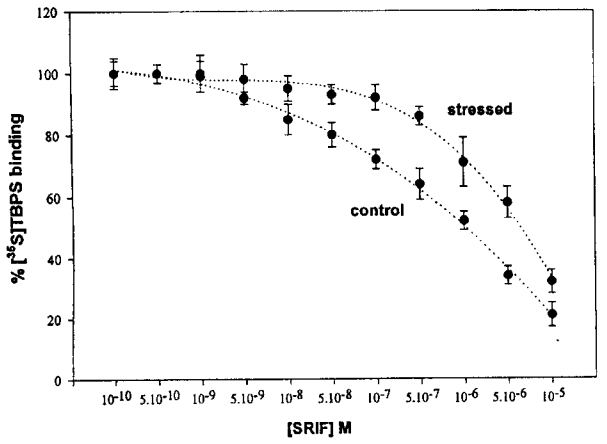

Fig. 2. Inhibition of [^{35}S]TBPS binding with increasing concentrations of somatostatin (SRIF) in *substantia nigra* of stressed and unstressed rats. Each point corresponds to the mean ± S.E.M. of 8–10 densitometric measurements of four separate experiments, each performed with five rats per group, and expressed as a percentage of maximal [^{35}S]TBPS-specific binding in the absence of SRIF.

Furthermore, in contrast to unstressed controls, submicromolar concentrations of the neuropeptide did not significantly affect the binding of [^{35}S]TBPS (Fig. 1B). Finally, in the presence of GABA (10^{-6} M), the ability of somatostatin-14 to inhibit the specific binding of [^{35}S]TBPS did not differ between stressed and unstressed control rats (data not shown).

Discussion

The present study examined the effects of stress immobilization on the *in vitro* autoradiography [^{35}S]TBPS binding at or near the chloride channel. The experiments presented herein reveal that the $GABA_A$ receptor complex as labelled by [^{35}S]TBPS was affected by a single exposure to stress (1-h immobilization) in the rat brainstem. These findings show that stress effects were highly localized. Indeed, from the brainstem structures analysed, the SN is the only structure which was sensitive to stress immobilization. This is to our knowledge the first reported alteration of [^{35}S]TBPS binding in this structure after stress. These findings reinforce the idea that the GABAergic system seems to be differentially affected by stress according to the brain area studied (Otero Losada, 1989); the relevance of these regional changes requires further studies. The enhancement of [^{35}S]TBPS binding could be explained by reduced concentrations of endogenous GABA, occurring specifically in SN after stress, which in turn enhanced the $GABA_A$ receptor sensitivity to its endogenous ligand. The mechanism whereby stress alters TBPS binding sites also allows other hypotheses. Thus, it is possible that the individual brain region differences observed could be due to the subunit heterogeneous distribution in these brain structures (Fritschy and Mohler, 1995). As a consequence, the potency and magnitude of the stress effect could be influenced by the subunit composition of the $GABA_A$ receptor. In this sense, the selectivity of the stress effect for certain subunit assemblies, as those probably present in the SN, could not be excluded. The observed alterations might also result from changes in brain neuroactive steroid levels. These compounds are now well known to exert anxiolytic (Bitran et al., 1991) and anti-stress (Purdy et al., 1991; Barbaccia et al., 1996) effects in brain. Furthermore, regional differences in brain concentrations of neurosteroids have been well evidenced and in this respect, it is interesting to note that SN contains the highest levels of several neurosteroids such as allopregnanolone (Bixo et al., 1997). Therefore, it is likely that acute stress reduced the synthesis and the release of these neurosteroids, which may result in an enhancement of [^{35}S]TBPS binding in the SN. Finally, it is not excluded that the observed changes could be due to the fact that in the stress paradigm used, the concentration range over which several neurosteroids were able to counteract the impairment in GABAergic transmission (Barbaccia et al., 1996) has not yet been reached in the SN.

The present findings are different from previous data showing decreases in the binding of [^{35}S]TBPS in many brain structures (Drugan et al., 1993). The discrepancy may be due to several procedural factors or the fact that the GABAergic system is differentially affected by stress, according to the brain area studied or the stress type applied.

Recently, the neuropeptide somatostatin-14 has been shown to reduce the binding of [^{35}S]TBPS to the $GABA_A$ receptor complex in forebrain structures with similar efficacy in each structure analysed (Vincens et al., 1998; Chigr et al., 1999). In the present study, we have been able to evidence such an effect in the rat brainstem for the first time. The sensitivity of the $GABA_A$ receptor complex to the allosteric action of somatostatin in rat brainstem structures, as shown in these data, reinforces the fact that this somatostatin modulation is not region-specific in the rat central nervous system (Chigr et al., 1999). Furthermore, these studies suggest that somatostatin-14 may naturally influence the central GABAergic tone.

The current data show that the ability of somatostatin-14 to allosterically modulate the binding of [^{35}S]TBPS is altered 10 min after a single session of immobilization stress. Whereas submicromolar concentrations of somatostatin significantly decreased the binding of [^{35}S]TBPS to SN in control rats, these concentrations did not show any efficacy in stressed rats. With respect to the lack of effect in the other brainstem structures, no changes in the ability of somatostatin-14 to displace [^{35}S]TBPS binding have been observed either in stressed or unstressed rats. Interestingly, the ability of the tetradecapeptide to distinguish between the binding of

[^{35}S]TBPS to SN in stressed and unstressed rats was eliminated in the presence of GABA. Similar findings were shown for the ability of the inhibitory effect of neurosteroid 5α-pregnane-3α,21-diol-20-one (5α-THDOC) on the binding of [^{35}S]TBPS (Deutsch et al., 1994). This effect has been explained to be due to the robust effect of the GABA itself on the inhibition of [^{35}S]TBPS binding and by consequence may mask the significant effect of the neuropeptide on the inhibition of [^{35}S]TBPS binding.

The mechanism for the observed decrease in somatostatin effect on [^{35}S]TBPS binding after immobilization stress is not clear; however, a stress-induced uncoupling of somatostatin and TBPS binding sites in the $GABA_A$ receptor complex seems likely, as suggested previously for other $GABA_A$ receptor modulators.

Acknowledgments

We are grateful to Drs. M. Vincens (Lab. Pharmaco. Endocrin., Paris, France) and M.-N. Montagne for their assistance in the elaboration of some parts of this work.

References

Abel, M. S., Carney, D. E. (1993) Alterations in t-butylbicyclophosphorothionate binding in the brains of lidocaine-kindled rats. *Met. Brain Dis.* **8,** 235-244.

Arancibia, S., Estupina, C., Pesco, J., Belmar, J., Tapia-Arancibia, L. (1997) Responsiveness to depolarization of hypothalamic neurons secreting somatostatin under stress and estrous cycle conditions: involvement of GABAergic and steroidal interactions. *J. Neurosci. Res.* **50,** 575-584.

Banerjee, P. K., Olsen, R. W., Tillakaratine, N. J. K., Brailowsky, R., Tobin, A. J., Carter Snead, III. O. (1998) Absence seizures decrease steroid modulation of t-[^{35}S]butylbicyclophosphorothionate binding in thalamic relay nuclei. *J. Pharmacol. Exp. Ther.* **287,** 766-72.

Barbaccia, M. L., Roscetti, G., Trabucchi, M., Mostallino, M. C., Concas, A., Purdy, R. H., Biggio, G. (1996) Time-dependent changes in rat brain neuroactive steroid concentrations and $GABA_A$ receptor function after acute stress. *Neuroendocrinology* **63,** 166-172.

Benyassi, A., Gavalda, A., Armario, A., Arancibia, S. (1993) Role of somatostatin in the acute immobilization stress-induced GH decrease in rat. *Life Sci.* **52,** 361-370.

Bitran, D., Hilvers, R. J., Kellog, C. K. (1991) Anxiolytic effects of 3α-hydroxy-5α[β]-pregnan-20-one: endogenous metabolites of progesterone that are active at the $GABA_A$ receptor. *Brain Res.* **561,** 157-161.

Bixo, M., Andersson, A., Winblad, B., Purdy, R. H., Bäckström, T. (1997) Progesterone, 5α-pregnane-3,20-dione and 3α-hydroxy-5α-pregnane-20-one in specfic regions of the human brain in different endocrine states. *Brain Res.* **764,** 173-178.

Bonham, A. C. (1995) Neurotransmitters in the CNS control of breathing. *Respir. Physiol.* **101,** 219-230.

Chigr, F., Ba M'hamed, S., Najimi, M., Vincens, M. (1999) Modulation of central $GABA_A$ receptor complex by somatostatin: a pharmacological study. *Therapie* **54,** 579-584.

Concas, A., Sanna, E., Cuccheddu, T., Mascia, M. P., Santoro, G., Maciocco, E., Biggio, G. (1993) Carbon dioxide inhalation, stress and anxiogenic drugs reduce the function of the $GABA_A$ receptor complex in the rat brain. *Prog. Neuropsychopharmacol. Biol. Psychiatry* **17,** 651-661.

Concas, A., Mostallino, M. C., Perra, C., Lener, R., Roscetti, G., Barbaccia M. L., Purdy, R. H., Biggio, G. (1996) Functional correlation between allopregnanolone and [^{35}S]TBPS binding in the brain of rats exposed to isoniazid, pentylenetetrazol or stress. *Br. J. Pharmacol.* **118,** 839-846.

Concas, A., Mostallino, M. C., Porcu, P., Follesa, P., Barbaccia, M. L., Trabucchi, M., Purdy, R. H., Grisenti, P., Biggio, G. (1998) Role of brain allopregnanolone in the plasticity of γ-aminobutyric acid type A receptor in rat brain during pregnancy and after delivery. *Proc. Natl. Acad. Sci. USA* **95,** 13284-13289.

Deutsch, S. I., Park, C. H., Hitri, A. (1994) Allosteric effects of a GABA receptor-active steroid are altered by stress. *Pharmacol. Biochem. Behav.* **47,** 913-917.

Drugan, R. C., Paul, S. M., Crawley, J. N. (1993) Decreased forebrain [^{35}S]TBPS binding and increased [^{3}H]muscimol binding in rats that do not develop stress-induced behavioral depression. *Brain Res.* **631,** 270-276.

Foddi, M. C., Cinquanta, M., Mennini, T. (1997) Effect of psychological stress on [^{35}S]TBPS binding in rat brain. *Pharmacol. Biochem. Behav.* **58,** 373-377.

Fritschy, J.-M., Mohler, H. (1995) $GABA_A$-receptor heterogeneity in the adult rat brain: differential regional and cellular distribution of seven major subunits. *J. Comp. Neurol.* **359,** 154-194.

Kvetňanský, R., Mikulaj, L. (1970) Adrenal and urinary catecholamines in rats during adaptation to repeated immobilization stress. *Endocrinology* **87,** 738-743.

Otero Losada, M. E. (1989) Acute stress and GABAergic function in the rat brain. *Br. J. Pharmacol.* **96,** 507-512.

Miller, J. A. (1991) The calibration of ^{35}S or ^{32}P with ^{14}C-labeled brain paste or ^{14}C plastic standards for quantitative autoradiography using LKB Ultrafilm or Amersham Hyperfilm. *Neurosci. Lett.* **121,** 211-214.

Nicholson, L. F. B., Faull, R. L. M., Waldvogel, H. J., Dragunow, M. (1992) The regional, cellular and subcellular localization of $GABA_A$/benzodiazepine receptors in the substantia nigra of the rat. *Neuroscience* **50,** 355-370.

Purdy, R. H., Morrow, A. L., Moore, P. H., Paul, S. M. (1991) Stress-induced elevations of γ-aminobutyric acid type A receptor-active steroids in the rat brain. *Proc. Natl. Acad. Sci. USA* **88,** 4553-4557.

Serra, M., Sanna, E., Concas, A., Foddi, C., Biggio, G. (1991) Foot-shock stress enhances the increase of [^{35}S]TBPS binding in the rat cerebral cortex and the convulsions induced by isoniazid. *Neurochem. Res.* **16,** 17-22.

Serra, M., Pisu, M. G., Littera, M., Papi, G., Sanna, E., Tuveri, F., Usala, L., Purdy, R. H., Biggio, G. (2000) Social isolation-induced decreases in both the abundance of neuroactive steroids and $GABA_A$ receptor function in rat brain. *J. Neurochem.* **75,** 732-740.

Sved, A. F. (1994) GABA-mediated neural transmission in mechanisms of cardiovascular control by the NTS. In: *Nucleus of the Solitary Tract,* ed. Barraco, I. R. A., pp. 245-253, CRC Press, Ann Arbor, MI.

Vincens, M., Mauvais-Jarvis, F., Behar, S. (1998) A novel recognition site for somatostatin-14 on the $GABA_A$ receptor complex. *Eur. J. Pharmacol.* **344,** R1-R2.

Zhang, K., Hamanaka, K., Soya, H., Nakase, S, Uesugi, Y., Inui, K., Nomura, J., Okasaki Y. (1999) Decreased expression of the mRNA for somatostatin in the periventricular nucleus of depression-model rats. *Life Sci.* **65,** PL87-94.