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Short Communication

Endogenous synthesis of N-methylnorsalsolinol in rat brain during *in vivo* microdialysis with epinine

Mitsuharu Kajita*[,a], Toshimitsu Niwa[b], Wakako Maruyama[c], Daiichiro Nakahara[d], Naohito Takeda[e], Hideo Yoshizumi[e], Akira Tatematsu[e], Kazuyoshi Watanabe[a], Makoto Naoi[f], Toshiharu Nagatsu[g]

[a]*Department of Pediatrics, Nagoya University School of Medicine, Showa-ku, Nagoya 466, Japan*
[b]*Department of Internal Medicine, Nagoya University Branch Hospital, Higashi-ku, Nagoya 461, Japan*
[c]*Department of Neurology, Nagoya University School of Medicine, Showa-ku, Nagoya 466, Japan*
[d]*Department of Psychology, Nagoya University College of Medical Technology, Higashi-ku, Nagoya 461, Japan*
[e]*Faculty of Pharmacy, Meijo University, Tempaku-ku, Nagoya 468, Japan*
[f]*Department of Biosciences, Nagoya Institute of Technology, Showa-ku, Nagoya 466, Japan*
[g]*Division of Genetics (II) Neurochemistry, Institute for Comprehensive Medical Science, Fujita Health University School of Medicine, Toyoake, Aichi 470-11, Japan*

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Abstract

The *in vivo* metabolic pathway for the synthesis of N-methylnorsalsolinol, an analogue of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), was studied in the rat brain. N-Methyldopamine (epinine) was perfused at the striatum of the rat brain by *in vivo* microdialysis. N-Methylnorsalsolinol (NMNSAL) was identified in the brain dialysate after epinine perfusion using gas chromatography–selected-ion monitoring mass spectrometry (GC–SIM-MS). We demonstrated that NMNSAL could be synthesized from epinine with an aldehyde by the Piclet–Spengler condensation reaction in the rat brain.

1. Introduction

Since 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) was found to cause parkinsonism in humans, monkeys and mice [1–3], an endogenous or exogenous neurotoxin structurally similar to MPTP has been sought. As endogenous MPTP-like compounds, 1,2,3,4-tetrahydroisoquinoline (TIQ) [4,5], salsolinol (1-methyl-6,7-dihydroxy-TIQ; SAL) [6], N-methylsalsolinol (1,2-dimethyl-6,7-dihydroxy-TIQ; NMSAL), and N-methylnorsalsolinol (2-methyl-6,7-dihydroxy-TIQ; NMNSAL) [7] were identified in brains. It was reported that NMSAL shows various forms of neurotoxicity *in vitro* [8,9]. Although NMSAL is synthesized by the N-methylation of SAL [10,11], it may also be synthesized from N-methyldopamine (epinine) and acetaldehyde by the Piclet–Spengler condensation reaction. In a previous report we demonstrated for the first time that epinine is

* Corresponding author.

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present in parkinsonian and normal human brains [12].

In this study, we have examined the possibility that NMNSAL and NMSAL are synthesized from epinine in the rat brain during *in vivo* microdialysis with epinine.

2. Experimental

2.1. Materials

NMSAL and NMNSAL were kindly supplied by Dr. P. Dostert, Farmitalia Carlo Erba (Milan, Italy). All other chemicals were of analytical grade.

2.2. Microdialysis

Microdialysis was performed according to the method of Nakahara *et al.* [13,14]. An adult Wistar rat (230–280 g, Japan SLC, Inc.) was anesthetized with sodium pentobarbital (40–50 mg/kg, i.p.). The 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 to the dura) according to the stereotaxic atlas of Paxinos and Watson [15]. Then Ringer's solution (147 m*M* NaCl, 2.3 m*M* $CaCl_2$, 4 m*M* KCl, pH 7.0) was perfused at a rate of 2 μl/min. After a 2-h stabilization period, Ringer's solution containing 1 m*M* epinine was perfused for 3 h. Two samples, one before and one 3 h after epinine perfusion, were used for analysis.

2.3. Sample preparation

The brain dialysate (40 μl) was brought to pH 8.5 with 4 ml of a solution prepared by dissolving 1 *M* $(NH_4)_2SO_4$ in 0.11 *M* NaOH (100 ml), and loaded onto a phenylboronic acid cartridge column (Analytichem International, 100 mg/ml). After washing with H_2O (4 ml) and methanol (4 ml), catechols were eluted with 4 ml 1 *M* acetic acid in methanol, and the eluate was evaporated to dryness under a stream of nitrogen. The dry residue was reacted with 20 μl of N,O-bis-(trimethylsilyl)trifluoroacetamide (BSTFA) containing 1% trimethylchrolosilane (TMCS) at 70°C for 30 min.

2.4. In vitro synthesis of NMSAL and NMNSAL

To examine if NMSAL and NMNSAL could be synthesized from epinine *in vitro*, 1 m*M* epinine in 2.5 ml of 0.1 *M* phosphate buffer saline (PBS) (pH 7.4) was incubated with 1 m*M* formaldehyde (HCHO) and/or 1 m*M* acetaldehyde (CH_3CHO) at 37°C for 3 h. As a control 1 m*M* epinine alone was incubated at the same time. Cathecols in these samples were extracted and derivatized as described above.

2.5. Gas chromatography–mass spectrometry

A Shimadzu gas chromatograph (GC-9A)–mass spectrometer (9020-DF) was used. The gas chromatograph was equipped with a DB-17 bonded fused-silica capillary column (30 m × 0.25 mm I.D., 0.25 μm film thickness). The GC–MS conditions were: injection temperature, 280°C; column temperature program, from 170°C to 206°C at 3°/min; ion-source temperature, 250°C; electron-impact ionization (EI) ionizing energy, 70 eV; trap current, 60 μA; accelerating voltage, 3 kV. Chemical-ionization (CI) mass spectrometry was performed using isobutane as a reactant gas. CI energy was 200 eV and the emission current was 200 μA. The other conditions were the same as for EI.

3. Results

Fig. 1 shows the EI mass spectra of the trimethylsilyl (TMS) derivatives of authentic NMNSAL (a) and NMSAL (c). NMNSAL-2TMS showed characteristic ions at *m/z* 322 ($[M-H]^+$) and 323 (M^+) and NMSAL-2TMS at *m/z* 322 ($[M-CH_3]^+$), 336 ($[M-H]^+$) and 337 (M^+). The *in vitro* reactant of epinine and HCHO (b) showed a peak with retention time and EI mass spectrum identical to those of authentic NMNSAL. The *in vitro* reactant of

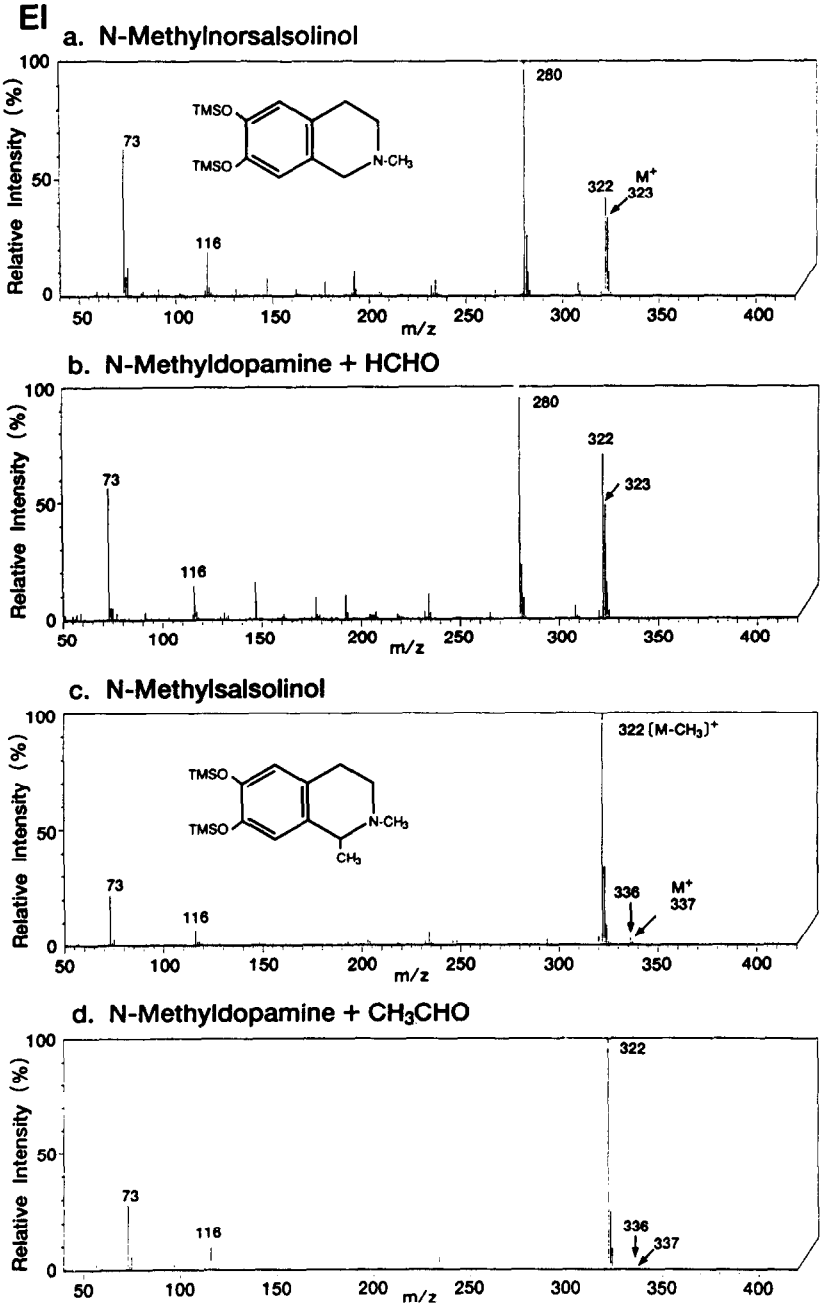

Fig. 1. EI mass spectra of the TMS derivatives of authentic NMNSAL (a), the extract from the *in vitro* reactant of epinine and HCHO (b), authentic NMSAL (c), and the extract from the *in vitro* reactant of epinine and CH_3CHO (d).

epinine and CH_3CHO (d) also showed a peak identified as NMSAL.

Selected-ion monitoring (SIM) was used to detect NMNSAL and NMSAL by monitoring *m/z* 322, 323, 336 and 337. Fig. 2 shows EI-SIM chromatograms of the TMS derivatives of au-

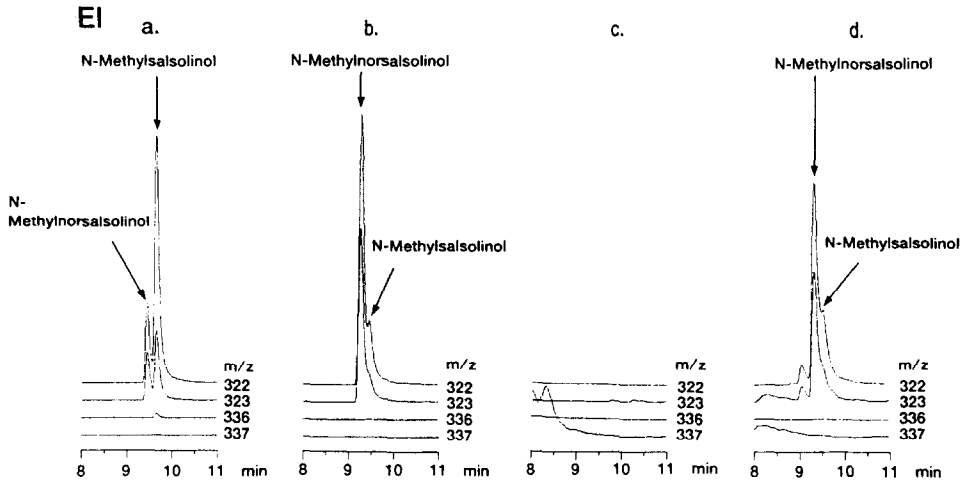

Fig. 2. EI-SIM chromatograms of the TMS derivatives of authentic NMNSAL and NMSAL (a), the extracts from the *in vitro* reactant of epinine, HCHO and CH_3CHO (b), and from the dialysates during *in vivo* microdialysis in the striatum without epinine (c) and with epinine (d).

thentic NMNSAL and NMSAL (a), the extracts from the *in vitro* reactant of epinine, HCHO and CH_3CHO (b), and from the dialysates during microdialysis in the striatum without epinine (c) and with epinine (d). NMNSAL and NMSAL were not detected in the dialysate during microdialysis without epinine. In the sample taken 3 h after perfusion with epinine, NMNSAL could be detected; the peak showed a retention time of 9.4 min and a peak-height ratio (*m/z* 323/322) identical to those of authentic NMNSAL. A very small peak in the SIM chromatogram of *m/z* 322 at 9.7 min suggests the presence of NMSAL.

Fig. 3 shows the CI mass spectra of the TMS derivatives of authentic NMNSAL (a) and NMSAL (c). NMNSAL-2TMS showed characteristic ions at *m/z* 324 ($[M+H]^+$) and NMSAL-2TMS at *m/z* 322 ($[M-CH_3]^+$) and 338 ($[M+H]^+$). The *in vitro* reactant of epinine with HCHO, CH_3CHO was identified as NMNSAL (b) and NMSAL (d), respectively. CI-SIM was performed by monitoring *m/z* 322, 324 and 338. Fig. 4 shows the CI-SIM chromatograms of the TMS derivatives of authentic NMNSAL and NMSAL (a), the extract from the *in vitro* reactant of epinine, HCHO and CH_3CHO (b), and the extracts from the dialysates during microdialysis without epinine (c) and with epinine (d). NMNSAL and NMSAL were not detected in the dialysate before perfusion of epinine, but NMNSAL was demonstrated in the sample taken 3 h after perfusion with epinine. NMSAL was also detected at 9.7 min with a peak-height ratio (*m/z* 338/322) identical to that of authentic NMSAL. The amount of NMNSAL produced *in vivo* during the administration of epinine was *ca.* 0.4 ng/μl dialysate and 10-fold that of NMSAL. In addition, the result of the *in vitro* experiment showed that the amount of NMNSAL production was *ca.* 0.6 μg/2.5 ml reaction mixture and 30-fold that of NMSAL. NMNSAL and NMSAL were not detected in the control reaction mixture in the *in vitro* experiment.

4. Discussion

It has been reported that brain tissue contains only small traces of CH_3CHO without ethanol administration [16,17]. Eriksson stated that reactions such as condensation of CH_3CHO with biogenic amines seem unlikely to occur within the brain because the CH_3CHO level is approximately zero when the arterial blood CH_3CHO concentration is lower than 200 nmol/ml [18]. In contrast, Heck *et al.* [19] demonstrated that the concentration of HCHO in the brain of normal

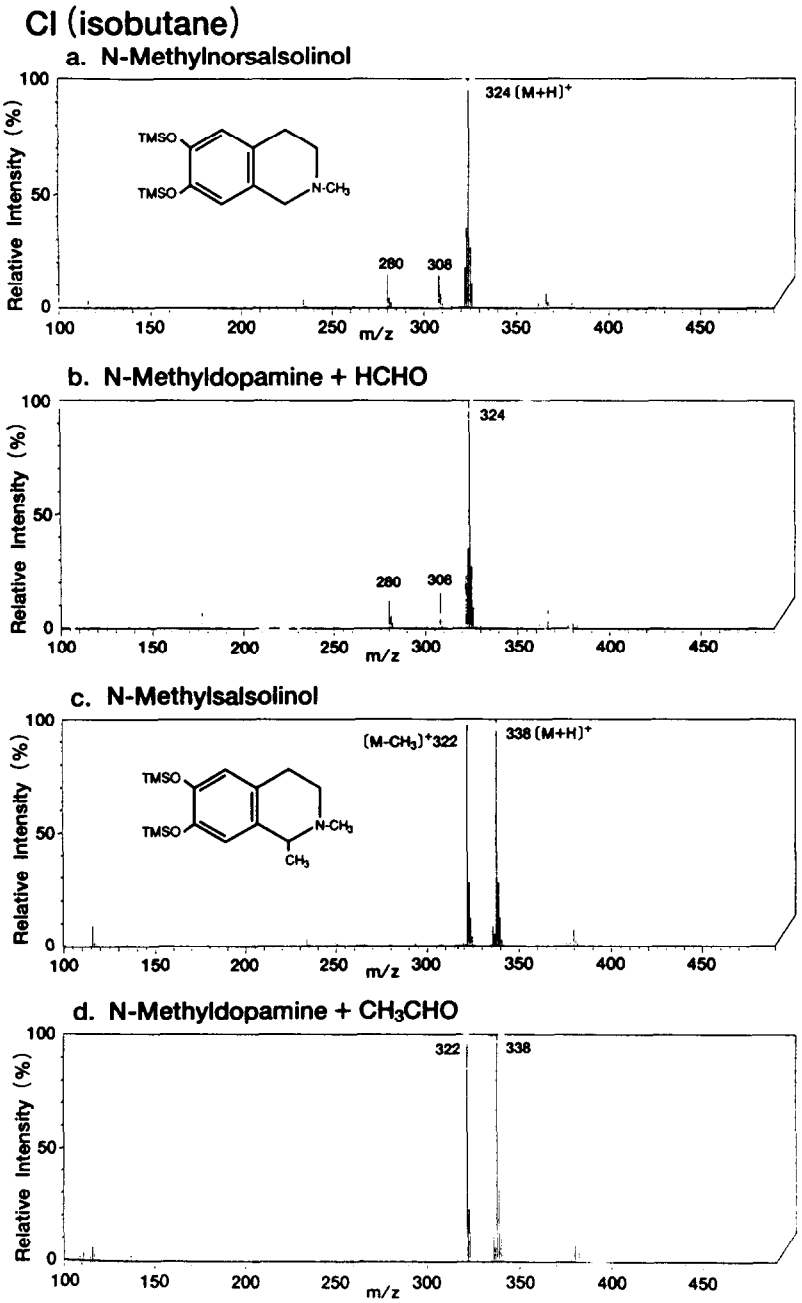

Fig. 3. CI mass spectra of the TMS derivatives of authentic NMNSAL (a), the extract from the *in vitro* reactant of epinine and HCHO (b), authentic NMSAL (c), and the extract from the *in vitro* reactant of epinine and CH_3CHO (d).

rat is 0.097 ± 0.014 μmol/g wet weight. As these aldehydes are volatile, their exact quantification in the organs such as brain is thought to be very difficult.

In the present study, we demonstrated that the synthesis of NMNSAL and NMSAL occurred in the rat brain during *in vivo* microdialysis with epinine. NMNSAL and NMSAL are formed

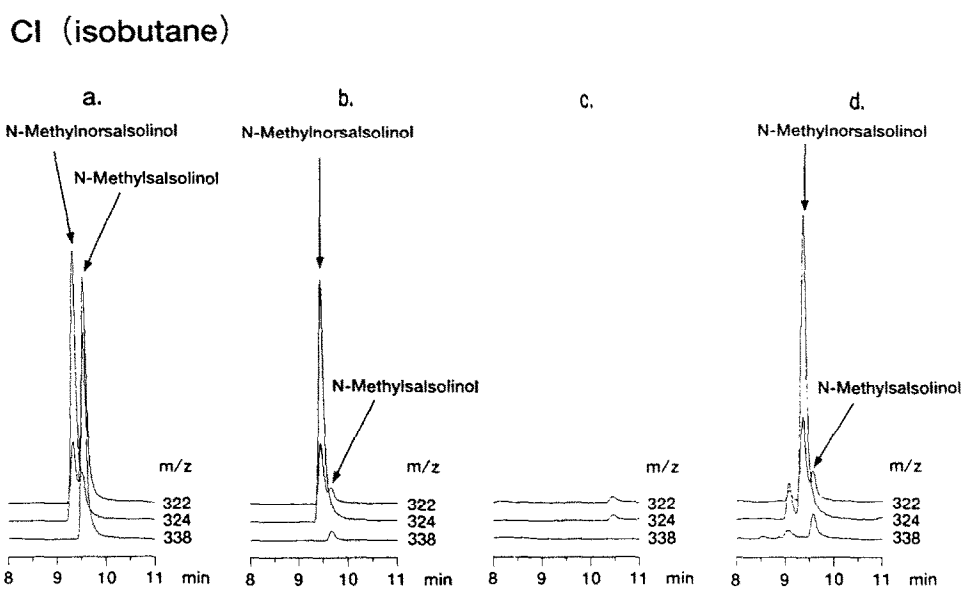

Fig. 4. CI-SIM chromatograms of the TMS derivatives of authentic NMNSAL and NMSAL (a), the extracts from the *in vitro* reactant of epinine, HCHO and CH_3CHO (b), and from the dialysates during *in vivo* microdialysis in the striatum without epinine (c) and with epinine (d).

probably non-enzymatically by the Piclet–Spengler condensation reaction of excessively given epinine with HCHO and CH_3CHO, respectively, which are present in the rat brain (Fig. 5). The occurrence of NMNSAL and NMSAL in the dialysate was no artifact of the work-up process, since NMNSAL and NMSAL could not be detected in the *in vitro* study.

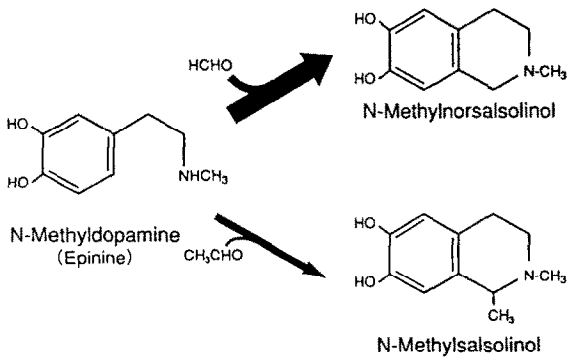

Fig. 5. Metabolic pathways of epinine to form NMNSAL and NMSAL in the brain *in vivo* as identified by microdialysis.

In this study the ratio of the production of NMNSAL to that of NMSAL in the microdialysis and in the *in vitro* study were *ca.* 10 and 30, respectively. This means that the reactivity of HCHO was much stronger than that of CH_3CHO.

In addition to via cyclization of epinine, NMSAL and NMNSAL can also be synthesized via N-methylation of SAL and norsalsolinol (NSAL), respectively, by N-methyltransferase. N-Methylation of these compounds is thought to increase their neurotoxicity [10,11]. Since cyclization of epinine is non-enzymatic, the significance of this route is totally dependent upon the content of epinine. We are now investigating quantitative analyses of epinine, NMNSAL and NMSAL in the various sections of parkinsonian and normal human brains, including the nigrostriatal regions, to clarify the significance of these two metabolic pathways in the brain.

The neurotoxicity of NMNSAL, NMSAL and their oxidized metabolites in relation to the etiology of Parkinson's disease should be further investigated.

5. Acknowledgement

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6. References

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