*Regulatory Peptides*, 48 (1993) 355–363



REGPEP 01542

# Brain somatostatinergic system at late pregnancy, parturition and the early postpartum period in the rat

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(Received 6 July 1992; revised version received 19 March 1993; accepted 20 May 1993)

*Key words:* Pregnancy; Somatostatin receptor; Frontoparietal cortex; Hippocampus; Rat

**Summary**

During pregnancy and postpartum rats experience a wide variety of behavioural changes. Since the somatostatinergic system has been implicated in the control of some of these changes, the present study examined somatostatin (SS) content and specific binding in the frontoparietal cortex and hippocampus of non-pregnant, pregnant (17 to 18 days), parturition and postpartum (10 and 30 days) rats as well as in ovariectomized rats which were or were not treated with estradiol valerianate. The content of somatostatin-like immunoreactivity (SSLI) was increased at 17 days of pregnancy in frontoparietal cortex and decreased at parturition and 10 days postpartum in that region and the hippocampus under study when compared with SSLI levels in non-pregnant rats. At 30 days postpartum the SSLI content returned to non-pregnant values in both brain regions. Scatchard analysis showed that the decrease in [$^{125}$I]Tyr$^{11}$-SS binding observed at 17 days of pregnancy in the frontoparietal cortex was due to the decrease in the number of SS receptors. In contrast, on the day of delivery the number of SS receptors in the same brain region increased. The affinity of the SS receptors was consistently unchanged in pregnant and non-pregnant rats in both regions. At 10 days postpartum the value of specific binding of the tracer to SS receptors in the frontoparietal cortex was not significantly different from that in the non-pregnant rats, although the actual number of receptors was slightly higher. Pregnancy did not change SS binding in the hippocampus. Neither ovariectomy nor the treatment of ovariectomized rats with estradiol valerianate affected cortical and hippocampal SS content and binding in the rats. These changes in the somatostatinergic system associated with late pregnancy, parturition and the early postpartum period may well be important because of their possible role in some of the behavioural changes observed during these periods.

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## Introduction

It has been observed that seizure susceptibility [1], conditioned avoidance learning [2] and general activity [3] vary significantly with the stage of pregnancy and postpartum. To date, the neurochemical bases which mediate these changes in maternal behavior are unclear. Nevertheless, the tetradecapeptide somatostatin (SS), which is distributed throughout the mammalian central nervous system [4–6], has been implicated in the control of these behaviours [7–8]. When applied intracerebroventricularly (i.c.v.), SS causes generalized tonic-clonic seizures [9], inhibits the extinction of active avoidance behaviour [7] and can influence locomotor activity [8]. Specific high affinity receptors for SS have been characterized in the rat brain [10–13] and there is strong evidence that these receptors mediate the biological effects of the neuropeptide [14–17]. The influence of late pregnancy, parturition and the early postpartum period on SS content and binding in rat frontoparietal cortex and hippocampus are investigated here. The paper also includes a study of the influence of ovariectomy and estrogen treatment of ovariectomized rats on the somatostatinergic system.

## Materials and Methods

*Chemicals*

Synthetic $Tyr^{11}$-SS and SS tetradecapeptide were purchased from Universal Biologicals Ltd. (Cambridge, UK); bacitracin and bovine serum albumin (fraction V) were purchased from Sigma (St. Louis, Mo. USA); and carrier-free $Na^{125}I$ (IMS 30, 100 mCi/ml) from the Radiochemical Centre (Amersham, UK). $Tyr^{11}$-SS was radio-iodinated by the chloramine-T method [18]. The tracer was purified in a Sephadex G-25 coarse column (1 × 100) which had been equilibrated with 0.1 M acetic acid containing bovine serum albumin 0.1% (w/v). Specific tracer radioactivity was about 350 Ci/g. The rabbit antibody used in the radioimmunoassay technique was purchased from the Radiochemical Centre (Amersham, UK). This antiserum was raised in rabbits against SS-14 conjugated to bovine serum albumin and is specific for SS, but since SS-14 also constitutes the C-terminal portions of both SS-25 and SS-28, the antiserum does not distinguish between these three forms. Cross-reactivity with other peptides was less than 0,5%. Cross-reaction with several SS analogues demonstrated that neither the N-terminal glycine nor the C-terminal cysteine residue is required for antibody binding, suggesting that the antigen site is directed towards the central part of the molecule containing the tryptophan residue. The binding of SS-14 to its antibody does not depend on an intact disulfide bond in the molecule, as breaking of the disulfide bond by reaction with 0.1% mercaptoethanol (boiling water bath, 5 min) did not change the immunoreactivity of the peptide.

*Experimental animals*

The animals used in this study were female Sprague Dawley rats ($n = 40$) weighing between 200 and 250 g. Rats were maintained on a 12 h light/dark cycle (07.00–19.00) and were allowed free access to food. For all experiments, controls ($n = 10$) were non-pregnant females of the same age as the experimental animals. The detection of sperm in the vaginal lavage the following morning was used to confirm the onset of pregnancy and that day was called day 1 of gestation. Mated females were separated from the males and housed in groups until the day they were used for the experiment. Pregnant rats were killed by decapitation on day 17 or 18 of gestation ($n = 5$). Other rats were decapitated on the day of delivery (usually day 20) ($n = 5$) and 10 ($n = 5$) and 30 ($n = 5$) days postpartum. One group of non-pregnant rats ($n = 8$) were anesthesized with diethyl ether and ovariectomized bilaterally through a low midline abdominal incision. One week later the rats were treated subcutaneously (s.c.) in the neck with either estradiol valerianate (50 μg/0.2 ml) in sesame oil or with sesame oil (0.2 ml, s.c.) ($n = 5$) once a day for two consecutive days. 24 h after the last injection the animals

were killed by decapitation. The brain was rapidly removed and the frontoparietal cortex and hippocampus were dissected over ice according to the method of Glowinski and Iversen [19].

*Tissue extraction and SS radioimmunoassay*

For somatostatin-like immunoreactivity (SSLI) measurements, frontoparietal cortex and hippocampus were rapidly homogenized using a Brinkman polytron (setting 5, 30 s), in 1 ml 2 M acetic acid. Extracts were boiled for 5 min in a water bath, chilled in ice, and aliquots (100 $\mu$l) were removed for protein determination [20]. Subsequently, homogenates were centrifuged at 15,000 *g* for 15 min at 4°C, and the supernatant was neutralized with 2 M NaOH. Extracts were immediately stored at −70°C until assay. SSLI level was determined in tissue extracts by a modified radioimmunoassay method [21], with a sensitivity limit of 10 pg/ml. Incubation tubes prepared in duplicate contained 100 $\mu$l samples of unknown or standard solutions of 0–500 pg cyclic SS tetradecapeptide diluted in phosphate buffer (0.05 M, pH 7.2 containing 0.3% bovine serum albumin, 0.01 M EDTA), 200 $\mu$l of appropriately diluted anti-SS serum, 100/$\mu$l of freshly prepared [$^{125}$I]Tyr$^{11}$-SS diluted in buffer to give 6000 cpm/assay tube (equivalent to 5–10 pg), and enough buffer to give a final volume of 0.8 ml. All reagents as well as the assay tubes were kept chilled on ice, before incubation at 4°C for 48 h. Separation of bound and free hormone was accomplished by the addition of 1 ml dextran-coated charcoal (dextran T70: 0.2% (w/v), Pharmacia, Uppsala, Sweden; charcoal: Norit A 2% (w/v), Serva, Feinbiochemica, Heidelberg, Germany). Dilution curves for each brain area were parallel to the standard curve. The intra- and inter-assay variation coefficients were 6.4% and 8.3%, respectively.

*Binding assay on membrane preparations*

Membranes from frontoparietal cortex and hippocampus were prepared as described by Reubi et al. [10]. Frontoparietal cortex and hippocampus were homogenized in 10 mM Hepes-KOH buffer (pH 7.6) (10 w/v) with a Brinkmann polytron homogenizer (setting 5, 15 s). The homogenate was spun at 600 *g* for 5 min at 4°C and the supernatant centrifuged at 48,000 *g* for 30 min at 4°C. The resulting pellet was suspended in 10 mM Hepes-KOH pH 7.6 (10 w/v) and centrifuged as before. The new pellet was then resuspended in 50 mM Tris-HCl buffer (pH 7.5). Samples were stored at −70°C until assay. Protein was determined by the method of Lowry et al. [20].

Specific SS binding was measured according to the modified method of Czernik and Petrack [11]. Brain membranes (about 0.15 mg protein/ml) were incubated in 250 $\mu$l of a medium containing 50 mM Tris-HCl buffer (pH 7.5), 5 mM $MgCl_2$, 0.2% (w/v) bovine serum albmin and 0.1 mg/ml bacitracin with 250 pM [$^{125}$I]Tyr$^{11}$-SS either in the absence or presence of 0.01–10 nM unlabelled SS. After a 60 min incubation at 30°C, the free radioligand was separated from the bound radioligand by centrifugation at 12,000 *g* (Beckman microcentrifuge) for 1.5 min and the resultant pellet was counted in a Beckman gamma counter. Nonspecific binding, i.e., binding occurring in the presence of a high concentration ($10^{-7}$ M) of unlabelled SS, represented about 20% of the binding observed in absence of native peptide and was subtracted from the total bound radioactivity in order to obtain the corresponding specific binding.

*Evaluation of radiolabelled peptide degradation*

The incubation medium after the binding study was subjected to Sephadex G-25 (fine) gel filtration. The column was developed in 6 M urea and 0.05 M sodium phosphate, pH 7.5, as described by Patel [22]. The radioactivity corresponding to the salt volume of the column was considered to be degraded.

*Data analysis*

Binding parameters for SS receptor binding were determined by least squares analysis of Scatchard plots [23]. Statistical comparisons of all data were carried out with one way analysis of variance and the Student's Newman-Keuls test. Means among groups

were considered significantly different when the $P$ value was less than 0.05. All points on each figure represent the mean of duplicate determinations in each individual experiment.

## Results

Fig. 1 shows the variation in concentrations of SSLI in frontoparietal cortex and hippocampus at late pregnancy, parturition and the postpartum period. The concentration of SSLI was increased at 17 days of pregnancy in the frontoparietal cortex but not in the hippocampus and decreased at parturition and 10 days postpartum in frontoparietal cortex and hippocampus. At 30 days postpartum the SSLI content had returned to non-pregnant values.

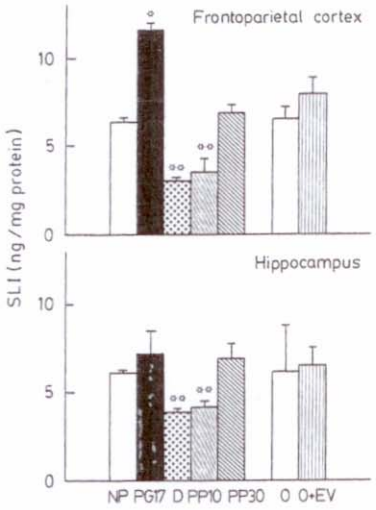


Fig. 1. Somatostatin-like immunoreactivity (SSLI) content in frontoparietal cortex and hippocampus at late pregnancy, parturition and the early postpartum period and after estradiol valerianate administration to ovariectomized rats. The experimental groups are abbreviated as follows: NP, non-pregnant ($n = 10$); PG 17, day 17 to 18 of pregnancy ($n = 5$); D, day of delivery ($n = 5$); PP10, day 10 of postpartum ($n = 5$); PP30, day 30 of postpartum ($n = 5$); O, ovariectomized ($n = 5$); O + V, ovariectomized plus estradiol valerianate ($n = 5$). Values are expressed as the mean ± S.E.M. of five separate experiments. In each of the experiments, determinations were made in duplicate. Asterisks indicate significant differences between the pregnancy (day 17), delivery or postpartum (day 10) group and the non-pregnant group: $^{*}P < 0.0007$, $^{**}P < 0.001$, $^{***}P < 0.0001$.

Although [$^{125}$I]Tyr$^{11}$-SS binding in membrane preparations from the frontoparietal cortex and hippocampus was time-dependent in the experimental groups, an equilibrium was reached and maintained between 50–180 min at 30 °C (data not shown). All subsequent binding experiments were therefore conducted at 30 °C for 60 min. Peptide degradation was determined in all the membrane preparations to rule out the possibility of different SS degrading activities which might have affected the interpretation of the results. Analysis of the free labelled supernatant SS by column chromatography revealed that 6.0% of the radioactivity was degraded after 60 min incubation in both brain regions.

Increasing concentrations of unlabelled SS competitively inhibited the specific binding of [$^{125}$I]Tyr$^{11}$-SS to cortical and hippocampal membranes in the preparations from all the experimental groups (Figs. 2 and 3, left panels). The specific binding of [$^{125}$I-Tyr$^{11}$]-SS to membranes prepared from the frontoparietal cortex was decreased at 17 days of pregnancy and rose on the day of delivery. There were no changes in the hippocampus (Figs. 2 and 3, left panels). Scatchard analysis showed that the decrease in [$^{125}$I]Tyr$^{11}$-SS binding observed at 17 days of pregnancy in the frontoparietal cortex was due to the decrease in the number of SS receptors. In contrast, on the day of delivery there was an increase in the number of SS receptors in the same brain region. The affinity of the receptors for SS was consistently unchanged in both cases. At 10 days postpartum the number of SS receptors in the frontoparietal cortex returned to non-pregnant values (Tables I and II).

The changes in receptor binding correlate inversely with tissue levels of immunoreactivity. This raises the possibility that the decreased receptor number is simply secondary to masking by endogenous material. The washing during membrane collection may be inadequate if unlabelled SS is tightly bound. There-

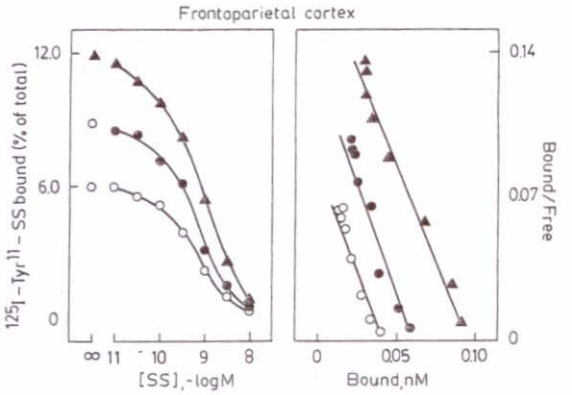


Fig. 2. (Left) Effect of pregnancy on competitive inhibition of specific [$^{125}$I]Tyr$^{11}$-somatostatin ([$^{125}$I]Tyr$^{11}$-SS, 250 pM) binding to membranes of the frontoparietal cortex. Membranes (0.15 mg protein/ml) were incubated for 60 min at 30 °C in the presence of 250 pM [$^{125}$I]Tyr$^{11}$-SS and increasing concentrations of native peptide. Points correspond to values of animals in the non-pregnant (●, $n = 10$), pregnant (day 17 to 18 of pregnancy) (○, $n = 5$) and parturition (△, $n = 5$) groups. Each point is the mean of five replicate experiments. For the sake of clarity, the other experimental groups studied are not represented, but the corresponding equilibrium binding parameters are included in Tables I and II. (Right) Scatchard analysis of the same data.

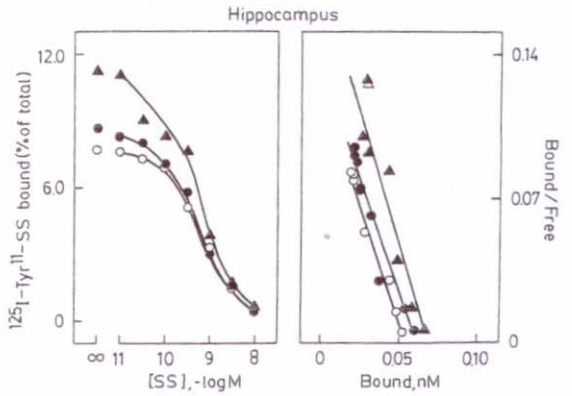


Fig. 3. (Left) Effect of pregnancy on competitive inhibition of specific [$^{125}$I]Tyr$^{11}$-somatostatin ([$^{125}$I]Tyr$^{11}$-SS, 250 pM) binding to membranes of the hippocampus. Membranes (0.15 mg protein/ml) were incubated for 60 min at 30 °C in the presence of 250 pM [$^{125}$I]Tyr$^{11}$-SS and increasing concentrations of native peptide. Points correspond to values of animals in the non-pregnant (●, $n = 10$), pregnant (day 17 to 18 of gestation) (○, $n = 5$) and parturition (△, $n = 5$) groups. Each point is the mean of five replicate experiments. For the sake of clarity, the other experimental groups studied are not represented but the corresponding equilibrium binding parameters are included in Tables I and II. Right panel: scatchard analysis of the same data.

fore, we compared receptor binding in control brains with that in brains to which some unlabelled SS was added to the homogenate fluid. Both groups gave similar SS receptor numbers (404 ± 12 and 448 ± 17 fmol/mg protein in frontoparietal cortex and hippocampus, respectively, of control brains versus 398 ± 10 and 362 ± 24 fmol/mg protein in frontoparietal cortex and hippocampus, respectively, of brains to which unlabelled somatostatin was added).

Ovariectomy as well as the treatment of ovariectomized rats with estradiol valerianate had no effect on cortical and hippocampal somatostatin content and binding (Tables I and II).

## Discussion

In the present study, we have used [$^{125}$I]Tyr$^{11}$-SS as tracer. Tyr$^{11}$-SS has been reported to retain the full biological activity of native SS [24,25]. This analog possesses almost the same potency to inhibit the release of both growth hormone (GH) stimulated by nembutal and thyrotropin (TSH) stimulated by thyrotropin-releasing hormone (TRH) as that of native SS in vivo. In the case of [$^{125}$I]Tyr$^{11}$-SS, the radiolabel is present in a region of the molecule well removed from the N-terminal end and, thus, is protected from aminopeptidase-like enzymes. Furthermore, the localization of the radiolabel at position 11 offers the advantage of labelling the active core (amino acid residues 7–11) of the SS sequence [25].

The results reported in this study show that the content of SSLI increased at 17 days of pregnancy in frontoparietal cortex but not in the hippocampus and decreased at parturition and 10 days postpartum in both brain regions. At 30 days postpartum, the SSLI content had returned to non-pregnant values.

TABLE I

Effect of pregnancy and estradiol valerianate (EV) on [$^{125}$I]Tyr$^{11}$-somatostatin equilibrium binding parameters in the frontoparietal cortex

Binding parameters were obtained by Scatchard [24] analysis of data from Figs. 2 and 3, right panels. $K_d$ is the dissociation constant; $B_{max}$ is the maximum binding capacity. Each value is the mean ± S.E.M. of the five replicate experiments.

| Groups | Hippocampus | | |
|---|---|---|---|
| | $K_d$ (nM) | $B_{max}$ (fmol/mg protein) | $n$ |
| Non-pregnant | 0.40 ± 0.04 | 390 ± 14 | 10 |
| Day 17 to 18 of pregnancy | 0.41 ± 0.04 | 266 ± 13* | 5 |
| Day of delivery | 0.55 ± 0.19 | 657 ± 51** | 5 |
| 10 Days postpartum | 0.53 ± 0.02 | 440 ± 28 | 5 |
| 30 Days postpartum | 0.52 ± 0.06 | 406 ± 30 | 5 |
| Ovariectomized | 0.41 ± 0.02 | 367 ± 22 | 5 |
| Ovariectomized + E.V. | 0.38 ± 0.07 | 413 ± 20 | 5 |

Statistically significant differences between non-pregnant and the other experimental groups were as follows: *$P < 0.0001$, ***$P < 0.0001$. $n$ = number of animals.

The SSLI levels in both brain regions in the control (non-pregnant) rats were similar to those previously reported by others [12,26]. The changes in SSLI content in the frontoparietal cortex and the hippocampus during late pregnancy, on the day of delivery and at early postpartum may have resulted from changes in synthesis, storage, release and/or degradation of SS. Since norepinephrine (NE) and 5-hydroxytryptamine (5-HT) stimulate brain SS release and since NE and 5-HT levels in cerebral cortex fall from normal levels during late pregnancy and rise in the early postpartum period [27–29], it is

TABLE II

Effect of pregnancy and estradiol valerianate (EV) on [$^{125}$I]Tyr$^{11}$-somatostatin equilibrium binding parameters in the hippocampus

Binding parameters were obtained by Scatchard [24] analysis of data from Figs. 2 and 3, right panels. $K_d$ is the dissociation constant; $B_{max}$ is the maximum binding capacity. Each value is the mean ± S.E.M. of the five replicate experiments.

| Groups | Hippocampus | | |
|---|---|---|---|
| | $K_d$ (nM) | $B_{max}$ (fmol/mg protein) | $n$ |
| Non-pregnant | 0.44 ± 0.02 | 399 ± 11 | 10 |
| Day 17 to 18 of pregnancy | 0.44 ± 0.17 | 368 ± 36 | 5 |
| Day of delivery | 0.35 ± 0.10 | 450 ± 38 | 5 |
| 10 Days postpartum | 0.53 ± 0.06 | 391 ± 38 | 5 |
| 30 Days postpartum | 0.49 ± 0.06 | 374 ± 29 | 5 |
| Ovariectomized | 0.41 ± 0.03 | 322 ± 37 | 5 |
| Ovariectomized + E.V. | 0.38 ± 0.07 | 354 ± 45 | 5 |

No statistically significant differences are obtained when compared with the control animals (non-pregnant or ovariectomized), respectively. $n$ = number of animals.

tempting to speculate that the increase of SSLI content in frontoparietal cortex during late pregnancy and the subsequent decrease on the day of delivery and at day 10 during postpartum could be a secondary and subsequent phenomenon to changes in the monoamine metabolism associated with pregnancy and its termination.

The fact that ovariectomy or estradiol administration to ovariectomized rats changed neither SSLI levels nor SS binding agrees with the fact that cerebral cortex and hippocampus have minimal quantities of intracellular estrogen receptors [30].

Scatchard analysis demonstrated the existence of only one type of SS receptor. This finding agrees with some reports [11–13] but not with other previously reported data [31–37]. Both Reubi [31] and Tran et al. [32] have shown that the newly developed SS analog, SMS 201–995, is able to identify subtypes of SS receptors in the brain. The photocrosslinking studies of Thermos et al. [33] suggest that the brain may express at least two molecularly distinct types of SS receptors. Raynor and Reisine [34,35] have identified two SS analogs (CGP 23996 and MK 678) that label pharmacologically distinct subclasses of SS receptors in the brain ($SS_1$ and $SS_2$) Recently, Kluxen et al. [36] have isolated a rat brain SS receptor cDNA and Yamada et al. [37] have reported the cloning and structural characterization of two subtypes of SS receptor. Given the multiplicity of SS receptor subtypes, the changes observed in the present studies could have been in one or more of the receptor species.

Opposite changes in SSLI and SS receptors were found in frontoparietal cortex at 17 days of pregnancy and at parturition. These changes in the somatostatinergic system do not appear to be under estradiol regulation, since ovariectomy and the exposure of ovariectomized rats to estradiol valerianate did not modify SS content and binding in the frontoparietal cortex and hippocampus.

Recently, we have reported an increase in the number of SS receptors in frontoparietal cortex caused by $\alpha_2$-adrenergic agonists (unpublished data). These data together with the changes described by Desan et al. [29] in cerebral cortex noradrenaline levels during late pregnancy and delivery allow us to speculate on the possible participation of noradrenergic neurons in the mediation of the changes found in the somatostatinergic system at late pregnancy and parturition.

Whether the changes in the somatostatinergic system shown in the present experiments are reflected by altered physiological function has not been determined. However, it is possible that seizure susceptibility [1], conditioned avoidance learning [2] and general activity [3], which all vary significantly with the stage of pregnancy and postpartum, may at least partly depend on changes in the somatostatinergic system since SS influences these behavioural activities [7–9]. We conclude that late pregnancy, parturition and the early postpartum period are associated with significant changes in the cerebral somatostatinergic system and therefore it is possible that this system may play a role in some of the behavioural changes that occur during these periods.

## Acknowledgements

We thank Carol F. Warren from the Alcalá de Henares University Institute of Education Sciences for her linguistic assistance. This work was supported by a grant from the Direccion General de Investigacion Cientifica y Tecnica (PM92-0049).

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