somatostatin receptor function by CGP-36742. In contrast, CGP-55845A, a high-affinity $GABA_B$ receptor antagonist (Froestl et al., 1992), did not change the postsynaptic effect of somatostatin, supporting the notion that the modulation of somatostatin*ergic* neurotransmission achieved via a CGP-36742-sensitive $GABA_B$ receptor subtype.

Previous results showed controversial somatostatin effects. Somatostatin was ineffective in modifying excitatory synaptic potentials but decreased GABAergic potentials in slices from the CA1 area of the hippocampus (Scharfman and Schwartzkroin, 1989; Xie and Sastry, 1992). In contrast, Tallent and Siggins (1997) reported that somatostatin reduced the pharmacologically isolated AMPA and NMDA receptor-mediated excitatory synaptic currents in the CA1 region of the rat hippocampus. Similarly, Boehm and Betz (1997) found that somatostatin inhibited excitatory synaptic currents, without changing GABAergic currents in single hippocampal neuron microcultures. However, even within one structure the effect of somatostatin on the glutamate peak current may range from a decrease to an increase as well as no effect at all (Gardette et al., 1995). Our proposal that CGP-36742 acts via the stimulation of pre- and postsynaptic somatostatin receptors (Fig. 5) suggests that the overall effect of somatostatin depends on the contribution of pre- and postsynaptic mechanisms in the rat hippocampus.

We suggest that activation of somatostatin receptors can modulate the effect of $GABA_B$ receptors, indicating a disinhibitory relationship between somatostatin and GABA

in the hippocampus. Allosteric sensitisation of somatostatin receptors by CGP-36742 assumes modes of action other than mere $GABA_B$ receptor inhibition and discloses 'cross-talk' between $GABA_B$ and somatostatin receptors that could account, at least in part, for the 'cognition enhancing' properties of CGP-36742. The unique mechanism by which increased levels of extracellular somatostatin modulate $GABA_B$ receptor function remains to be determined. Because $GABA_B$ and somatostatin receptors are both coupled to heterotrimeric G proteins, possibilities for the indirect modulation of such a system are multiplied by the number of potential coupling pathways. For direct modes of interaction, we may conjecture the existence of a chimera dimer consisting of CGP-36742-sensitive $GABA_B$ and somatostatin subunits, similar to the chimeric dimer of somatostatin and dopamine receptor subunits which has enhanced functional activity (Rocheville et al., 2000). We conclude that inhibition of $GABA_B$ receptors by CGP-36742 increases extracellular levels of somatostatin, which may in turn reduce GABA levels in the hippocampus.

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