Neuroscience Letters, 134 (1991) 37–40

NSL 08264

Changes in striatal somatostatin receptors in pups after cocaine administration to pregnant and nursing dams

M.N. Rodríguez-Sánchez and E. Arilla

Molecular Neuroendocrinology Unit, Department of Biochemistry and Molecular Biology, Medical School, University of Alcalá, Alcalá de Henares, Madrid (Spain)

(Received 5 February 1991; Revised version received 2 August 1991; Accepted 18 September 1991)

Key words: Cocaine; Somatostatin; Receptor; Striatum; Offspring; Rat

Primiparous female Wistar rats were injected subcutaneously with single daily doses of 40 mg of cocaine hydrochloride/kg from day 7 to 19 of gestation, from day 7 of gestation to day 15 postpartum or from parturation to day 15 postpartum. At birth, some of the offspring were fostered to control mothers to limit the effect of cocaine to the prenatal period and some were left with their mothers with the aim of studying prenatal plus postnatal exposure to cocaine. Prenatal and/or postnatal cocaine exposure did not affect the content of somatostatin (SS)-like immunoreactivity (SLI) in the striatum of the offspring as compared with the control groups on day 15 in all experimental groups. Prenatal and prenatal-plus-postnatal exposure to cocaine increased the total number of binding sites for ^{125}I-Tyr^{11}-SS in the rat striatum at 15 days of age. Prenatal exposure to cocaine also decreased the apparent affinity of the receptors. Postnatal exposure to cocaine alone had no such post-treatment effect on ^{125}I-Tyr^{11}-SS binding. These results suggest that the development of SS receptors in the rat striatum can be altered by prenatal exposure to cocaine.

A number of animal studies have described the effects of prenatal cocaine exposure on abnormalities in the motor control of the offspring [3, 25]. This effect of cocaine is thought to be mediated by the dopaminergic system [1]. However, motor control does not depend exclusively on the dopaminergic system, since other neurotransmitters such as somatostatin (SS) [18] and TRH [30] also influence motor control. In addition, if we accept that cocaine is an indirect dopaminergic agonist which blocks dopamine reuptake in the adult [10] and neonatal brain [11] and that dopamine plays a role in the regulation of somatostatinergic system [1, 22, 27], it is possible that cocaine, by affecting the dopaminergic system, might be able to influence the development of somatostatinergic neurons or receptors in the inmature brain. As an initial approach to examining this possibility, we studied the effects of chronic prenatal and/or postnatal cocaine exposure on SS binding to dissociated cells from the striatum, a nucleus involved primarily in the control of movement [13], in developing 15-day-old offspring. This study also includes the determination of somatostatin-like immunoreactivity (SLI) content in this brain area.

Correspondence: E. Arilla, Molecular Neuroendocrinology Unit, Department of Biochemistry and Molecular Biology, Medical School, University of Alcalá, 28871, Alcalá de Henares, Madrid, Spain.

Synthetic Tyr^{11}-SS and SS-14 were purchased from Universal Biologicals Ltd. (Cambridge, U.K.), cocaine hydrochloride was from Alcaliber S.A. (Madrid, Spain). The origin of the other chemicals was as in ref. 14. Synthetic Tyr^{11}-SS was radioiodinated at a specific radioactivity of about 400 Ci/g.

Cocaine is known to cross the placenta rapidly and also passes from the mother's blood into breast milk [29]. Therefore, for prenatal and/or postnatal drug application, primiparous female Wistar rats (230–250 g) were injected subcutaneously with single daily doses of 40 mg of cocaine hydrochloride/kg from day 7 to 19 of gestation, from day 7 of gestation to day 15 or from parturition to day 15 postpartum [5]. At birth, some offspring were fostered to control mothers to limit the effect of cocaine to the prenatal period and some were left with their mothers with the aim of studying prenatal plus postnatal exposure to cocaine. Since females are more sensitive to cocaine than males [6], the present study was limited to females. The female offspring of the treated and control rats were sacrificed by decapitation at 15 days of age, their brains were removed at 4°C, and the striatum dissected according to Glowinski and Iversen [7]. The striatum was excised from 14 rat pups and pooled in 7 groups of 2 rats prior to mincing.

SS was extracted from the striatum following the method of Patel and Reichlin [12]. SLI content was

measured by a modified specific radioimmunoassay method [12], with a sensitivity limit of 10 pg/ml. Protein concentration was estimated by the method of Lowry et al. [9] using bovine serum albumin (BSA) as a standard. Separation of bound and free hormone was accomplished by addition of 1 ml dextran-coated charcoal (dextran 0.2% w/v; charcoal 2% w/v). The dilution curve of the brain area studied was parallel to the standard curve. The intra- and inter-assay variation coefficients were 6.2% and 8.9%, respectively.

Cells from the striatum of 15-day-old offspring were prepared as described by Roeder et al. [20]. Cell protein was estimated by the method of Lowry et al. [9] using BSA as a standard. Experimental conditions for SS binding were essentially as previously described [4]. Briefly, dissociated cells from rat striatum (1 mg protein/ml) were incubated in 0.5 ml of 50 mM Tris-HCl buffer (pH 7.5) containing 5 mM $MgCl_2$, 30 mM $CaCl_2$, 1% BSA, 0.1% bacitracin and 10 pM (^{125}I-Tyr^{11}-SS) in the absence or presence of 0.01–10 nM unlabelled SS. After 60 min incubation at 25%, cell-bound peptide was isolated by centrifugation at 13,000 *g* for 1.5 min, and radioactivity determined in a kontron gamma counter. Non-specific binding was obtained from the amount of radioactivity bound in the presence of 10^{-6} M SS and represented about 20% of the binding observed in the absence of unlabelled peptide. This non-specific component was subtracted from the total bound radioactivity in order to obtain the corresponding specific binding. To determine the extent of tracer degradation during incubation, we measured the ability of preincubated peptide to bind to fresh cells as previously described [24]. Results were given in all cases as the mean ± S.E.M. The Student's *t*-test for unpaired variables was employed to assess differences between control and experimental groups, as indicated in the text and figures. The number of receptors and affinity constants in Scatchard plots [23] were calculated by linear regression analysis.

Cocaine administration to pregnant and/or nursing rats produced no changes in SLI content in the striatum of the offspring as compared with the control groups on day 15 (Table I). Since cocaine is a dopaminergic agonist [10] the present results are in apparent conflict with other findings that suggest an influence of dopamine on striatal SS function [1, 22 , 27]. This conflict is probably due to the measurement of peptide levels being a rather insensitive index of release. However, Salin et al. [21] have recently demonstrated that lesions in nigrostriatal dopaminergic neurons, which markedly reduce striatal dopamine content, do not affect the number of neurons expressing SLI in the striatum ipsilateral to the lesion. In addition, the hypothesis of Salin et al. [22] that dopamine exerts opposite effects on the content of preproSS messenger RNA encoding SS in the rat striatum through an action on D_1 and D_2 receptors is compatible with our observation that cocaine, which is a dopaminergic agonist [10], does not affect the content of SLI in this brain area.

Dissociated striatal cells from both control and cocaine-exposed 15-day-old rats bound ^{125}I-Tyr^{11}-SS in a time-dependent process; an apparent equilibrium was observed between 50 and 120 min at 25% (data not shown). All subsequent binding experiments were therefore conducted at 25°C for 60 min. The degradation of tracer by dissociated cells was similar (10%) in all the experimental groups. Fig. 1 shows the competitive effect of unlabelled SS upon the specific binding of ^{125}I-Tyr^{11}-SS to dissociated striatal cells in the preparations from the offspring of both control and cocaine-exposed rats. These data, analyzed by the Scatchard method [23], revealed that prenatal and prenatal-plus postnatal expo-

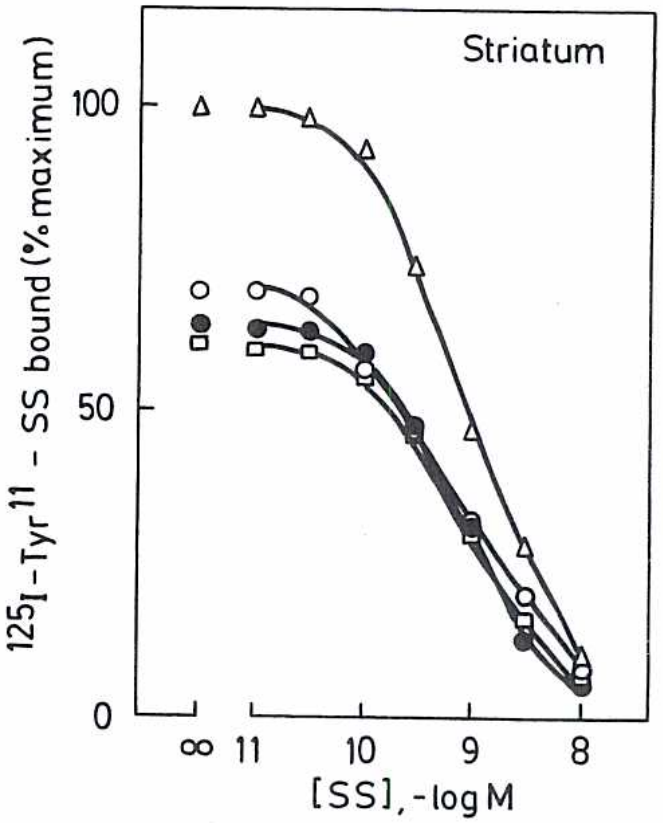

Fig. 1. Competitive inhibition of specific ^{125}I-Tyr^{11}-somatostatin (^{125}I-Tyr^{11}-SS, 100 pM) binding by unlabelled somatostatin to dissociated cells of the striatum of 15-day-old offspring of control (●), rats treated with cocaine during pregnancy and sacrificed at 15 days of age (○) or during 15 days postpartum (□) or during pregnancy plus 15 first days postpartum (△). Dissociated cells (1 mg protein/ml) were incubated for 60 min at 25°C in the presence of 100 pM ^{125}I-Tyr^{11}-SS and increasing concentrations of native peptide. Each point represents the mean obtained from 7 experiments in which duplicate determinations were made. For the sake of clarity, standard error is not represented but it was always below 10% of the mean values.

TABLE I

EFFECT OF PRENATAL AND/OR POSTNATAL COCAINE EXPOSURE ON THE SOMATOSTATIN-LIKE IMMUNOREACTIVITY (SLI) IN STRIATUM OF THE OFFSPRING ON DAY 15

For details of treatment see text. Determinations were made in duplicate for each experiment. The striatum was excised from 10 rat pups and pooled in 5 groups of 2 rats prior to mincing. The results are expressed as ng somatostatin/mg protein and as the means ± S.E.M. No statistically significant differences were obtained when compared with the control animals.

Groups	Striatum
Saline (controls)	5.83 ± 0.48
Cocaine-treated during pregnancy	4.98 ± 0.33
Cocaine-treated during 15 first days postpartum	5.09 ± 0.30
Cocaine-treated during pregnancy plus 15 first days postpartum	5.08 ± 0.43

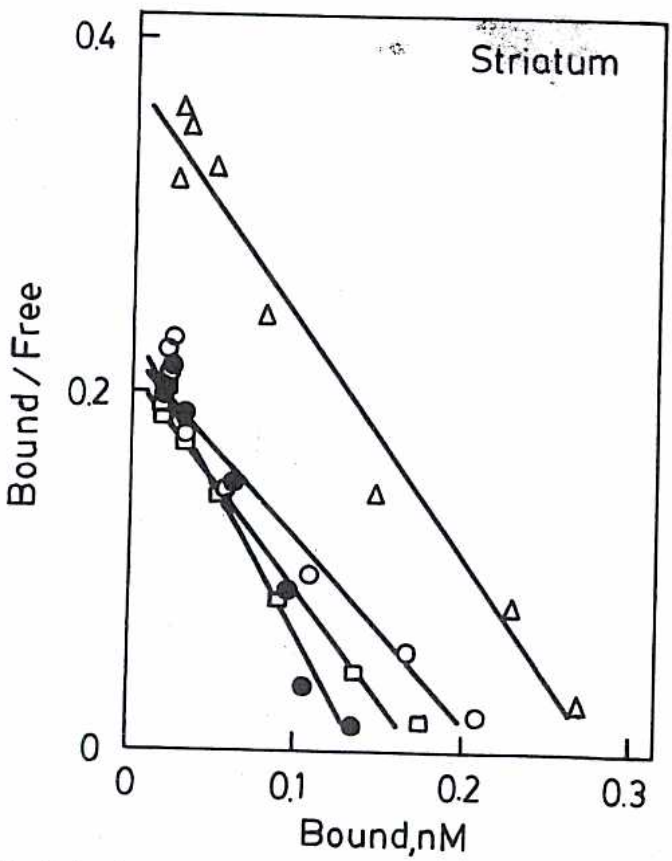

Fig. 2. Scatchard plots of specific ^{125}I-Tyr^{11}-somatostatin (^{125}I-Tyr^{11}-SS) binding to dissociated cells (1 mg protein/ml) of the striatum 15-day-old offspring of controls (●), rats treated with cocaine during pregnancy and sacrificed at 15 days of age (○) or during 15 first days postpartum (□) or during pregnancy plus 15 first days postpartum (△). The corresponding equilibrium-binding parameters are included in Table II.

sure to cocaine increased the total number of receptors for ^{125}I-Tyr^{11}-SS in the rat striatum at 15 days of age. Prenatal exposure to cocaine also decreased the apparent affinity of the receptors. Postnatal exposure to cocaine alone had no such posttreatment effects of ^{125}I-Tyr^{11}-SS binding (Fig. 2 and Table II). Scatchard analysis demonstrated the existencence of only one type of SS receptor. This feature agrees with some studies [26] but differs from others [17]. It is conceivable that use of small SS analogs [17] could explain the differences.

The mechanism responsible for the change in SS binding to its own receptors observed in the present study is unknown. Several studies have shown anatomical and functional interconnections between dopaminergic and somatostatinergic systems [2, 27]. We have recently reported that haloperidol, a dopamine receptor blocker, gave rise to a decrease in the number of SS receptors in the rat brain [14]. After haloperidol is withdrawn, the number of dopamine receptors rises and the number of SS receptors returns to control values, although the actual number of SS receptors remains slightly higher [15]. These previous findings together with the present results may suggest that the effects of prenatal and/or postnatal cocaine exposure on SS receptors may be mediated in part through the effects of the drug on dopaminergic receptors, although they do not preclude the possibility that other factors may be involved as well.

Previous studies from this laboratory have indicated

TABLE II

EFFECT OF PRENATAL AND/OR POSTNATAL COCAINE EXPOSURE IN THE RAT ON EQUILIBRIUM PARAMETERS OF SOMATOSTATIN (SS) BINDING TO DISSOCIATED CELLS FROM STRIATUM OF THE OFFSPRING ON DAY 15

The striatum was excised from 14 rat pups and pooled in seven groups of two rats prior to mincing. Binding parameters were obtained by Scatchard [23] analysis of data from Fig. 2. K_d, dissociation constant; B_{max}, binding capacity. Results are expressed as means ± S.E.M.

Groups	Striatum	
	B_{max} (fmol/mg protein)	K_d (nM)
Saline (controls)	139 ± 27	0.60 ± 0.10
Cocaine-treated during pregnancy	217 ± 15*	0.99 ± 0.14*
Cocaine-treated during 15 first days postpartum	177 ± 20	0.84 ± 0.25
Cocaine-treated during pregnancy plus 15 first days postpartum	281 ± 41**	0.76 ± 0.15

*$P < 0.05$, **$P < 0.02$, statistically significant compared with control.

that acute cocaine administration to mature rats decreased SS binding in rat brain [19]. This apparent discrepancy with the results obtained in the present paper may be explained in that in the acute study the rats were sacrificed 15–20 min after cocaine administration, when cocaine would be present and synaptic concentrations of dopamine would be maximal and all decreases dopamine receptor number [8]. Prenatal exposure to cocaine not only leads to an increase in the number of SS receptors in the rat striatum at 15 days of age, but also to a concomitant rise in the number of dopamine receptors in the striatum [28].

These results suggest that the development of SS receptors in the rat striatum can be altered by prenatal exposure to cocaine.

This work was supported by grants from the Comisión Interministerial de Ciencia y Tecnología (PB87-0753) and the Fondo de Investigaciones Sanitarias de la Seguridad Social of Spain (88/0903). The authors thank Carol F. Warren, from the Alcalá de Henares University Institute of Education Sciences for her editorial help.

1 Beal, M.F. and Martin, J.B., Effects of neuroleptic drug on brain somatostatin-like immunoreactivity, Neurosci. Lett., 47 (1984) 125–130.

2 Chesselet, M. and Reisine, J.D., Somatostatin regulates dopamine release in rat striatal slices and cat caudate nuclei, J. Neurosci., 3 (1983) 232–236.

3 Church, M.W. and Overbeck, G.W., Prenatal cocaine exposure in the Long-Evans rat: II. Dose-dependent effects on offspring behavior, Neurotoxicol. Teratol., 12 (1990) 335–343.

4 Colás, B., Prieto, J.C. and Arilla, E., Somatostatin binding to dissociated cells from rat cerebral cortex, Peptides, 11 (1990) 1109–1112.

5 Fantel, A.C. and MacPhail, B.J., The teratogenicity of cocaine, Teratology, 26 (1982) 17–19.

6 Glick, S.D. and Hinds, P.A., Sex differences in sensitization to cocaine-induced rotation, Eur. J. Pharmacol., 99 (1984) 119–121.

7 Glowinski, J. and Iversen, L.L., Regional studies of catecholamines in the rat brain. I. The disposition of [^{3}H]norepinephrine, [^{3}H]dopamine and [^{3}H]DOPA in various regions of the brain, J. Neurochem., 13 (1966) 655–669.

8 Goeders, N.E. and Kuhar, M.J., Chronic cocaine administration induces opposite changes in dopamine receptors in the striatum and nucleus accumbens, Alcohol Drug Res., 7 (1987) 207–216.

9 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J., Protein measurement with the Folin phenol reagent, J. Biol. Chem., 193 (1951) 265–275.

10 Missale, C., Castelletti, L., Govoni, S., Spano, P.F., Trabucchi, M. and Hanbauer, I., Dopamine uptake is differentially regulated in rat striatum and nucleus accumbens, J. Neurochem., 45 (1985) 51–56.

11 Nomura, Y., Naitoh, F. and Segawa, T., Regional changes in monoamine content and uptake of the rat brain during postnatal development, Brain Res., 101 (1976) 305–315.

12 Patel, J.C. and Reichlin, S., Somatostatin in hypothalamus, extrahypothalamic brain, and peripheral tissues of the rat, Endocrinology, 103 (1978) 523–531.

13 Penney, J.B. and Young, A.B., Striatal inhomogeneities and basal ganglia function, Movem. Disord., 1 (1986) 3–15.

14 Perez-Oso, E., Colás, B., López-Ruiz, M.P. and Arilla, E., Long-term haloperidol treatment decreases somatostatin binding in rat brain, Neuropeptides, 13 (1989) 157–163.

15 Perez-Oso, E., López-Ruiz, M.P. and Arilla, E., Effect of haloperidol withdrawal on somatostatin level and binding in rat brain, Biosci. Rep., 10 (1990) 15–22.

16 Reith, M.E., Meisler, B.E., Sershen, H. and Lajtha, A., Structural requirements for cocaine congeners to interact with dopamine and serotonin uptake sites in mouse brain and to induce stereotyped behavior, Biochem. Pharmacol., 35 (1986) 1123–1129.

17 Reubi, J.C., Evidence for two somatostatin-14 receptor types in rat brain cortex, Neurosci. Lett., 49 (1984) 259–263.

18 Rezek, M., Havlicek, V., Leybin, L., Pinsky, C., Kroeger, E.A., Hughes, K.R. and Friesen, H., Neostriatal administration of somatostatin: differential effect of small and large doses on behavior and motor control, Can. J. Physiol. Pharmacol., 55 (1977) 234–242.

19 Rodríguez-Sánchez, M.N. and Arilla, E., Effects of acute and chronic cocaine administration on somatostatin level and binding in the rat brain, Neuropeptides, 16 (1990) 1–7.

20 Roeder, L.M., Tildon, J.T. and Wiliams, I.B., Transport of 2-deoxy-D-glucose by dissociated brain cells, Brain Res., 345 (1985) 298–305.

21 Salin, P., Kerkerian-Le Goff, L., Heidet, V., Epelbaum, J. and Niloullon, A., Somatostatin-immunoreactive neurons in the rat striatum: effects on corticostriatal and nigrostriatal dopaminergic lesion, Brain Res., 521 (1990) 23–32.

22 Salin, P., Mercugliano, M. and Chesselet, M.F., Differential effects of chronic treatment with haloperidol and clozapine on the level of preprosomatostatin mRNA in the striatum, nucleus accumbens, and frontal cortex of the rat, Cell. Mol. Neurobiol., 10 (1990) 127–144.

23 Scatchard, G., The attractions of proteins for small molecules and ions, Ann. N.Y. Acad. Sci., 51 (1949) 660–671.

24 Schonbrunn, A., Rorstad, O.P., Westendorg, J.M. and Martin, J.B., Somatostatin analogs: correlation between receptors binding affinity and biological potency in GH pituitary cells, Endocrinology, 113 (1983) 1559–1567.

25 Spear, L.P., Kierstein, C.L., Bell, J., Yoottanasumpun, V., Greenbaum, R.M., O'shea, J., Hoffmann, H. and Spear, N.E., Effects of prenatal cocaine exposure on behavior during the early postnatal period, Neurotoxicol. Teratol., 11 (1989) 57–63.

26 Srikant, C.B. and Patel, Y.C., Somatostatin receptors: identification and characterization in rat brain membranes, Proc. Natl. Acad. Sci. U.S.A., 78 (1981) 3930–3934.

27 Thal, L.J., Laing, K., Horowith, S.G. and Makman, M.H., Dopamine stimulates rat cortical somatostatin release, Brain Res., 372 (1986) 205–209.

28 Trulson, M.E. and Ulissey, M.J., Chronic cocaine administration decreases dopamine synthesis rate and increases [^{3}H]spiroperidol binding in rat brain, Brain Res. Bull., 19 (1987) 35–38.

29 Wiggens, R.C., Rolsten, C., Ruiz, B. and Davis, C.M., Pharmacokinetics of cocaine: basic studies of route, dosage, pregnancy, and lactation, Neurotoxicology, 10 (1989) 367-382.

30 Vogel, R.A., Cooper, B.R., Barlow, T.S., Prange, A.J. Jr., Mueller, R.A. and Breese, G.R., Effects of TRH on locomotor activity, operant performance and ingestive behavior, J. Pharmacol. Exp. Ther., 208 (1978) 161–168.