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Human brain somatostatin release from isolated cortical nerve endings and its modulation through $GABA_B$ receptors

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1 The release of somatostatin-like immunoreactivity (SRIF-LI) in the human brain was studied in synaptosomal preparations from fresh neocortical specimens obtained from patients undergoing neurosurgery to remove deeply sited tumours.

2 The basal outflow of SRIF-LI from superfused synaptosomes was increased about 3 fold during exposure to a depolarizing medium containing 15 mM KCl. The K^+-evoked overflow of SRIF-LI was almost totally dependent on the presence of Ca^{2+} in the superfusion medium.

3 The $GABA_B$ receptor agonist, (−)-baclofen (0.3−100 μM), inhibited the overflow of SRIF-LI in a concentration-dependent manner ($EC_{50} = 1.84 \pm 0.20$ μM; maximal effect: about 50%). The novel $GABA_B$ receptor ligand, 3-aminopropyl(difluoromethyl)phosphinic acid (CGP 47656) mimicked (−)-baclofen in inhibiting the SRIF-LI overflow ($EC_{50} = 3.06 \pm 0.52$ μM; maximal effect: about 50%), whereas the $GABA_A$ receptor agonist, muscimol, was ineffective up to 100 μM.

4 The inhibition by 10 μM (−)-baclofen of the K^+-evoked SRIF-LI overflow was concentration-dependently prevented by two selective $GABA_B$ receptor antagonists, 3-amino-propyl (diethoxymethyl)-phosphinic acid (CGP 35348) ($IC_{50} = 24.40 \pm 2.52$ μM) and [3-[[(3,4-dichlorophenyl) methyl]amino]propyl] (diethoxymethyl) phosphinic acid (CGP 52432) ($IC_{50} = 0.06 \pm 0.005$ μM).

5 The inhibition of SRIF-LI overflow caused by 10 μM CGP 47656 was abolished by 1 μM CGP 52432.

6 When human synaptosomes were labelled with [^{3}H]-GABA and depolarized in superfusion with 15 mM KCl, the inhibition by 10 μM (−)-baclofen of the depolarization-evoked [^{3}H]-GABA overflow was largely prevented by 10 μM CGP 47656 which therefore behaved as an autoreceptor antagonist.

7 In conclusion: (a) the characteristics of SRIF-LI release from synaptosomal preparations of human neocortex are compatible with a neuronal origin; (b) the nerve terminals releasing the neuropeptide possess inhibitory receptors of the $GABA_B$ type; (c) these receptors differ pharmacologically from the $GABA_B$ autoreceptors present on human neocortex nerve terminals since the latter have been shown to be CGP 35348-insensitive but can be blocked by CGP 47656.

Keywords: Human cerebrocortex; somatostatin release; $GABA_B$ receptors; presynaptic receptors; (−)-baclofen; CGP 35348; CGP 52432; CGP 47656; epilepsy; cognition.

Introduction

Somatostatin (SRIF) was first characterized as a growth hormone release-inhibiting factor from bovine hypothalamus (Brazeau *et al.*, 1973). Subsequently, it has been shown that SRIF is widely distributed throughout the mammalian CNS (Brownstein *et al.*, 1975; Hendry *et al.*, 1984b). The different regional distribution of SRIF in the animal brain, its concentration in nerve endings (Epelbaum *et al.*, 1977) from which the peptide can be released by depolarizing stimuli *in vitro* (Iversen *et al.*, 1978; Bennet *et al.*, 1979; Bonanno *et al.*, 1991a,b) and *in vivo* (Vezzani *et al.*, 1993) and the existence of SRIF receptor subtypes (Hoyer *et al.*, 1994; Reisine & Bell, 1995) support the notion that SRIF may play a role as neurotransmitter in addition to a more classical neuroendocrine role.

SRIF has been implicated in a number of CNS physiological functions, including cognition, and in some neurological and neuropsychiatric disorders such as Alzheimer's disease and epilepsy. In rats, depletion of SRIF induced by cysteamine is known to produce cognitive impairment (Haroutunian *et al.*, 1987; DeNoble *et al.*, 1989; Schettini, 1991). Reduction of SRIF levels in the cortical areas is an autoptic finding typical of Alzheimer's disease (Davies *et al.*, 1980; Dawbarn *et al.*, 1986; Chan-Palay, 1987; Gabriel *et al.*, 1993), a condition characterized by profound cognitive deficits. As to epilepsy, whether the augmentation of SRIF release seen in slices from kindled rats (Vezzani *et al.*, 1992) reflects a proconvulsive action of the neuropeptide (Havlicek & Friesen, 1979; Marksteiner & Sperk, 1988) or a 'defence' mechanism, as proposed by Manfridi *et al.* (1991), remains to be established.

Somatostatin is colocalized with GABA in subgroups of neurones in many regions of the CNS including the cerebral cortex and the hippocampus (Hendry *et al.*, 1984a; Somogyi *et al.*, 1984). The study of GABA-SRIF interactions is therefore of interest, also in view of the recently proposed involvement of $GABA_B$ receptors in epilepsy and in cognitive processes. The $GABA_B$ receptor agonist, (−)-baclofen, has been reported to display either anticonvulsant or proconvulsive effects depending on the experimental conditions (see review by Mott and Lewis, 1994). Some $GABA_B$ receptor antagonists have been found to facilitate seizures, while others are ineffective (Fröstl and Marescaux, personal communication). $GABA_B$ receptor antagonists endowed with remarkable cognition enhancing activities have been described (Mondadori *et al.*, 1993; Fröstl *et al.*, 1995b).

The release of SRIF-like immunoreactivity (SRIF-LI) from superfused rat cerebrocortex synaptosomes was found to be inhibited by GABA or (−)-baclofen acting at $GABA_B$ receptors localized on SRIF-releasing axon terminals (Bonanno

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et al., 1991a). More recently, these receptors were classified as a pharmacological subtype different from other release-regulating $GABA_B$ receptors present on rat neocortex nerve endings releasing GABA, glutamate or cholecystokinin (Gemignani *et al.*, 1994).

It had been shown that, in the human brain, the majority of SRIF is present in the cerebral cortex (Emson *et al.*, 1981). However, no information is available on SRIF release and its modulation in the human brain. It seemed therefore important to establish: (a) whether SRIF release could be monitored using human brain tissue; (b) if a $GABA_B$ receptor-mediated interaction between GABA and SRIF also exists in the human brain; (c) if the GABA-SRIF interaction involves pharmacologically distinct subtypes of the $GABA_B$ receptor.

Methods

Human specimens

Human cerebral cortex specimens were obtained from patients undergoing neurosurgery to reach deeply located tumours. Samples represented parts of frontal (10), temporal (5), parietal (4) and occipital (2) lobes and were obtained from 8 male and 13 female patients (aged 43–75 years). The tissues were processed separately on the day of surgery. See Bonanno *et al.*, (1989) for premedication and anaesthesia details.

Synaptosome preparation

Immediately after removal, the tissue was placed in a physiological salt solution (see below) kept at 0–4°C and a crude synaptosomal preparation obtained within 60–90 min. The tissue was homogenized in 40 volumes of 0.32 M sucrose, buffered at pH 7.4 with phosphate, using a glass-Teflon tissue grinder (clearance 0.25 mm, 12 up-down strokes in about 1 min, 900 r.p.m.). The homogenate was first centrifuged at 1000 *g* for 5 min; synaptosomes were isolated from the supernatant by centrifugation at 12000 *g* for 20 min. The synaptosomal pellet was then resuspended in a physiological medium having the following composition (mM): NaCl, 125, KCl, 3, $MgSO_4$, 1.2, $CaCl_2$ 1.2, NaH_2PO_4, 1, $NaHCO_3$ 22, glucose 10 (aeration with 95% O_2 and 5% CO_2); pH 7.2–7.4. Protein was determined according to Bradford (1976).

Release experiments

Identical aliquots of the synaptosomal suspensions (0.6–0.8 mg of protein in the different experiments) were distributed on microporous filters placed at the bottom of 20 parallel superfusion chambers maintained at 37°C (Raiteri *et al.*, 1974). Superfusion was then started with standard medium supplemented with 0.1% Polypep (Sigma) at a rate of 0.5 ml min^{-1} and continued for a total of 48 min. After 36 min to equilibrate the system, fractions were collected according to the following scheme: two 3-min fractions (basal release) before and after one 6-min fraction (evoked release). A 90 s period of depolarization was applied after the first fraction had been collected. KCl (15 mM) was used to depolarize synaptosomes, NaCl substituting for an equimolar concentration of KCl. Synaptosomes were exposed to (−)-baclofen, muscimol or to 3-amino-propyl(difluoromethyl)phosphinic acid (CGP 47656) at the end of the first fraction collected. 3-Amino-propyl(diethoxymethyl)phosphinic acid (CGP 35348) or 3-[[((3,4-dichlorophenyl)methyl)amino]propyl]-(diethoxymethyl)phosphinic acid (CGP 52432) was added to the superfusion medium 8 min before (−)-baclofen. Superfusate fractions were collected into vials containing acetic acid (final concentration 1 M) and aliquots were boiled to ensure peptidase inhibition. Superfusate fractions were freeze dried and analyzed for their SRIF-LI content by radioimmunoassay. The SRIF antiserum used was characterized in details previously (Dawbarn *et al.*, 1985). The freeze dried samples were reconstituted in phosphate buffer (50 mM; pH 7.4) containing 0.1% Polypep and radioimmunoassayed. The assay sensitivity was 0.5 fmol per tube and the curve was linear between 1 and 150 fmol using [^{125}I]-Tyr^{11}-somatostatin-14 as a tracer.

In one set of experiments the effect of CGP 47656 on the depolarization-evoked release of [^{3}H]-GABA was assayed. Synaptosomes were incubated (15 min at 37°C) with 0.04 μM [^{3}H]-GABA and then layered on microporus filters (0.2–0.4 mg of protein in the different experiments). Superfusion was carried out as in the case of SRIF-LI release with the superfusion medium not containing Polypep. Aminooxyacetic acid (50 μM) was present throughout to prevent [^{3}H]-GABA metabolism. CGP 47656 was added to the superfusion medium either at the end of the first fraction collected, when tested as an agonist, or 8 min before (−)-baclofen, when tested as an antagonist. Radioactivity was measured in each fraction collected and in the superfused filters.

The radioactivity released has been assumed to consist largely of authentic [^{3}H]-GABA considering that the experiments were performed in the presence of aminooxyacetic acid and that the K^+ (15 mM)-evoked tritium overflow from human cortex synaptosomes prelabelled with [^{3}H]-GABA was 90% Ca^{2+}-dependent (Bonanno *et al.*, 1989). As to somatostatin, the term SRIF-LI has been used throughout the text although the human neocortex was reported to contain exclusively SRIF-14 (Emson *et al.*, 1981).

Calculation

The amount of SRIF-LI released in each fraction was expressed as fmol mg^{-1} protein. The radioactivity in the fractions collected in the experiments of [^{3}H]-GABA release was expressed as a percentage of the total tritium content at the start of the respective collection period. The depolarization-evoked overflow was estimated by subtracting the peptide or the percentage tritium content of the basal release from the release evoked in the 6-min fraction collected during and after the depolarization period. The drug effects were evaluated as the ratio of the depolarization-evoked overflow calculated in the presence of the drugs vs. that calculated under control conditions. Appropriate controls with antagonists were always run in parallel. The effects of the $GABA_B$ receptor antagonists were expressed as a percentage of the inhibitory effect of (−)-baclofen. EC_{50} and IC_{50} values were determined by analyzing the experimental data using a function fitting routine provided by the software Sigma Plot, Windows version 2.0. Student's two-tailed *t* test was used for comparison of two mean values.

Chemicals

[^{125}I]-Tyr^{11}-somatostatin-14 and [^{3}H]-GABA (specific activity, 90 Ci $mmol^{-1}$) were obtained from Amersham Radiochemical Centre (Buckinghamshire). Muscimol was purchased from Sigma Chemical (St. Louis, MO, U.S.A.). CGP 35348, CGP 52432, CGP 47656 and (−)-baclofen were kindly given by Ciba Geigy (Basel, Switzerland).

Results

When human neocortical synaptosomes were exposed in superfusion to 15 mM K^+ a large increase of SRIF-LI efflux was observed. The basal release of the neuropeptide in the two 3-min fractions collected before and after the 6-min fraction comprising the evoked release amounted to 4.20 ± 0.39 and to 3.12 ± 0.24 fmol mg^{-1} protein ($n = 21$), respectively. The SRIF-LI content in the 6-min fraction of the evoked release was 22.95 ± 1.71 fmol mg^{-1} protein ($n = 21$). Thus, according to the calculation described in the Methods section, the K^+-evoked overflow amounted to 15.63 ± 1.21 fmol mg^{-1} protein ($n = 21$).

A set of experiments was performed to assess the Ca^{2+}-dependency of the K^+-evoked SRIF-LI overflow. As shown in

Figure 1, removal of Ca^{2+} ions from the superfusion medium completely prevented the SRIF-LI overflow elicited by depolarization with 15 mM K^+.

Addition to the medium of (−)-baclofen (0.3−100 μM), concomitantly with high K^+, inhibited concentration-dependently the SRIF-LI overflow (Figure 2). The maximal effect (about 50%) was produced by 10 μM of the agonist. The EC_{50} value was 1.84±0.20 μM. Muscimol, tested at 100 μM, did not affect the SRIF-LI overflow. Figure 2 also shows that the K^+-evoked overflow of SRIF-LI was inhibited by CGP 47656 (EC_{50} = 3.06±0.52 μM); the efficacy of CGP 47656 was almost identical to that of (−)-baclofen.

The inhibition by (−)-baclofen (10 μM) of the K^+-evoked SRIF-LI overflow was then tested against increasing concentrations of two selective $GABA_B$ receptor antagonists, CGP 35348 (Olpe *et al.*, 1990) and CGP 52432 (Fröstl *et al.*, 1992). As shown in Figure 3, both compounds antagonized (−)-baclofen in a concentration-dependent manner, although their affinities differed widely from each other (CGP 35348, IC_{50} = 24.40±2.52 μM; CGP 52432, IC_{50} = 0.06±0.005 μM).

Figure 4 shows that the inhibition of the K^+-evoked overflow of SRIF-LI produced by the maximally effective concentration of CGP 47656 (10 μM) was abolished by 1 μM CGP 52432.

Finally, a set of experiments was performed in which CGP 47656 was tested at the $GABA_B$ autoreceptors. Previously, the depolarization-evoked release of [^{3}H]-GABA from human neocortex synaptosomes prelabelled with the radioactive amino acid had been found to be inhibited by GABA or (−)-baclofen acting at $GABA_B$ autoreceptors (Bonanno *et al.*, 1989). These autoreceptors were then found to be blocked by CGP 52432 but not by CGP 35348 (Fassio *et al.*, 1994). As shown in Table 1, CGP 47656 did not affect significantly the K^+-evoked overflow of [^{3}H]-GABA, up to 100 μM. However, when added at 10 μM to the superfusion medium, CGP 47656 antagonized by about 70% the inhibition of [^{3}H]-GABA overflow caused by 10 μM (−)-baclofen.

None of the compounds used, at the concentrations indicated, modified the basal release of SRIF-LI or of [^{3}H]-GABA. Neither CGP 35348 nor CGP 52432, up to 100 or 3 μM, respectively, affected the K^+-evoked overflow of the neuropeptide (data not shown).

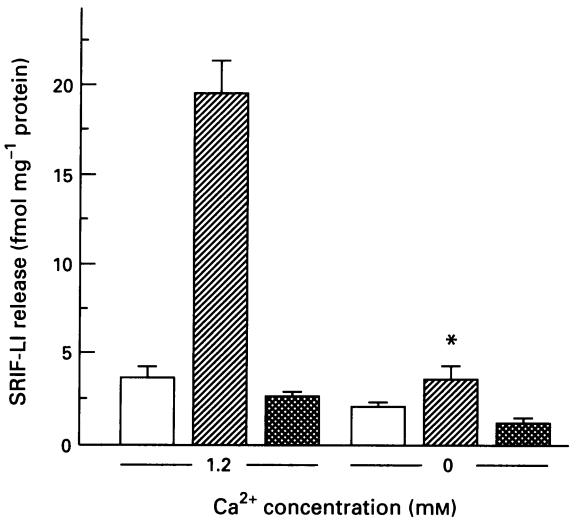

Figure 1 Ca^{2+}-dependence of the SRIF-LI release from human cortical brain synaptosomes. A 90 s period of stimulation was applied after 39 min of superfusion. The Ca^{2+}-free medium was introduced 18 min before. Columns represent the mean of 3 separate experiments run in triplicate with s.e. mean. Open columns: SRIF-LI released in the 3-min fraction collected before the onset of stimulation; hatched columns: SRIF-LI released in the 6-min fraction collected during and after the stimulation period; cross hatched columns: SRIF-LI released in the 3-min fraction collected 6 to 9 min after the onset of stimulation. Statistically significant when compared to the depolarization-evoked release in the presence of 1.2 mM $CaCl_2$ (Student's two-tailed *t* test): $*P<0.001$.

Discussion

In the last decade an increasing number of investigations have focused on neurotransmitter release and its regulation in the human CNS (see review by Raiteri, 1994). SRIF release has however not been investigated in any of these works. Studies on the regional distribution of SRIF in the human brain had

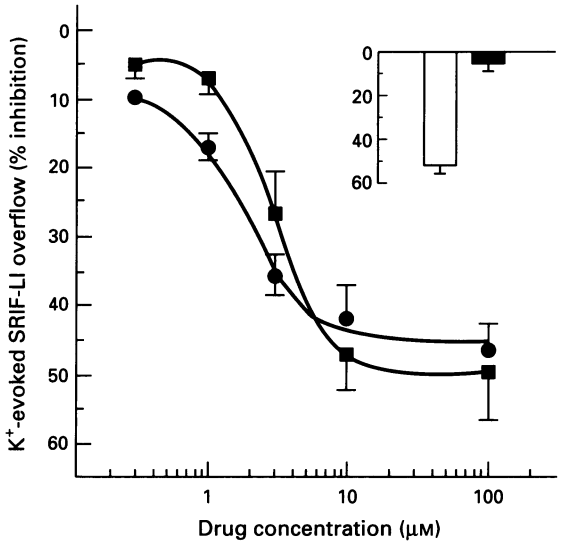

Figure 2 Effect of (−)-baclofen, CGP 47656 or muscimol on the overflow of SRIF-LI evoked by 15 mM K^+ depolarization of human brain cortical synaptosomes. Drugs were added to the superfusion medium concomitantly with the depolarizing stimulus. See Methods for other technical details. Points represent the mean±s.e.mean of 4−12 separate experiments run in triplicate. (●): (−)-baclofen; (■) CGP 47656. *Inset*: Open column: 10 μM (−)-baclofen; solid column: 100 μM muscimol.

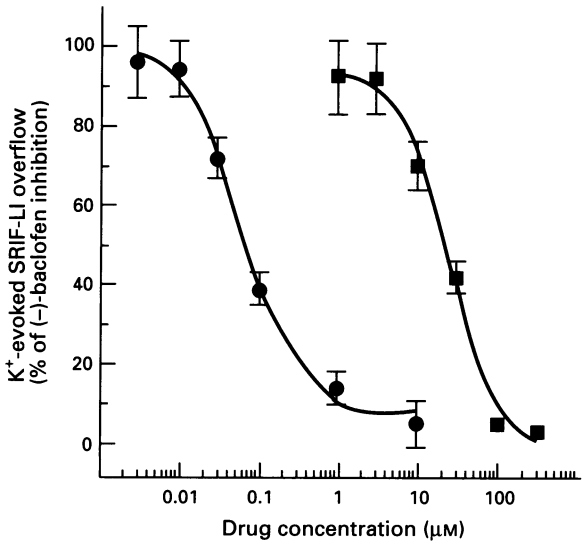

Figure 3 Antagonism by CGP 52432 or CGP 35348 of the 10 μM (−)-baclofen-induced inhibition of the K^+-evoked overflow of SRIF-LI. (−)-Baclofen was added to the superfusion medium concomitantly with the depolarizing stimulus; antagonists 8 min before. See Methods for other technical details. Points represent the mean± s.e.mean of 4−6 separate experiments run in triplicate. (●) CGP 52432; (■) CGP 35348.

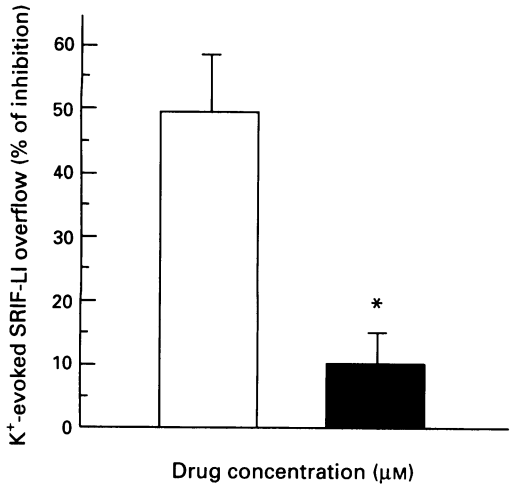

Figure 4 Antagonism by CGP 52432 of the 10 μM CGP 47656-induced inhibition of the K^+-evoked overflow of SRIF-LI. CGP 47656 was added to the superfusion medium concomitantly with the depolarizing stimulus; CGP 52432 8 min before. See Methods for other technical details. Columns represent the mean ± s.e.mean of 3 separate experiments run in triplicate. Open column: 10 μM CGP 47656; solid column: 10 μM CGP 47656 + 1 μM CGP 52432. Statistically significant when compared to the effect of CGP 47656 alone (Student's two-tailed *t* test): * $P<0.001$.

Table 1 Effects of (−)-baclofen, CGP 35348, CGP 47656 and CGP 52432 on the K^+-evoked overflow of [^{3}H]-GABA from human cortical brain synaptosomes

	% inhibition	n
(−)-Baclofen 10 μM	44.3 ± 3.4	5
(−)-Baclofen 10 μM + CGP 52432 1 μM	6.01 ± 0.89*	5
(−)-Baclofen 10 μM	46.6 ± 4.21	6
(−)-Baclofen 10 μM + CGP 35348 100 μM	47.2 ± 2.46	6
(−)-Baclofen 10 μM	40.1 ± 3.12	3
(−)-Baclofen 10 μM + CGP 47656 10 μM	13.8 ± 4.03*	3
CGP 47656 10 μM	5.23 ± 2.98	3
CGP 47656 100 μM	3.09 ± 2.06	3

Values are means ± s.e.means of *n* experiments in triplicate. Statistical significance of changes versus the effect of (−)-baclofen alone were determined by Student's two-tailed *t* test: *$P<0.001$. The effects of CGP 52432 and CGP 35348 were taken from Fassio *et al.* (1994).

shown that the majority of the neuropeptide is present in the cerebral cortex (Emson *et al.*, 1981). This is particularly propitious since the bioptic material that can become available from the neurosurgery largely consists of fresh samples of neocortex.

In this study we monitored release of SRIF-LI from synaptosomes of human neocortex in superfusion. Synaptosomes were chosen instead of slices essentially for the following reasons: (a) nerve terminals are the major sites of transmitter release; (b) superfused synaptosomes lend themselves particularly well to studying mechanisms of transmitter release regulation such as those involving presynaptic receptors; (c) the release of a peptide from stimulated brain slices can be affected through indirect mechanisms inherent in the complexity of the structure, such as the presence of extracellular proteolytic enzymes or of modulatory inputs brought about by other transmitters released during the stimulation of the tissue: these events should be minimized in a thin layer of synaptosomes up-down superfused in which the released substances are rapidly removed; (d) previous works from this laboratory (Bonanno *et al.*, 1991a,b; Gemignani *et al.*, 1994) indicate that, in rats, SRIF-LI release can be conveniently studied from synaptosomal preparations. It was clearly important to compare data from human tissue with those obtained in the rat also in order to establish the validity of the rat as an animal model in future studies.

The first results obtained show that the release of SRIF-LI was significantly enhanced when synaptosomes were exposed to mild depolarization, i.e. to 15 mM KCl. Moreover, the overflow of SRIF-LI elicited by depolarization was strictly dependent on the presence of Ca^{2+} ions in the external medium. These characteristics are very similar to those of the overflow of SRIF-LI evoked by depolarizing stimuli from rat brain tissues (Iversen *et al.*, 1978; Bonanno *et al.*, 1991b). It should be added that the overflow of SRIF-LI provoked by 15 mM K^+ in rat neocortex synaptosomes was in part (50%) sensitive to tetrodotoxin (Bonanno *et al.*, 1991b). Altogether these data support the idea that SRIF can be neuronally released as a neurotransmitter also in the human brain.

The depolarization-evoked overflow of SRIF-LI was inhibited by the selective $GABA_B$ receptor agonist, (−)-baclofen. The characteristics of the superfusion technique employed (a thin layer of synaptosomes up-down superfused) suggest a direct action of (−)-baclofen at SRIF-releasing nerve terminals. The blockade of the (−)-baclofen effect by CGP 35348 and CGP 52432, two selective $GABA_B$ receptor antagonists (Olpe *et al.*, 1990; Fröstl *et al.*, 1992), indicates that (−)-baclofen acts at receptors of the $GABA_B$ type. Thus SRIF-releasing nerve terminals in the human neocortex appear to possess $GABA_B$ receptors mediating inhibition of SRIF release evoked by depolarization. The data are compatible with the existence, in the human cerebrocortex, of a functional link between GABA and SRIF mediated through $GABA_B$ receptors. As previously mentioned, GABA and SRIF coexist in some brain cortical neurones (Hendry *et al.*, 1984a); therefore, whether the GABA possibly regulating *in vivo* the release of SRIF originates from SRIF-negative GABAergic neurones or from neurones co-storing GABA and SRIF is difficult to say. Moreover, the possibility that the release of GABA and SRIF from the neurones co-storing the two transmitters is differentially regulated by two $GABA_B$ receptors sited on the same terminal cannot be excluded. However, it should be considered that the total population of GABAergic neurones is substantially larger than the SRIF-containing subgroup. In any case, the term heteroreceptor will be used in the remainder of the text to indicate the $GABA_B$ receptor regulating the evoked release of SRIF.

It is now well recognized that $GABA_B$ receptors are pharmacologically heterogeneous (Bonanno and Raiteri, 1993; Bowery, 1993; Mott and Lewis, 1994; Banerjee and Snead, 1995). As shown in Figure 2, the $GABA_B$ heteroreceptors regulating SRIF release in human neocortex can be activated not only by (−)-baclofen, but also by the novel selective $GABA_B$ receptor 'ligand' CGP 47656 (Gemignani *et al.*, 1994; Fröstl *et al.*, 1995a). This compound appears therefore to be a full heteroreceptor agonist. However, CGP 47656 was unable to inhibit the K^+-evoked overflow of [^{3}H]-GABA from human synaptosomes; instead it prevented the inhibitory effect of (−)-baclofen on the [^{3}H]-GABA overflow, thus behaving as a $GABA_B$ autoreceptor antagonist. Previously, the human autoreceptors had been shown to be CGP 35348-insensitive (Fassio *et al.*, 1994). Thus, in the human neocortex, the $GABA_B$ autoreceptors sited on GABAergic terminals (insensitive to CGP 35348; blocked by CGP 47656) and the $GABA_B$ heteroreceptors sited on SRIF-releasing terminals (blocked by CGP 35348; activated by CGP 47656) appear to represent two pharmacologically distinct subtypes of the $GABA_B$ receptor. This is the first work showing the existence in the human brain of two $GABA_B$ receptors having distinct function and pharmacological profiles.

It seems important to note that human $GABA_B$ autoreceptors and heteroreceptors on SRIF terminals do not seem to differ from the corresponding receptors found in rats (cf. with Gemignani *et al.*, 1994). The rat may therefore continue to be considered a convenient model in further understanding the mechanisms of GABA and SRIF release and the physiological role of the GABA-SRIF interaction.

The existence in the human brain of a GABA-SRIF interaction involving two pharmacologically distinct $GABA_B$ receptors opens various possibilities for modulating SRIF transmission by use of selective $GABA_B$ receptor ligands. Reduced release of SRIF may play some role in the cognitive impairment typical of Alzheimer's disease or accompanying other pathologies. Selective $GABA_B$ heteroreceptor antagonists, able to interrupt the inhibitory action of endogenous GABA, could lead to increased SRIF release and to cognitive improvement. Interestingly, (−)-baclofen was reported to cause amnesia in rats (Carletti *et al.*, 1993) and this effect could be prevented by $GABA_B$ receptor antagonists. Some of these antagonists behaved on their own as memory-enhancing agents with diverse effects on learning and memory and in different species, including monkeys (Mondadori *et al.*, 1993; Fröstl *et al.*, 1995b).

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