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THE INFLUENCE OF SALSOLINOL ON BASIC RAT METABOLISM

Abstract: *The influence of salsolinol on basic rat metabolism*

Parkinson's disease (PD) is associated with a broad spectrum of non-motor symptoms, which are poorly understood and foremost, may precede motor impairment. These symptoms include weight changes and gastrointestinal dysregulation. In our experiment, we applied salsolinol given peripherally and continuously in rats to induce changes in the enteric nervous system, which might be similar to those observed in PD patients. Surprisingly, we noted decrease in body weight and alteration in body fat contents of the animals during salsolinol exposure. The blood glucose levels, lipid profile and hepatic enzymes levels were assessed as well.

While lipid profile, postprandial blood glucose and hepatic enzymes levels remained indifferent, postprandial triglyceridemia was significantly lower in all salsolinol-treated rats in comparison with the control, which might be related to disturbed absorption. We also suggest that diminished body weight gain and lower adipose tissue accumulation in salsolinol-treated animals were due to delayed gastric emptying together with disturbed gut function resulting in absorptive dysfunction.

Key words: salsolinol, rat, epididymal fat pad, weight loss, Parkinson's disease, glucose, triglycerides, cholesterol

INTRODUCTION

Although still considered as a movement disorder, Parkinson's disease (PD) is associated with a broad spectrum of non-motor symptoms, which are poorly understood and foremost, may precede motor impairment. These symptoms include weight changes and gastrointestinal dysregulation, such as dysphagia, delayed gastric emptying, nausea, constipation and faecal incontinence [1].

Weight loss is frequent in patients with Parkinson's disease. Dysphagia, anorexia, sense of smell and appetite loss as well as gastrointestinal dysfunction may be possible causes of reduced energy intake; while rigidity, tremor, and levodopa-induced dyskinesia may increase energy expenditure. Moreover levodopa may enhance glucose metabolism resulting in enhanced energy expenditure. Depression, antiparkinsonian drugs, medical complications such as pneumonia and malignancies also may facilitate weight loss in PD. Weight loss is associated

with malnutrition and may precipitate infection, accelerate motor, behavioral and autonomic impairment in PD patients [2–4]. The mechanism of weight loss in these patients, however, remains still undefined.

The catechol isoquinoline derivatives are compounds widely present in the mammalian brain and the most investigated one is referred to as salsolinol (1-methyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline). It was detected for the first time in rat and human brain tissue samples [5, 6]. It seems to exert a wide range of effects on the catecholaminergic transmission and dopaminergic neurons. For instance, it has been proposed that salsolinol can cause the release of stored catecholamines, the inhibition of catecholamine reuptake, the inhibition of monoamine oxidase, catechol-O-methyltransferase and tyrosine hydroxylase [7–9]. In addition, it has been implicated in the development of alcoholism, in the release of the hormone prolactin [10, 11] and in the etiopathogenesis of Parkinson's disease [12–15].

Salsolinol can be either endogenously synthesized from dopamine and acetaldehyde by salsolinol synthase and alternatively, from dopamine and pyruvic acid [16] or delivered exogenously with food [17]. Various toxins, tetrahydroizochinolines among them, may easily cross the intestinal barrier and reach the intestinal, neural pathways, initiate the processes of neurodegeneration in enteric nervous system and subsequently, via dorsal motor nucleus of the vagus nerve (dmX), in the central nervous system. According to Braak's hypothesis, the gastric mucosa appears to be one of the likely sites for such entries as (1) a very large segment of the dmX innervates that part of the gastrointestinal tract; (2) the chymus remains in the stomach for a long period of time; (3) the epithelial lining of the stomach is thin, consists of only a single cell layer, and is susceptible to lesions and chronic infection; and (4) large numbers of pathological inclusions such as Lewy Bodies have been found in the enteric nervous system of the stomach [18].

Uptake of exogenous substances from the extraneuronal space is known to occur at the axon terminal and such material is to be transferred to the cell soma via retrograde axonal transport. Lots of neuroactive substances are taken up in this way, usually through receptor-mediated endocytosis by the presynaptic membrane of the next nerve cell in the neuronal chain. And, such a neurotropic constituent might induce conformational changes in normal α-synuclein molecules, provoking their aggregation and formation of Lewy Bodies [18].

The vagus nerve consists of axons, which emerge from or converge onto three nuclei of the medulla, including the dorsal nucleus of vagus — which sends parasympathetic output to the viscera, especially the intestines. The vagus nerve contributes to the bidirectional communications between the gastrointestinal tract and central nervous system. Afferent neurons of the vagus nerve are important targets of gut hormones, particularly the hormones involved in controlling food intake. Vago-vagal reflexes are involved in feeding homeostasis, making neuromodulation an attractive method for managing obesity [19].

In animal models with vagal nerve stimulation (VNS) decreased food intake and weight loss are considered as the result of the stimulation of brain centers, the peripheral action of vagal stimulation via short cholinergic reflexes, and the combination of central and peripheral signals [20]. It is also hypothesized that the VNS decreases both food intake and body weight gain by mimicking the "satiety" signals transmitted from the gut to the brain, leading to the activation of the hypothalamic neurons that initiate the state of satiety. VNS also exerts anorexigenic effects on food intake and body weight gain in rats with high-fat diet-induced obesity [21–24].

Body weight, food intake and body fat content are regulated by multiple factors [20, 25, 26]. Despite fluctuations in the amount of food consumed, body weight remains stable within a relatively narrow range. Food intake together with body mass is controlled by both short- and long-term regulatory mechanisms [27]. Hypoglycemia increases food intake and stimulates vagal nerve activity [28]. Food transported into the stomach and duodenum activates both chemo- and mechanoreceptors. These signals are also transmitted via the vagus nerve to the hind brain where they are integrated and play a major role in short-term regulation by limiting the size of the meal consumed [25]. The long-term control of food intake involves many mediators (ghrelin, leptin, nesfatin-1, orexins and neuropeptide Y) and structures (nucleus of the solitary tract, arcuate nucleus of vagus nerve and hypothalamus) [26].

In our experiment, we applied salsolinol given peripherally and continuously in rats to induce changes in the enteric nervous system, which might be similar to those observed in PD patients. Surprisingly, we noted significant decrease in body weight of the animals during salsolinol exposure. We can hypothesize that salsolinol given peripherally can diminish food consumption and body weight via vagus afferents since it targets vagus nerve fibers and other centers responsible for appetite controlling. Thus, the aim of this work was to evaluate the effects of salsolinol on body mass and food intake. The blood glucose levels, lipid profile and hepatic enzymes levels were assessed as well.

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 $23 \pm 2^{\circ}C$, 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.

Prior to implantation rats were randomly divided into the following eight 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).

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.

One-hour stool frequency was measured twice a week after the start of salsolinol infusion. Assays were performed between 10:00 and 12:00 on each day. Each animal was removed from its home cage and placed in a clean, clear plastic cage without food nor water for 1 h. Stools were collected immediately after expulsion and placed in sealed tubes. The total stools were weighed to provide a wet weight, then dried overnight at 65°C and weighed again to provide a dry weight. Results were normalized to body weight. Stool water content was then calculated to indicate colon water absorption [29].

At the end of the experiment (day 15 or 29), following a 12-hour fast, rats were allowed free access to food (a mixture of high fat food and charcoal, 10 : 1) for 1 h. Half an hour later, animals were euthanized by decapitation and the stomach contents were weighed to assess solid gastric emptying (results not presented in this study). Both epididymal fat pads, located between the cauda epididymis and the distal extremity of the testis, were dissected from each rat and weighed. The epididymal fat pad/body weight ratio was calculated by dividing the fat pad weight by the final body weight.

Blood samples collected at the end of the experiment were taken into tubes containing aprotinin (0.6 TIU per 1 ml of blood) (Sigma-Aldrich, USA) 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 glucose, hepatic enzymes (aspartate and alanine transaminases), triglycerides, total cholesterol as well as LDL and HDL levels were measured with chemistry immune-analyser Olympus AU 600. All measurements were performed in duplicate. Additionally, salsolinol serum level was measured by liquid chromatography–mass spectrometry technique.

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

RESULTS

FOOD INTAKE, BODY WEIGHT AND EPIDIDYMAL FAT PAD WEIGHT

The mean food consumption was alike however the mean weight gain was significantly diminished in the salsolinol-treated groups of rats. Epididymal fat pad weight, which reflects total body fat content, was significantly higher in the control groups compared with the salsolinol-treated groups.

Table 1

Mean food intake, body weight and epididymal fat pad weight in salsolinol-treated (S1, SF1 — rats subjected to continuous dosing of salsolinol for two weeks and fed with standard or high-fat diet, respectively) and control group of rats (C1, CF1 — control rats fed with standard or high-fat diet, respectively). Asterisks (*) indicate significant differences between the salsolinol-treated and control groups.

	Group			
	S1	C1	SF1	CF1
Total food intake (g)	340.4 ± 15.2	360.2 ± 24.6	238.7 ± 26.6	253.5 ± 36.3
Total food intake (kcal)	973.5 ± 43.5	1030.2 ± 70.4	1035.9 ± 115.4	1100.2 ± 157.5
Initial body weight (g)	253.7 ± 9.9	251.5 ± 9.1	243.2 ± 4.7	220.0 ± 9.4
Final body weight (g)	312.8 ± 14.1	334.0 ± 8.9	314.1 ± 19.8	318.0 ± 4.9
Body weight gain (g)	59.1 ± 5.9*	81.6 ± 2.7	70.9 ± 18.2*	98.0 ± 13.9

Table 1 cont.

Group			
S1	C1	SF1	CF1
Epididymal fat pad weight (EFP) (g)	3.4 ± 0.3	3.8 ± 0.4	4.1 ± 0.4*	5.1 ± 0.2
Body weight gain/initial weight (%)	23.3	32.4	29.1	44.5

Table 2

Mean food intake, body weight and epididymal fat pad weight in salsolinol-treated (S2, SF2 — rats subjected to continuous dosing of salsolinol for four weeks and fed with standard or high-fat diet, respectively) and control group of rats (C2, CF2 — control rats fed with standard or high-fat diet, respectively). Asterisks (*) indicate significant differences between the salsolinol-treated and control groups.

|	Group			
	S2	C2	SF2	CF2
Total food intake (g)	644.3 ± 55.1	696.9 ± 39.1	429.3 ± 36.3	509.3 ± 24.1
Total food intake (kcal)	486.9 ± 157.6	993.1 ± 111.8	863.2 ± 157.5	210.4 ± 104.6
Initial body weight (g)	23.9 ± 4.9	232.5 ± 11.9	234.2 ± 8.5	225.7 ± 7.6
Final body weight (g)	351.1 ± 33.5	366.8 ± 18.4	331.8 ± 24.9	387.5 ± 17.9
Body weight gain (g)	98.3 ± 30.5*	120.4 ± 19.7	97.6 ± 28.9*	161.8 ± 13.8
Epididymal fat pad weight (EFP) (g)	3.7 ± 0.5	5.1 ± 0.5	4.9 ± 1.3*	7.8 ± 1.0
Body weight gain/ initial weight (%)	42.2	51.8	41.7	71.7
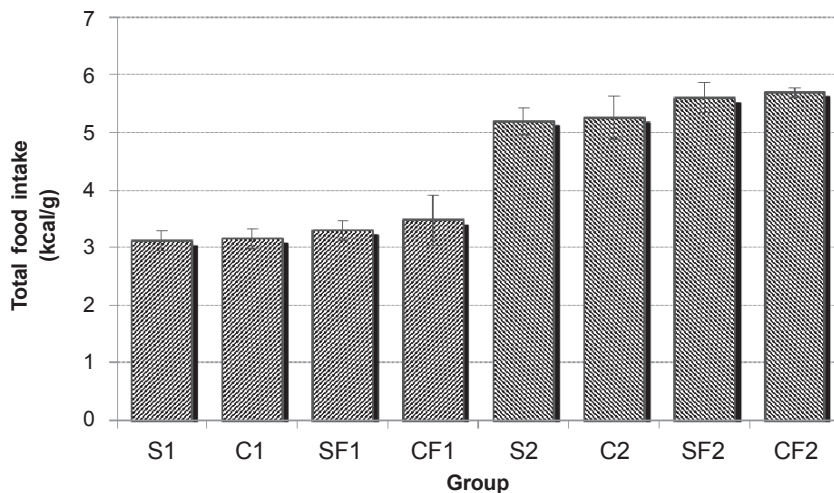
Fig. 1. Total food intake (kcal/g) during the experiment in salsolinol-treated (S1, SF1 — rats subjected to continuous dosing of salsolinol for two weeks and fed with standard or high-fat diet, respectively; S2, SF2 — rats subjected to continuous dosing of salsolinol for four weeks and fed with standard or high-fat diet, respectively) and control rats C1, CF1 — control rats fed with standard or high-fat diet for two weeks, respectively; C2, CF2 — control rats fed with standard or high-fat diet for four weeks, respectively). No statistically significant differences between the salsolinol-treated and control groups were observed.
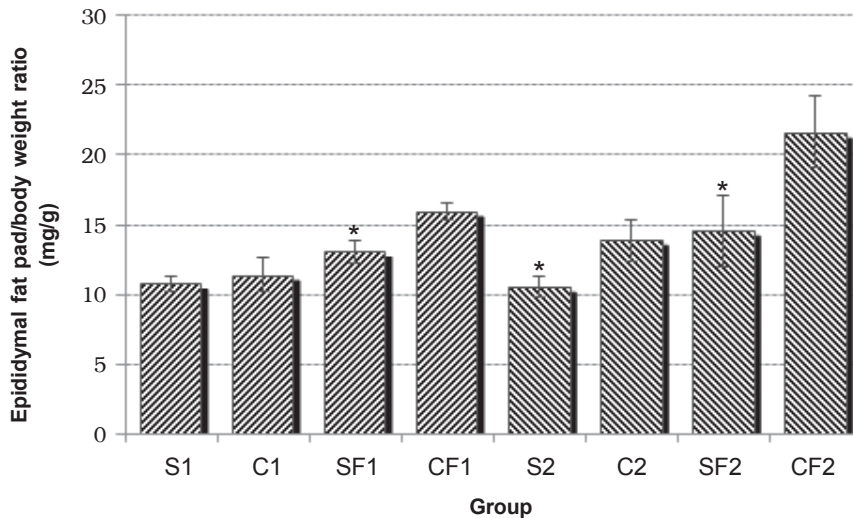

Fig. 2. Epididymal fat pad/body weight ratio (mg/g) in salsolinol-treated (S1, SF1 — rats subjected to continuous dosing of salsolinol for two weeks and fed with standard or high-fat diet, respectively; S2, SF2 — rats subjected to continuous dosing of salsolinol for four weeks and fed with standard or high-fat diet, respectively) and control rats (C1, CF1 — control rats fed with standard or high-fat diet for two weeks, respectively; C2, CF2 — control rats fed with standard or high-fat diet for four weeks, respectively). Asterisks (*) indicate significant differences between the salsolinol-treated and control groups.

GLUCOSE

There was no statistically significant difference between the salsolinol-treated and control groups in the level of postprandial glycemia.
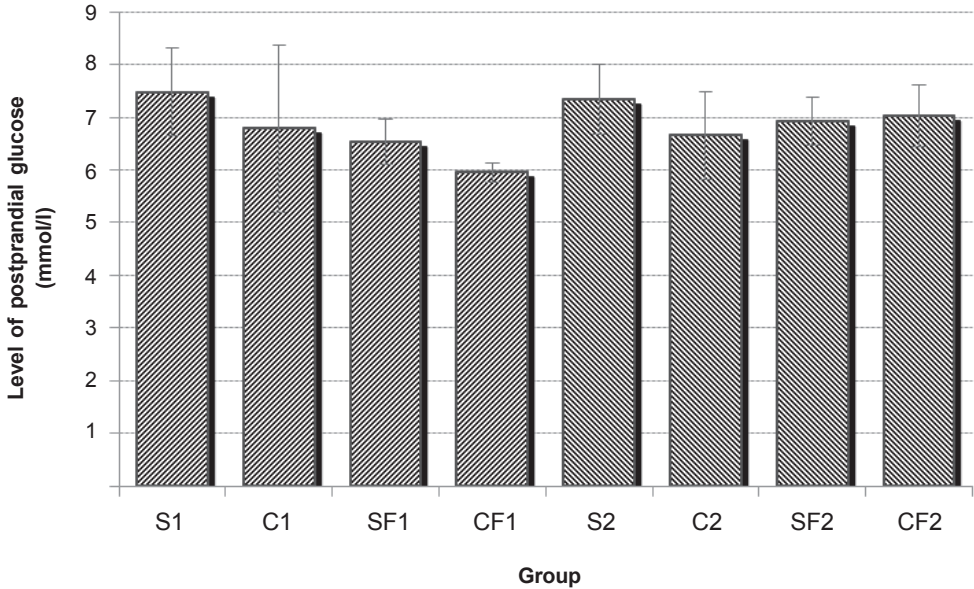

Fig. 3. Blood serum levels of postprandial glucose (mmol/l) in salsolinol-treated (S1, SF1 — rats subjected to continuous dosing of salsolinol for two weeks and fed with standard or high-fat diet, respectively; S2, SF2 — rats subjected to continuous dosing of salsolinol for four weeks and fed with standard or high-fat diet, respectively) and control rats (C1, CF1 — control rats fed with standard or high-fat diet for two weeks, respectively; C2, CF2 — control rats fed with standard or high-fat diet for four weeks, respectively). No statistically significant differences between the salsolinol-treated and control groups were observed.

TRIGLYCERIDES

Postprandial triglyceridemia was significantly lower in all salsolinol-treated rats in comparison with the control.
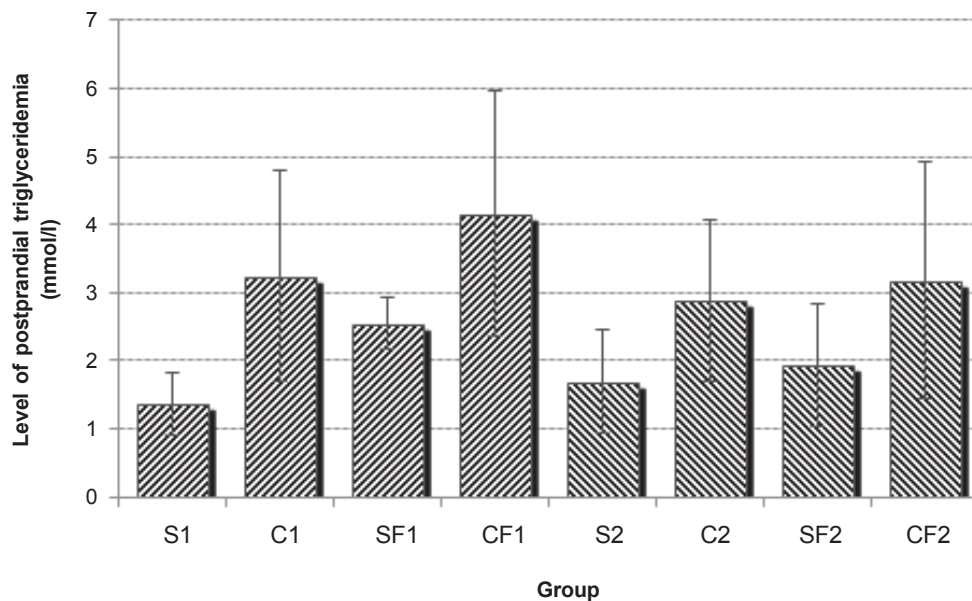

Fig. 4. Blood serum levels of postprandial triglycerides (mmol/l) in salsolinol-treated (S1, SF1 — rats subjected to continuous dosing of salsolinol for two weeks and fed with standard or high-fat diet, respectively; S2, SF2 — rats subjected to continuous dosing of salsolinol for four weeks and fed with standard or high-fat diet, respectively) and control rats (C1, CF1 — control rats fed with standard or high-fat diet for two weeks, respectively; C2, CF2 — control rats fed with standard or high-fat diet for four weeks, respectively). Asterisks (*) indicate significant differences between the salsolinol-treated and control groups.

LIPIDS PROFILE

There was no statistically significant difference between the salsolinol-treated and control groups in the level of total cholesterol, high-density lipoproteins as well as low-density lipoproteins.

<div align="right">T a b l e 3</div>

Blood serum levels (mmol/l) of total cholesterol (TC), high-density lipoproteins (HDL) as well as low-density lipoproteins (LDL) in salsolinol-treated (S1, SF1 — rats subjected to continuous dosing of salsolinol for two weeks and fed with standard or high-fat diet, respectively; S2, SF2 — rats subjected to continuous dosing of salsolinol for four weeks and fed with standard or high-fat diet, respectively) and control rats (C1, CF1 — control rats fed with standard or high-fat diet for two weeks, respectively; C2, CF2 — control rats fed with standard or high-fat diet for four weeks, respectively). No statistically significant differences between the salsolinol-treated and control groups were observed.

	Group			
	S1	C1	SF1	CF1
TC	1.87 ± 0.43	1.93 ± 0.33	2.07 ± 0.18	1.80 ± 0.31
HDL	0.55 ± 0.08	0.61 ± 0.10	0.63 ± 0.08	0.59 ± 0.07
LDL	0.78 ± 0.32	0.35 ± 0.30	0.28 ± 0.24	0.16 ± 0.02
	Group			
	S2	C2	SF2	CF2
TC	1.93 ± 0.36	2.14 ± 0.31	1.96 ± 0.23	1.82 ± 0.38
HDL	0.64 ± 0.09	0.67 ± 0.07	0.61 ± 0.50	0.52 ± 0.05
LDL	0.58 ± 0.44	0.36 ± 0.35	0.48 ± 0.51	0.17 ± 0.07

HEPATIC ENZYMES

There was no statistically significant difference between the salsolinol-treated and control groups in the level of aspartate and alanine transaminases.

Table 4

Blood serum levels of aspartate (AspAT) and alanine (AlAT) transaminases in salsolinol-treated (S1, SF1 — rats subjected to continuous dosing of salsolinol for two weeks and fed with standard or high-fat diet, respectively; S2, SF2 — rats subjected to continuous dosing of salsolinol for four weeks and fed with standard or high-fat diet, respectively) and control rats (C1, CF1 — control rats fed with standard or high-fat diet for two weeks, respectively; C2, CF2 — control rats fed with standard or high-fat diet for four weeks, respectively). Asterisks (*) indicate significant differences between the salsolinol-treated and control groups.

Group			
S1	C1	SF1	CF1
AspAT	156.3 ± 41.8	125.9 ± 14.2	121.3 ± 20.4	101.5 ± 10.5
AlAT	40.1 ± 13.9	39.9 ± 6.5	27.0 ± 2.2	27.3 ± 3.6

Group			
S2	C2	SF2	CF2
AspAT	134.6 ± 25.2	124.3 ± 17.5	$140.3 \pm 3.8*$	74.5 ± 9.7
AlAT	55.6 ± 26.0	46.7 ± 14.1	31.5 ± 5.2	24.6 ± 4.6

ONE-HOUR STOOL COLLECTION

Stool water content, an indicator of colon peristalsis and absorption, was indifferent between groups during the first two weeks after the mini-pump implantation. The difference was noted from the third week of experiment in rats treated with salsolinol for four weeks and fed with a standard diet — S2 = 47.70% ± 7.22, C2 = 59.95% ± 10.97 as well as with high-fat diet SF2 = 55.88% ± 0.05, CF2 = 63.18% ± 5.6.

SERUM LEVEL OF SALSOLINOL

The limit of detection was set at 0.86 ng/l. Salsolinol was not detected in serum samples, which suggests the compound did not reach the systemic blood.

DISSCUSSION

Gastrointestinal dysfunction has been recognized as one of the most frequent manifestations of autonomic dysfunction in PD and may consist of disordered, excessive salivation, dysphagia, gastroparesis, decreased bowel movement frequency

and defecatory dysfunction characterized by excessive straining and incomplete evacuation. And the enteric nervous system abnormalities have to play a role in the pathogenesis of the disease [30].

Salsolinol due to its structural similarity to MPTP (1-methyl-4-phenyl-1,2,3, 6-tetrahydropyridine) might be a useful substance in modeling enteric pathologies similar to those in PD. It is already well known that metabolism of salsolinol occurs through two reactions, N-methylation and oxidation. N-methylation of (R)-salsolinol into N-methyl-(R)-salsolinol was demonstrated in the rat striatum [31]. It was shown that methylation of (R)-salsolinol is catalyzed by two N-methyltransferases with different optimum pH at 7.0 and 8.4. N-methyl-(R)-salsolinol is further oxidized into 1,2-dimethyl-6,7-dihydroxyisoquinolinium by means of an oxidase [16, 32]. A non-enzymatic process of autoxidation has also been demonstrated [33]. The distribution of the N-methylated and oxidized derivatives of salsolinol in the human nigrostriatal pathway seems to follow a specific pattern [31], which suggests that these derivatives may be involved in the function of dopamine neurons under physiological and pathological conditions. Notably, neurotoxic effects have been attributed to these metabolites, highlighting their role in the etiology of Parkinson's disease [34–36]. At the same time, these metabolites might also be neuroprotective due to their role as hydroxyl radical scavengers. Therefore, it has been suggested that salsolinol might represent a "double faced" molecule [36, 37].

However little is known about the general influence of salsolinol on short-term lipids' and carbohydrates' absorption, their utilization as well as on body weight and energy storing. At the end of our experiment salsolinol was not detected in serum samples, which suggests the compound did not reach the systemic blood and must have exerted its influence through local mechanisms in the gastrointestinal tract.

Meal absorption is a complex phenomenon. Postprandial hyperlipidemia and hyperglycemia are simultaneously present in the post-absorptive phase [38]. Maintenance of a normal plasma glucose concentration requires precise matching of glucose utilization and endogenous glucose production or dietary glucose delivery. Glucose is derived from three sources: the intestinal absorption that follows the digestion of dietary carbohydrates, glycogenolysis and gluconeogenesis. The profile of postprandial hyperglycemia is determined by many factors, including the timing, quantity and composition of the meal [39]. The amount of cholesterol absorbed from the diet in humans is a major contributor to levels of cholesterol in circulation. Early lipid digestion, from the oral cavity to the duodenum, produces emulsions consisting of free cholesterol, triglycerides, free fatty acids and phospholipids. Bile salt emulsified triglycerides and cholesteryl esters are hydrolyzed by pancreatic lipase and carboxyl ester lipase. Cholesterol absorption is achieved through passage across brush border membranes and into intestinal enterocytes in the jejunum. The appeal of HDL lies in the ability to undo cholesterol accumulation in tissues [40]. Plasma triglyceride (TG) level is regulated by

both synthesis and degradation of both very low density lipoprotein (VLDL) and chylomicron particles. The clearance of TG-rich lipoproteins from the circulation is controlled by the actions of lipoprotein lipase and hepatic lipase, and by the interlipoprotein exchange of TG by cholesteryl ester transfer protein [41]. In our experiment, there was no statistically significant difference between the salsolinol -treated and control groups in the level of postprandial glycemia. Moreover, since there was no significant difference in the levels of aspartate and alanine transaminases between the salsolinol-treated and control groups it seems unlikely that salsolinol exerts toxic effects on hepatocytes or influences glucose metabolism.

Postprandial triglyceridemia, however, was significantly lower in all salsolinol -treated rats in comparison with the control. In our opinion the elevated level of postprandial triglycerides might be due to the access to high-fat food prior to decapitation. Postprandial increase of TG was markedly lower in animals exposed to salsolinol in comparison with the control animals which might be related to the delayed gastric emptying (unpublished data) or disturbed absorption. Nevertheless, this still needs to be clarified.

Fatty acids deposited as triacylglycerols in adipose tissue represent the primary energy store in animals. In periods of increased energy demand, such as fasting and cold exposure, stored fat is mobilized by lipolytic enzymes, which hydrolyze adipose triacylglycerols and release non-esterified fatty acids into the circulation [42]. When an energy deficit occurs (during fasting or exercise) triglycerides are broken down and fatty acids and glycerol are released into circulation. This release helps to supply the organism's increased energy demands. Balance usually exists between these two states; but dysregulation can occur, leading to either an excessive storage of lipid within adipose tissue or an excessive depletion in states of catabolism [43]. Adipose tissue is an endocrine organ, composed of several cell types, that affects multiple other systems in a body as well as an organ that secretes numerous paracrine factors [44]. In rodents, fat tissue is localized in different depots and visceral fat easily accumulates in the epididymal fat pad, which is well delimitated and easy to excise. Although the epididymal fat pad represents only a small percentage of the total body weight, previous studies showed that the epididymal fat pad weight calculated as a proportion of total body weight highly correlates with the total body fat in mice and rats [45–47]. Moreover, the caudal epididymal fat pad is under the strong hormonal and nervous influence [48–50]. In the present study, body composition was remarkably altered by salsolinol. Epididymal fat pad and body weight ratio was significantly lower in salsolinol-treated rats compared to the control animals.

This observation correlates with the results of body weight assessment. Although salsolinol treated animals consumed similar amount of food there was significant decrease in body weight gain in animals exposed to salsolinol. Therefore, we conclude that salsolinol may not influence the appetite regulating mechanism and the reduction of body weight was due to other, probably peripheral effects.

We further evaluated colonic transit, which was disturbed in salsolinol-treated rats. Stool water content, an indicator of colon motility and absorption, was lower in salsolinol-treated rats after two weeks prior to the mini-pump implantation. Slower transit and decreased stool water content might be responsible for diminished absorption.

Similar results were obtained by Zhu et al. [51] in rat model using 6-hydroxydopamine (6-OHDA). The fecal outputs of the 6-OHDA group of rats were significantly lower than the output of the control group. Stool water content of the 6-OHDA group of rats was also lower than that of control group, which indicates that the colon motility rats was significantly disturbed by 6-OHDA. Moreover, compared with control group, the percentage of residual solid food in the stomach of 6-OHDA group of rats was significantly higher than that of control group, indicating that stomach emptying of 6-OHDA group of rats is delayed.

Furthermore, Greene et al. [29] described delayed gastric empting related to dysfunction in the intrinsic enteric nervous system in rats during rotenone treatment. Despite persistent neural dysfunction in the colonic enteric nervous system, he reported no lasting abnormality of stool frequency detected in rotenone-treated rats evaluated for three weeks. The transient decrease in stool output associated with rotenone infusion appeared to be correlated with body weight. Weight loss caused by rotenone infusion has been previously described, and was related to the fact that animals do not eat and drink sufficiently during early time points [52]. Green attribute the early decrease in weight and stool frequency observed in his study to decreased food and water intake; however, he acknowledged that it is possible that the order of events is reversed, and that abnormal GI motility is a cause, rather than a result of decreased intake and weight loss. Our results seem to confirm these observations since salsolinol reduced body weight gain while food consumption remained not diminished.

We conclude that absorptive dysfunction, of rather peripheral than central origin, resulted from delayed gastric emptying together with disturbed colon function, might be responsible for diminished body weight gain and slower adipose tissue accumulation in salsolinol-treated animals.

CONFLICT OF INTEREST STATEMENT

None declared.

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