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# A novel recognition site for somatostatin-14 on the $GABA_A$ receptor complex

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**Abstract**

Functional interactions between γ-aminobutyric acid (GABA) and somatostatin are suggested by the presence of synaptic contacts between GABA and somatostatin neurons, colocalisation of GABA and somatostatin and reciprocal modulation of somatostatin and GABA release. Nevertheless, a direct interaction of somatostatin with the $GABA_A$ receptor complex has not yet been investigated. A quantitative autoradiographic technique was used to determine the ability of somatostatin to interact with the [$^{35}$S]*t*-butylbicyclophosphothionate [$^{35}$S]TBPS binding sites of the $GABA_A$ receptor complex: somatostatin inhibited [$^{35}$S]TBPS binding with $IC_{50}$ values in the micromolar range in all brain regions studied. These results demonstrate for the first time a direct interaction between somatostatin and the $GABA_A$ receptor complex. © 1998 Elsevier Science B.V.

*Keywords:* Somatostatin; [$^{35}$S]TBPS ([$^{35}$S]*t*-butylbicyclophosphothionate) binding site; $GABA_A$ receptor complex

Somatostatin-14 is a biologically active tetradecapeptide known to mediate various biological actions through its own specific membrane receptors on target cells. To date, five distinct members of the somatostatin receptor family have been identified, and all are putative seven-transmembrane G protein-coupled receptors (Viollet et al., 1997). In addition, considerable evidence suggests the existence of a functional interaction between γ-aminobutyric acid (GABA) and somatostatin (Epelbaum et al., 1994). Moreover, somatostatin has been reported to exert a direct analgesic effect (Bloom and Polak, 1987) and this effect could involve an enhancement of $GABA_A$ activity (Frye and Duncan, 1994). However a direct interaction of somatostatin with the $GABA_A$ receptor complex has not yet been demonstrated.

In order to investigate the ability of somatostatin to interact with the $GABA_A$ receptor complex, competition between somatostatin and [$^{35}$S]*t*-butylbicyclophosphothionate ([$^{35}$S]TBPS) binding was studied in rat brain. It is known (Olsen et al., 1990) that all natural $GABA_A$ receptors recognize the cage convulsant [$^{35}$S]TBPS.

The autoradiographic technique described by Vincens et al. (1993) was used: mounted brain sections were incubated with 3 nM [$^{35}$S]TBPS in the absence (total binding) or presence of somatostatin 14 (Sigma, range of concentrations assayed, $10^{-8}$ M to $10^{-4}$ M). Somatostatin 14 was dissolved ($10^{-3}$ M) in acetic acid 0.01 N and then in buffer solution containing bacitracine 3‰. Alternate sections were incubated with $10^{-5}$ M picrotoxin to assess nonspecific binding.

The autoradiographic distribution of [$^{35}$S]TBPS specific binding in the absence of somatostatin (total binding Fig. 1, upper panel) showed that the highest densities of [$^{35}$S]TBPS binding sites were in layer IV of the cerebral cortex, in the globus pallidus, and in some thalamic nuclei. Intermediate densities of binding sites were found in superficial and deep layers of the cerebral cortex, in the molecular layer of the dentate gyrus, in the strata oriens and radiatum of the CA1 field of the hippocampus and in the caudate putamen. High concentrations of somatostatin ($10^{-5}$ M), inhibited [$^{35}$S]TBPS binding throughout the brain (Fig. 1, lower panel).

Somatostatin ($10^{-8}$, $10^{-4}$ M) produced a dose-dependent inhibition of specific [$^{35}$S] TBPS binding. In all brain regions analyzed, somatostatin inhibited [$^{35}$S]TBPS specific binding with $IC_{50}$ values in the micromolar range (for

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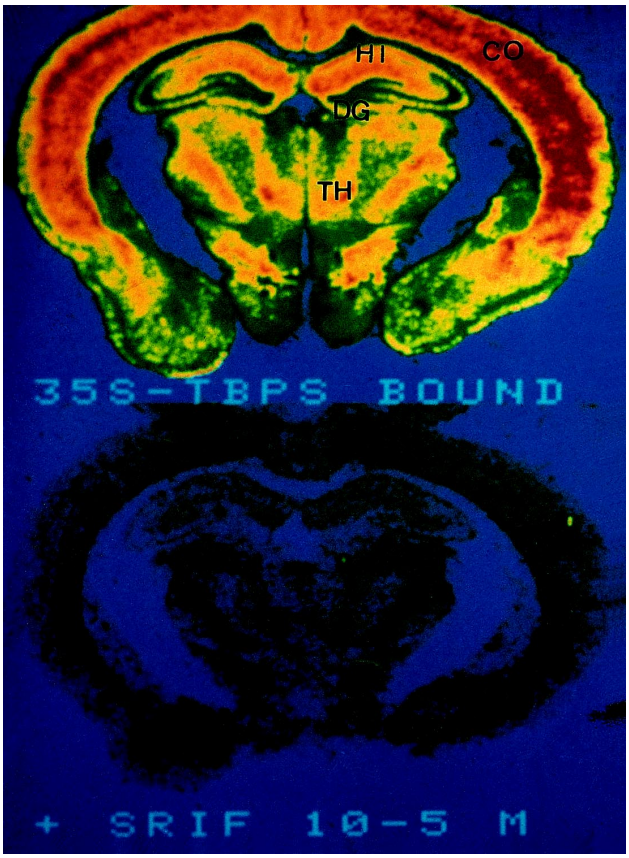


Fig. 1. The digitalized image (Biocom, les Ulis, France, RAG-200) of an autoradiographic film showing the density of [$^{35}$S]TBPS binding sites in rat midbrain (coronal section at level p21 of the Paxinos and Watson atlas). (upper panel) Total [$^{35}$S]TBPS binding. (lower panel) Inhibition of [$^{35}$S]TBPS binding by somatostatin $10^{-5}$ M. (CO cerebral cortex, HI hippocampus (field CA1), DG dentate gyrus, TH thalamus).

all the brain regions studied the corresponding $IC_{50}$ values were $1.8 \pm 0.10$; $2.9 \pm 0.2$; $3.0 \pm 0.3$; $2.7 \pm 0.1$; $3.8 \pm 0.4 \times 10^{-6}$ M for cortex layer I–III, IV, V, CA1 and dentate gyrus, respectively). No significant interstructural variability was observed with the brain regions investigated.

In this study, we demonstrated that somatostatin directly interacts with [$^{35}$S]TBPS binding, a ligand that labels the barbiturate and the neurosteroid sites on the $GABA_A$ receptor complex. These results are similar to those observed for various mammalian species (Vincens et al., 1993, 1995) with 3$\alpha$-OH-5$\alpha$-pregnan-20-one (3$\alpha$5$\alpha$P), using autoradiography of brain tissues. The $IC_{50}$ values of somatostatin to inhibit [$^{35}$S]TBPS binding were similar to those obtained with 3$\alpha$5$\alpha$P which is the most potent neurosteroid for inhibiting [$^{35}$S]TBPS binding, but lower than the $IC_{50}$ values observed ($10^{-4}$ M) by Vincens et al. (1995), for the barbiturate, pentobarbital. Nevertheless, these $IC_{50}$ values (micromolar range) were higher than those corresponding to the affinity of somatostatin (nanomolar range) for its own specific membrane receptors (Viollet et al., 1997).

Many substances, including benzodiazepines, barbiturates, steroids, general anaesthetics, avermectin, propofol and $Zn^{2+}$ (Sieghart, 1995) have been reported to act as modulators of $GABA_A$ receptors, but it is the first time that the $GABA_A$ receptor complex is reported to be a target for a neuropeptide.

In conclusion, the present data demonstrated for the first time a direct interaction between somatostatin in micromolar concentrations and the $GABA_A$ receptor complex, and suggest that somatostatin possesses a specific recognition site on the $GABA_A$ receptor complex. These results show that somatostatin, as well as steroids or barbiturates, can be considered as a potent inhibitory modulator of [$^{35}$S]TBPS binding. The mechanism and the physiological relevance of this interaction still remain to be determined.

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