

The peripheral noradrenergic terminal as possible site of action of salsolinol as prolactoliberin

D. Székács^a, I. Bodnár^a, B. Mravec^b, R. Kvetnansky^b,
E.S. Vizi^c, G.M. Nagy^a, M.I.K. Fekete^{a,*}

^aNeuroendocrine Research Unit, Hungarian Academy of Sciences, Department of Human Morphology, Semmelweis University, Budapest, Hungary

^bInstitute of Experimental Endocrinology, Slovak Academy of Sciences, Bratislava, Slovakia

^cInstitute of Experimental Medicine, Hungarian Academy of Sciences, Budapest, Hungary

Received 27 September 2006; accepted 3 October 2006

Available online 1 December 2006

Abstract

Salsolinol, an endogenous isoquinoline, induces selective prolactin release in rats [Tóth, B.E., Homicskó, K., Radnai, B., Maruyama, W., DeMaria, J.E., Vecsernyés, M., Fekete, M.I.K., Fülöp, F., Naoi, M., Freeman, M.E., Nagy, G.M., 2001. Salsolinol is a putative neurointermediate lobe prolactin releasing factor. *J. Neuroendocrinol.* 13, 1042–1050]. The possible role of dopaminergic and adrenergic signal transduction was investigated to learn the mechanism of this action. The effect of salsolinol (10 mg/kg i.v.) was inhibited by reserpine treatment (2.5 mg/kg i.p.) and reinstated by pretreatment with monoamine oxidase inhibitor (pargyline 75 mg/kg i.p.). Salsolinol did not affect the *in vitro* release of dopamine (DA) in the median eminence, and did not inhibit the L-DOPA induced increase of DA level in the median eminence. 1-Methyl dihydroisoquinoline (1MeDIQ) is an antagonist of salsolinol induced prolactin release and causes increase in plasma NE level [Mravec, B., Bodnár, I., Fekete, M.I.K., Nagy, G.M., Kvetnansky, R., 2004. An antagonist of prolactoliberin induces an increase in plasma catecholamine levels in the rat. *Autonom. Neurosci.* 115, 35–40]. Using tissue catecholamine contents as indicators of the interaction between salsolinol and 1MeDIQ we found no interaction between these two agents to explain the changes in prolactin release in the median eminence, lobes of the pituitary, superior cervical and stellate ganglion. Increasing doses of salsolinol caused a dose dependent decrease of tissue dopamine concentration and increase of NE/DA ratio in the salivary gland, atrium and spleen. These changes of DA level and NE/DA ratio run parallel in time with the increase of prolactin release. 1MeDIQ antagonized the increase of prolactin release and decrease of tissue DA content caused by salsolinol. Neither this increase of prolactin secretion nor the decrease of DA level in spleen could be demonstrated in NE transporter (NET) knock out mice. The results presented argue for the possible role of peripheral norepinephrine release as a target for salsolinol in its action releasing prolactin. The dominant role of norepinephrine transporter may be suggested.

© 2006 Elsevier Ltd. All rights reserved.

Keywords: Prolactin; Salsolinol; Norepinephrine; Peripheral sympathetic terminals

1. Introduction

Salsolinol is an endogenous tetrahydroisoquinoline derivative (Sandler et al., 1973; Naoi et al., 1996), which induces a selective increase of prolactin secretion (Tóth et al., 2001). In the mechanism of suckling or stress induced prolactin release salsolinol seems to play a pivotal role (Bodnár et al., 2004). The

generally accepted dopaminergic inhibition of prolactin secretion is not influenced by this isoquinoline derivative (Tóth et al., 2001, 2002). Studies on the binding site of salsolinol have shown a characteristic antagonism of the binding by L-DOPA, and agents acting on aromatic amino acid decarboxylase (Homicskó et al., 2003). Therefore attempts were made to investigate the possible effect of salsolinol on dopamine release with special emphasis on the decarboxylase activity. Furthermore, the salsolinol induced changes in the catecholamine content of median eminence and pituitary were measured as well as the *in vitro* release of dopamine from the median eminence of the rat. Finally, the catecholamine content of sympathetic ganglia and of peripheral organs were determined as affected by salsolinol and

* Corresponding author at: Department of Human Morphology and Developmental Biology, Semmelweis University, Tüzoltó u. 58, Budapest 1094, Hungary. Fax: +36 1 215 3064.

E-mail address: fek61@ella.hu (M.I.K. Fekete).

it's antagonist 1-methyl dihydroisoquinoline (1MeDIQ; Bodnár et al., 2004; Mravec et al., 2004). Indirect and direct evidences suggested that the peripheral norepinephrine release is deeply affected by salsolinol.

2. Methods and materials

Male (measurements of monoamine content and prolactin) and female (reserpine–pargyline interaction, *in vitro* release studies) Sprague–Dawley rats of 250–350 g body weight were used. The animals were kept in an air-conditioned room with regular lighting. The experiments were done in the light period, between 8 and 12 a.m. Indwelling venous catheter was implanted in the jugular vein (for details see the Ref. Tóth et al., 2001) 1 day before experiments with reserpine treated rats. In the other experiments the treatments were done *i.p.* as indicated in the figures or legends. All procedures were done under the control and permission of the Ethical Committee of the Semmelweis University in accordance with the Hungarian law on animal care.

NE transporter KO animals: Breeding pairs of NET knock out (KO) and wild type mice (C57BL/6J based) were transferred from Institute of Experimental Medicine, Hungarian Academy of Sciences (Budapest), originating from Duke University, Medical Center (Durham, NC). The animals contained the DNA construct previously shown to produce genetic deletion of the NET (Xu et al., 2000). The experiments were done on 3–5 months' male homozygous (NET^{-/-}) mice and their wild type littermates (NET^{+/+}).

The animals were killed by decapitation when tissue samples were taken for HPLC analysis; the tissue samples were dissected and put on dry ice immediately. Trunk blood was collected for hormone measurements. The determination of catechol derivatives was done by high performance liquid chromatography (HPLC) within 2 weeks following the experiments, using the method described (DeMaria et al., 1998). Homogenization was done in 200–500 μ l of 0.2N perchloric acid containing EGTA (0.025 mM) by ultrasound. After centrifugation (10,300 \times g, 15 min, 4 °C) 20 μ l of supernatant was injected into the HPLC. The protein content of the pellet was determined by Lowry's method (Lowry et al., 1951); ng/mg protein values are given in the results, individual values of NE/DA ratios were calculated.

Shimadzu HPLC apparatus was used with auto sampler and built-in integrator program, combined with electrochemical detection (ESA Coulochem II). Reverse phase column (Wakosil II C18, 150 mm \times 4.6 mm, 5 μ m, SGE) was employed; the eluent was 5% acetonitrile in water containing sodium phosphate (NaH₂PO₄) 0.075 M, octanesulfonic acid sodium salt 1.7 mM, EDTA 0.1 mM and triethylamine (100 μ l/l). The pH of the mobile phase was adjusted to 3.0 with phosphoric acid. The interassay variation of the determinations was 8–15% for the measured catechols (norepinephrine, dopamine).

2.1. Hormone analysis

Prolactin was measured by radioimmunoassay (RIA), with kits kindly provided by NHPP, NIDDK and Dr. A.F. Parlow. The RIA procedure was similar to the instructions supplied with the kit, with modifications as described previously (Kacsóh et al., 1993). We used the Chloramine-T method for iodination and protein A (BactASorb, Human Rt, Gödöllő, Hungary) for separation of bound and free hormone. Data collection and calculations for curve fitting were made using LKB Clinigamma software. The data were expressed in terms of NIAMDD-Rat-RP-3. The within- and between-assay variances were 10% and 14%, respectively. The sensitivity of the prolactin assay was 0.5 ng/ml rat plasma, expressed in terms of the rat prolactin RP-3 standard (or 25 pg PRL). We used 50 μ l plasma per samples. All samples were measured in duplicates.

2.2. Release studies

2.2.1. [7,8-³H]-Dopamine release from rat median eminence

Female rats were killed by decapitation, the brain was rapidly removed and the median eminence was prepared under a ZEISS SV 11 stereomicroscope and

immediately placed into ice-cold Krebs solution continuously gassed with a mixture of 95% O₂ and 5% CO₂.

The median eminences were washed in 1 ml Krebs solution, and loaded at 37 °C for 45 min with [7,8-³H]-dopamine at a concentration of 155 KBq/ml Krebs solution (specific activity 1147 GBq/mmol).

After the incubation the tissue pieces were washed three times with 1 ml of Krebs solution and transferred into a four-channel micro volume perfusion system of a cell volume of 200 μ l. A single tissue piece was put into each chamber; the preparation was superfused with Krebs solution at 37 °C and at a rate of 0.4 ml/5 min for 45 min and the effluent was discarded.

Subsequently 5 min fractions were collected for 100 min. Supramaximal (30 V) field stimulation (3 ms, 5 Hz for 2 min = 600 shocks) was applied during the collection of the 3rd (S₁) and 13th (S₂) fractions, the S₂/S₁ ratios were calculated counting the integrated stimulation induced release of labeled dopamine. Drugs were usually added from the 8th fraction till the end of the experiments. At the end of the experiments the median eminences were taken off from the chambers and were homogenized in 100 μ l 10% trichloroacetic acid. A 350 μ l aliquot of the superfusate and 80 μ l of the tissue supernatant was added to 2 ml of scintillation cocktail (Ultima Gold, Packard). Tritium was measured with a Packard 1900 TR liquid scintillation counter.

All chemicals were of HPLC grade pure, the ion free water was purified on Simplicity 185 (UV) Water System (Millipore Co., Bedford, MA). Salsolinol and 1MeDIQ were synthesized by F. Fülöp (Department of Chemistry, University of Szeged, Faculty of Pharmacy). L-DOPA was obtained from Sigma.

2.3. Statistical analysis

The statistical analysis of data was performed using Statistica program (StatSoft Inc., Tulsa, OK, USA): ANOVA followed by Dunnett's test.

3. Results

3.1. Effect of reserpine

Attempts to learn the mechanism of action of salsolinol has been started with the investigation of interaction of a monoamine oxidase inhibitor (pargyline) and reserpine with salsolinol measuring prolactin release. Reserpine (2.5 mg/kg *i.p.* 45 min before salsolinol injection) increased the basal level of plasma prolactin. After reserpine treatment salsolinol (10 mg/kg *i.v.* at 0 min) caused no increase of plasma prolactin concentration. The effect of salsolinol was reestablished by pargyline pretreatment (75 mg/kg *i.p.* 120 min before salsolinol, Fig. 1).

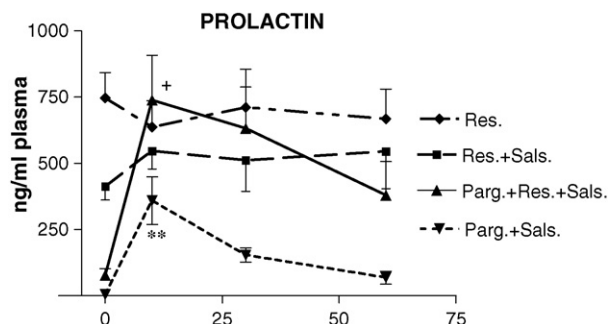


Fig. 1. The effect of salsolinol (Sals.) on plasma prolactin level in reserpine (Res.; 2.5 mg/kg *i.p.* 45 min before salsolinol) and pargyline (Parg. 75 mg/kg *i.p.* 2 h before salsolinol treatment); $n = 6$, means \pm S.E.M. Ordinate: plasma prolactin level ng/ml, abscissa: time in min after salsolinol injection. ** $P < 0.01$, * $P < 0.05$ compared to the respective 0 min value.

Table 1

The effect of salsolinol on stimulation evoked ^3H -DA release in individual median eminences in vitro

Suckling dams		Weaning
Control	Salsolinol, 50 μM	Salsolinol, 50 μM
0.82 \pm 0.15 (3)	0.86 \pm 0.18 (6)	0.75 \pm 0.05 (3)

Ratios of DA overflow induced by two stimulations (S_2/S_1), means \pm S.E.M. (n). Control: S_2/S_1 ratio without in vivo or in vitro treatment; salsolinol was added to the perfusion fluid after the first stimulation; weaning, suckling: the dams were separated or not, respectively, from the pups for 4 h before tissue dissection.

3.2. Studies of in vitro release of dopamine from the median eminence

The prolactin release induced by salsolinol is independent of the gender of the animals. However, the sharpest salsolinol induced prolactin release was demonstrable after a 4 h weaning period in rats (Tóth et al., 2001). Therefore, the in vitro release studies were carried out in female rats using normal adults, suckling and weaning dams. The release of dopamine was measured in vitro from single median eminences. Under control condition the dopamine release was stable: the S_2/S_1 ratios were between 0.8 and 0.9. Salsolinol did not change the stimulation induced dopamine release neither in median eminences obtained from control female rats (not shown), nor in those got from suckling dams or from dams after a 4 h weaning (Table 1). In separate experiments no relevant changes in endogenous dopamine, norepinephrine were found following salsolinol treatment in the median eminence, or in the neurointermediate lobe of the pituitary, neither there was an interaction between salsolinol and its antagonist 1MeDIQ in these tissues (not shown).

3.3. Effect of salsolinol–L-DOPA combined administration

In course of the salsolinol-binding site studies (Homicskó et al., 2003) results suggested a possible action of salsolinol on aromatic amino acid decarboxylase activity. L-DOPA in dose of 25 mg/kg i.p. 30 min after injection caused a minimal increase of dopamine content in the median eminence of male rats ($P > 0.05$). In salsolinol treated rats (20 mg/kg i.p.) a non-significant decrease of dopamine concentration was seen and L-DOPA significantly increased the tissue dopamine level (Fig. 2). The experiment was done in pargyline pretreated (70 mg/kg i.p. 120 min before measurements) animals.

3.4. Effect of salsolinol and 1MeDIQ on dopamine concentration of peripheral organs

A synthetic isoquinoline derivative 1-methyl-dihydroisoquinoline (1MeDIQ), which causes an increase of norepinephrine release in the periphery, antagonizes the prolactin release induced by salsolinol (Bodnár et al., 2004; Mravec et al., 2004). This finding led us to investigate the changes of catecholamine levels in peripheral organs and in sympathetic ganglia. Four doses of salsolinol from 0.2 to 25 mg/kg i.p. were

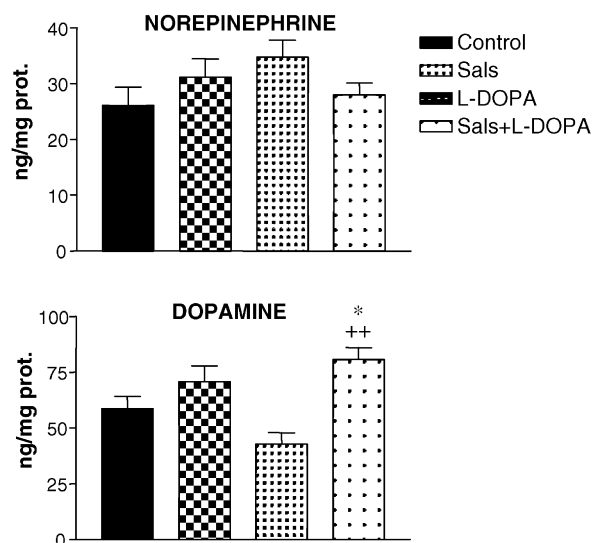


Fig. 2. NE, DA levels in the median eminence of salsolinol (20 mg/kg i.p. 20 min before decapitation) and L-DOPA (25 mg/kg i.p. 15 min before decapitation) treated male rats; $n = 6$, means \pm S.E.M. * $P < 0.05$ compared to the control, ** $P < 0.01$ compared to the group treated with salsolinol only. The animals received pargyline treatment (70 mg/kg i.p.) 2 h before dissection.

injected. The norepinephrine, and dopamine levels were measured and the norepinephrine/dopamine (NE/DA) ratios were calculated. There was a dose related decrease in dopamine concentration in the submandibular gland, spleen (Fig. 3) as well as in the atrium. Similar decrease of tissue dopamine content was measured in the pancreas and vas deferens. The noradrenalin concentration in these organs was not changed by salsolinol, or there was a slight non-significant decrease of the norepinephrine content leading to an increase in NE/DA ratio.

The increase of prolactin secretion as induced by salsolinol reaches its maximum 15 min after the administration. The increase of NE/DA ratio measured simultaneously in peripheral organs runs in close parallelism in time with the changes of plasma prolactin concentration (Fig. 4).

The interaction of salsolinol and 1MeDIQ has been measured in the superior cervical and stellate ganglia and in the atria of rats. Simultaneously the plasma prolactin levels were also measured. In the superior cervical and stellate ganglia a slight non-significant increase in dopamine content due to 1MeDIQ treatment and a decrease of DA level following salsolinol administration ($P > 0.05$) was observed. The results obtained in stellate ganglion are shown in Fig. 5. A decrease of dopamine concentration and consecutive increase of NE/DA ratio induced by salsolinol were clearly demonstrable in the atrium, spleen, salivary gland, and vas deferens. 1MeDIQ injected 10 min before salsolinol antagonized the induced prolactin secretion. 1MeDIQ induced a significant decrease of NE/DA ratio, and it inhibited the decrease of dopamine level and the increase of NE/DA ratio caused by salsolinol in the atrium (Fig. 6).

3.5. Effect of salsolinol in NET KO mice

The effect of salsolinol was measured in norepinephrine transporter knock out (NET KO) mice. Heterozygous animals

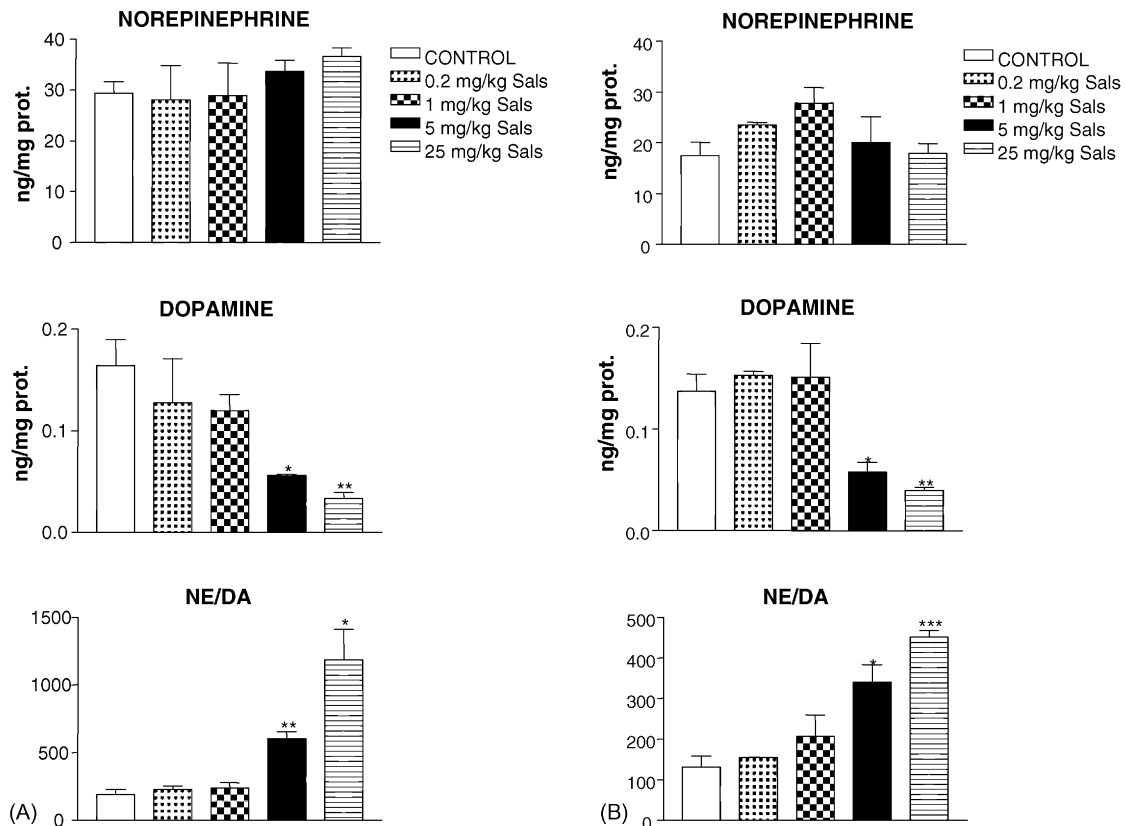


Fig. 3. NE, DA levels and NE/DA ratios in the submandibular gland (A) and in the spleen (B) as affected by increasing doses of salsolinol (Sals). Measurements were done 30 min after treatment; $n = 5$, means \pm S.E.M. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ compared to the control values.

of the same strain were used as controls. The prolactin secretion was highly augmented in these latter animals, however, no significant change in the prolactin release of NET KO animals was seen after salsolinol treatment. The dopamine content in the NET KO mice was also measured in the atrium (not shown) and in the spleen. No change in the concentration of this monoamine could be measured in KO mice, while in BALB C mice the salsolinol induced dopamine decrease was clearly demonstrable (Fig. 7).

4. Discussion

Series of experimental results presented suggest the participation of monoamines in the prolactin releasing activity of salsolinol. Reserpine inhibited the effect of salsolinol on prolactin release, which may be the consequence of the high plasma prolactin concentration, when salsolinol was injected. The reappearance of salsolinol's effect due to the pretreatment with a monoamine oxidase inhibitor argues for the role of an aromatic monoamine (substrate of monoamine oxidase) in the effect of salsolinol. A series of pharmacological investigations show this typical interaction of reserpine and monoamine oxidase inhibitors. Gudelsky and Metzger (1984) employing the same reserpine and pargyline treatment concluded that dopamine release is dependent of its synthesis and of monoamine oxidase activity. In rats treated with pargyline at a dose of 75 mg/kg, a salsolinol caused altered MAO activity

is not probable: either the inhibition of synthesis or the altered release may be responsible for the effect of salsolinol. The dominant role of monoamines in reserpine–MAO inhibitor interaction was proved (Spector, 1963; Shore, 1966). Serotonin might have been a possible candidate targeted by salsolinol (Clemens and Shaar, 1980), however, while the effect of salsolinol on prolactin release was consequently inhibited by 1MeDIQ, this inhibitor did not affect the 5-hydroxytryptophan induced prolactin release (unpublished observation).

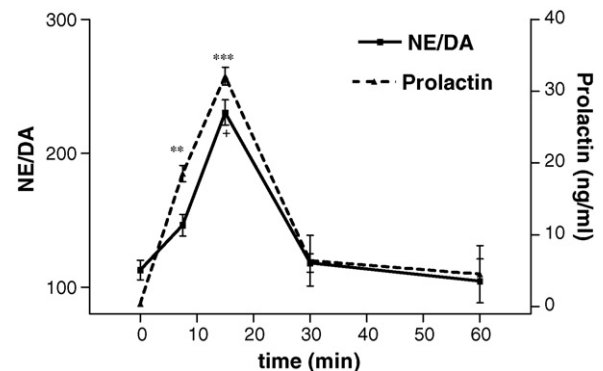


Fig. 4. Time course of prolactin release (broken line) and NE/DA ratios in the atrium (solid line) following salsolinol (25 mg/kg i.p.) treatment in male rats; $n = 3$, means \pm S.E.M. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ compared to the 0 min value.

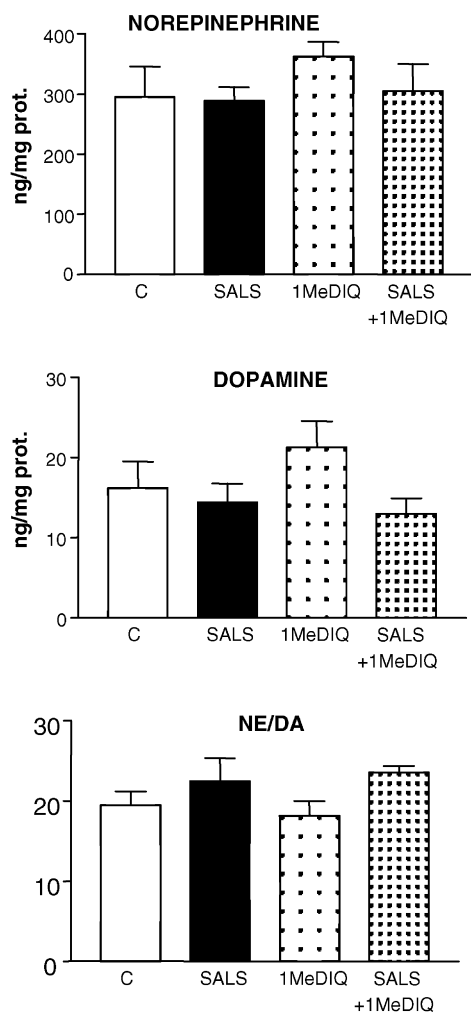


Fig. 5. Interaction of salsolinol and 1MeDIQ (both 40 mg/kg i.p. 20 and 30 min before sampling, respectively) measuring NE and DA levels as well as NE/DA ratios in the stellate ganglion of male rats; $n = 6$, means \pm S.E.M.

Salsolinol has been shown to induce a selective prolactin releasing activity; the release of other pituitary hormones was not altered by salsolinol (Tóth et al., 2001). Most of the changes of prolactin secretion are due to an altered dopaminergic inhibition of the secretory activity of pituitary lactotroph cells (Meites and Clemens, 1972; Ben-Jonathan et al., 1977; Plotsky et al., 1978). Salsolinol, however, does not affect the D2 dopaminergic receptor sensitivity or the effect of the inhibitors of this receptor site, neither it has affinity to adrenergic or serotonergic receptors on the basis of the data obtained so far. The ligands of dopamine receptors could not compete the binding of labeled salsolinol (Tóth et al., 2001, 2002; Homicskó et al., 2003). Substrates or inhibitors of aromatic amino acid decarboxylase proved to be competitors of salsolinol binding (Homicskó et al., 2003). The result that the L-DOPA induced increase in dopamine content was not inhibited, but augmented in the pituitary strongly suggests that the mentioned result of in vitro binding studies have no in vivo relevance regarding decarboxylase activity.

The possible role of dopamine in the salsolinol induced prolactin release seems to be excluded also by the result of in vitro release studies. Electrical stimulation as well as amphetamine like drugs induce increase in the dopamine release from the median eminences in vitro (Vizi et al., 1985); salsolinol was without effect in median eminences obtained from lactating and/or weaning dams.

The isoquinoline derivative 1MeDIQ inhibits the prolactin release induced by salsolinol. 1MeDIQ depresses the increase of prolactin induced by suckling and stressful stimuli, too (Bodnár et al., 2004; Mravec et al., 2004). Measuring the plasma catecholamine concentration, it turned out that 1MeDIQ induces a sharp increase in plasma level of norepinephrine and epinephrine. Pharmacological manipulations as well as lesions of peripheral sympathetic innervation indicate a principal role of norepinephrine released from the peripheral sympathetic terminal in the action of 1MeDIQ (Mravec et al., 2004).

Investigating the interaction of 1MeDIQ and salsolinol using the measurement of tissue catecholamine content in parallel with the plasma prolactin level the question was whether we can demonstrate changes in catecholamine metabolism parallel with the actual secretory activity of lactotrophs. In cycling and in lactating rats sharp changes in dopamine metabolism were shown in different lobes of the pituitary gland in parallel with changes of prolactin secretion (DeMaria et al., 1998; Nagy et al., 1998). In contrast, neither in the median eminence nor in the neuro-intermediate and anterior lobe of the pituitary could we find changes in catecholamine levels, which could explain the antagonism between salsolinol and 1MeDIQ. On the other side in the peripheral organs: e.g. in the spleen and in the atrium the salsolinol induced decrease of dopamine concentration and its inhibition by 1MeDIQ showed clear parallelism with the changes of prolactin secretion. The existence of dopaminergic neurons in the periphery (Hadjiconstantinou and Neff, 1987) is generally questioned (Bell and Gillespie, 1981; Eisenhofer et al., 2004; Mezey et al., 1996); therefore the dopamine content found in the tissues examined represents mainly the precursor pool of norepinephrine in its synthetic process. The low value of NE/DA ratio may show a fast release of the norepinephrine formed recently (this is the situation e.g. after 1MeDIQ treatment), in contrary the increase of NE/DA ratio may indicate a reduced release of newly formed NE (as caused by salsolinol).

These results seem to indicate an important role of peripheral norepinephrine release in the effect of salsolinol as prolactoliberin. The close parallelism of NE/DA ratio with the changes of prolactin secretion induced by increasing doses of salsolinol or measuring the time curve of prolactin secretion after salsolinol strengthens this suggestion. The crucial role of peripheral sympathetic neurons was proved measuring the daily rhythm of prolactin secretion (Kizer et al., 1975; Castrillon et al., 2001; Esquifino et al., 2004) in rats submitted to ganglionectomy of the superior cervical ganglion. In this ganglion similarly as in the stellate ganglion we could not find changes in catecholamine levels following salsolinol treatment

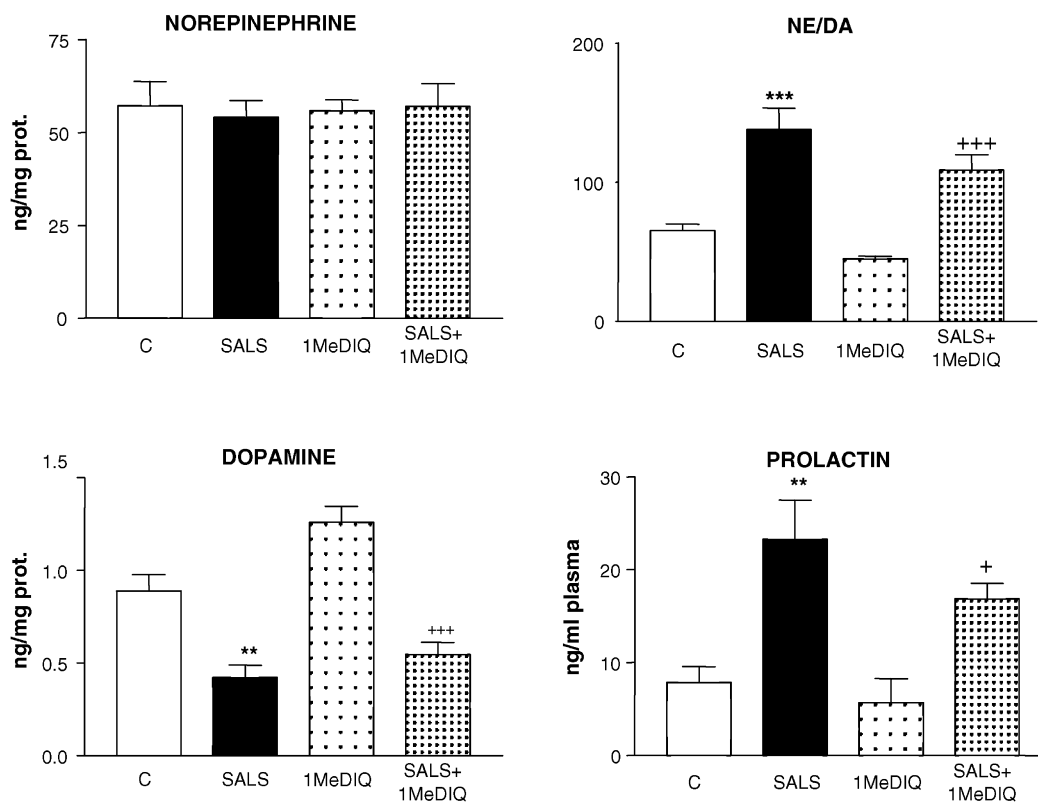


Fig. 6. Interaction of salsolinol and 1MeDIQ (both 40 mg/kg i.p. 20 and 30 min before sampling, respectively) measuring plasma prolactin, NE and DA levels as well as NE/DA ratios in the atria of male rats; $n = 6$, means \pm S.E.M. ** $P < 0.01$, *** $P < 0.001$ compared to the control; significant interaction between salsolinol and 1MeDIQ at the level of $^+P < 0.05$, at the level of $^{+++}P < 0.001$.

similar to those found in terminal region. This result emphasizes the primary role of the sympathetic terminals and seems to exclude that the dopamine containing paraganglionic SIF cells (Eränkő, 1976) would play a role in the changes of dopamine content caused by salsolinol.

Monoamine transporters have great functional and therapeutic importance (Iversen, 2000; Vizi, 2000). In that sense was it crucial to employ NET KO mice described by Bohn et al. (2000) and Xu et al. (2000). The prolactin releasing activity as well as the decrease of dopamine concentration in the atrium was clearly demonstrable in wild type of KO as well as in Balb C mice. In contrast the KO mice lacking the NE transporter did not respond to salsolinol: no prolactin release and no changes in the atrial dopamine level could be measured. The lack of dopamine response could be putatively explained by the lack of prolactin response (DeMaria et al., 2000), however, our preliminary data clearly show that in hypophysectomized rats the salsolinol induced decrease of dopamine content was comparable to that in the intact animals. Recent data show that the neuronal release of NE is almost completely inhibited in NET KO mice (Vizi et al., 2004). It is concluded that releasable NE pool, as well as NE transporter seems to be absolute prerequisite of the action of salsolinol. Quite recently in support to the role of norepinephrine in prolactin release nisoxetine the selective inhibitor of NE transport counteracted the salsolinol induced prolactin release (result to be published).

Preliminary experiments done in DA transporter and VMAT KO mice (courtesy of Wetsel, W.C., Duke University, Medical Center) showed unaffected activity of salsolinol in regard to prolactin release. These results argue for the possible specific role of NE transporter in the salsolinol induced prolactin release.

Noradrenergic terminals of inhibitory character regarding prolactin release were described in the paraventricular nucleus of the rat (Daftary et al., 2000). Although the effect of 1MeDIQ in the hypothalamic nuclei cannot be excluded, salsolinol most probably is acting in the periphery. Data in the literature (Origitano et al., 1981) as well as our unpublished investigation using tritium labeled salsolinol prove that salsolinol given parenterally does not pass the blood–brain barrier. An inhibition of prolactin secretion by direct pituitary action of NE is described (Friend et al., 1978).

Salsolinol inhibits tyrosine hydroxylase at relatively high concentration in vitro (Patsenka and Antkiewicz-Michaluk, 2004). Further investigations are needed to exclude this mechanism to explain our results. Salsolinol is fairly active to induce prolactin release following an i.v. bolus of 10 mg/kg α -methyltyrosine (unpublished observations), and in course of the present studies no significant decrease of norepinephrine level was found in the organs investigated after injection of salsolinol, suggesting no or at least minimal inhibition of the rate limiting enzyme of catecholamine synthesis.

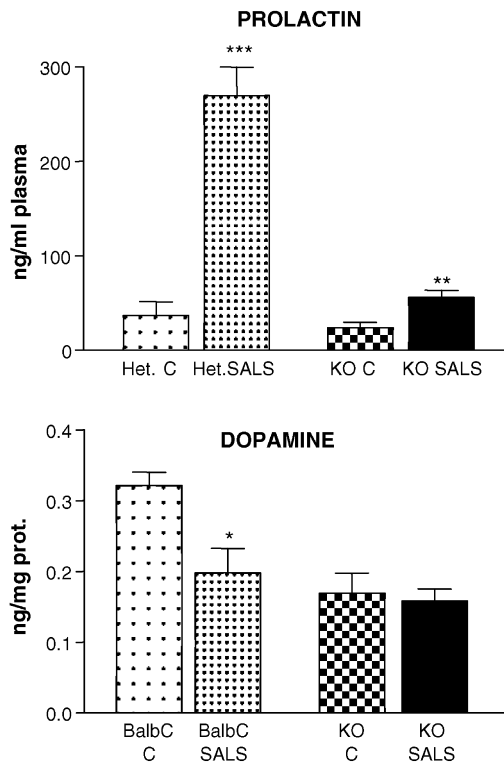


Fig. 7. The effect of salsolinol on the plasma prolactin level and on the DA content in the spleen of male mice. Het., heterozygous C57 Black; KO, norepinephrine transporter (NET) knock out C57 Black mice; Balb C mice were treated with saline (C, controls) or salsolinol (SALS) 10 mg/kg i.p. 20 min before sampling; $n = 3-5$. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ compared to the respective control.

5. Conclusion

The results presented argue for the possible role of peripheral norepinephrine release as a target for salsolinol in its action releasing prolactin. Measurement of in vitro release of dopamine, ligand binding, as well as interaction studies done with dopaminergic agents contradicted the possibility of an interaction of salsolinol with dopaminergic signal transduction. In accordance with this result we could not find changes in dopamine release measured in vitro from the median eminence in the presence of salsolinol. 1MeDIQ, the antagonist of salsolinol's action as prolactoliberin, turned out to be a norepinephrine releaser, and salsolinol acted as a possible inhibitor of this norepinephrine release in peripheral organs as indicated by the NE/DA ratio in peripheral organs. The dominant role of norepinephrine transporter was underlined by the result showing that neither prolactin release nor changes in norepinephrine metabolism in peripheral organs were affected by salsolinol in NET knock out mice.

References

Bell, C., Gillespie, J.S., 1981. Dopamine and noradrenaline levels in peripheral tissues of several mammalian species. *J. Neurochem.* 36, 703–706.

- Ben-Jonathan, N., Oliver, C., Weiner, H.J., Mical, R.S., Porter, J.C., 1977. Dopamine in hypophysial portal plasma of the rat during the estrous cycle and throughout pregnancy. *Endocrinology* 100, 452–458.
- Bodnár, I., Mravec, B., Kubovcakova, L., Tóth, E.B., Fülöp, F., Fekete, M.I.K., Kvetnansky, R., Nagy, G.M., 2004. Stress-, as well as suckling-induced prolactin (PRL) response is blocked by a structural analogue of the putative hypophysiotrophic PRL releasing factor, salsolinol (SAL). *J. Neuroendocrinol.* 16, 208–213.
- Bohn, L.M., Xu, F., Gainetdinov, R.R., Caron, M.G., 2000. Potentiated opioid analgesia in norepinephrine transporter knock-out mice. *J. Neurosci.* 20, 9040–9045.
- Castrillon, P.O., Cardinali, D.P., Pazo, D., Cutrera, R.A., Esquifino, A.I., 2001. Effect of superior cervical ganglionectomy on 24-h variations in hormone secretion from the anterior hypophysis and in hypothalamic monoamine turnover during the preclinical phase of Freund's adjuvant arthritis in rats. *J. Neuroendocrinol.* 13, 288–295.
- Clemens, J.A., Shaar, C.J., 1980. Control of prolactin secretion in mammals. *Fed. Proc.* 39, 2588–2592.
- Daftary, S.S., Boudaba, C., Tasker, J.G., 2000. Noradrenergic regulation of parvocellular neurons in the rat hypothalamicparaventricular nucleus. *Neuroscience* 96, 743–751.
- DeMaria, J.E., Livingstone, J.D., Freeman, M.E., 1998. Characterization of the dopaminergic input to the pituitary gland throughout the estrous cycle of the rat. *Neuroendocrinology* 67, 377–383.
- DeMaria, J.E., Nagy, G.M., Freeman, M.E., 2000. Immunoneutralization of prolactin prevents the stimulatory feedback of prolactin on hypothalamic neuroendocrine dopaminergic neurons. *Endocrine* 12, 333–337.
- Eisenhofer, G., Kopin, I.J., Goldstein, D.S., 2004. Catecholamine metabolism: a contemporary view with implications for physiology and medicine. *Pharmacol. Rev.* 56, 331–349.
- Eränkő, O., 1976. SIF cells, chromaffin cells, granule-containing cells and interneurons. In: Eränkő, O. (Ed.), *Structure and Function of the Small Intensive Fluorescent Cells*. Fogarty International Center Proceedings No. 30, NIH, Bethesda, MD, USA, pp. 1–7.
- Esquifino, A.I., Alvarez, M.P., Cano, P., Jimenez, V., Duvilanski, B., 2004. Superior cervical ganglionectomy differentially modifies median eminence and anterior and mediobasal hypothalamic GABA content in male rats: effects of hyperprolactinemia. *Exp. Brain Res.* 157, 296–302.
- Friend, W.C., Brown, G.M., Kirpalani, S., Wilson, D., 1978. Hypothalamic catecholaminergic effects on prolactin release in vitro. *Can. J. Physiol. Pharmacol.* 56, 304–309.
- Gudelsky, G.A., Metzger, H.Y., 1984. Function of tuberoinfundibular dopamine neuron sin pargyline- and reserpine-treated rats. *Neuroendocrinology* 38, 51–55.
- Hadjiconstantinou, M., Neff, N.H., 1987. Is dopamine a transmitter in the periphery? *Neuropharmacology* 26, 809–814.
- Homicskó, K.G., Kertész, I., Radnai, B., Tóth, B.E., Tóth, G., Fülöp, F., Fekete, M.I.K., Nagy, G.M., 2003. Binding site of salsolinol: its properties in different regions of the brain and the pituitary gland. *Neurochem. Int.* 42, 19–26.
- Iversen, L., 2000. Neurotransmitter transporters: fruitful targets for CNS drug discovery. *Mol. Psychiatr.* 5, 357–362.
- Kacsóh, B., Veress, Z., Tóth, B.E., Avery, L.M., Grosvenor, C.E., 1993. Bioactive, immunoreactive variants of prolactin in milk and serum of lactating rats and their pups. *J. Endocrinol.* 138, 243–257.
- Kizer, J.S., Zivin, J.A., Jacobowitz, D.M., Kopin, I.J., 1975. The nyctohemeral rhythm of plasma prolactin: effects of ganglionectomy, pinealectomy, constant light, constant darkness or 6-OH-dopamine administration. *Endocrinology* 96, 1230–1240.
- Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J., 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* 193, 265–275.
- Meites, J., Clemens, J.A., 1972. Hypothalamic control of prolactin secretion. *Vitam. Horm.* 30, 165–221.
- Mezey, E., Eisenhofer, G., Harta, G., Hansson, S., Gould, L., Hunyady, B., Hoffman, B.J., 1996. A novel non-neuronal catecholaminergic system: exocrine pancreas synthesizes and releases dopamine. *Proc. Natl. Acad. Sci. U.S.A.* 93, 10377–10382.

- Mravec, B., Bodnár, I., Fekete, M.I.K., Nagy, G.M., Kvetmanský, R., 2004. An antagonist of prolactin-releasing hormone induces an increase in plasma catecholamine levels in the rat. *Autonom. Neurosci.* 115, 35–40.
- Nagy, G.M., DeMaria, J.E., Freeman, M.E., 1998. Changes in the local metabolism of dopamine (DA) in the anterior (AL) and neural (NL) lobes but not in the intermediate lobe (IL) of the pituitary gland during nursing. *Brain Res.* 790, 315–317.
- Naoi, M., Maruyama, W., Dostert, P., Kohda, K., Kaiya, T., 1996. A novel enzyme enantio-selectively synthesizes (*R*)salsolinol, a precursor of a dopaminergic neurotoxin, *N*-methyl(*R*)salsolinol. *Neurosci. Lett.* 212, 183–186.
- Origitano, T., Hannigan, J., Collins, M.A., 1981. Rat brain salsolinol and blood-brain barrier. *Brain Res.* 224, 446–451.
- Patsenka, A., Antkiewicz-Michaluk, L., 2004. Inhibition of rodent brain monoamine oxidase and tyrosine hydroxylase by endogenous compounds—1,2,3,4-tetrahydro-isoquinoline alkaloids. *Pol. J. Pharmacol.* 56, 727–734.
- Plotsky, P.M., Gibbs, D.M., Neill, J.D., 1978. Liquid chromatographic-electrochemical measurement of dopamine in hypophysial stalk blood of rats. *Endocrinology* 102, 1887–1894.
- Sandler, M., Carter, S.B., Hunter, K.R., Stern, G.M., 1973. Tetrahydroisoquinoline alkaloids: in vivo metabolites of L-DOPA in man. *Nature* 241, 439–443.
- Shore, P.A., 1966. The mechanism of norepinephrine depletion by reserpine, metaraminol and related agents. The role of monoamine oxidase. *Pharmacol. Rev.* 18, 561–568.
- Spector, S., 1963. Monoamine oxidase in control of brain serotonin and norepinephrine content. *Ann. NY Acad. Sci.* 107, 856–864.
- Tóth, B.E., Bodnár, I., Homicskó, K.G., Fülöp, F., Fekete, M.I.K., Nagy, G.M., 2002. Physiological role of salsolinol: its hypophysiotrophic function in the regulation of pituitary prolactin secretion. *Neurotoxicol. Teratol.* 24, 655–666.
- Tóth, B.E., Homicskó, K., Radnai, B., Maruyama, W., DeMaria, J.E., Vecsernyés, M., Fekete, M.I.K., Fülöp, F., Naoi, M., Freeman, M.E., Nagy, G.M., 2001. Salsolinol is a putative neurointermediate lobe prolactin releasing factor. *J. Neuroendocrinol.* 13, 1042–1050.
- Vizi, E.S., Harsing Jr., L.G., Zimanyi, I., Gaal, G., 1985. Release and turnover of noradrenaline in isolated median eminence: lack of negative feedback modulation. *Neuroscience* 16, 907–916.
- Vizi, E.S., 2000. Role of high-affinity receptors and membrane transporters in non-synaptic communication and drug action in the central nervous system. *Pharmacol. Rev.* 52, 63–89.
- Vizi, E.S., Zsilla, G., Caron, M.G., Kiss, J.P., 2004. Uptake and release of norepinephrine by serotonergic terminals in norepinephrine transporter knockout mice: implications for action of selective serotonin reuptake inhibitors. *J. Neurosci.* 24, 7888–7894.
- Xu, F., Gainetdinov, R.R., Wetsel, W.C., Jones, S.R., Bohn, L.M., Miller, G.W., Wang, Y.M., Caron, M.G., 2000. Mice lacking the norepinephrine transporter are supersensitive to psychostimulants. *Nat. Neurosci.* 3, 465–471.