

# The role of catecholamines in the prolactin release induced by salsolinol

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## Abstract

Salsolinol (1,2,3,4-tetrahydro-6,7-dihydroxy-1-methylisoquinoline) is an endogenous prolactin releasing agent. Its action can be inhibited by another isoquinoline, 1-methyl-3,4-dihydroisoquinoline (1MeDIQ), which has a strong norepinephrine releasing activity. Salsolinol does not alter the dopamine release in median eminence *in vitro*, providing evidence for the lack of interaction with presynaptic D2 dopamine receptors. At the same time, lack of norepinephrine transporter abolishes salsolinol's action. Salsolinol decreases tissue level of dopamine and increases norepinephrine to dopamine ratio in organs innervated by the sympathetic nervous system indicating a possible decrease of norepinephrine release. Enzymes of catecholamine synthesis and metabolism are probably also not the site of action of salsolinol. In summary, based upon all of these observations a physiologically relevant interplay might exist between the sympatho-neuronal system and the regulation of prolactin release.

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Our group has recently identified an isoquinoline in the extract of the neuro-intermediate lobe of pituitary gland (Tóth *et al.*, 2001). Detailed analysis of this isoquinoline revealed that the dopamine-derived endogenous substance is salsolinol, which has been first described in the seventies of the last century (Sandler *et al.*, 1973).

Examining the biological effect of salsolinol, a sharp and dose-dependent increase of prolactin secretion has been observed both in male and lactating female rats (Tóth *et al.*, 2001). The salsolinol induced elevation of plasma prolactin could be prevented by an other isoquinoline derivative 1-methyl-3,4-dihydroisoquinoline (1MeDIQ). Moreover, this exogenous substance could block the secretory response of prolactin induced by suckling stimulus, and by immobilization stress in lactating and in male rats, respectively (Bodnár *et al.*, 2004). The structure of salsolinol and 1MeDIQ, and the possible formation of salsolinol through the condensation of dopamine (DA) and acetaldehyde are shown in Fig. 1. In our

present paper, we are presenting a summary of the results obtained so far on the mechanism of action of salsolinol.

One of the first questions has been addressed in our studies was how far the biogenic amines, first of all the well characterized hypothalamic prolactin inhibiting dopaminergic neurons and/or their terminals in the medial basal hypothalamus are taking part in the effect of salsolinol. The reserpine—monoamine oxidase inhibitor (MAOI) interaction is a classical pharmacological paradigm: any response depending on the release of biogenic amines (catecholamines or serotonin) is inhibited by reserpine (inhibitor of vesicular monoamine transport), and this inhibition is counteracted by MAOI pretreatment. Employing this model, we have found an inhibition of salsolinol's action by reserpine which inhibition could not be shown in MAOI pretreated animals (Székács *et al.*, 2007a). The role of serotonin in the effect of salsolinol seemed to be improbable since the 5-hydroxy-tryptophan induced increase of plasma prolactin level was not changed by 1MeDIQ (unpublished result). Therefore the possible role of DA was examined first.

It has been previously shown that binding of D<sub>2</sub> antagonists (like haloperidol) has been not affected by salsolinol excluding the possibility of an interaction between salsolinol and

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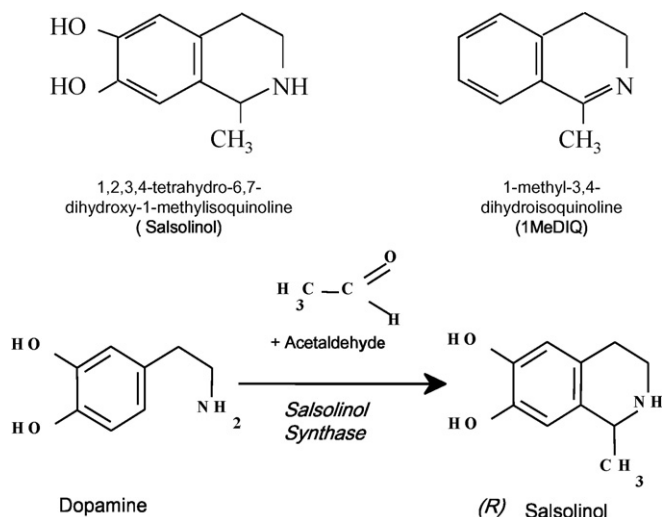


Fig. 1. The structure of salsolinol and 1MeDIQ and the formation of salsolinol.

postsynaptic  $D_2$  receptors (Tóth et al., 2001). On the other hand, the binding of salsolinol could not be affected by the DA receptor ligands examined (Homicskó et al., 2003). The basal and stimulation induced release of DA from individual median eminences obtained from lactating rats was also examined. As it is shown on Fig. 2A, neither the basal nor the stimulus (due to electrical stimulation) induced dopamine release has been altered by salsolinol treatment. Under similar conditions the stimulus induced release of dopamine was reduced by the  $D_2$  dopamine receptor ( $D_2R$ ) agonist bromocriptine (Fig. 2B). It should be mentioned that  $D_2R$  exists in two isoforms with distinct localization and physiological role. The long form of this receptor ( $D_{2L}R$ ) is principally postsynaptic, while the short one ( $D_{2S}R$ ) is presynaptic and functions as an autoreceptor allowing DA to inhibit its own release (Sarkar et al., 1983; Khan et al., 1998). The median eminence can easily be considered as a presynaptic area containing the neuronal endings of the hypothalamic neuroendocrine dopaminergic (NEDA) neurons. It has been already shown that the  $D_{2S}R$  isoform is present in the median eminence (Khan et al., 1998). We can conclude that salsolinol does not interfere with the presynaptic subtype of the  $D_2R$  (Fig. 2).

Preliminary experiments performed on DA transporter (DAT), and vesicular monoamine transporter (VMAT) gene deficient mice (provided by Wetsel W.C., Duke University, USA) have shown unaltered activity of salsolinol in regard to prolactin release. In norepinephrine (NE) transporter (NET) knock out mice, however, a considerable loss of the prolactin releasing activity of salsolinol has been observed. These results argue for the possible role of NET in salsolinol induced prolactin release (Székács et al., 2007a).

In cooperation with Richard Kvetnansky's group, we have measured the effect of 1MeDIQ on plasma NE level and found that this compound induces a marked increase of plasma NE (Mravec et al., 2004). These results together with those data obtained in transporter knock out mice led us to investigate the effect of salsolinol on the catecholamine levels of peripheral organs.

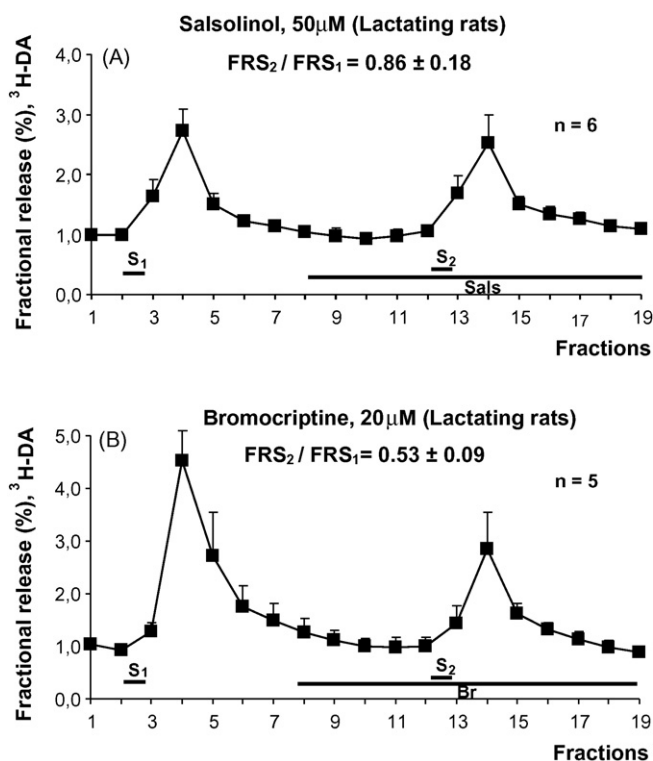


Fig. 2. The effect of salsolinol (A,  $n = 6$ ) and bromocriptine (B,  $n = 5$ ) on stimulation evoked [ $7,8^3H$ ]-dopamine release in individual median eminences in vitro. Abscissa: number of fractions; 5 min fractions were collected for 100 min. Supramaximal (30 V) field stimulation (3 ms, 5 Hz for 2 min = 600 - shocks) was applied during the collection of the 3rd (S1) and 13th (S2) fractions, the FRS<sub>2</sub>/FRS<sub>1</sub> ratios were calculated counting the integrated stimulation induced release of labeled dopamine (means  $\pm$  S.E.M.). FRS<sub>2</sub>/FRS<sub>1</sub> in the control was 0.82  $\pm$  0.15 ( $n = 3$ ). Salsolinol or bromocriptine was added to the perfusion fluid after the first stimulation.

It has been found that in peripheral organs of rats treated with salsolinol there was a dose related decrease of DA concentration and an even more prominent dose related increase of NE/DA ratio. This could be shown in the salivary gland, atrium, spleen, ovaries and vas deferens. The changes of catecholamine concentration run in parallel with the increase of plasma prolactin level (Székács et al., 2007a).

The parallel changes of peripheral catecholamine metabolism with prolactin release has been further strengthened by the result showing that in 1MeDIQ pretreated animals not only the prolactin release but also the increase of NE/DA ratio in the atrium or in the spleen is inhibited (Székács et al., 2007a).

In ganglionectomized rats a disturbed regulation of prolactin release has been described (Kizer et al., 1975). When we measured the catecholamine levels in the superior cervical and stellate ganglia no salsolinol induced alteration could be seen (Székács et al., 2007a). This seems to exclude a direct ganglionic effect of salsolinol, while the NE release induced by 1MeDIQ is largely dependent of intact ganglionic neurotransmission (Mravec et al., 2004).

Studies where circulating prolactin was immunoneutralized using specific antibody and the change in the metabolism of

hypothalamic dopaminergic system were investigated clearly indicate that endogenous prolactin regulates its own secretion by activating hypothalamic neuroendocrine dopaminergic neurons (DeMaria et al., 2000). Therefore we have decided to see the possible effect of prolactin on catecholamines and their metabolites following hypophysectomy. It has been found that in the absence of prolactin, peripheral tissue DA concentrations decreased and NE/DA ratios increased in rats after salsolinol treatment compared to that in intact animals (Székács et al., 2007b). These findings seem to exclude the role of prolactin in the salsolinol induced changes of peripheral catecholamine metabolism.

The adrenal gland is the main source of peripheral catecholamines. Therefore it has seemed to be obvious to look at the effect of salsolinol on tissue catecholamine levels following medullectomy. Salsolinol injection resulted in a comparable elevation of prolactin release as well as increase of

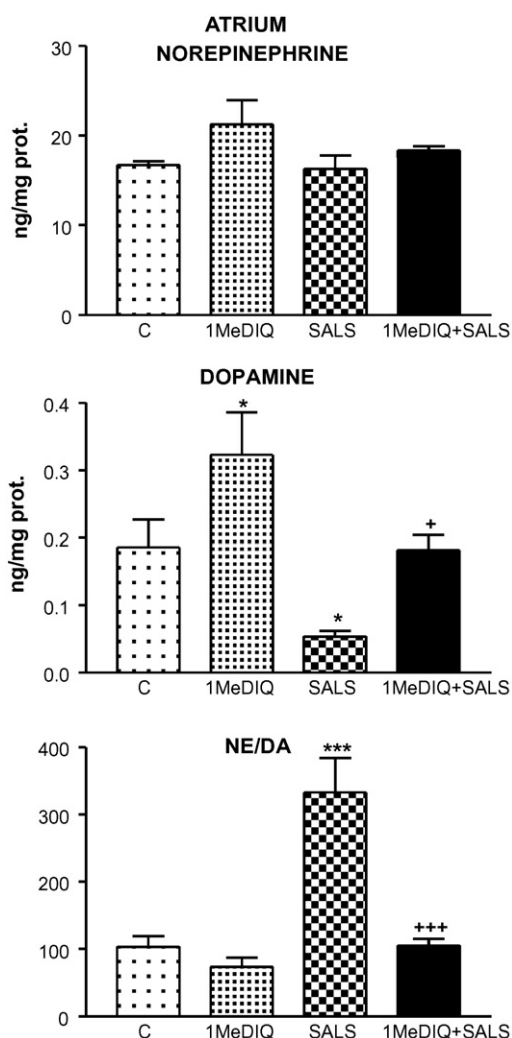


Fig. 3. Interaction of salsolinol and 1MeDIQ (both 25 mg/kg i.p. 20 and 25 min before sampling, respectively) measuring NE and DA levels as well as NE/DA ratios in the atria of adrenalectomized (ADX) male rats;  $n = 6$ , means  $\pm$  S.E.M. \* $P < 0.05$  \*\*\* $P < 0.001$  compared to ADX control (C), significant interaction between salsolinol and 1MeDIQ at the level of + $P < 0.05$ , and at +++ $P < 0.001$  (One-way ANOVA and Newman–Keuls test).

NE/DA ratio in several tissues (spleen, salivary gland and atrium) of medullectomized rats compared to intact animals (Székács et al., 2007b). Moreover, following adrenalectomy not only the effect of salsolinol but the effect of its antagonist, 1MeDIQ was unaltered (Fig. 3). We can conclude that neither the medullary catecholamines nor the adrenal steroids are important sites for the action of salsolinol or 1MeDIQ.

In relation with the prolactin releasing effect of salsolinol, another possibility, namely the alteration of the enzyme activity at the level of the enzymatic steps of the NE synthesis has also been investigated. In our previous studies, it has been shown that several inhibitors of aromatic amino acid decarboxylase could interfere with the binding of salsolinol to homogenates of pituitary gland (Homicskó et al., 2003). When changes in DA concentration of the median eminence in pargyline pretreated male rats were examined using L-Dopa (25 mg/kg i.p.), which in itself is not able to increase DA level, a concomitant injection of salsolinol significantly elevated the concentration of DA, therefore decarboxylase activity was certainly not inhibited (Székács et al., 2007a).

The effect of a possible alteration of dopamine  $\beta$ -hydroxylase (DBH) in the salsolinol-induced prolactin response was also investigated by injecting a known DBH blocker, disulfiram (Goldstein et al., 1964) at a dose of 200 mg/kg intraperitoneally. It has been found that it could not alter the prolactin response, making the role of DBH improbable as a site of action for salsolinol (Fig. 4).

Treatment of the rats with an inhibitor of tyrosine hydroxylase, alpha-methyl-para-tyrosine, caused a significant decrease of DA concentration in the atrium. This decrease was partially prevented by salsolinol. Results, summarized on Fig. 5, indicate that the prolactin releasing activity of salsolinol is possibly not due to an inhibition of the enzyme tyrosine hydroxylase. The observed change may indicate a decreased turnover of DA in salsolinol treated animals.

In summary, we have found an endogenous prolactin releasing substance in the extract of the neurointermediate lobe of the pituitary. Its action can be inhibited by another isoquinoline, 1MeDIQ, which also releases NE in the periphery.

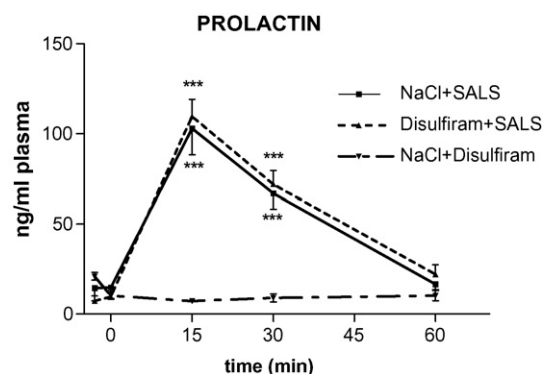


Fig. 4. Plasma prolactin level as affected by salsolinol (SALS, 10 mg/kg i.v.) in saline (NaCl) or disulfiram (200 mg/kg i.p.) treated male rats;  $n = 5$ , means  $\pm$  S.E.M. \*\*\* $P < 0.001$  compared to NaCl + disulfiram group (One-way ANOVA and Newman–Keuls test).

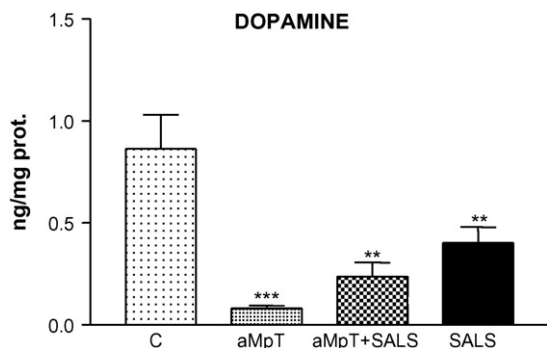


Fig. 5. Dopamine concentration in the atrium in salsolinol (SALS, 25 mg/kg i.p. 20 min before decapitation), and alpha-methyl-para-tyrosine (aMpT, 100 mg/kg i.p. 45 min before salsolinol administration) treated male rats;  $n = 5$ , means  $\pm$  S.E.M. \*\* $P < 0.01$ , \*\*\* $P < 0.001$  compared to control (C) group (One-way ANOVA and Newman–Keuls test).

Salsolinol does not alter DA release in the median eminence in vitro. However the lack of NE transporter abolishes salsolinol's action. At the same time, salsolinol decreases tissue DA level and increases NE/DA ratio indicating a possible decrease of NE release in vivo. Enzymes of the catecholamine metabolism examined so far are probably not affected by salsolinol. As described previously (Origitano et al., 1981), salsolinol does not cross the blood brain barrier, which was confirmed in our laboratory using i.v. administration of  $^3\text{H}$ -salsolinol. Therefore the target of salsolinol's action should be at the periphery. The most probable site of its action is the transport of NE in the sympathetic nerve terminals.

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