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Short Communication

Endogenous synthesis of N-methylsalsolinol, an analogue of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, in rat brain during *in vivo* microdialysis with salsolinol, as demonstrated by gas chromatography–mass spectrometry

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ABSTRACT

N-Methylsalsolinol, an analogue of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, is present in the brains of patients with Parkinson's disease. To determine the metabolic pathway for the synthesis of N-methylsalsolinol in the brain, salsolinol was perfused through the striatum or the substantia nigra of the rat brain by *in vivo* microdialysis. N-Methylsalsolinol was detected in the brain dialysate samples during microdialysis with salsolinol using gas chromatography–mass spectrometry with selected-ion monitoring. These results demonstrate that endogenous N-methylation of salsolinol into N-methylsalsolinol occurs in the brain *in vivo*.

INTRODUCTION

Since the discovery of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) as a highly selective, irreversible neurotoxin that produces parkinsonism in humans, monkeys and mice [1–3], structurally similar compounds that may accumulate in the brain and induce Parkinson's disease during ageing have been extensively searched for. 1,2,3,4-Tetrahydroisoquinoline (TIQ) has emerged as one of the candidates. TIQ was detected in human brains [4–6], and the subcutaneous injection of TIQ produced parkinsonism, which was treated by L-DOPA, in primates with a reduction of dopamine and biopterin concentrations and tyrosine hydroxylase activity in the nigro-striatal regions [7,8] and with an accumulation of TIQ in the brain [9]. Since TIQ is commonly found in various foods [10,11], TIQ from foods may accumulate in the human brain over a long period, and may have some relation to Parkinson's disease.

Besides TIQ, N-methyl-1,2,3,4-tetrahydroisoquinoline (NMTIQ) was suggested to be a possible neurotoxin after screening of various compounds structurally related to MPTP for neurotoxicity [12]. NMTIQ was detected in the brain of primates with parkinsonism after systemic administration of TIQ [13]. NMTIQ may be oxidized to the N-methylisoquinolinium ion ($NMIQ^+$), a more neurotoxic compound.

While searching for other endogenous MPTP-like compounds, TIQs with catechol structure, 1,2-dimethyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline (N-methylsalsolinol) and 2-methyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline (N-methylnorsalsolinol), were identified in the brains of patients with Parkinson's disease [14]. However, there has been no evidence that demonstrates the metabolic pathway for the synthesis of N-methylsalsolinol and N-methylnorsalsolinol in the brain. 1-Methyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline (salsolinol) has been reported to be present in human brains [15]. Two enantiomers of salsolinol, (1*R*) and (1*S*), have been detected, and the (*R*) enantiomer predominates in the human brain.

In this study we demonstrated, by detecting N-methylsalsolinol in the dialysate using gas chromatography–mass spectrometry (GC–MS), that endogenous synthesis of N-methylsalsolinol from salsolinol occurred in the rat brain during *in vivo* microdialysis with salsolinol.

EXPERIMENTAL

Materials

N-Methyl-(1*R*)-salsolinol and (1*R*)-salsolinol were synthesized by P. Dostert, Farmitalia Carlo Erba (Milan, Italy). N,O-Bis(trimethylsilyl)trifluoroacetamide (BSTFA) containing 1% trimethylchlorosilane (TMCS) was purchased from Pierce (Rockford, IL, USA). All other chemicals used were of analytical grade.

Microdialysis

Microdialysis in the rat brain was carried out as reported by Nakahara and co-workers [16,17]. Male adult Wistar rats weighing 260–340 g were anesthetized with sodium pentobarbital. The guide cannula for microdialysis was implanted in the left striatum (coordinates: A-0.5 to the bregma, L-3.0 to the midline, V-3.0 from the dura) or substantia nigra (A-5.3, L-2.2, V-6.4), according to the stereotaxic atlas of Paxinos and Watson [18]. Dummy probes were inserted into the guide

cannula until the experiment started. Then, 24–48 h after surgery, rats were transferred from their home cages to transparent acrylic boxes (30 cm depth × 30 cm length × 35 cm height), which were located on animal activity monitors (Automex II, Columbus, OH, USA) to measure gross body movement. Then the dialysis probe was inserted instead of the dummy probe, and the cannula was perfused with Ringer's solution (147 m*M* NaCl, 2.3 m*M* $CaCl_2$, 4 m*M* KCl, pH 7.0); the perfusion flow-rate was 2 μl/min. The dialysis sample was collected at 20-min intervals, after a 2-h stabilization period. The first three samples were used to determine the baseline levels of compounds in the dialysate. Then the perfusion of 1 m*M* (1*R*)-salsolinol in the Ringer solution was started and continued for 2 h. Six samples were collected. All samples were frozen immediately and stored at −80°C until analysis.

Sample preparation

The dialysate (70 μl) was brought to pH 8.5 with 1 ml of a solution prepared by dissolving 13.21 g of $(NH_4)_2SO_4$ in 0.11 *M* NaOH (100 ml) and loaded into a phenylboronic acid cartridge (Analytichem International, 100 mg/ml) for a selective liquid–solid extraction. After washing with water (4 ml) and methanol (4 ml), diols were eluted with 4 ml of 1 *M* acetic acid in methanol, and the eluate was evaporated to dryness with a nitrogen stream. The residue was trimethylsilylated with BSTFA (20 μl) containing 1% TMCS at 70°C for 30 min.

Gas chromatography–mass spectrometry

A Shimadzu GC-9A gas chromatograph combined with a double-focusing mass spectrometer (Shimadzu 9020-DF) was used. The gas chromatograph was equipped with a moving-needle type solventless injector, and a DB-17 open tubular capillary column (25 m × 0.25 mm I.D.). The conditions were: injection temperature, 270°C; column temperature programme, from 130°C to 169°C at 3°C/min; ion-source temperature, 250°C; electron-impact (EI) ionization energy, 70 eV; trap current, 60 μA; accelerating voltage, 3.0 kV.

RESULTS

Fig. 1 shows electron-impact (EI) mass spectra of the trimethylsilyl (TMS) derivatives of N-methyl-(1*R*)-salsolinol (a) and (1*R*)-salsolinol (b). N-Methyl-(1*R*)-salsolinol-2TMS showed a base peak ion at *m/z* 322 ($[M - CH_3]^+$), a weak ion at *m/z* 336 ($[M - H]^+$) and a weak molecular ion at *m/z* 337 (M^+). (1*R*)-Salsolinol-3TMS showed a base peak at *m/z* 380 ($[M - CH_3]^+$), a weak ion at *m/z* 394 ($[M - H]^+$) and a weak molecular ion at *m/z* 395 (M^+). Two enantiomers, (1*R*) and (1*S*), showed the same retention times and the same mass spectra, and could not be differentiated from each other by GC–MS. Selected-ion monitoring (SIM) was used to detect N-methylsalsolinol at very low concentration in the brain dialysate by monitoring *m/z* 322, 323, 336 and 337.

Fig. 2 shows SIM chromatograms of the TMS derivatives of N-methyl-(1*R*)-salsolinol (a), (1*R*)-salsolinol (b), the extract from dialysate during microdialysis without (1*R*)-salsolinol in the striatum (c) and the extract from dialysate during microdialysis with (1*R*)-salsolinol in the striatum (d). N-Methyl-(1*R*)-salsolinol was detected at 11.0 min in the SIM chromatogram and (1*R*)-salsolinol at 11.4 min, although the relative intensities of the peaks at *m/z* 322, 323, 336 and 337 were very low (Fig. 1b). N-Methylsalsolinol was not detected in the dialysate during microdialysis without (1*R*)-salsolinol (Fig. 1c). During microdialysis with (1*R*)-salsolinol, however, N-methylsalsolinol could be detected in the SIM chromatogram (Fig. 1d), since the peak showed an identical retention time on the chromatogram at 11.0 min and almost identical peak-height ratios such as *m/z* 323 to 322, *m/z* 336 to 322 and *m/z* 337 to 322, to those of the TMS derivative of the authentic compound. The concentration of N-methylsalsolinol in the dialysate was estimated as approximately 0.1% of that of (1*R*)-salsolinol.

Fig. 3 shows SIM chromatograms of the TMS derivatives of N-methyl-(1*R*)-salsolinol (a), (1*R*)-salsolinol (b), the extract from dialysate during microdialysis without (1*R*)-salsolinol in the substantia nigra (c) and the extract from dialysate

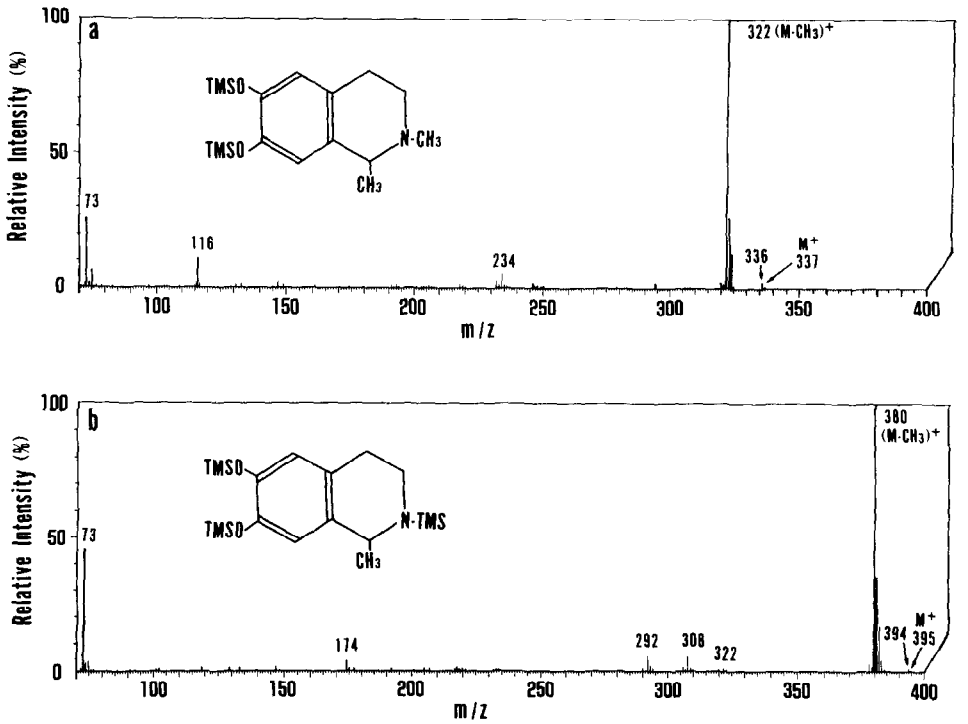

Fig. 1. EI mass spectra of the trimethylsilyl (TMS) derivatives of (1*R*)-N-methylsalsolinol (a) and (1*R*)-salsolinol (b).

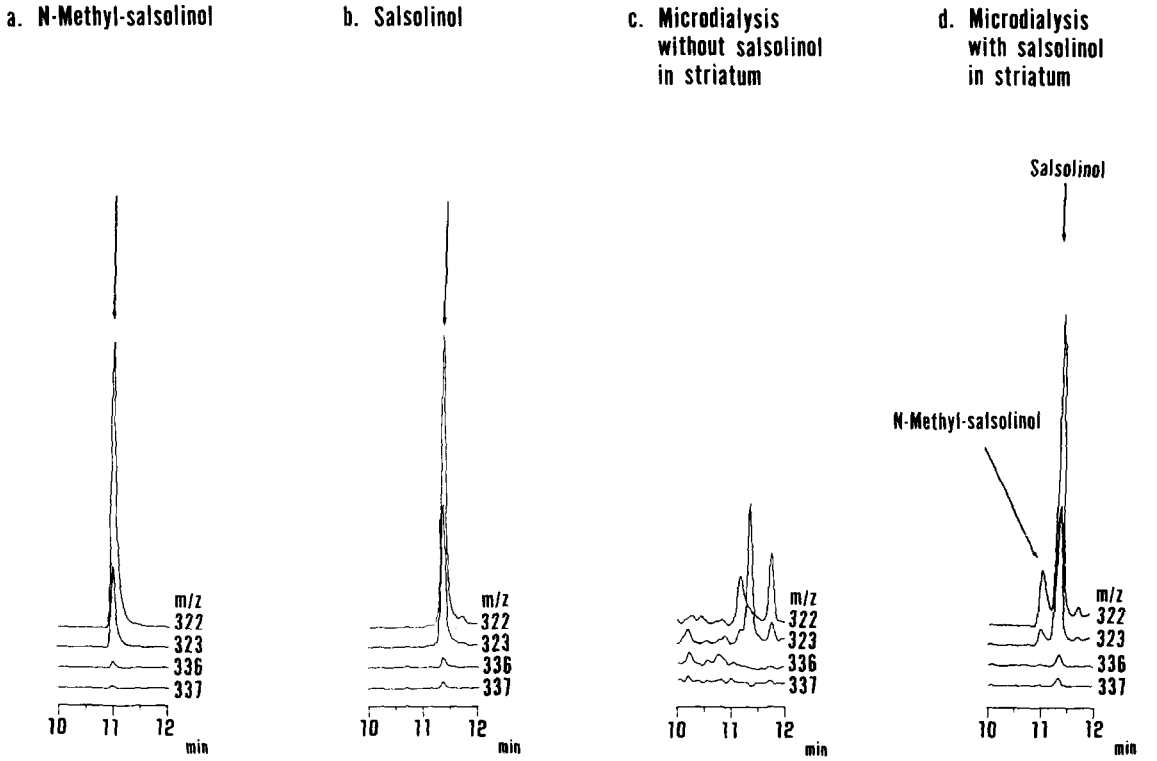

Fig. 2. SIM chromatograms of the TMS derivatives of N-methyl-(1*R*)-salsolinol (a), (1*R*)-salsolinol (b), the extract from dialysate during microdialysis without (1*R*)-salsolinol in the striatum (c) and the extract from dialysate during microdialysis with (1*R*)-salsolinol in the striatum (d).

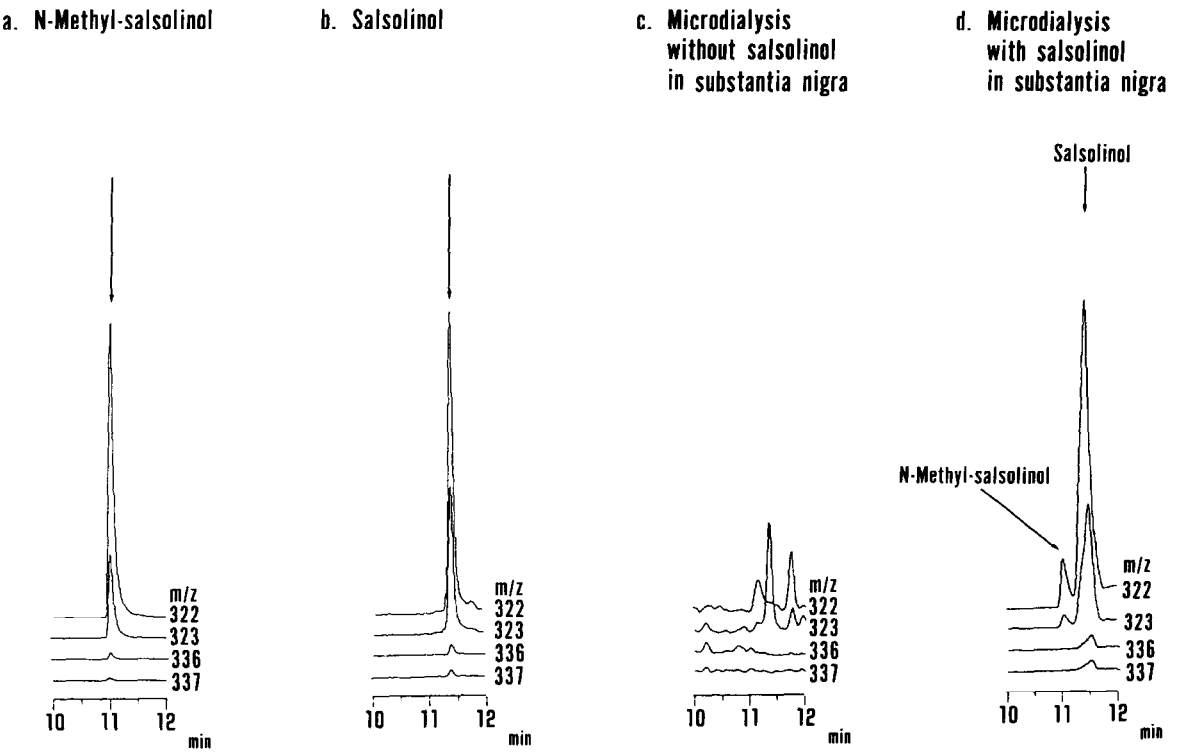

Fig. 3. SIM chromatograms of the TMS derivatives of N-methyl-(1*R*)-salsolinol (a), (1*R*)-salsolinol (b), the extract from dialysate during microdialysis without (1*R*)-salsolinol in the substantia nigra (c) and the extract from dialysate during microdialysis with (1*R*)-salsolinol in the substantia nigra (d).

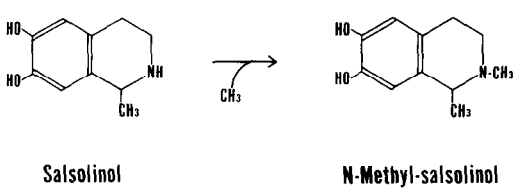

Fig. 4. Endogenous N-methylation of salsolinol into N-methylsalsolinol in the brain.

during microdialysis with (1*R*)-salsolinol in the substantia nigra (d). N-Methylsalsolinol was also demonstrated in the dialysate during microdialysis with (1*R*)-salsolinol in the substantia nigra, but not in the dialysate during microdialysis without (1*R*)-salsolinol.

DISCUSSION

In this study, N-methylsalsolinol was detected in the brain dialysate during *in vivo* microdialysis with salsolinol, and thus endogenous N-methylation of salsolinol into N-methylsalsolinol in the brain *in vivo* was demonstrated (Fig. 4). N-Methylsalsolinol has a molecular structure similar to that of MPTP, and may be a neurotoxin that causes Parkinson's disease.

Salsolinol was first detected in the urine of a parkinsonian patient on L-DOPA medication [19], and then in human brains [15]. The concentration of salsolinol was reported to be markedly increased in the brains of alcoholism patients [15]. Salsolinol may be non-enzymically formed by the *in vivo* Pictet–Spengler condensation of dopamine with acetaldehyde or with pyruvic acid. Salsolinol is present in various foods, such as banana, soy sauce, wine and beer. The (*R*) enantiomer of salsolinol predominates in human urine, the (*S*) enantiomer predominates in port wine, and an (*R*)/(*S*) ratio very near to 1 was found in dried banana [20–22]. The (*R*)

enantiomer of salsolinol may represent endogenous salsolinol, which is formed by ring cyclization of dopamine with pyruvic acid followed by decarboxylation and reduction [20].

Salsolinol inhibits the uptake of catecholamines, causes the release of stored catecholamines [23] and inhibits tyrosine hydroxylase [24] and monoamine oxidase [25]. N-Methylsalsolinol is thought to be more neurotoxic than salsolinol, since N-methylation of TIQ and oxidation of NMTIQ was demonstrated to increase its neurotoxicity [26–28]. N-Methylsalsolinol, which is endogenously formed from salsolinol, may accumulate in the brain, resulting in cell death over a long period. The number of dopaminergic neurons in the substantia nigra decreases markedly in Parkinson's disease, and even in a normal ageing process. The endogenous synthesis of N-methylsalsolinol may play a role in the decrease in the number of the dopaminergic neurons.

6,7-Dihydroxy-1,2,3,4-tetrahydroisoquinoline (norsalsolinol) was detected in normal rat brain using GC–MS [29]. Norsalsolinol may be formed non-enzymically by *in vivo* Pictet–Spengler condensation of dopamine and formaldehyde. N-Methylnorsalsolinol, which is present in the human brain [14], may be synthesized by a similar mechanism, *i.e.* endogenous N-methylation of norsalsolinol in the brain.

N-Methylsalsolinol is structurally similar to MPTP and may be oxidized by monoamine oxidase to the 1,2-dimethyl-6,7-dihydroxyisoquinolinium ion, which is thought to be more neurotoxic. The neurotoxicity of N-methylsalsolinol and its oxidized metabolite, 1,2-dimethyl-6,7-dihydroxyisoquinolinium ion, should be studied to establish their possible involvement in Parkinson's disease.

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