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MINIREVIEW

Salsolinol, a Derivate of Dopamine, is a Possible Modulator of Catecholaminergic Transmission: a Review of Recent Developments

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Summary

Catecholamine (dopamine, norepinephrine and epinephrine) synthesizing neurons are widely distributed in the brain, sympathetic ganglia and throughout peripheral organs. Results of several recent experiments clearly suggest that many of these neurons can also contain 1-methyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline (salsolinol), a derivate of dopamine. However, direct proof of salsolinol synthesis in those neurons is still missing. The data obtained with administration of exogenous salsolinol strongly indicate that it may play an important role in catecholaminergic regulatory processes, such as the regulation of prolactin release and/or neuronal transmission in sympathetic ganglia. Several recent data have also indicated a relationship between salsolinol or its metabolites and the etiology of Parkinson's disease or neuropathology of chronic alcoholism. These seemingly different roles of salsolinol will be discussed separately, but some common features will also be highlighted. Based on all of the discussed data the existence of a "salsolinolergic" system using salsolinol as a neuromodulator, which may be present in catecholamine synthesizing neurons, is postulated.

Key words

Salsolinol • Dopamine • Prolactoliberin • Parkinson's disease • 1MeDIQ

Introduction

The neurotransmitters dopamine, norepinephrine and epinephrine are widely distributed in the brain and periphery (Hokfelt *et al.* 1974, Lindvall and Bjorklund 1978, Bjorklund and Lindvall 1984). Fundamental work on dopamine function by A. Carlsson was awarded the Nobel Prize in 2000 (Carlsson 2001). The brain dopaminergic system is implicated in a variety of physiological and pathophysiological processes. It regulates prolactin secretion, motion, emotion, cognition and functional neuromodulation at many levels of the visual system (Weinberger *et al.* 1988, Masson *et al.* 1993, Nieoullon 2002). An imbalance between

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dopaminergic neurotransmission and dopamine receptors is known to be associated with the symptomatology of numerous neuropsychiatric disorders, like schizophrenia, psychosis, mania and depression as well as neuropathological disorders, like Parkinson's disease (PD), neurolathyrism, Huntington's disease (Carlsson 1988, Bermanzohn and Siris 1992, Brown and Gershon 1993, Jakel and Maragos 2000, Kostrzewa and Segura-Aguilar 2003).

Previous studies evaluating the function of salsolinol (1-methyl-6,7-dihydroxy-1,2,3,4-tetrahydro-isoquinoline), a dopamine-derived endogenously synthesized compound, revealed its involvement in the progression of disease characterized by dysfunction of dopaminergic neurons, as in the case of PD (Moser *et al.* 1995).

More recent data, however, have indicated that salsolinol might represent a neuromodulator of dopaminergic neurotransmission. These findings suggest that salsolinol may serve as a neuromodulator in the tuberoinfundibular and nigrostriatal dopaminergic systems (Tóth *et al.* 2001, Naoi *et al.* 2002). Moreover, there are indications that salsolinol may participate in the regulation of neurotransmission of small intensely fluorescent (SIF) cells in the sympathetic ganglia as well (Bodnár *et al.* 2004a, Mravec *et al.* 2004).

This article summarizes the data that suggest the role of salsolinol in catecholaminergic (especially dopaminergic) transmission as well as in the processes that are characterized by a dysfunction of catecholaminergic neurons.

Biosynthesis of salsolinol

Salsolinol is an endogenously synthesized catechol isoquinoline that has been detected in rat and human brain tissue samples (Sandler *et al.* 1973, Collins and Bigdeli 1975). Salsolinol can be synthesized from dopamine and acetaldehyde by the enzyme salsolinol synthase. Alternatively, it can also be synthesized from dopamine and pyruvic acid by forming an intermediate metabolite, salsolinol-1-carboxylic acid. Salsolinol-1-carboxylic acid can be directly metabolized by an unknown enzyme to salsolinol or at first to 1,2-dehydrosalsolinol and then to salsolinol (Naoi *et al.* 1996, 2002) (Fig. 1).

Therefore, it is not surprising that salsolinol and its metabolites can be detected in many areas of the brain that are also rich in dopamine. The highest concentration

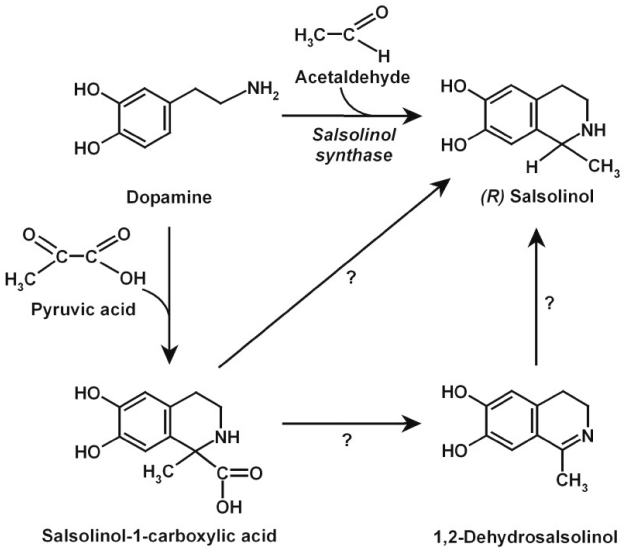

Fig. 1. Biosynthesis of salsolinol (adapted from Naoi *et al.* 2002). ? - unknown enzyme.

of salsolinol and its metabolites has been detected in the basal ganglia, especially in the striatum (Musshoff *et al.* 1999). It has also been found in the substantia nigra, frontal cortex (Naoi *et al.* 2002), hypothalamus (Musshoff *et al.* 2000), median eminence and in neuro-intermediate lobe of the pituitary gland (Tóth *et al.* 2001).

Interestingly enough, only (*R*) enantiomer of salsolinol is present in the brain, however, both (*R*) and (*S*) enantiomers are found in human plasma and urine (Naoi *et al.* 2004). The enantiomer selective occurrence of salsolinol suggests that it is endogenously synthesized in nerve bodies or synaptic terminals of dopamine neurons. Nevertheless, it must be emphasized that the direct evidence is still missing. Moreover, it is known that glial cells are able to take up a wide range of neurotransmitters (Hansson 1985, Inazu *et al.* 1999, Takeda *et al.* 2002). Therefore, at present it is not possible to exclude the synthesis and/or uptake of salsolinol by glial cells as well.

Biodegradation of salsolinol

Salsolinol is metabolized by the enzyme N-methyltransferase to N-methyl-salsolinol and consequently by amine oxidase to 1,2-dimethyl-6,7-dihydroxyisoquinolinium ion (Naoi *et al.* 2002, 2004) (Fig. 2). It is thought that some metabolites of salsolinol are involved in the etiopathogenesis of Parkinson's disease (Maruyama and Naoi 2002; for details see below).

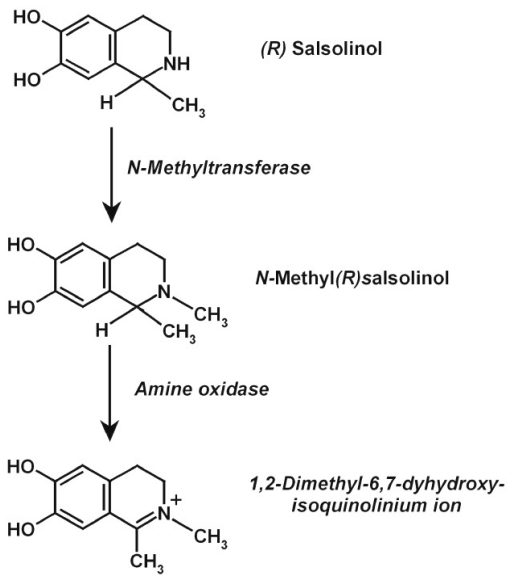

Fig. 2. Biodegradation of salsolinol (adapted from Naoi *et al.* 2004).

Role of salsolinol in catecholamine system dysfunctions

Neurotoxicity of the salsolinol

Neurotoxins, e.g. 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPPT), 6-hydroxydopamine (6-OHDA), are chemical substances which have an active role in promoting neuronal necrosis, apoptosis or neurodegenerative processes. Moreover, neurotoxins might also impair nervous system functions by their deleterious effect on neuronal satellite cells (Kostrzewa 1999, Segura-Aguilar and Kostrzewa 2004).

Properties of salsolinol, as a neurotoxin, are intensively studied. Salsolinol has a molecular structure similar to MPPT and also to 6-OHDA, which are known to induce loss of catecholaminergic cells. Salsolinol and/or its methylated derivates have been suggested to act as endogenous dopaminergic neurotoxins, inducing selective neuronal cell death and eliciting symptoms almost identical to idiopathic Parkinson's disease (Martinez-Alvarado *et al.* 2001; for details see next section).

Salsolinol could lead to neurotoxicity in dopaminergic cells by inhibition of mitochondrial complex II (succinate-Q reductase) activity (Storch *et al.* 2000). *In vitro* studies have shown that incubation of dopaminergic neuroblastoma SH-SY5Y cells with N-methyl-salsolinol caused cell apoptosis. Moreover, it has been shown that copper accelerates salsolinol-induced PC 12 cells death (Kim *et al.* 2001).

Salsolinol can trigger typical apoptotic dopaminergic cell death, which appears to by mediated, at least in part, through a reactive oxygen species-activated cascade (Chun *et al.* 2001). The data indicate that mitochondria are the site which decides the cell death induced by N-methyl-salsolinol (Storch *et al.* 2000, Naoi *et al.* 2002). Moreover, it is suggested that salsolinol and its derivates might induce alteration of protein synthesis in the endoplasmic reticulum (Kheradpezhouh *et al.* 2003).

The role of salsolinol in Parkinson's disease

The cause of chronic nigral cell death in PD and the underlying mechanisms remain elusive. The data suggest that exogenous and endogenous neurotoxic substances (e.g. MPTP, 1,1'-dimethyl-4,4'-bipyridium (paraquat), isoquinoline derivates) can participate in nigral dopaminergic cell loss (Antkiewicz-Michaluk 2002, Kostrzewa and Segura-Aguilar 2003). Hence, a great part of research dealing with the isoquinoline derivate salsolinol and its metabolites is focused on its involvement in the etiopathogenesis of PD (for review see Dostert *et al.* 1988, Nagatsu 1997, 2002, Naoi *et al.* 1997, Antkiewicz-Michaluk 2002).

The data suggest that especially N-methylated derivates of salsolinol, N-methyl-salsolinol and its metabolite products, might be importantly involved in etiopathogenesis of PD (Maruyama *et al.* 1997). It has been demonstrated that patients with PD have an increased activity of N-methyltransferase, catalyzing the synthesis of N-methyl-salsolinol from salsolinol, in lymphocytes (Naoi and Maruyama 1999, Maruyama and Naoi 2002, Naoi *et al.* 2002, 2004). Animal studies showed that the activity of a neutral N-methyltransferase in the striatum was found to determine the level of 1,2-dimethyl-6,7-dihydroxyisoquinolinium ion, an oxidation product of N-methyl-salsolinol in the substantia nigra (Maruyama *et al.* 2000).

It has also been observed that endogenously synthesized salsolinol and its derivatives (e.g. norsalsolinol, N-methyl-norsalsolinol, N-methyl-salsolinol) are increased in the cerebrospinal fluid (Maruyama *et al.* 1996) and the urine (Moser *et al.* 1996) of patients with idiopathic PD. Because norsalsolinol derivates are found in low or undetectable concentrations in healthy subjects (Niwa *et al.* 1991) the role as a biological marker for PD has been proposed (Moser *et al.* 1995). However, recent data indicate that the observed

increase of systemic levels of norsalsolinol derivatives are induced by levodopa treatment and do not represent an accurate biological marker of PD (Scholz *et al.* 2004).

It can be supposed that salsolinol is synthesized in terminals of dopaminergic cells of the substantia nigra, where its presence has already been proven (Naoi *et al.* 2002). Thus, it can participate in the regulation of the nigrostriatal system activity. During unfavorable conditions, salsolinol and/or one of its metabolites can participate in the etiopathogenesis of PD (Maruyama *et al.* 1997). Therefore, as far as the search and development of new drugs for the treatment of PD is now focusing on compounds exhibiting neuroprotective and anti-apoptotic influence against N-methyl-salsolinol (Naoi *et al.* 2000, Maruyama *et al.* 2004, Yi *et al.* 2005).

In addition to the loss of dopaminergic cells in substantia nigra (Bernheimer *et al.* 1973, Damier *et al.* 1999), reduction of the number of catecholaminergic neurons in the locus coeruleus, subcoeruleus, retrorubral nucleus and ventral tegmental areas was found (German *et al.* 1989, 1992). Whether salsolinol, or its metabolites, are responsible for the loss of catecholaminergic neurons in these brain areas remains to be answered.

The role of salsolinol in autonomic dysfunction in PD

Variable dysfunctions of autonomic system have been recognized in patients with PD, including cardiovascular symptoms, gastrointestinal, urogenital, sudomotor and thermoregulatory dysfunction, papillary abnormalities as well as sleep and respiratory disorders (Micieli *et al.* 2003).

It is believed that orthostatic hypotension, common in PD patients, is a consequence of chronic L-DOPA treatment. Recent studies showed that orthostatic hypotension is most likely the result of cardiac sympathetic denervation in PD (Li *et al.* 2002, Goldstein 2003). Patients with PD having sympathetic neurocirculary failure also show a significant decrease in 6-[^{18}F]fluorodopamine-derived radioactivity in the heart that is a marker for the reuptake activity of sympathetic nerve endings. These data clearly indicate a reduction of sympathetic terminals in the heart of patients with PD (Goldstein *et al.* 2000).

The heart is not the only destination of axons of catecholaminergic neurons. It also contains cells, synthesizing catecholamines called small intensely fluorescent (SIF) cells (Slavíková *et al.* 2003). A population of dopaminergic SIF cells can be detected in the heart atria (Baptista and Kirby 1997).

A possible involvement of salsolinol in the physiological regulation of heart function has already be evaluated in *in vitro* experiments. Salsolinol produces a dose-dependent positive inotropic effect on isolated guinea pig myocardium and a positive chronotropic effect on isolated and perfused rat heart. These data suggest that salsolinol might influence heart rate and contractility acting as β-receptor agonist, especially because this effect could be antagonized by propranolol (10 μg/ml). At the same time the chronotropic effect of salsolinol is potentiated by naloxone, an opioid receptor antagonist (Chavez-Lara *et al.* 1989, Sokolova *et al.* 1990). Therefore, it can also be hypothesized that the decreased 6-[^{18}F]fluorodopamine-derived radioactivity in the heart of patients with PD is due to the reduction of dopaminergic SIF cell population, and that salsolinol or one of its derivates may participate in this process by a similar effect to that of different neurotoxins that results in a loss of dopaminergic cells of substantia nigra.

Interestingly enough, it has been observed that salsolinol and some of its metabolites may increase or decrease the formation of hydroxyl radicals, so that they might be neuroprotective or neurotoxic, respectively, and thus might represent a "double faced" molecule. It has been already hypothesized that a disproportion in the ratio of the neuroprotective to the neurotoxic effect of salsolinol might participate in the pathogenesis of PD (Maruyama *et al.* 1995).

The role of salsolinol in addiction

Dopaminergic neurons of the ventral tegmental area (constituting mesocorticolimbic systems) together with endogenous opiates and gama-aminobutyric acid play an important role in the resulting addiction (Koob 1992, Schultz *et al.* 1997, Kelley and Berridge 2002). It has been suggested that salsolinol, a condensation product of the alcohol metabolite acetaldehyde and dopamine (Fig. 1), may be involved in the balance of the reward systems. The intracranial self-administration technique has shown that salsolinol induced reinforcement in the nucleus accumbens shell of rats at concentrations that are pharmacologically possible. These reinforcing actions are mediated in part by D_2/D_3-like receptors (Rodd *et al.* 2003).

In vitro data showed a significant decrease of pro-opiomelanocortin gene expression caused by salsolinol. This suggests a possible involvement of salsolinol in the establishment of opioid deficiency in

alcoholism (Putscher *et al.* 1995). These findings indicate that the rewarding effect of salsolinol may involve mu-opioid receptors (Matsuzawa *et al.* 2000). Salsolinol can also induce a reduction of receptor affinity for its ligand in the opioid system by a down regulation process due to the continuous opiate receptor stimulation, occurring after ethanol administration (Lucchi *et al.* 1982). The data suggest that salsolinol may also have a modulatory role on benzodiazepine receptors in the brain (Kuriyama *et al.* 1987).

Elevated concentrations of salsolinol have been determined in the blood plasma of alcoholics. However, due to a high inter-individual variance in urine salsolinol concentration, it is not a sufficient marker for distinguishing between alcoholics and a non-alcoholics (Musshoff 2002). Similarly, a recent finding has shown the lack of a significant association between alcohol consumption and salsolinol formation (Musshoff *et al.* 2005).

The role of salsolinol in physiological regulatory processes

Release of prolactin

Prolactin (PRL) secretion is under a dominant and tonic inhibitory control of dopamine released from terminals of the hypothalamic neuroendocrine dopaminergic (NEDA) system (Freeman 2000).

Several studies performed by Nagy and his associates during the last few years have clearly indicated that salsolinol may represent at least one of a long suspected neuro-intermediate lobe (NIL) derived prolactoliberin, detected in the perchloric acid extracts of the NIL (Tóth *et al.* 2001). They have also shown that salsolinol is present in the median eminence and also in the anterior lobe of the pituitary gland. It is well known that these regions are the terminal fields of the NEDA system (Tóth *et al.* 2001). Furthermore, the salsolinol concentration is elevated during situations when PRL secretion is increased from the anterior lobe of the pituitary gland (Tóth *et al.* 2001). Moreover, administration of salsolinol to rats and/or mice significantly increases plasma levels of PRL without having any effect on other known pituitary hormones (Tóth *et al.* 2001).

Molecular structure of the receptor that might mediate the prolactoliberin effect of salsolinol is not yet fully known. However, it has been shown that salsolinol is unable to displace D_1 and D_2 antagonists (^{3}H-SCH23390, ^{3}H-spiperone). At the same time, it is able to displace some agonists of the α_2-adrenoceptors, such as ^{3}H-clonidine as well as ^{3}H-apomorphine, a ligand of the D_2 dopamine receptor family in the nanomolar range (Antkiewicz-Michaluk *et al.* 2000, Tóth *et al.* 2002, Vetulani *et al.* 2003). These data suggest that the salsolinol-induced increase of PRL secretion is mediated through its binding to a specific binding sites, which can also recognize dopamine as a signaling molecule, although, its property differs from any of the known dopaminergic receptors (Tóth *et al.* 2002, Homicsko *et al.* 2003). More recently it has been shown that a cAMP-coupled mechanism is probably involved in the prolactin releasing action of salsolinol (Radnai *et al.* 2005), suggesting a receptor-mediated change in cAMP.

The regulation of PRL secretion is rather interesting. On one hand, dopamine acts as a "prolactostatin", on the other hand its metabolite, salsolinol, acts as a "prolactoliberin". Consequently it can be supposed that the ratio of dopamine to salsolinol synthesis and release from the NEDA system represents a sophisticated and physiologically economic mechanism for the regulation of PRL release.

Inhibition of sympathoadrenal system activity during stress

Intraperitoneal application of salsolinol effectively reduces both plasma epinephrine (EPI) and norepinephrine (NE) levels during stressful situations in rats (Bodnár *et al.* 2004a). The immobilization procedure represents one of the strongest stressors, eliciting a huge increase in plasma levels of both EPI and NE (Kvetňanský *et al.* 1978). However, salsolinol administration before or during immobilization almost completely prevents the immobilization-induced increase in plasma catecholamines (Bodnár *et al.* 2004a). The results obtained from these experiments suggest that salsolinol may be able to act at the level of sympathetic ganglia (Mravec *et al.* 2004). It can probably influence the dopaminergic SIF cells regulating synaptic transmission between preganglionic and postganglionic neurons (Matthews 1989, Heym *et al.* 1993, 1994, Tanaka and Chiba 1991, 1996).

Therefore, it can be hypothesized that salsolinol participates in the physiological regulation of the sympathoadrenal system activity and prevents over-activation of this system during episodes of acute stress.

Salsolinol might potentially influence sympathoadrenal system activity also *via* modification of

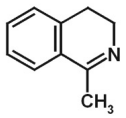

Fig. 3. Structure of 1MeDIQ (1-methyl-3,4-dihydroisoquinoline).

catecholaminergic transmission in areas of central nervous system. Hypothalamic paraventricular nucleus (PVN) represents a brain area that participates importantly in the regulation of sympathoadrenal system activity (Kenney *et al.* 2003). Exposure of animals to stress is accompanied by an increased release of catecholamines in PVN (Pacák *et al.* 1995, Pacák 2000). Whether endogenously synthesized salsolinol might influence NE-mediated processes in PVN and consequently in the activity of the sympathoadrenal system needs further investigation.

1-methyl-3,4-dihydroisoquinoline (1MeDIQ) – a useful tool for study of salsolinol functions in the organism

1MeDIQ (Fig. 3) is a structural analogue of salsolinol that dose-dependently antagonizes salsolinol-induced PRL release. The administration of 1MeDIQ blocks stress and suckling-induced PRL release (Bodnar *et al.* 2004b). Moreover, it has an opposite effect on catecholamine secretion than salsolinol. Administration of 1MeDIQ increases both EPI and NE secretion from the adrenal medulla and sympathetic terminals of otherwise non-stressed animals (Mravec *et al.* 2004).

The administration of 1MeDIQ elicits an increase in motor activity of rats and mice (Fekete *et al.* unpublished observations). It can be hypothesized that the observed increase in motor activity may be a result of the antagonisms of 1MeDIQ on salsolinol-mediated regulation of the motor system in basal ganglia (Vetulani *et al.* 2001).

Based on the above described data, it seems that 1MeDIQ is a potent antagonist of most of the known salsolinol-induced biological responses. Therefore, 1MeDIQ might offer an important tool for studying the site and mechanism of action of salsolinol.

Conclusions

It has been shown that salsolinol, a derivative of dopamine, is related to nervous structures of catecholamine neurons and their terminal fields

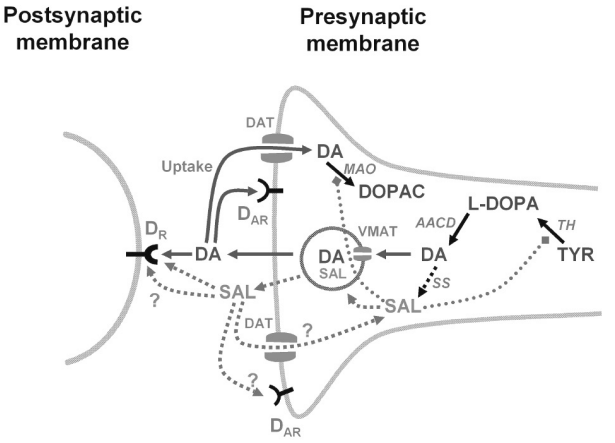

Fig. 4. Schematic representation of proposed salsolinol role as neuromodulator of dopaminergic transmission. Salsolinol might inhibit enzymes related to the biosynthesis and biodegradation of catecholamines (Naoi *et al.* 2004). The reuptake of salsolinol from synaptic cleft to dopaminergic terminals via dopamine transporter remains questionable (Storch *et al.* 2002). AADC – aromatic amino acid decarboxylase; DA - dopamine; DAT – dopamine transporter; D_{AR} – dopamine autoreceptors; DOPAC – 3,4-dihydroxyphenylacetic acid; D_R – dopamine postsynaptic receptors; L-DOPA (L-dihydroxyphenylalanine); MAO – monoamine oxidase; SAL – salsolinol; SS – salsolinol synthase; TH – tyrosine hydroxylase; TYR – tyrosine; VMAT – vesicular monoamine transporter (adapted from Tóth *et al.* 2002).

(Musshoff *et al.* 1999, 2000, Tóth *et al.* 2001, Naoi *et al.* 2002) (Fig. 4). Convincing experimental data suggest that salsolinol may be involved in the dopaminergic regulatory processes of both prolactin secretion and sympathoadrenal system activity (Bodnár *et al.* 2004a, 2004b).

There are indications that salsolinol may play a role in the regulation of other processes and may also be influenced by the dopaminergic system (Antkiewicz-Michaluk *et al.* 2000, Vetulani *et al.* 2003). Salsolinol, therefore, can potentially represent a neuromodulator, which participates in the equilibrium of transmission of information at synapses composed of presynaptic neurons synthesizing dopamine as their primary neurotransmitter. In addition, salsolinol can affect levels of monoamine neurotransmitters by inhibiting enzymes related to the metabolism of catecholamines and indoleamines (Naoi *et al.* 2004). Whether salsolinol might be synthesized in other than dopaminergic neurons (e.g. noradrenergic or adrenalinergic) remains to be answered.

Remarkably, there are some similarities between the neuromodulator role of salsolinol and L-DOPA (Misu *et al.* 1995, 1996, Tedroff 1997). Both affect receptor status, enzyme activity of the catecholamine biosynthesis as well as mitochondrial metabolism. Similar to the above discussed effects of salsolinol, administration of

exogenous L-DOPA affects dopamine receptor status, aromatic amino acid decarboxylase (AADC) activity and mitochondrial oxidation in experimental animals (Opacka-Juffry and Brooks 1995). Nevertheless, further similarity that receptor and/or transporter for either L-DOPA or salsolinol have not yet been unequivocally determined (Misu *et al.* 1996, Ishiia *et al.* 2000, Sugaya *et al.* 2001).

It is also supposed that salsolinol may be involved in processes characterized by an altered function of dopaminergic cells, as in the case of Parkinson's disease or alcoholism (Dostert *et al.* 1988, Putscher *et al.* 1995, Naoi *et al.* 1997, Antkiewicz-Michaluk 2002). There is also an important question whether salsolinol can participate in the pathogenesis of other diseases characterized by dysfunctions of dopaminergic neurotransmitter systems in the brain or at certain peripheral locations, as in schizophrenia or Huntington's disease. Based upon the findings that salsolinol may represent a "double-faced" molecule having neuroprotective as well as neurotoxic properties, the manifestation of its "good or bad" influence may depend on hitherto non-specified factor (Maruyama *et al.* 1995).

At the same time, there is no doubt that salsolinol meets some of the criteria that are necessary for fulfilling the definition of a neurotransmitter/neuromodulator function (Schwartz 2000, Deutch and Roth 2003). It is synthesized in neurons, exogenous application elicits a specific effect and is present a biodegradation pathway for termination of its effect and elimination (Toth *et al.* 2001, Naoi *et al.* 2002). In addition, the effect of salsolinol on prolactin secretion as well as on sympathoadrenal system activity can be blocked by an antagonist of the transmitter (1MeDIQ) in a dose-dependent manner (Bodnár *et al.* 2004a, Mravec *et al.* 2004). It must be emphasized that many questions still remain to be answered for salsolinol to be considered as a neurotransmitter or neuromodulator. For example, at present it is difficult to localize the distribution of salsolinol and its relationship with the known catecholaminergic transmitters (dopamine, norepinephrine). The gene sequence of the enzyme responsible for salsolinol synthesis is still unknown. However, it is possible to study the alteration of salsolinol-regulated functions after application of its antagonist (1MeDIQ), which can help to highlight the possible site of salsolinol action.

The consideration of salsolinol as a neuromodulator of catecholaminergic neurotransmission might stimulate further research. It could have a positive influence on the development of a new generation of drugs affecting salsolinol-modulated catecholaminergic functions.

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