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Presence of N-methyldopamine in parkinsonian and normal human brains

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ABSTRACT

N-Methyldopamine (epinine) has been identified for the first time in parkinsonian and normal human brains by gas chromatography–mass spectrometry. N-Methylsalsolinol and N-methylnorsalsolinol, which are analogues of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, which produces parkinsonism in humans, may be synthesized from N-methyldopamine by the Pictet–Spengler condensation reaction as an alternative metabolic pathway.

INTRODUCTION

Since the discovery that a dopaminergic neurotoxin, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), produces parkinsonism in humans, monkeys and mice [1–3], endogenous or environmental neurotoxins structurally similar to MPTP causing Parkinson's disease have been examined. 1,2,3,4-Tetrahydroisoquinoline (TIQ) was discovered in parkinsonian human brains [4], and subcutaneous injection of TIQ produced parkinsonism in monkeys [5]. 1-Methyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline (salsolinol; SAL) has been detected in the human brain [6,7]. However, induction of parkinsonism by intraventricular administration of SAL in mammals has not yet been demonstrated.

During our search for other endogeneous TIQ-

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like compounds, we identified 1,2-dimethyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline (N-methylsalsolinol; NMSAL) and 2-methyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline (N-methylnorsalsolinol; NMNSAL) in parkinsonian brains and various foods [8,9]. NMSAL shows various neurotoxicities *in vitro* [10]. Although NMSAL is thought to be synthesized from SAL by an N-methyltransferase in the human brain [7,11], it may also be metabolically synthesized from N-methyldopamine (epinine) and acetaldehyde by the Pictet–Spengler condensation reaction [12] (Fig. 1). In this study, we first identified N-methyldopamine in parkinsonian and normal human brains by using selected-ion monitoring (SIM) of gas chromatography–mass spectrometry (GC–MS), and then demonstrated that NMSAL and NMNSAL are synthesized from N-methyldopamine *in vitro*.

EXPERIMENTAL

Materials

Human brains were obtained at autopsy from five patients with Parkinson's disease, and five patients who had no neurodegenerative disorders. The samples were kept at − 80°C until analysis.

N-Methyldopamine was purchased from Sigma (St. Louis, MO, USA). Pentafluoropropionic anhydride (PFPA), heptafluorobutyric anhydride (HFBA) and N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) containing 1% trimethylchlorosilane (TMCS) were purchased from Pierce (Rockford, IL, USA). NMSAL and NMNSAL were kindly supplied by Dr. P. Dostert (Farmitalia Carlo Erba, Italy). All other chemicals used were of analytical grade.

Sample preparation

The frontal lobe (1 g) of parkinsonian or normal human brain was homogenized at 0°C for 10 min with 0.1 *M* HCl (2.5 ml) containing EDTA (0.1%, w/v). The homogenate was centrifuged at 12 000 *g* for 15 min at 4°C. The pellet was homogenized with 0.1 *M* HCl (2.5 ml) containing EDTA (0.1%, w/v), and centrifuged again. The combined supernatant was evaporated to dry-

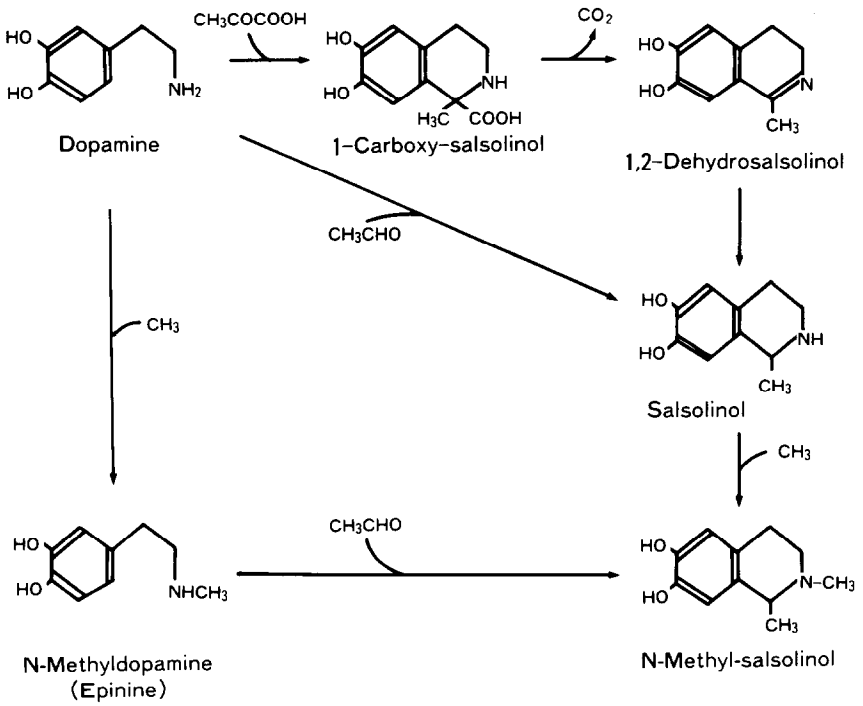

Fig. 1. Probable biosynthetic pathways of N-methylsalsolinol (NMSAL) in the human brain.

ness, and the residue was dissolved in 0.5 ml of ethanol and brought to pH 8.5 with 4 ml of a solution prepared by dissolving 13.2 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). After washing with water (4 ml) and methanol (4 ml), catechols were eluted with 4 ml of 1 *M* acetic acid in methanol, and the eluate was evaporated to dryness under a nitrogen stream. The residue was dissolved in 20 μl of acetonitrile–PFPA (1:1) or acetonitrile–HFBA (1:1), and derivatized at 70°C for 30 min.

In vitro synthesis of NMSAL and NMNSAL

To determine whether NMSAL and NMNSAL could be synthesized from N-methyldopamine, a reaction mixture (2.5 ml final volume) of 1 m*M* formaldehyde (for NMNSAL) or 1 m*M* acetaldehyde (for NMSAL), respectively, in 0.1 *M* phosphate buffer saline (PBS) (pH 7.4) containing 1 m*M* N-methyldopamine was incubated at 37°C for 24 h. As a blank, 1 m*M* N-methyldopamine alone in 0.1 *M* PBS (pH 7.4) was incubated at the same time. From these samples, diols were extracted by the method described above. The dry residue was treated with 20 μl of BSTFA containing 1% TMCS at 70°C for 30 min.

Gas chromatography–mass spectrometry

We used a Shimadzu GC-9A gas chromatograph combined with a Shimadzu 9020-DF double-focusing mass spectrometer. The chromatograph was equipped with a DB-17 bonded fused-silica capillary column (30 m × 0.32 mm I.D.). For the analysis of N-methyldopamine in the brain, an SIM method was used. The injection temperature was 280°C, and the column temperature was programmed from 110°C at 5°C/min for the PFP derivatives and from 120°C at 5°C/min for the HFB derivatives. The ion-source tem-

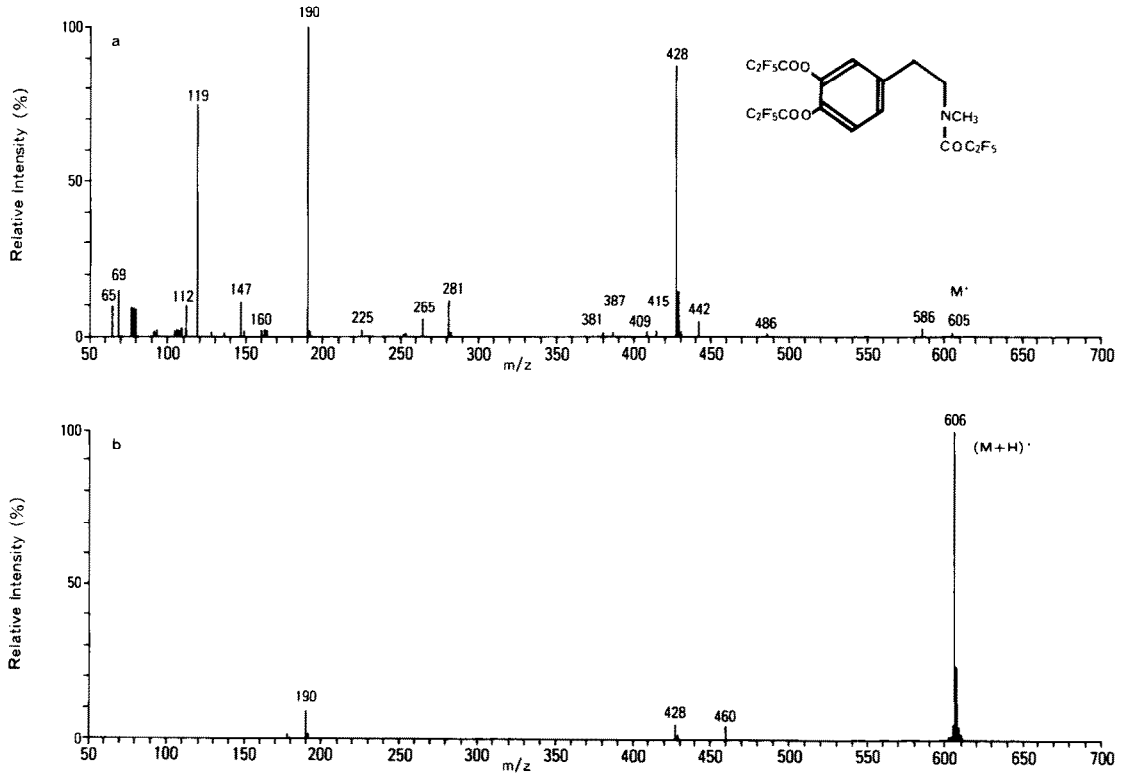

Fig. 2. (a) EI and (b) CI (isobutane) mass spectra of the PFP derivative of N-methyldopamine.

perature was 250°C, the electron-impact (EI) ionization energy 70 eV, the trap current 60 μA and the accelerating voltage 3 kV. Chemical ionization (CI) mass spectrometry was performed using isobutane as a reagent gas. The CI energy was 200 eV and the emission current 200 μA. The other conditions were the same as for EI.

For the analysis of NMSAL and NMNSAL, EI mass spectrometry was performed using a DB-17 capillary column (30 m × 0.25 mm I.D.). The column temperature was programmed from 170°C at 3°C/min. The other conditions were the same as for N-methyldopamine.

RESULTS

The EI mass spectrum of the pentafluoropropionic (PFP) derivative of authentic N-methyldopamine showed characteristic ions at m/z 428 ($[M - NHCH_3PFP]^+$), 429, and 442 ($[M - OPFP]^+$) as shown in Fig. 2a. Fig. 3 shows EI-SIM chromatograms of PFP-derivatized authentic N-methyldopamine (a), and the PFP-derivatized extract from the frontal lobe of a parkinsonian patient (b) and a control patient (c). N-Methyldopamine was detected in the both brains; the peaks showed identical retention times at 6.2 min and identical peak-height ratios (m/z 429/428, 442/428) to those of authentic N-methyldopamine.

The EI mass spectrum of HFB-derivatized authentic N-methyldopamine showed characteristic ions at m/z 528 ($[M - NHCH_3HFB]^+$), 529, and 542 ($[M - OHFB]^+$). The peaks detected in EI-SIM chromatograms of the HFB derivatized extracts from both brains showed identical retention times (5.8 min) and peak-height ratios to those of authentic N-methyldopamine.

In Fig. 2b, CI mass spectrum of the PFP derivative of N-methyldopamine shows an intense protonated molecular ion at m/z 606, $[M + H]^+$, indicating the molecular mass to be m/z 605. CI-SIM was performed to confirm the molecular ions of the peaks in the brain extracts. Fig. 4 shows the CI-SIM chromatogram of the PFP derivatives of authentic N-methyldopamine (a) and extracts from the brains of a parkinsonian patient (b) and a control patient (c). Since both brain extracts exhibited an intense ion peak at m/z 606, and the peaks showed identical reten-

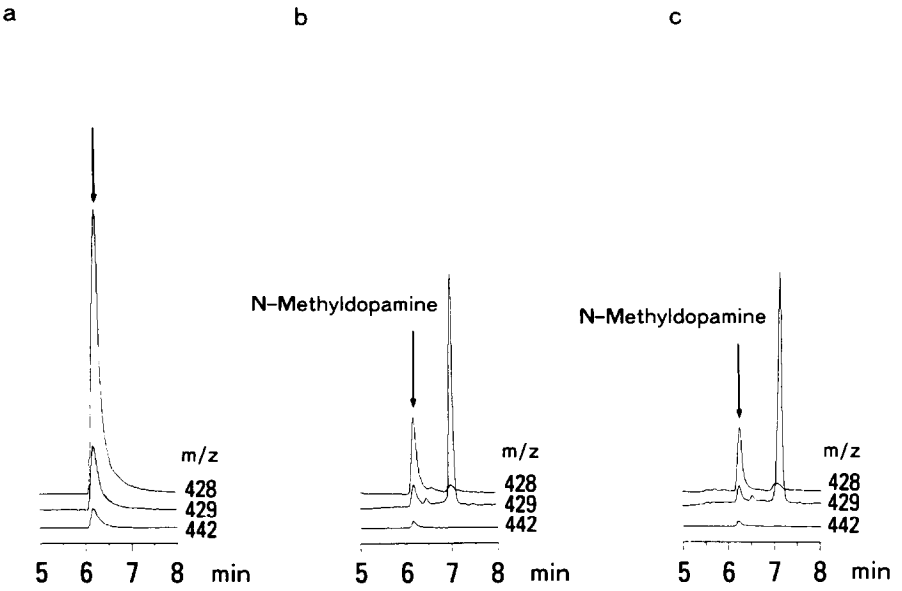

Fig. 3. EI-SIM chromatograms of (a) the PFP derivative of N-methyldopamine, and PFP-derivatized extracts from (b) parkinsonian frontal lobe and (c) normal frontal lobe.

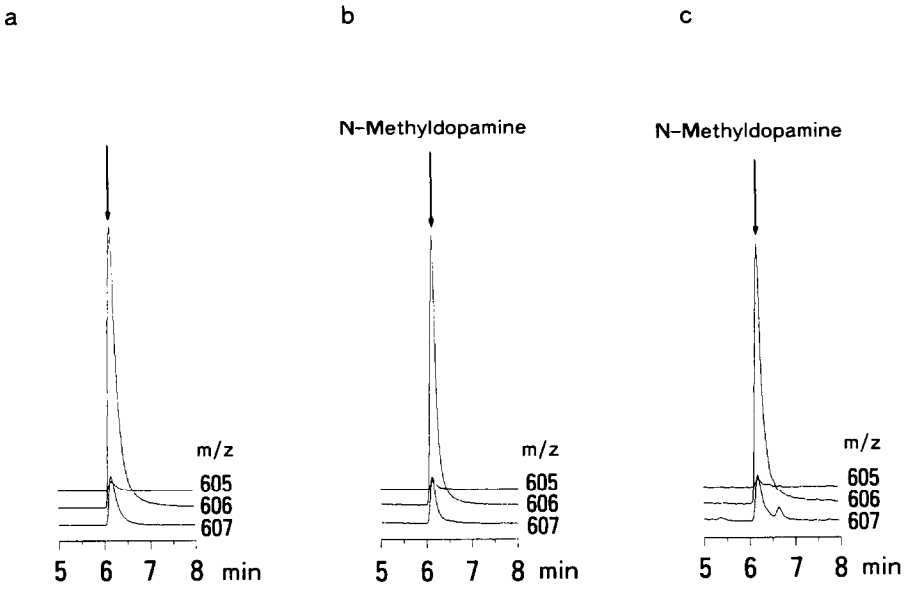

Fig. 4. CI (isobutane)-SIM chromatograms of (a) the PFP derivative of N-methyldopamine, and PFP-derivatized extracts from (b) parkinsonian frontal lobe and (c) normal frontal lobe.

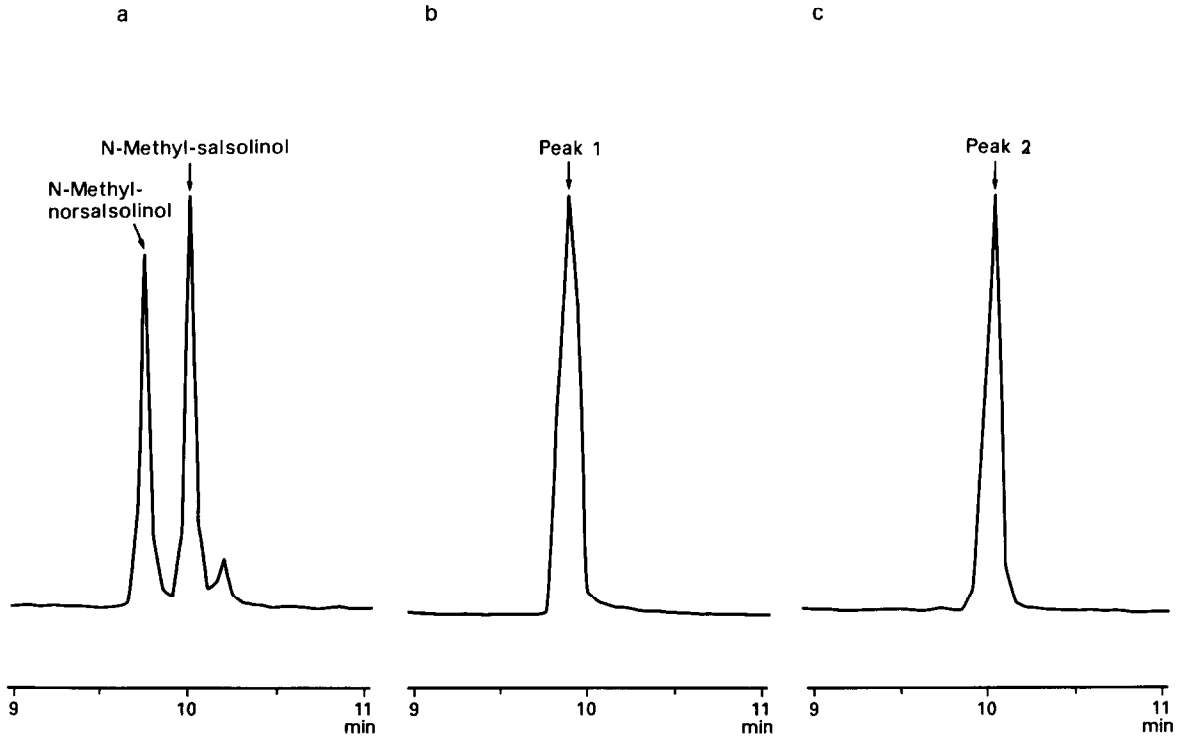

Fig. 5. EI total ion chromatograms of (a) the TMS derivatives of NMNSAL and NMSAL (standards), (b) the extract from the reaction of N-methyldopamine and formaldehyde (37°C, 24 h), and (c) that of N-methyldopamine and acetaldehyde (37°C, 24 h).

tion times (6.2 min) to that of N-methyldopamine, the molecular ions of the peaks were confirmed to be 605. By EI-SIM and CI-SIM, the peaks detected in the brain extracts were confirmed to be N-methyldopamine. N-Methyldopamine was detected in all five parkinsonian and all five control brains, and the approximate amounts of N-methyldopamine in the frontal lobes were 1 ng/g wet tissue.

Fig. 5 shows the EI total ion chromatograms of the trimethylsilyl (TMS) derivatives of NMNSAL and NMSAL (a), the extract from the

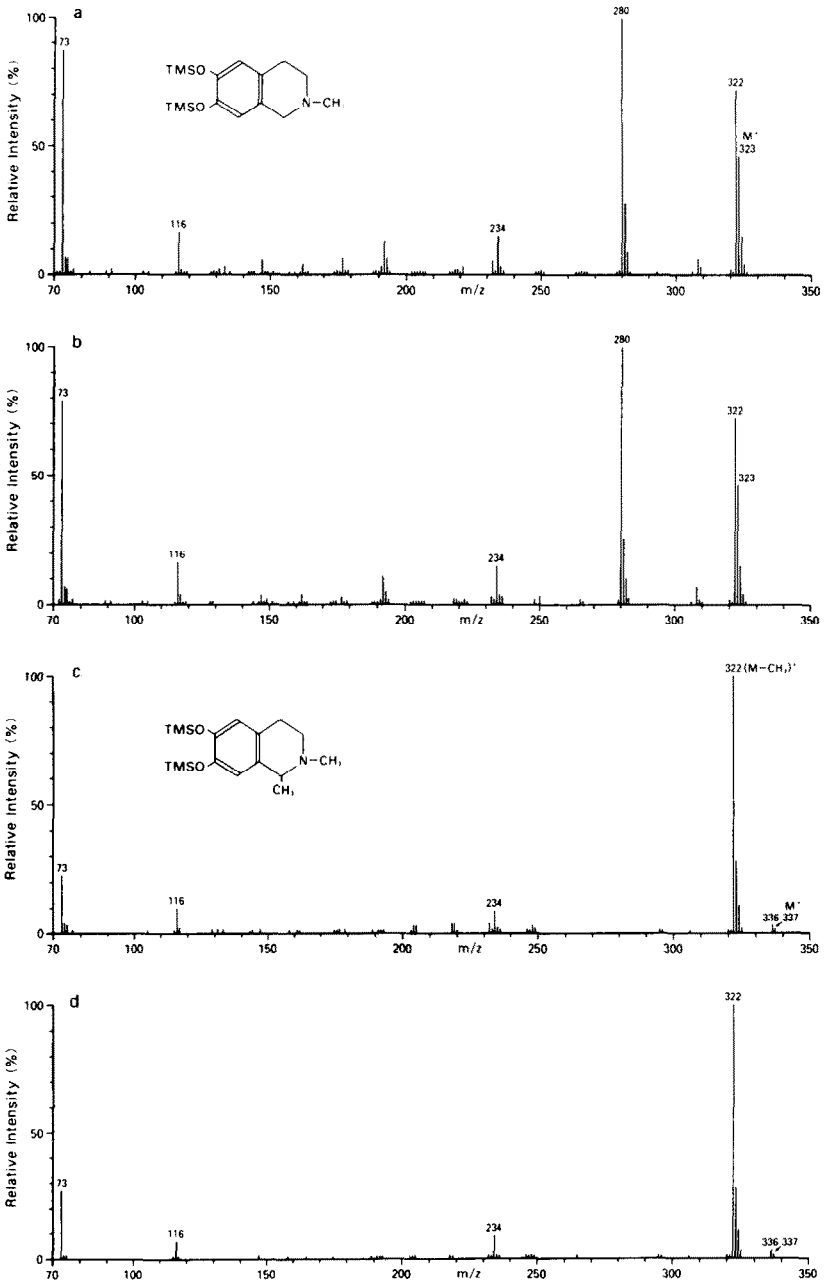

Fig. 6. EI mass spectra of (a) the TMS derivative of NMNSAL, (b) peak 1 in Fig. 5, (c) the TMS derivative of NMSAL, and (d) peak 2 in Fig. 5.

reaction of N-methyldopamine and formaldehyde (b), and that of N-methyldopamine and acetaldehyde (c). In the extract from the blank, no peak was detected. Fig. 6 shows EI mass spectra of the TMS derivatives of NMNSAL (a) and NMSAL (c). Peak 1 from the reaction of N-methyldopamine and formaldehyde was identified as NMNSAL; it showed an identical retention time (9.8 min) and almost an identical EI mass spectrum (b) to those of the TMS derivative of authentic NMNSAL. Peak 2 from the reaction of N-methyldopamine and acetaldehyde was confirmed to be NMSAL; it had an identical retention time (10.0 min) and almost an identical mass spectrum (d) to those of the TMS derivative of authentic NMSAL.

DISCUSSION

N-Methyldopamine was proposed as a potential precursor of epinephrine by Halle in 1906 [13], and first found in the parotid gland of *Bufo marinus* by Maerki *et al.* in 1962 [14]. Since then, it has been detected only in animal tissues, such as rat superior cervical ganglia [15], hypothalamus and brainstem [16], but the amounts were so small that the formation of epinephrine from N-methyldopamine was thought to be a minor pathway [15]. N-Methyldopamine has not been found in any human tissues. We have demonstrated in this study for the first time that N-methyldopamine is present in parkinsonian and normal human brains.

Recently we identified NMSAL and NMNSAL, structually similar to MPTP, in parkinsonian and control human brains and some foods [8,9], and reported that NMSAL was synthesized from SAL *in vivo* in the rat brain [17]. In this study, we demonstrated that NMNSAL and NMSAL were synthesized from N-methyldopamine *in vitro*. NMSAL is formed from N-methyldopamine and acetaldehyde by the Pictet–Spengler condensation reaction. NMNSAL is also formed from N-methyldopamine and formaldehyde by the same reaction. N-Methyldopamine displays dopaminergic and α- and β-adrenoreceptor agonist properties [18]. Its prodrug, ibopamine (the isobutyric ester of N-methyldopamine), has been used for the treatment of congestive heart failure [19]. Ibopamine taken orally is hydrolysed to N-methyldopamine, which is thought to be a therapeutically active moiety of the drug, and excreted in the urine after being either sulphate-conjugated or oxidized to homovanillic acid and 3,4-dihydroxyphenylacetic acid [20].

Further investigations are required into the biological role and the distribution of N-methyldopamine in the human brain. To determine its relation to Parkinson's disease, we are now trying quantitative analyses of N-methyldopamine and other parkinsonism-related compounds, such as NMSAL and NMNSAL, in the various sections of brain including substantia nigra. We intend to investigate whether endogeneous NMSAL and NMNSAL formation form N-methyldopamine occurs in the brain *in vivo*, and whether it causes parkinsonism in animals.

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