Allosteric Modulation of $GABA_A$ Receptor by Somatostatin Is Altered under Stress in Rat Brainstem

(stress / somatostatin / rat / brainstem / $GABA_A$ receptor / [^{35}S]TBPS)

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Abstract. This study was conducted to investigate somatostatin modulation of $GABA_A$ receptor binding in several rat brainstem structures, located principally in the mesencephalon, after exposure to acute immobilization stress (single 1-hour session). Animals were randomly assigned to either control or stress conditions and changes in specific binding of the $GABA_A$ receptor as labelled with TBPS were assessed by *in vitro* quantitative autoradiography with the aid of a computer-assisted image analysis system. Exposure to immobilization stress led to a significant increase in [^{35}S]TBPS binding site density in the SN of stressed rats compared to controls. In the other brainstem structures analysed, specific binding of [^{35}S]TBPS remained unchanged in stressed rats. Furthermore, the results of the present *in vitro* study demonstrate an alteration of the modulatory effect of somatostatin on the $GABA_A$ receptor complex in the SN of stressed rats as compared to controls. This apparent alteration of allosteric effects of GABA receptor-somatostatin in the SN of stressed rats was eliminated in the presence of 1 micromolar concentration of GABA. Taken together, these data provide the first evidence of stress-induced alteration of allosteric effects of GABA-somatostatin in the rat mesencephalon. Furthermore, they also demonstrate that the tetradecapeptide somatostatin is particularly effective in modifying the [^{35}S]TBPS binding to the $GABA_A$ receptor in this cerebral region.

The ability of stress to engage a gamma-aminobutyric acid (GABA)-mediated mechanism is well known (Serra et al., 1991; Foddi et al., 1997). Recent reports suggest that GABA receptors may mediate responses to stressors and the physiological control of stress (Drugan et al., 1993). Indeed, stress has been shown to be associated with alterations in the capacity of ligand binding to the $GABA_A$ receptor complex (Concas et al., 1993; Barbaccia et al., 1996; Serra et al., 2000). Furthermore, receptor binding studies show a significant alteration in the capacity of allosteric modulators of the $GABA_A$ receptor complex to influence [^{35}S]t-butylbicyclophosphorothionate ([^{35}S]TBPS) binding in the rat brain after stress (Deutsch et al., 1994; Banerjee et al., 1998; Concas et al., 1998). Whereas the alteration of the binding and the pharmacological properties of the $GABA_A$ receptor complex have been extensively studied in the forebrain and diencephalic structures (Otero Losada, 1989; Purdy et al., 1991; Concas et al., 1996; 1998; Serra et al., 2000), this has received little attention in the brainstem. In fact, only few binding studies have measured the alteration of the $GABA_A$ receptor complex in this brain region (Abel and Carney, 1993). $GABA_A$ receptors in the brainstem play important roles in a variety of physiological functions. For instance, activation and inhibition of these receptors alter respiratory rhythms, blood pressure and motor controls (Nicholson et al., 1992; Sved, 1994; Bonham, 1995). The same stress paradigms have been reported to influence the somatostatinergic system as evidenced biochemically (Benyassi et al., 1993; Arancibia et al., 1997; Zhang et al., 1999). Interestingly, this tetradecapeptide has been shown to allosterically modulate the binding of [^{35}S]TBPS to the $GABA_A$ receptor complex in the central nervous system (Vincens et al., 1998; Chigr et al., 1999). Indeed, somatostatin produced a dose-dependent inhibition of [^{35}S]TBPS-specific binding on the $GABA_A$ receptor complex in rat forebrain regions (Vincens et al., 1998; Chigr et al., 1999). Taken together, these observations have now prompted us to investigate whether stress was associated with an alteration of the *in vitro* autoradiographic labelling of the [^{35}S]TBPS site on the benzodiazepine/GABA chloride ionophore receptor complex as well as with a modulation of the $GABA_A$ receptor complex by somatostatin in different rat brainstem structures, particularly those located in mesencephalon.

Material and Methods

Male Wistar rats (220–240 g upon delivery) purchased from IFFA-CREDO, were maintained in 12-h light/dark cycles (light on at 7.00 h) with free access to

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Abbreviations: GABA – gamma-aminobutyric acid, SN – *substantia nigra*, TBPS – [^{35}S]t-butylbicyclophosphorothionate.

water and food, except during experimentation. Animals were handled for 5 days (20 min/day) to habituate them to the stress of handling and assigned to either a control (no stress) or immobilization stress (one 1-h stress session). At the time of experiments, the rats of the experimental group were picked up from their cages and were immediately subjected to stress immobilization. Immobilization stress conducted under this protocol was done as previously described by Kvetňanský and Mikulaj (1970) with slight modifications. Stress was applied by placing the rats on a piece of wood, and the front and hind legs of the rats were immobilized with plastic collars and adhesive tape on the boards of the wood. This allowed normal breathing and only restricted movements were possible (free movement of the head and tail). The animals were kept immobilized for 1 hour. These experimental measures minimized the pain of the rats. Ten minutes after the single immobilization stress session animals were killed by decapitation. Control rats were killed after removal from the home cages without prior manipulations. In all experiments, the immobilization procedure and decapitation were performed between 10 h and 12 h, to avoid possible circadian variations in the receptor function. After decapitation, the brains were removed, snap-frozen in dry ice, and kept at –80°C until sectioning. Serial coronal 20-μm thick sections from each brain were cut at –20°C in a cryostat (Frigocut 2880, Reichert Jung), collected onto 2% gelatin-coated slides and kept at –20°C until used. [^{35}S]TBPS autoradiography was conducted as we described previously (Chigr et al., 1999). Briefly, after a preincubation step of 30 min in 50 mM Tris-HCl, pH 7.4, sections were incubated with 3 nM [^{35}S]TBPS (100–140 Ci/mmol; NEN, Boston, MA) in 50 mM Tris-HCl buffer, pH 7.4, containing 500 mM NaCl and 10^{-4} M ascorbic acid, for 3 h at room temperature, in the absence (total binding) or in the presence of the unlabelled compound (picrotoxin, 5-pregnane-3αol-20-one:5α3αP, somatostatin, Sigma Chemical Co., St Louis, MO). The sections were then rinsed, dried out in ambient air, transferred to cardboard film cassettes along with slides containing labelled plastic standard (Amersham, Les Ulis, France) and apposed onto [^{3}H]Hyperfilm (Amersham). After an exposure period of 36 h at 4°C, the films were developed and fixed. The autoradiographic labelling obtained was quantified in many brainstem structures by computerized densitometry (Biocom, Les Ulis, France). Data were measured in relative optical density (OD) units for displacement studies. Drug-inhibited [^{35}S]TBPS binding was then plotted as a percentage of the specific binding obtained in the absence of the drugs. For non-displacement studies, the optical densities of each structure were measured from the exposed film and converted into femtomoles/mg (fmol/mg), based on calibration curves of standards with known radioactivity (Miller, 1991). Specific binding was defined as total binding minus non-specific binding (binding in the presence of 10^{-5} M picrotoxin). For all experiments, each point was the mean of measurements in five to eight sections for each individual animal, repeated with at least five animals for either control or stressed rats. The data were analysed by analysis of variance (ANOVA) followed by Scheffe's test. A P value of < 0.05 was considered statistically significant.

Results

The distribution of $GABA_A$ receptors, using [^{35}S]TBPS as a ligand in the rat mesencephalon, showed a heterogeneous distribution. From all the structures examined, the *substantia nigra* (SN) was the most densely labelled. As shown in Fig. 1, only in the SN (*pars reticulata* and *compacta*), the [^{35}S]TBPS binding (3 nM) was increased significantly ($P < 0.05$) by 1 h of immobilization stress. No changes were observed in the binding of this channel ligand in any of the areas studied, though a tendency for the binding to be increased was observed in the *raphe nucleus* (Fig. 2). Since the alterations in [^{35}S]TBPS binding in the SN were the most reliable phenomena, the other procedures were conducted in this structure. As depicted in Fig. 2, *in vitro* addition of increasing concentrations of somatostatin produced a dose-dependent decrease in the specific binding of [^{35}S]TBPS in the non-stressed rats. The efficacy of somatostatin-14 to inhibit the [^{35}S]TBPS binding was evidenced in every structure examined, the average IC50 obtained was 10^{-6} M. The application of 1-h immobilization stress altered the modulatory effect of somatostatin-14 on the [^{35}S]TBPS binding to a picrotoxin-sensitive site in the rat SN. Indeed, the dose-dependent inhibition of [^{35}S]TBPS binding by somatostatin-14 relationship was shifted to the right in a parallel manner by a factor of ten.

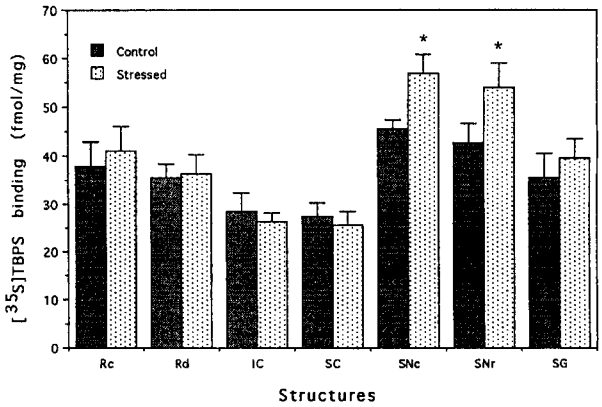

Fig. 1. Effect of immobilization stress on [^{35}S]TBPS-specific binding in several brainstem structures (Rc, Rd, IC, SC, SNc, SNr, and SG respectively: *raphe centralis*, *raphe dorsalis*, *inferior colliculus*, *superior colliculus*, *substantia nigra pars compacta*, *substantia nigra pars reticulata*, and *substantia griseum*). Each bar in the figure represents the mean ± S.E.M. expressed in fmol/mg and obtained from 5 to 7 separate experiments. *$P < 0.05$ compared with the control value.