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ELEVATED INTERLEUKIN-1β SERUM LEVEL AFTER CHRONIC PERIPHERAL SALSOLINOL ADMINISTRATION

Abstract: The catechol isoquinoline derivatives are endogenous compounds present in the mammalian brain and the representative one is referred to as salsolinol. It may be formed from aromatic amines leading to neurotoxic N-methyltetrahydroquinolinium ions that may play a role in the etiology of Parkinson's disease (PD). Neuroinflammation and apoptosis is thought to be a major contributor to the neuronal degeneration in PD. The alteration of inflammatory cytokines in the brain, cerebral spinal fluid and plasma of PD patients supports the existence of functional interconnections between the immune and nervous systems. In animal studies, chronic administration of salsolinol induced parkinsonian-like symptoms, both peripherally and centrally. However, still little has been known about the effects of salsolinol on the pro-inflammatory cytokine production or mast cells activation in the gastrointestinal tract.

Male Wistar rats were subjected to continuous intraperitoneal dosing of salsolinol (200 mg/kg in total) with osmotic mini-pumps for two or four weeks and fed with either standard or high fat diet. An equivalent group of rats served as the appropriate controls. At the end of the experiment animals were decapitated and blood samples as well as tissue fragments were collected. Serum samples were assayed immunoenzymatically for IL-1β and by liquid chromatography–mass spectrometry for histamine. Tissue fragments from gastric antrum, duodenum and proximal colon were formalin fixed, paraffin-embedded and stained with either hematoxylin and eosin or toluidine blue.

Once activated, mast cells might secrete a range of neurosensitizing and pro-inflammatory molecules, increasing gut-blood and blood-brain barrier permeability. Cytokines mediate the activity of immune cells and may affect brain neurochemistry. The results of the present work serve as an additional support for the existence of an interrelationship between the nervous and immune system.

Key words: salsolinol, rat, interleukin-1β, histamine, mast cells, inflammation, Parkinson's disease.

INTRODUCTION

The catechol isoquinoline derivatives are endogenous compounds present in the mammalian brain and the representative one is referred to as salsolinol (1-methyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline). It may be formed by non-enzymatic or enzymatic pathways from aromatic amines leading to neurotoxic N-methyltetrahydroquinolinium ions that may play a role in the etiology of Parkinson's

disease (PD) [1–3]. In animal studies, chronic administration of salsolinol induced parkinsonian-like symptoms in the gastrointestinal tract [3–5].

So far, neuroinflammation and apoptosis is thought to be a major contributor to the neuronal degeneration in the PD. The alteration of inflammatory cytokines in the brain, cerebral spinal fluid and plasma of PD patients supports the existence of functional interconnections between the immune and nervous systems [6, 7]. The microenvironment of the central and peripheral immune system is modified during the pathological process of PD, although it is not clear if these changes are secondary to the process of cell degeneration, or if they may play an active role in neurodegeneration. Changes in the brain could affect immune cells in vivo and these effects could be subsequently transferred to the peripheral immune system. The question of the existence of a systemic inflammation in PD, in which cytokines may be possibly involved, might be relevant to the better understanding of the pathophysiology of this disease [8].

Cytokines in the gastrointestinal tract are synthesized by activated enterocytes and immune cells in the intestinal mucosa as well as by immune and enteric glial cells in the muscle layers of the gut. Released cytokines influence the activity of many cells, including neurons. Host defense mechanisms involving the enteric innervation are not necessarily present under physiological conditions. Inflammatory mediators, such as IL-1β and IL-6 enhance gastrointestinal motility through direct excitatory actions on a subset of myenteric neurons and through the presynaptic inhibition of transmitter release in the enteric nervous system (ENS). Once these pro-inflammatory mediators are produced, they can activate primary afferent nerves, as well [9]. Recent reports indicate that a pro-inflammatory event in the periphery can induce chronic, self-propelling central neuroinflammation. The entry of systemic pro-inflammatory factors to the brain may result in the activation of microglia to produce more inflammatory factors, which may be responsible for neuronal death, suggesting a clinical implication for the link between peripheral and central inflammation [8].

Information input processed by the enteric nervous system is derived from local sensory receptors, the central nervous system and immune cells including mast cells. Mast cells (MC) are important effectors of brain-gut axis that translate the stress signals into the release of a wide range of neurotransmitters and cytokines, which may profoundly affect the gastrointestinal physiology [10]. Excessive activation of mast cells is positively correlated with the severity of allergic and inflammatory conditions in the gut. Accumulation of activated or degranulated MCs and increased levels of tryptase derived from MCs are found in the intestines of patients with inflammatory bowel disease, for example. These results imply that activation of mast cells is required for the maintenance of a basal level of mucosal homeostasis, but abnormal activation induces intestinal inflammation [11]. What is more, nerve-mast cell communication is bi-directional. Antigen-stimulated or IgE-activated mast cells act on the ENS. In allergic and inflammatory conditions,

a positive feedback loop is established in which mast cells activate nerves that further enhance mast cell activity [9].

Immune activation in neurodegenerative diseases is not restricted to brain sites and abnormalities in peripheral immune functions in patients with Parkinson's disease have already been reported [8]. Early involvement of the ENS is proposed in the pathogenesis of idiopathic Parkinson's disease, too [12]. Thus the aim of this study was primarily, to assess the influence of exogenous salsolinol on the serum level of the pro-inflammatory cytokine IL-1β and histamine and secondary, to morphologically analyze the gastric and intestinal wall.

MATERIAL AND METHODS

Thirty-two adult male Wistar rats were studied. The animals were housed in individual cages. All animals were housed in the same optimal conditions, and food and water were provided ad libitum. During experiment, rats were fed with either standard diet (2.86 kcal/g, Labofeed, Poland) or with obesity-inducing high-fat diet (4.34 kcal/g, Bento Kronen Products, Belgium). The temperature was maintained at 23 ± 2°C, and animals were placed on a 12:12 h dark/light cycle. The Jagiellonian University Bioethical Committee approved the care and use of the animals (ethical approval number — 67/2009).

Rats were either subjected to continuous dosing of salsolinol or served as control. Salsolinol (salsolinol hydrochloride, Sigma, USA) in the total dose of 200 mg/kg was dissolved in 200 μL of 0. 9% solution of salt and delivered by ALZET osmotic mini-pumps (Durtec, USA) implanted intraperitoneally. Control groups were implanted with ALZET osmotic mini-pumps filled with physiological solution of salt (Fig. 1).

Prior to implantation rats were randomly divided into the following groups (n = 4 each): (1) rats subjected to continuous dosing of salsolinol for two weeks (delivery rate 0.5 μL/h, S1 group) and fed with standard diet throughout the entire experimental period; (2) rats subjected to continuous dosing of salsolinol for two weeks (delivery rate 0.5 μL/h, SF1 group) and fed with high-fat diet throughout the entire experimental period; (3) rats subjected to continuous dosing of salsolinol for four weeks (delivery rate 0.25 μL/h, S2 group) and fed with standard diet throughout the entire experimental period; (4) rats subjected to continuous dosing of salsolinol for four weeks (delivery rate 0.25 μL/h, SF2 group) and fed with high-fat diet throughout the entire experimental period; (5) a control group fed with standard diet for two weeks (C1 group); (6) a control group fed with high-fat diet for two weeks (CF1 group); (7) a control group fed with standard diet for four weeks (C2 group); (8) a control group fed with high fat-diet for four weeks (CF2 group). Rats were starved for 12 hours and operated under general anesthesia induced with sodium pentobarbital given intraperitoneally at a dose of 0.25 mg/kg (Vetbutal, Biowet, Poland).

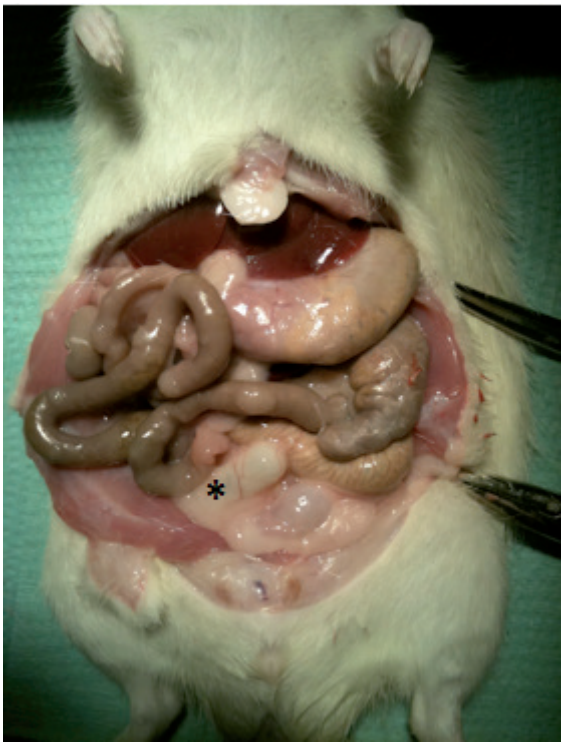

Fig. 1. Implanted osmotic mini-pump *in situ* (*— mini-pump).

On the last day of the experiment animals were sacrificed and stomachs, small and large intestines were removed. All tissue fragments were formalin fixed and then paraffin-embedded 5 μm slices were prepared. Specimens were stained with either hematoxylin and eosin for the estimation of the severity of inflammation or toluidine blue (metachromatic staining) for mast cells analysis [13].

During the study, daily food intake and body weight were measured each morning. For each rat, the amount of daily food intake was determined by weighting the amount of food remaining from that given 24 h before.

The severity of inflammation was examined under optical microscope Axiophot (Zeiss, Germany) in each section according to the four criteria, including mucosal abrasion, hemorrhage, leukocyte infiltration and edema, adapted from [14]. Mucosal abrasion was defined as erosion of the mucosa. The presence or absence of mucosal abrasion per individual field was determined at 100× magnification on a scale of 0 — no abrasion and 1 — visible abrasion. The presence or absence of hemorrhage per individual field was determined at 100× magnification on a scale of 0 — no hemorrhage and 1 — visible hemorrhage. Leukocyte infiltrations (neutrophils and mononuclear cells) were evaluated in each of the view fields at 400× magnifications on a scale of 0 — no extravascular leukocytes, 1 — less than 20, 2 — 20 to 45 and 3 — greater than 45 leukocytes per high power field.

Edema in each view fields was scored at 200× magnification on a scale of 0 — no edema, 1 — mild edema (no alteration in the width of the submucosa), 2 — moderate edema (an increase of less than twice the width of the submucosa) and 3 — severe edema (an increase of more than twice the width of the submucosa). The total score of all view fields was divided by the maximal possible score and multiplied by 100, in each category respectively.

The number of mast cells were counted semi-automatically in eight consecutive visual fields in five serial slides of stomach (antrum), duodenum and proximal colon prepared from every animal, under magnification 125×. Images were collected using light microscope Axiophot (Zeiss) equipped with colour camcorder ProgRes C12 plus (Jenoptik) and quantified using Multiscan 18.03 Software (CSS, Warszawa).

Immediately after decapitation all blood samples were collected and incubated for 30 minutes at 4°C for clotting formation. After centrifugation at 1500 g for 20 min at 4°C (Megafuge 1.0R, Heraeus Instruments), serum samples were collected and frozen at –80°C until further analysis.

Serum aliquots were prepared from each sample and the level of IL-1β was assayed by a quantitative ELISA kit (R&D Systems Europe Ltd) according to the manufacturer's instruction. All measurements were performed in duplicate.

For histamine assessment, samples of rat sera were purified and concentrated using a solid phase extraction (SPE) method for liquid chromatography–mass spectrometry (LC/MS). 500 µl of serum was diluted with water to final volume of 3ml and few portions of formic acid were added to obtain pH = 3. Then samples were applied on columns (LiChrolut Merck, C-18, 200 mg, 3 ml). SPE columns were washed with 3 ml of 0,1% trifluoroacetic acid (TFA) and were eluted with 3 ml of 0,1% TFA in 40% acetonitrile. Obtained sample's eluates were lyophilized overnight and dry remainings were reconstituted in 0,1% formic acid for further LC/MS analysis. Samples for calibration curve of histamine were prepared in the same mode as above.

High-performance liquid chromatography (HPLC) separation was performed on a reversed-phase HPLC system (Agillent 1100 series, USA), using a BDS Hypersil C18 column (100 mm × 3 mm ID, 3 µm particle size) with a guard column (5 mm × 3 mm, 3 µm) (Thermo Scientific, USA). The mobile phase solvents were: 0,1% formic acid in water (phase A) and 0,1% formic acid in acetonitrile (phase B). The HPLC separations were carried out at a flow rate of 300 µl/min with a linear gradient of B in A: from 10% to 95% in 1,5 min, next to 10% in 5 min. Injection volume was 25 µl.

Mass spectrometric detection was performed using an API 2000 triple-quadrupole mass spectrometer (Applied Biosystem, USA), with electrospray ionization source (temperature 350°C, positive ion mode). For histamine detection multiple reaction monitoring (MRM) mode was used with the reaction m/z 113.0–96.0. All acquired data were evaluated by Analyst Software (AB Sciex, USA). Concentrations

of histamine in rat serum samples were calculated using the standard calibration curves, constructed by linear regression analysis plotting of peak area versus histamine concentration. The limit of detection of histamine was set at 2 pg/ml.

All data presented are expressed as the mean and standard deviation (SD). The results were analyzed using a one-way analysis of variance (ANOVA) followed by post hoc tests. All statistical tests were performed using STATISTICA software package (StatSoft, Tulsa, USA). Statistical significance was set at $p < 0.05$.

RESULTS

1. BODY WEIGHT AND FOOD INTAKE

Chronic intraperitoneal treatment with salsolinol significantly diminished the mean weight gain and the mean food consumption in rats fed with both standard and high fat diet, in comparison with relevant control group of animals ($p < 0.05$) (Table 1).

Table 1

Total food intake and body mass gain in animals fed with standard and high-fat diet after intraperitoneal salsolinol administration.

	Groups			
	S1	C1	SF1	CF1
Body mass gain (%) over initial body weight	23.3 *	32.4	29.1 *	44.5
Total food intake (g)	340.4 ± 15.2	360.2 ± 24.6	238.7 ± 26.6	253.5 ± 36.3
	S2	C2	SF2	CF2
Body mass gain (%) over initial body weight	42.2 *	51.8	41.7 *	71.7
Total food intake (g)	644.3 ± 55.1	696.9 ± 39.1	429.3 ± 36.3	509.3 ± 24.1

Two weeks of salsolinol exposure: S1 — standard diet, SF1 — high fat diet, together with control groups; C1 — standard diet, CF1 — high fat diet; four weeks of salsolinol exposure: S2 — standard diet, SF2 — high fat diet, together with control groups; C2 — standard diet, CF2 — high fat diet; * $p < 0.05$.

2. HISTOLOGICAL ANALYSIS

Neither chronic intraperitoneal treatment with salsolinol nor the high fat diet led to formation of inflammatory lesions along the gastrointestinal tract of rats. There were no signs of pathological patterns, such as mucosal abrasion and edema, hemorrhage or leukocyte infiltration in the routine biopsies from gastric antrum, duodenum or proximal colon stained with hematoxylin and eosin. There were no visible changes between all the experimental groups (Table 2, Fig. 1).

Table 2

Inflammatory index score after intraperipheral chronic salsolinol administration.

Groups	Mean (%) ± SD	Groups	Mean (%) ± SD
S1	21.01 ± 10.61	S2	23.44 ± 13.78
C1	25.52 ± 11.95	C2	22.40 ± 14.34
SF1	27.78 ± 9.26	SF2	19.62 ± 11.72
CF1	25.35 ± 12.72	CF2	24.48 ± 22.63

Two weeks of salsolinol exposure: S1 — standard diet, SF1 — high fat diet, together with control groups; C1 — standard diet, CF1 — high fat diet; four weeks of salsolinol exposure: S2 — standard diet, SF2 — high fat diet, together with control groups; C2 — standard diet, CF2 — high fat diet.

3. HISTOLOGICAL ANALYSIS OF MAST CELLS

The total number of metachromatic cells (mast cells) was evaluated in every histological sample from gastric antrum, duodenum and proximal colon (Table 3).

Table 3

Mast cells count in the stomach, duodenum and proximal colon in animals fed with standard and high-fat diet after intraperitoneal salsolinol administration.

Groups	Stomach	Duodenum	Proximal colon
	Mean ± SD	Mean ± SD	Mean ± SD
S1	169.0 ± 100.8	14.0 ± 9.1	18.7 ± 13.4
C1	115.4 ± 62.2	5.2 ± 2.1	12.8 ± 14.0
SF1	192.3 ± 103.7	16.0 ± 10.5	17.3 ± 11.6
CF1	110.9 ± 78.2	7.8 ± 7.8	16.7 ± 11.2
S2	212.6 ± 84.3	18.4 ± 8.6	22.3 ± 22.1
C2	133.9 ± 73.3	11.7 ± 8.9	16.4 ± 8.6
SF2	188.6 ± 107.2	23.7 ± 22.8	23.7 ± 22.8
CF2	145.7 ± 122.6	16.3 ± 11.5	19.4 ± 15.4

Two weeks of salsolinol exposure: S1 — standard diet, SF1 — high fat diet, together with control groups; C1 — standard diet, CF1 — high fat diet, four weeks of salsolinol exposure: S2 — standard diet, SF2 — high fat diet, together with control groups; C2 — standard diet, CF2 — high fat diet.

The total number of MC in the gastrointestinal wall was increased significantly in all salsolinol-treated rats, especially in the gastric antral region (Fig. 2). We

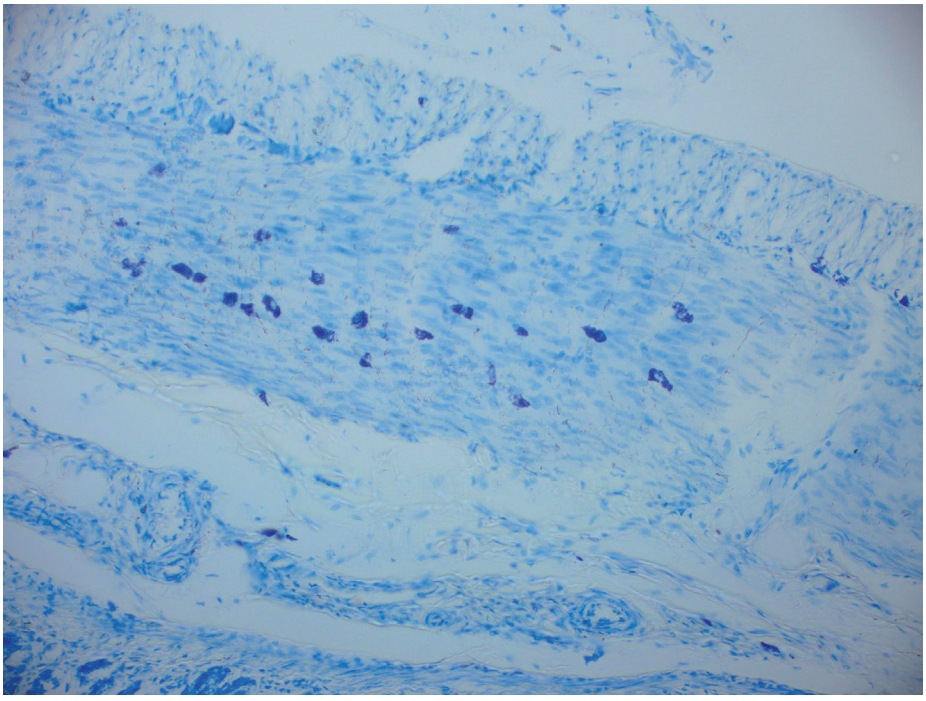

Fig. 2. Mast cells in the rat stomach wall (cross-section) after toluidine blue staining. Mast cells red-purple (metachromatic staining) and the background blue (orthochromatic staining). Magnification 400×.

assumed that high fat diet was responsible for the increase in the number of mast cells within the gastrointestinal wall, however not statistically significant.

4. SERUM LEVEL OF HISTAMINE

The serum levels of histamine were elevated in both groups of salsolinol-treated rats (S1 and S2) in comparison with the appropriate control groups (Table 4).

Table 4

The serum level of histamine (ng/ml) in animals fed with standard diet after intraperitoneal salsolinol administration.

Groups	Mean (%) ± SD	Groups	Mean (%) ± SD
S1	591.837 ± 488.414	S2	405.376 ± 222.508
C1	121.473 ± 79.428	C2	313.156 ± 184.936

Two weeks of salsolinol exposure: S1 — standard diet together with control group C1 — standard diet; four weeks of salsolinol exposure: S2 — standard diet together with control group C2 — standard diet.

5. SERUM LEVEL OF INTERLEUKIN-1β

Chronic intraperitoneal salsolinol administration increased pro-inflammatory IL-1β levels in all experimental groups compared to relevant controls (Fig. 3). The highest values were present in animals given salsolinol for four weeks (S2 group). The high-fat diet did not influence the IL-1β levels in rats.

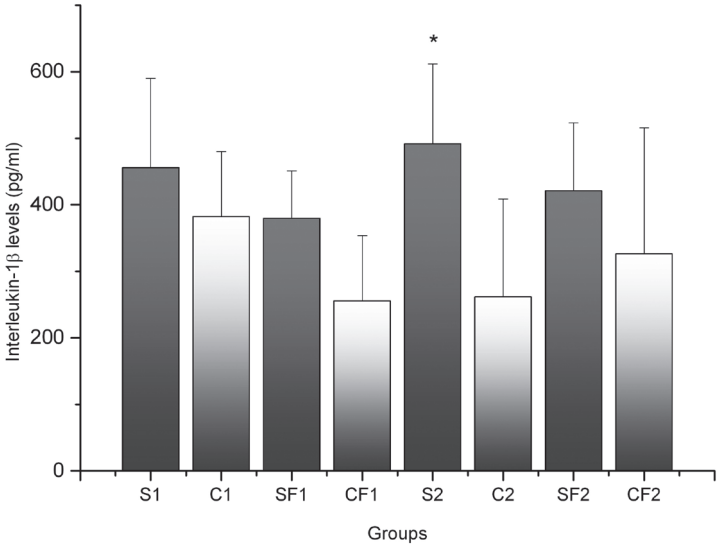

Fig. 3. The serum level of interleukin-1β (pg/ml); two weeks of salsolinol exposure: S1 — standard diet, SF1 — high fat diet, together with control groups; C1 — standard diet, CF1 — high fat diet; four weeks of salsolinol exposure: S2 — standard diet, SF2 — high fat diet, together with control groups; C2 — standard diet, CF2 — high fat diet; *p <0.05.

DISCUSSION

The course of PD progression is hugely debated, although one leading hypothesis postulates that PD pathology begins in the enteric plexus or olfactory bulb and works its way into the central nervous system in appropriate stages. Neuroactive substances are usually taken up at synapses, where they are controlled by receptor-mediated endocytosis and transported to the cell body via the axon. The absence of a myelin sheath around axons of the first neuron in the potential chain of vulnerable projections may facilitate entrance and damage by unknown toxins or pathogens. Most of the neurons located within the gastrointestinal wall, intrinsic enteric neurons, extrinsic preganglionic parasympathetic and sympathetic fibres, lack such a shielding barrier [12]. Salsolinol and its active metabolites may as well, for example, participate in the equilibrium of information transmission at synapses [3].

Jang *et al.* have recently supported the Braak-like progression of pathology. They demonstrated that a pathogenic virus was sufficient to induce toxicity in nigral DA neurons. The study also implicated inflammation as a significant factor in extra-nigral and Lewy body histopathology. Data such as these indicate the potential of inflammation to contribute to non-motor symptoms in PD [15]. Inflammation is a vital cellular response, however, when prolonged, it overrides the bounds of physiological control, eventually becomes destructive and may permeate all levels of dysfunction associated with PD, including non-motor symptoms [16].

Recognition that an extensive communication exists between the immune system and the nervous system is one of the most fundamental advances in the recent neuroscience. In fact, mast cells may represent such an undervalued peripheral link to the nervous tissue in an inflammatory setting. Mast cells participate in innate host defense reactions, are capable of phagocytosis and antigen presentation and can modulate the adaptive immune response [17]. In our experiment, neither chronic intraperitoneal treatment with salsolinol (total dose of 50 mg of racemic salsolinol hydrochloride) nor the high fat diet led to formation of inflammatory lesions along the gastrointestinal tract of rats. However, the total number of MC in the gastrointestinal wall was significantly increased in all salsolinol-treated rats, especially in the gastric antral region. We assumed that high fat diet was responsible for the increase in the number of mast cells within the gastrointestinal wall. Our previous results showed mucosal mast cells depletion and increase in the degranulated cells, which we suggest should be due to excessive mast cells degranulation caused by acute salsolinol treatment (50 mg of salsolinol hydrochloride daily) [18]. Mast cells, once activated may secrete a range of neurosensitizing compounds [10]. In our experiment, the serum levels of histamine were significantly elevated in all salsolinol-treated rats in comparison with the control groups. However, histamine is not the only MC-derived mediator taking part in the interaction between MCs and ENS. A whole array of cytokines plays a central role in this interaction, but such data are only beginning to accumulate. The mast cells-enteric nerves interaction is not a one-sided relationship [19]. Indeed, the consequences of vagus nerve stimulation, for example, and mediator release on intestinal mast cells function have caught researchers' interest in recent years [13, 20].

Morphological and functional studies have provided evidence that apart from the interaction between the enteric nervous system and mast cells there is also a functional communication between the central nervous system and mast cells [10]. Communication between the central nervous system and mast cells in the gut mucosa may occur through various pathways, of which the most important occurs to be through intrinsic reflexes. Both mast cells and sensory neurons code information by releasing chemical message that is decoded by numerous processing circuits in the nervous system [21]. Reports of Pavlovian conditioning

of enteric mast cell degranulation give direct evidence for a brain-mast cell connection [22].

Chen *et al.* reported that men with elevated plasma levels of interleukin-6 have an increased risk of developing PD. IL-6 has both pro-inflammatory and anti-inflammatory activities [23]. IL-1β is a potent pro-inflammatory cytokine that acts through IL-1 receptors found on numerous cell types including neurons and microglia. IL-1 signaling leads to NF-κB mediated expression of pro-inflammatory cytokines. In PD patients, IL-1β is elevated in cerebrospinal fluid, striatum and substantia nigria. Over-expression of IL-1β in these patients may lead to an increased neuron's, probably especially dopaminergic, vulnerability to exogenous toxins and an increased risk for PD onset [24]. What is more, blood brain barrier disruption has already been demonstrated in different systemic infections as a result of the activation of various mediators that cause multiple organ failure including the brain. Microglia of the blood origin may also activate the immune system and lead to central damage [25]. For example, it has been reported that bone marrow-derived microglial cells add to the neuroinflammatory response and express iNOS in the MPTP mouse model of PD [26]. These findings indicate that systemic infection may be a significant risk factor in the genesis of Parkinson's disease. Neurons have been shown to be particularly susceptible to IL-1β-mediated toxicity [27]. In our experiment, intraperitoneal salsolinol treatment increased peripheral IL-1β production. However, the relevance of these findings to PD pathogenesis should be further evaluated in every detail.

Significant evidence linking systemic inflammation with neurodegeneration comes mainly from epidemiological studies. Changes in peripheral immune factors can undoubtedly influence neurodegenerative processes [27]. What is more, neuroinflammation may also raise the brain's sensitivity to stress [17]. The role of inflammation in PD has been suggested over the last 20 years by a number of studies, showing microglia activation, cytokine production and oxidative damage in vivo and post-mortem, although the precise role of inflammation in the pathogenesis of PD remains unclear [28]. The results of the present work serve as an additional support for the existence of an interrelationship between the nervous and immune system. We are convinced that the emerging information on the brain and systemic immune as well as nervous system communication, especially early in the disease progression, will provide us with a window of opportunity to target early symptoms of Parkinson's disease.

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