MODULATION OF THE GABA$_A$ RECEPTOR BY SRIF IN RAT THALAMUS

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Received 2012-08-20, Revised 2012-09-08; Accepted 2012-09-14

ABSTRACT

Somatostatin has been reported to modulate GABA$_A$ receptor complex in many brain structures. This study was conducted to investigate somatostatin modulation of the GABA$_A$ receptor binding in several rat diencephalic structures, focusing primarily on the thalamus, as this structure plays important roles. Animals were assigned to control conditions. Changes in specific binding of GABA$_A$ receptor as labelled with $^{35}$S-Tertiary Butylbicyclophosphorothionate (TBPS) were assessed by in vitro quantitative autoradiography with the aid of a computer assisted image analysis system. Our results reveal the presence of higher densities in several thalamic structures located principally in the part of thalamus. We demonstrate for the first time the presence of a modulatory effect of somatostatin on the GABA$_A$ receptor complex in this brain region in rats. Indeed, the peptide affected in a concentration-dependent manner; the binding of $^{35}$S-Tertiary Butylbicyclophosphorothionate (TBPS) to the convulsant site of the GABA$_A$ receptor complex in thalamic structures with an affinity in the micromolar range ($10^{-3}$ to $3.10^{-6}$M). The inhibitory effect of somatostatin is observed in all thalamic structures analyzed. The absence of a specific region effect of somatostatin on the binding of $^{35}$S-TBPS, suggests the presence of a homogenous subunit GABA$_A$ receptor composition. Furthermore, GABA and muscimol, a GABA$_A$ receptor agonist, enhanced the affinity of somatostatin effect on $^{35}$S-TBPS. This suggests that somatostatin allosterically modifies $^{35}$S-TBPS binding through a mechanism similar to that of GABA.

Keywords: Somatostatin, In Vitro Quantitative Autoradiography, $^{35}$S-Tbps Binding, GABA$_A$ Receptor Complex

1. INTRODUCTION

Gamma-Amino Butyric Acid (GABA) is widely accepted as an inhibitory neurotransmitter in the mature central nervous system (Purves et al., 2012; Bowery and Smart, 2006). GABA-mediated inhibition passes via two major types of receptors, GABA$_A$ and GABA$_B$ receptors (Mehta and Ticku, 1999; Bowery, 2010). GABA$_A$ are hetero-oligomeric ligand-gated ion channel proteins. They are distributed widely in the central nervous system of many vertebrate species (Edgar and Schwartz, 1990; Fubara et al., 1996). These receptors are primarily responsible for fast inhibitory neurotransmission in the central nervous system (Macdonald and Olsen, 1994). One of the unique features of these receptors is that, in addition to the GABA binding site, the receptor has several allosteric modulatory sites. Indeed, they are regulated by many positive and negative allosteric modulators including neurotransmitters, neuromodulators and neurohormones (Majewska, 1992; Sapp et al., 1992; Macdonald and Olsen, 1994; Sieghart, 2012; Huidoboro-Toro et al., 1996). Interestingly, we
reported for the first time that a neuropeptide i.e., somatostatin, was able to modulate allosterically the GABA_A receptor complex. Indeed, the neuropeptide induced a dose-dependent inhibition of [³⁵S]-Tertiary Butylbicyclophosphorothionate (TBPS) binding in several forebrain regions in rats (Vincens et al., 1998; Chigr et al., 1999; 2002). However, the effects of somatostatin on GABA_A receptors in other brain regions remain to be established. One region that has not been closely studied is the thalamus. This is surprising since this structure plays important functional roles (Saalmann and Kastner, 2011; Wang et al., 2011). Furthermore, this brain region is enriched in somatostatin containing neuronal elements (Wang et al., 2000) GABA (Geis and Borst, 2012) and GABA receptors (Edgar and Schwartz, 1990; Halonen et al., 2009). Somatostatin and GABA_A receptors in this region play important roles in a variety of physiological functions (Leresche et al., 2000; Wang, 2011; Wang et al., 2011). Therefore, the principal goal of the present study was the assessment of somatostatin effects on the in vitro autoradiographic labeling of [³⁵S]-TBPS site on the benzodiazepine/GABA chloride ionophore receptor complex by receptor autoradiography in rat thalamic structures.

2. MATERIALS AND METHODS

2.1. Animals

16 Male Wistar rats (200-220 g) were housed 4-5 per cage in an animal room maintained at 22±2 and 50±10%, relative humidity and on a 12light/12 dark cycle. Food and water were available ad libitum. The rats were sacrificed by decapitation, their brains rapidly removed. The brains were then frozen in isopentane and dry ice rinsed 1 h under tap water. The selected thalamic structures were evidence for somatostatin inhibition of [³⁵S]-TBPS binding in a dose-dependent manner in the rat thalamic structures (Fig. 1). The maximum of inhibition in [³⁵S]-TBPS binding i.e., 83.5±2.37% (mean ±SEM, n = 7) was obtained at 10⁻⁵M somatostatin, the highest concentration used. Within the thalamic structures investigated, no significant interregional differences were evidenced for somatostatin inhibition of [³⁵S]-TBPS specific binding. Thus, somatostatin inhibited [³⁵S]-TBPS binding with IC₅₀ values in the micromolar range.
range \((1.3 \times 10^{-6} \text{ to } 2.3 \times 10^{-6} \text{ M})\) (Table 1). Scatchard analysis of the displacement curves obtained with somatostatin evidenced the presence of a single high affinity site (data not shown). The apparent Dissociation constant (KD) is in the average of \(29 \pm 4 \text{nM}\). The inhibitory effect of somatostatin on the \([{}^{35}\text{S}]\)-TBPS binding was not nucleus specific in the thalamic region. The IC\(_{50}\) values did not show significant differences between the thalamic structures analyzed (Table 1).

In order to determine whether the inhibitory effect of SRIF occurs by acting at the GABA\(_A\) receptor, we tested the actions of GABA and muscimol (a GABA\(_A\) receptor agonist). Furthermore, we assayed the effect of a positive modulator of GABA\(_A\) receptor complex, i.e., the neurosteroid 5\(\alpha\)3\(\alpha\)P to determine any cooperativity between somatostatin and neurosteroids. Thus, in the presence of GABA \((10^{-6} \text{ M})\) in the medium incubation, the effect of somatostatin on the binding of \([{}^{35}\text{S}]\)-TBPS is more pronounced (Fig. 1a). Significant decrease of \([{}^{35}\text{S}]\)-TBPS specific binding was observed in all the thalamic structures analyzed in rats and for all the somatostatin concentrations used (Fig. 1a). The IC\(_{50}\) values were decreased tenfold \(0.12 \times 10^{-6} \text{ M}–0.23 \times 10^{-6} \text{ M}\) (Table 1). The addition of a muscimol, to the medium incubation at a concentration of \(10^{-6} \text{ M}\), enhances also the dose-dependent inhibitory effect of somatostatin on \([{}^{35}\text{S}]\)-TBPS binding.
Fig. 1. Effect of somatostatin alone (upper curves in a-c) or in the presence of GABA 1µM (Panel a), Muscimol 1µM (Panel b) and the neurosteroid 5α3αP 1µM (Panel c) on the specific binding of [³⁵S]-TBPS binding in rat mediiodorsal thalamic nucleus. Brain sections containing the thalamic level were incubated at room temperature for 180 min with 2nmol/L [³⁵S]TBPS and various concentrations of somatostatin (10⁻¹⁰ to 10⁻⁵ mol/L) in the absence or the presence of the modulators cited. For all experiments performed, each point corresponds to the mean ± S.E.M. of 8-10 densitometric measurements (4-6 independent experiments) and is expressed as a percentage of maximal [³⁵S] (TBPS) binding in the absence of drugs.

Table 1. Effect of GABA, muscimol and the neurosteroid 5α-pregnan-3αol-one on somatostatin modulation of [³⁵S]-tert-butylcyclophosphonothionate binding to different thalamic nuclei.

| Thalamic structure                  | +STIF (µmol/L) | +SRIF +GABA (10⁻⁶ mol/L) | IC₅₀ (µmol/L) | +SRIF +5α3αP (10⁻⁶ mol/L) |
|------------------------------------|----------------|---------------------------|---------------|---------------------------|
| Angular thalamic nucleus           | 3.3±0.4        | 0.23±0.02*                | 0.15±0.01*    | 0.13±0.02*                |
| Central medial thalamic nucleus    | 1.3±0.1        | 0.21±0.02*                | 0.19±0.03*    | 0.14±0.02*                |
| Centrolateral thalamic nucleus     | 2.3±0.4        | 0.23±0.04*                | 0.16±0.03*    | 0.17±0.04*                |
| Laterodorsal thalamic nucleus      | 1.8±0.3        | 0.26±0.02*                | 0.18±0.01*    | 0.15±0.01*                |
| Mediodorsal thalamic nucleus       | 1.3±0.1        | 0.15±0.01*                | 0.19±0.03*    | 0.19±0.02*                |
| Paracentral thalamic nucleus       | 2.1±0.1        | 0.26±0.04*                | 0.18±0.01*    | 0.15±0.01*                |
| Posterior thalamic nucleus         | 1.8±0.3        | 0.19±0.01*                | 0.21±0.04*    | 0.23±0.03*                |
| Submedial thalamic nucleus         | 1.4±0.2        | 0.21±0.02*                | 0.22±0.03*    | 0.24±0.02*                |
| Ventrolateral thalamic nucleus     | 1.3±0.2        | 0.25±0.01*                | 0.13±0.03*    | 0.21±0.03*                |
| Ventromedial thalamic nucleus      | 1.8±0.3        | 0.12±0.02*                | 0.19±0.02*    | 0.16±0.02*                |
| Ventral posteriomedial nucleus     | 1.7±0.2        | 0.15±0.02*                | 0.17±0.03*    | 0.11±0.02*                |
| Reticular thalamic nucleus         | 1.6±0.4        | 0.13±0.04*                | 0.18±0.03*    | 0.18±0.02*                |
| Rhomboid nucleus                   | 1.9±0.3        | 0.24±0.02*                | 0.19±0.01*    | 0.12±0.01*                |

Data are the mean ± SEM of six animals from four to six independent experiments. Each experiment was repeated three times. *P<0.01 compared with the effect of SRIF only, in post hoc analyses. Specific binding of [³⁵S]-tert-butylcyclophosphonothionate (TBPS) was measured in the presence of increasing concentrations of somatostatin (SRIF) in the absence or the presence of GABA, muscimol or 5α-pregnan-3αol-one (5α3αP) under equilibrium. In each case, the concentration required to achieve 50% of TBPS binding (IC₅₀) was determined.

The IC₅₀ values are in the range of 15 10⁻⁶ M to 0.22 10⁻⁶ M (Fig. 1b, Table 1). Equivalent pattern concerning the allosteric modulation of the tetradecapeptide was obtained when the neurosteroid 5α3αP (10⁻² M) was added to incubation medium (Fig. 1c). Similarly, the IC₅₀ values were decreased tenfold (0.11 10⁻⁶ M–0.24 10⁻⁶ M) (Table 1). Taken together, these data show that In all experimental situations, the dose-dependent inhibition effect of somatostatin on [³⁵S]-TBPS binding is enhanced tenfold and the average of is around of 10⁻⁷ M.
4. DISCUSSION

The present study was designed to investigate the regional distribution of \[^{35}\text{S}\]TBPS binding sites and to assess the effect of the tetradecapeptide somatostatin on the \(\text{GABA}_A\) receptor complex in the rat brain diencephalon and particularly in the thalamus. \[^{35}\text{S}\]TBPS was chosen as the ligand in this study for the following reasons. First, \[^{35}\text{S}\]TBPS is an ideal ligand for autoradiographic studies, because of its high specific activity, low non-specific binding, high resolution in autoradiographic analysis (Giorgi et al., 1994; Atack et al., 2007) and convenient quantification using commercially available standards. Second, modulation of \[^{35}\text{S}\]TBPS binding by many positive-GABA agonists such as neurosteroids has been used as of a highly of allosteric interactions of neurosteroids with the \(\text{GABA}_A\) receptor (Vincens et al., 1992; 1993; Atack et al., 2007; Halonen et al., 2009).

We have found that \[^{35}\text{S}\]TBPS bound in slide-mounted rat diencephalon to a single class of receptors with an equilibrium-dissociation KD value of 29±4 nM. The kinetic parameters and pharmacological specificity measured in mounted-tissue sections obtained from rat thalamic level are quite similar to those found with slide-mounted cerebral cortex in rat (Edgar and Schwartz, 1990; Chigr et al., 2002). Furthermore, these values are consistent with data obtained with cerebral homogenate membrane preparations (Atack et al., 2007).

The present study examined the effects of the tetradecapeptide somatostatin on the in vitro autoradiography \[^{35}\text{S}\]TBPS binding at or near the chloride channel. This investigation represents the first detailed investigation of somatostatin modulation of \[^{35}\text{S}\]TBPS binding to \(\text{GABA}_A\) receptors in the rat thalamus. Indeed, a gradually and significant decrease in the density of \[^{35}\text{S}\]TBPS binding sites, in the rat thalamic structures, following the addition of increasing concentrations of somatostatin was observed (Fig 1a).

The potentiating effect of exogenous GABA or the \(\text{GABA}_A\) receptor agonist, muscimol, required to produce this effect could suggest that somatostatin exerts its effects with more potent facilitatory effects which were GABA sensitive. These finding add experimental support to the multiplicity of allosteric modulators of the different components of the \(\text{GABA}_A\) receptor complex.

The inhibition of \[^{35}\text{S}\]TBPS binding observed at higher concentrations of somatostatin, may represent a direct activation of the GABA-dependent Cl\(^-\) channels, as suggested for some neuromodulators acting at the \(\text{GABA}_A\) receptor complex (Sieghart, 2012; Samochocki and Strosznjder, 1993; Concas et al., 1994; Quinn and Harris, 1995; Martin et al., 1996; Sanna et al., 1996). This suggestion is consistent with the evidence that somatostatin-induced inhibition of \[^{35}\text{S}\]TBPS binding, is blocked by bicuculline (Concas et al., 1994). Furthermore, the fact that in the presence of exogenous GABA, low concentrations of somatostatin were able to decrease \[^{35}\text{S}\]TBPS binding suggests that in the absence of GABA, these low concentrations do not directly open the \(\text{GABA}_A\) receptor-operated CI-channel as is the case for other neuromodulators (Concas et al., 1994). It appears from our experiments that the tetradecapeptide, affects the \[^{35}\text{S}\]TBPS binding in all thalamic structures analyzed. Furthermore, the peptide inhibition of the \[^{35}\text{S}\]TBPS binding is not region specific in thalamus. These data are in good accord with previous findings describing neurosteroids in forebrain regions in different mammalian species (Vincens et al., 1993). Such homogeneity suggests that the peptide binding site and the barbiturate binding site belong to the same functional entity and presumably are not depending on the demonstrated molecular heterogeneity of brain \(\text{GABA}_A\) receptors (Levitan et al., 1988; Simon et al., 2004). This absence of brain regional differences may favor the efficacy of allosteric coupling between GABA and channel sites, which could indicate the absence of differences in the agonist efficacy. This could be due to gamma 2 subunit, responsible for the coupling between GABA and ion channel. Indeed, a deficit in this subunit implies a deficient coupling (Sinkkonen et al., 2004). Furthermore, it well known that the gamma subunit is highly sensitive to GABA and neurosteroids (Brown et al., 2002; Smith et al., 2007). These two compounds inhibit the \[^{35}\text{S}\]TBPS binding in similar manner to somatostatin (Chigr et al., 2002). These findings are different from previous data showing that a proportion of \(\text{GABA}_A\) receptors in the thalamus displaying atypical allosteric coupling between the agonist and channel sites: the so-called GABA-insensitive \[^{35}\text{S}\]TBPS binding (Sinkkonen et al., 2001a; 2001b; 2004; Halonen et al., 2009).

It is well known that the \(\text{GABA}_A\) receptors can either be synaptic or extrasynaptic (Farrant and Nussser, 2005). The extrasynaptic receptors consist of specific subunit combinations including gamma subunit and are preferentially located in several brain regions including thalamus (Cope et al., 2005; Jia et al., 2005). This argues strongly that somatostatin modulates principally extrasynaptic \(\text{GABA}_A\) receptors at the thalamic level. Of interest, these receptors are responsible of tonic inhibition of GABA (Farrant and
Based on these results and the results we reported previously (Chigr et al., 1999; 2002), we postulate that somatostatin modulates both synaptic and extrasynaptic GABA<sub>A</sub> receptors in the central nervous system, with the same efficacy.

Inhibition of <sup>[35]S</sup>-TBPS binding by various concentrations of somatostatin and GABA yielded dose-response curves that were similarly shaped suggesting that the neuropeptide allosterically affects <sup>[35]S</sup>-TBPS binding through a mechanism similar to that of GABA (Chigr et al., 2002). However, other interpretations concerning the mechanism of somatostatin modulation of <sup>[35]S</sup>-TBPS binding could be also exist with regard to the specific peptide receptors. Indeed, the pharmacological action of somatostatin on GABA<sub>A</sub> receptor presents several similarities with other several neurotransmitters which influence the heterooligomeric complex. These neurotransmitters modulate the activity of GABA<sub>A</sub> receptor complex via the different signal transduction pathways (Huidoboro-Toro et al., 1996) generated by the activation of their specific receptors. Finally it is interesting to note that somatostatin effects could be a result of receptor-receptor interactions as shown for other neuromodulators (Ghosh et al., 1997; Agnati et al., 2003). Indeed, the action of the peptide passes via one of the five somatostatin receptor subtypes (Hoyer et al., 1994) which implicate a release of endogenous GABA or GABA agonists affecting by consequent the binding of <sup>[35]S</sup>-TBPS binding.

The presence of somatostatin modulation of GABA<sub>A</sub> receptor complex as shown in the present study is of interest in regard to the functional relevance of such interactions. It is suggested that the naturally occurring peptide could play an important role in modulating GABA-mediated synaptic inhibition in certain physiological states (Green and Mason, 1996; Leresche et al., 2000; Wang et al., 2000). Thus, in rat thalamus, the neuropeptide abolished spontaneous bursts of GABA(A) inhibitory postsynaptic potentials in 85% and decreased (40%) the amplitude of single spontaneous GABA(A) inhibitory postsynaptic potentials in 87% of thalamic neurons respectively (Leresche et al., 2000). Furthermore, the present pharmacological data are supported by previously reported anatomical findings. Immunohistochemical studies suggest the presence of dense somatostatinergic networks in GABAergic neuronal structures (Wang et al., 2000). Consequently, the tetradecapeptide could modulate directly the release of GABA and thereby cause an inhibitory effect in the central nervous system.

In summary, the findings of the present data indicate that somatostatin has a modulatory effect on the central GABA<sub>A</sub> receptor complex, which is GABA sensitive. Such findings will help to strengthen the relationship between GABAergic and somatostatinergic effects, which may be important in certain physiological conditions (Rage et al., 1993; Luddens et al., 1995). These findings illustrate also the importance of such interactions in somatostatin-mediated GABAergic transmission in the rat diencephalon.

5. CONCLUSION

The present results demonstrate the presence of a modulatory effect of somatostatin on the GABA<sub>A</sub> receptor complex in rat thalamus. Furthermore, the data suggest that somatostatin allosterically modifies <sup>[35]S</sup>-TBPS binding through a mechanism similar to that of GABA.

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