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Effect of (*R*)-Salsolinol and *N*-Methyl-(*R*)-Salsolinol on the Balance Impairment Between Dopamine and Acetylcholine in Rat Brain: Involvement in Pathogenesis of Parkinson Disease

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BACKGROUND: Parkinson disease (PD), a progressive neurodegenerative disease, affects at least 1% of population above the age of 65. Although the specific etiology of PD remains unclear, recently the endogenous neurotoxins such as (*R*)-salsolinol [(*R*)-Sal] and *N*-methyl-(*R*)-salsolinol [(*R*)-NMSal] have been thought to play a major role in PD. Much interest is focused on the degeneration of dopamine neurons induced by these neurotoxins. However, little literature is available on the impact of endogenous neurotoxins on the balance between dopamine (DA) and acetylcholine (ACh).

METHODS: After injection of (*R*)-Sal or (*R*)-NMSal into the rat brain striatum, the concentrations of DA and its metabolites were detected by HPLC with electrochemical detection. We assessed the influence of neurotoxins on acetylcholinesterase (AChE) activity and developed a microdialysis-electrochemical device to measure ACh concentrations with enzyme-modified electrodes.

RESULTS: (*R*)-Sal and (*R*)-NMSal led to concentration-dependent decreases in the activity of AChE. ACh concentrations in striatum treated with (*R*)-Sal or (*R*)-NMSal were increased to 131.7% and 239.8% of control, respectively. As to the dopaminergic system, (*R*)-NMSal caused a significant decrease in DA concentrations and (*R*)-Sal reduced the concentrations of DA metabolites in the striatum.

CONCLUSIONS: (*R*)-Sal and (*R*)-NMSal exerted a considerable effect on the balance between DA and ACh by impairing the cholinergic system as well as the dopaminergic system. It is likely that the disruption of balance between DA and ACh plays a critical role in the pathogenesis of neurotoxin-induced PD.

Parkinson disease (PD)[3] is a chronically progressive, age-related neurodegenerative disorder characterized by rest tremor, balance impairment, slowness of movement, and rigidity *(1)*. Although the main pathological feature of PD is the selective death of dopaminergic neurons in the substantia nigra, the etiology of this common neurodegenerative disease remains to be elucidated. However, since 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) was proved to induce an acute and permanent parkinsonian syndrome in intravenous drug users *(2)*, much research on the PD pathogenesis has focused on the neurotoxins that can cause clinical features similar to that seen in PD. Recently, 1(*R*)-methyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline [(*R*)-salsolinol or (*R*)-Sal] and 1(*R*),2-dimethyl-6,7-dihydroxy-1,2,3,4-tetra-hydroisoquinoline [*N*-methyl-(*R*)-salsolinol or (*R*)-NMSal], the endogenous MPTP-like biological alkaloids, have received increased attention in the study of pathogenesis of PD. The enantio-specific biosynthesis of salsolinol *(3)* has been proposed to occur by condensation

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[3] Nonstandard abbreviations: PD, Parkinson disease; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; (*R*)-Sal; 1(*R*)-methyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline or (*R*)-salsolinol; (*R*)-NMSal, 1(*R*),2-dimethyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline or *N*-methyl-(*R*)-salsolinol; DA, dopamine; ACh, acetylcholine; AChE, acetylcholinesterase; DTNB, 5,5′-dithiobis-2-nitrobenzoic acid; ChO, choline oxidase; Ch, choline; DOPAC, 3,4-dihydroxyphenylacetic acid; HVA, homovanillic acid; pmPD, poly(m-(1,3)-phenylenediamine); pTy, polytyramine (pTy); MAO, monoamine oxidase.

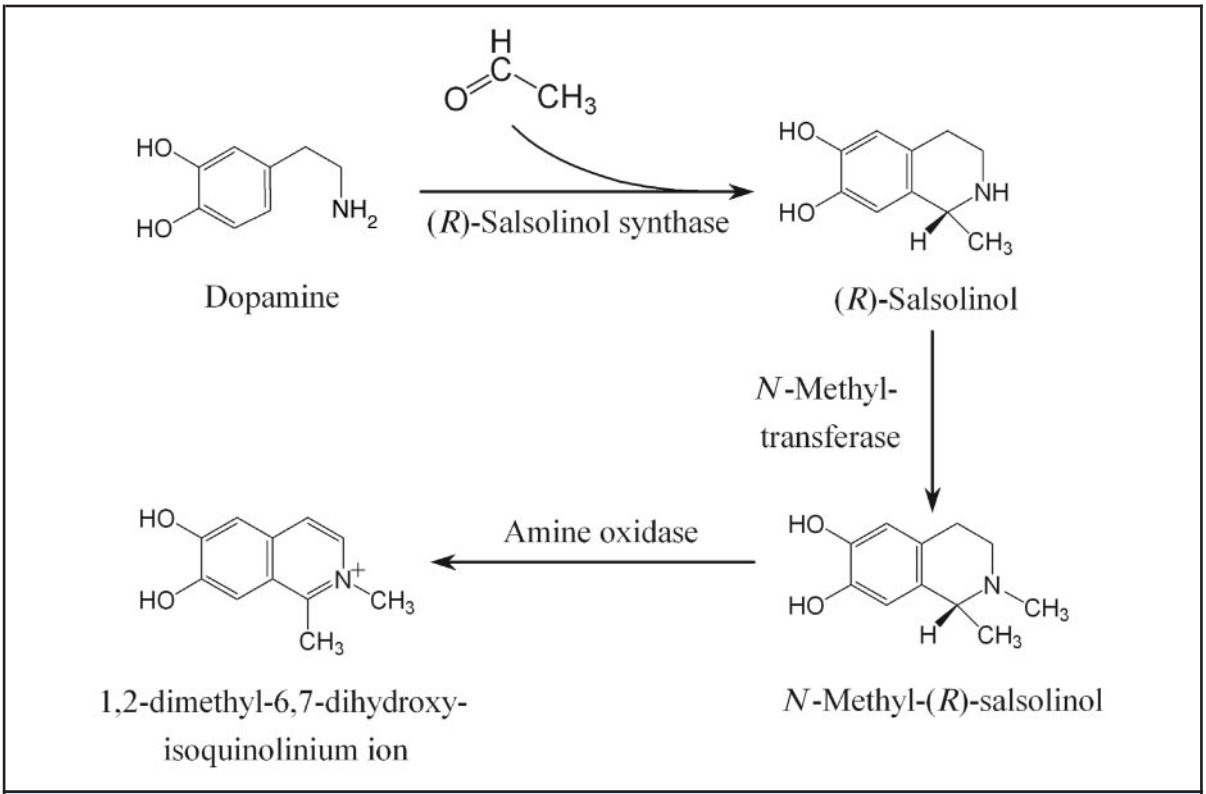

Fig. 1. Biosynthesis pathway of (*R*)-salsolinol and its derivatives.

The enzymatic condensation of dopamine with acetaldehyde is catalyzed by (*R*)-salsolinol synthase to yield (*R*)-Sal. Then *N*-methyltransferase catalyzes the *N*-methylation of (*R*)-Sal into (*R*)-NMSal, which is further oxidized into 1,2-dimethyl-6,7-dihydroxyisoquinolinium ion.

of dopamine (DA) with acetaldehyde under the catalysis of (*R*)-Sal synthase. Then *N*-methyltransferase catalyzes the synthesis of *N*-methyl-(*R*)-salsolinol *(4)*, which is further oxidized into 1,2-dimethyl-6,7-dihydroxyisoquinolinium ion *(5, 6)* (Fig. 1). There is evidence supporting the hypothesis that (*R*)-Sal and (*R*)-NMSal may be involved in the etiology of PD. For example, (*R*)-Sal concentrations in the urine and cerebrospinal fluid from parkinsonian patients were significantly higher than those from controls *(7, 8)*, and (*R*)-NMSal has been shown to elicit a parkinsonian syndrome in rats *(9)*. With recent advances in the understanding of PD, the crucial role of endogenous neurotoxins in the development of PD is increasingly apparent *(10)*. PD has been considered to undergo depletion of DA neurons in the substantia nigra, which connects with the striatum by the nigrostriatal pathway.

Normally, these neurons in the substantia nigra produce the important neurotransmitter DA, a chemical messenger responsible for transmitting signals between substantia nigra and striatum. Hence, the loss of dopaminergic neurons in the substantia nigra leads directly to DA deficiency in the striatum *(11)*. Consistent with this view, endogenous neurotoxins (*R*)-Sal and (*R*)-NMSal have been considered to be responsible for the massive depletion of dopaminergic neurons and participate in the pathogenesis of PD. However, it is clear that PD is more than just a syndrome of a dopaminergic deficiency, and that future research needs to pay more attention to the multiple neuronal systems affected in PD, such as cholinergic systems. It has been documented that DA from substantia nigra neurons excites striatonigral neurons, while another neurotransmitter, acetylcholine (ACh) from striatal cholinergic neurons, opposing DA action, inhibits striatonigral neurons *(12)*. Because striatal ACh and DA terminals are very dense and closely spaced, a strong interaction between DA and ACh occurs in the striatum. It has been shown that cholinergic activity acts locally and potently regulates DA release *(13)*, and DA likewise influences ACh release *(14)*. Normal function of striatonigral neurons depends primarily on the balance between DA and ACh. According to this concept, disruption of this balance, resulting from the im-

pairment of cholinergic system, might be a main contributor to the development of PD. In addition, acetylcholinesterase (AChE, EC 3.1.1.7) *(15)* is known to play a critical role in terminating acetylcholine-mediated neurotransmission. When the enzyme is blocked, it can no longer participate in the hydrolysis of ACh *(16)*. Thus, an excessive amount of ACh accumulates, and the balance between DA and ACh is disrupted. On the basis of this fact, we speculated that endogenous neurotoxins might be related not only to the loss of DA neurons in the substantia nigra, but also to the disruption of balance between DA and ACh via the inhibition of AChE. Consequently, knowledge of the imbalance caused by (*R*)-Sal and (*R*)-NMSal is essential for understanding the mechanism behind neurotoxin-induced PD. In this study, we evaluated the influence of (*R*)-Sal and (*R*)-NMSal on the imbalance between DA and ACh, which could be implicated in the pathogenesis of PD.

Materials and Methods

CHEMICALS

(*R*)-Sal, (*R*)-NMSal, acetylthiocholine iodide, 5,5′-dithiobis-2-nitrobenzoic acid (DTNB), tetraisopropyl pyrophosphoramide (*iso*-OMPA), AChE (type V-S, from electric eel, 1000 U/mg), choline oxidase (ChO, EC 1.1.3.17, from *Alcaligenes* species, 13 U/mg), m-(1,3)-phenylenediamine, tyramine, ACh, choline (Ch), DA, 3,4-dihydroxyphenylacetic acid (DOPAC), and homovanillic acid (HVA) were purchased from Sigma Chemicals. All other reagents used were of analytical grade.

ANIMALS AND SURGERY

Subjects were male Sprague-Dawley rats weighing between 250 and 350 g. Rats were housed individually in cages with free access to food and water in a temperature- and humidity-controlled room. Animals were maintained on a 12-h light/dark cycle, with lights on at 0700. All procedures were carried out in accordance with the Guide for the Care and Use of Laboratory Animals *(17)*.

On the day of surgery, animals were anesthetized with sodium pentobarbital (50 mg/kg intraperitoneally) and placed into a stereotaxic apparatus. A small hole was drilled into the skull and a syringe, used for injection of the endogenous neurotoxins, was lowered into the striatum (1.2 mm anterior to the bregma, −2.2 mm lateral from the midsagittal suture, and −3.4 mm ventral from the dura surface) *(18)*. (*R*)-Sal or (*R*)-NMSal, dissolved in saline solution (0.9% NaCl) in doses of 10.0, 20.0, 40.0, or 80.0 nmol/5 μL, was injected into the striatum at a rate of 1.0 μL/min. Control rats (n = 18) received only the saline solution, with an equal number of rats for microdialysis experiment and AChE analysis. In each experimental group, 29 animals were injected with various doses of (*R*)-Sal or (*R*)-NMSal, and 9 rats per group were used for microdialysis experiments; the other rats were for AChE analysis. The incision was sutured and treated with antibiotic ointment. After surgery, all rats were returned to cages and allowed free access to food and water.

IN VIVO MICRODIALYSIS

We performed in vivo microdialysis experiments 3, 5, and 7 days after neurotoxin treatment. Under sodium pentobarbital anesthesia, rats were implanted with the guide cannula (MD 2251; BAS) in the injection site, and the microdialysis probe (dialysis length 4 mm, diameter 0.24 mm; BAS) was secured into the guide cannula. Probes were equilibrated by perfusion with artificial cerebrospinal fluid (NaCl, 147 mmol/L; KCl, 3.0 mmol/L; $MgCl_2$, 1.0 mmol/L; $CaCl_2$, 1.2 mmol/L; sodium phosphate, pH 7.4, 1.5 mmol/L) at a flow rate of 1.0 μL/min for 60 min. The dialysis samples were collected into a refrigerated collector every 30 min. After the experiments, the brain was removed and the placement of the microdialysis probe was verified with a cryostat microtome and viewing lens.

ANALYSIS OF DOPAMINE AND ITS METABOLITES

We quantified concentrations of DA and its metabolites DOPAC and HVA by HPLC with electrochemical detection (CHI-832 electrochemical system; CHI Co.). Dialysis samples were separated on a C-18 reversed-phase column (Luna 5 μm C_{18}; Phenomenex) at a rate of 1.0 mL/min with a mobile phase consisting of 0.2 mol/L phosphate buffer solution and 10% methanol at pH 5.0. DA and its metabolites in the dialysis samples were oxidized on a polyvinyl alcohol–modified electrode *(19)*, at a potential of +0.6 V vs an Ag/AgCl reference electrode. We report the values obtained as a percent of saline-treated control concentrations.

ACETYLCHOLINE ANALYSIS

We measured ACh concentrations using a microdialysis-electrochemical device based on our previous work *(20)*, consisting of a microdialysis probe and a 3-electrode system. We prepared working electrodes by first sequentially electrodepositing poly(m-(1,3)-phenylenediamine) (pmPD) and polytyramine (pTy) from the monomers on the platinum microelectrode (see the Data Supplement that accompanies the online version of this article at http://www.clinchem.org/content/vol54/issue4). The pmPD layer functions as a permselective coating on the electrode *(21)*, and the pTy layer provides pendant amino groups for covalent attachment of AChE and ChO through a glu-

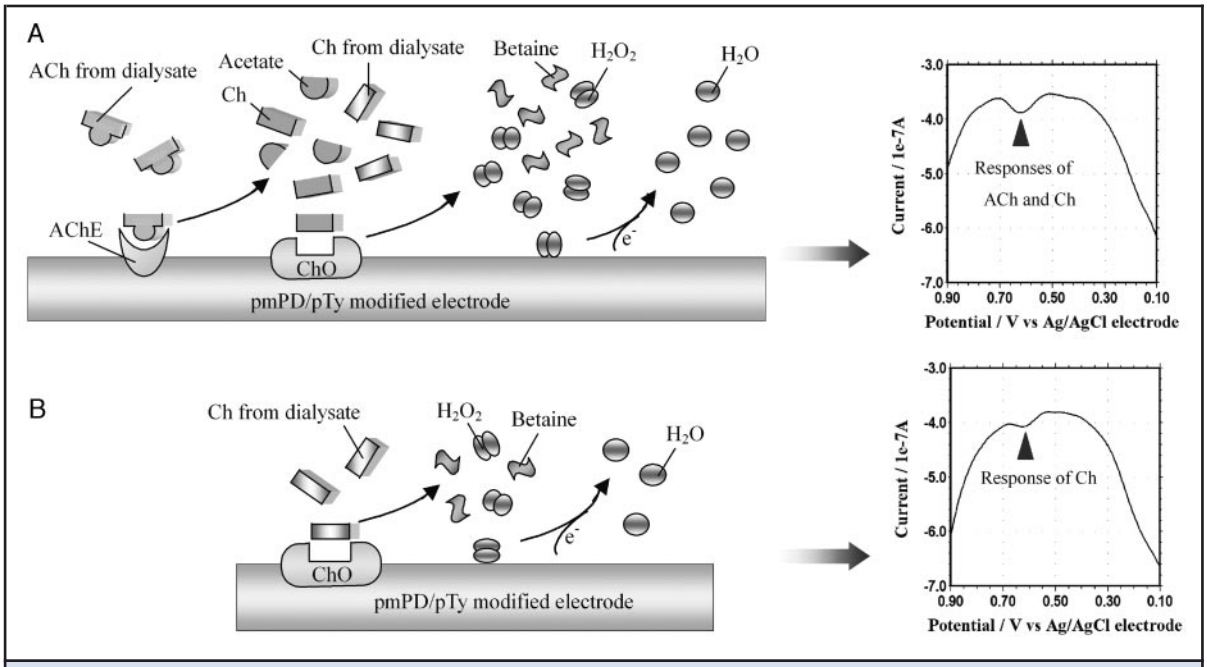

Fig. 2. Schematic diagram of sensors for the measurement of ACh.

(A), Responses of ACh and Ch at the AChE-ChO/pmPD/pTy–modified electrode. In the presence of AChE, ACh from dialysate hydrolyzed to yield acetate and Ch. Then the latter product, together with Ch from dialysate, was oxidized to betaine and H_2O_2, which is readily detected electrochemically. (B), Response of Ch at the ChO/pmPD/pTy–modified electrode. Only Ch from dialysate was determined through the measurement of H_2O_2 product.

taraldehyde linker *(22)*. We then used the AChE-ChO/pmPD/pTy– or ChO/pmPD/pTy–modified electrode as the working electrode. The Ag/AgCl electrode served as a reference electrode and the outlet of the microdialysis probe as an auxiliary electrode (see Supplemental Fig. 1 in the online Data Supplement). Data were acquired by differential pulse voltammetry measurement in the potential range of +0.1 to +0.9 V. The determination of ACh involves indirect measurement of hydrogen peroxide product as shown in the scheme in Fig. 2. The current response at the AChE-ChO/pmPD/pTy–modified electrode was due to the electrochemical reduction of H_2O_2 derived from both ACh and Ch, whereas the current response at the ChO/pmPD/pTy–modified electrode was ascribed only to the contribution of Ch. It should be noted that the response of ACh was estimated by subtraction of the Ch response at the AChE-ChO/pmPD/pTy–modified electrode rather than the response at the ChO/pmPD/pTy–modified electrode. With the use of the sensitivity of the AChE-ChO/pmPD/pTy–modified electrode to Ch and the Ch concentration determined at the ChO/pmPD/pTy–modified electrode, we could obtain the Ch response at the AChE-ChO/pmPD/pTy–modified electrode. To determine ACh concentrations, we calibrated the AChE-ChO/pmPD/pTy–modified electrode with ACh standard solutions.

ACETYLCHOLINESTERASE ASSAY

Immediately after the decapitation of animals, the striatum was removed on an ice-chilled plate, weighed, and stored at −70 °C. After thawing, tissues were homogenized in ice-cold saline solution, and homogenates were used for enzymatic analysis.

We determined AChE activity by the method of Ellman et al. *(23)*. We spectrophotometrically determined enzymatic activity by measuring the absorbance of sample at 412 nm in the presence of 2.5 mL 0.1 mol/L phosphate buffer (pH 8.0), 40 μL 0.075 mol/L acetylthiocholine iodide as substrate, 40 μL 0.01 mol/L *iso*-OMPA as an inhibitor of butyrylcholinesterase (BuChE, EC 3.1.1.8), 100 μL 0.01 mol/L DTNB, and 200 μL brain homogenate at 25 °C for 5 min. All samples were run in triplicate, and the enzyme activity was expressed as percent of control values.

STATISTICAL ANALYSIS

Student *t* test with *P* value was used for comparisons. Differences were regarded as statistically significant at

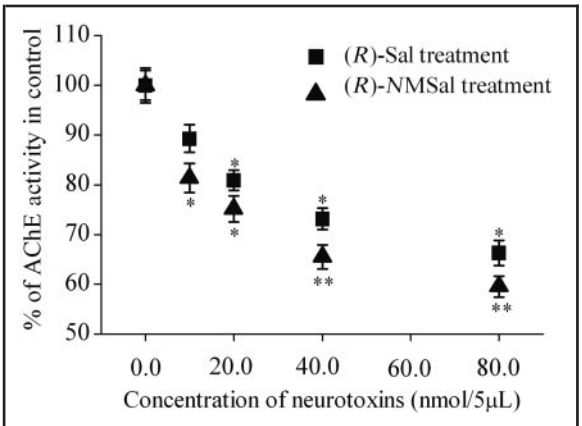

Fig. 3. Effects of (*R*)-Sal and (*R*)-NMSal concentrations on the activity of AChE in the rat brain striatum.

The activity of AChE was determined at day 3 after (*R*)-Sal or (*R*)-NMSal was injected into the rat brain striatum, and expressed as percent of control values (*$P < 0.05$; **$P < 0.01$; n = 5). Each data point and error bar represent the mean and SD of 15 replicate samples collected from 5 rats, triplicate measurement for each sample. In control rats, the activity of AChE is 20.5 (2.1) nmol/min/mg protein, consistent with the previous report *(24)*.

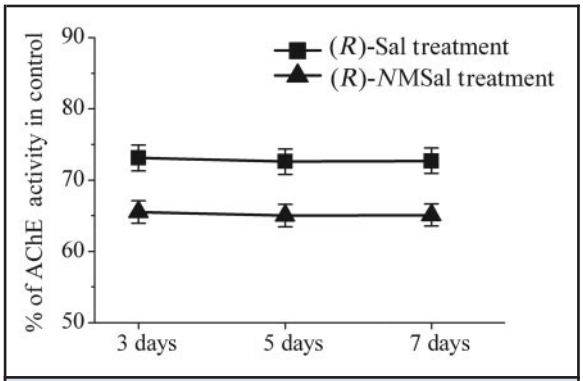

Fig. 4. Effect of time on the AChE activity in the rat brain striatum treated with 40.0 nmol/5 μL (*R*)-Sal or (*R*)-NMSal.

The activity of AChE was expressed as percent of control values. Each data point and error bar represent the mean and SD of 15 replicate samples from rats, with each sample tested in triplicate.

$P < 0.05$. All analyses were performed with the SPSS 10.0 statistical package program.

Results

Injection of (*R*)-Sal or (*R*)-NMSal into the striatum of rat brain led to a concentration-dependent decrease in the activity of AChE (Fig. 3). No inactivation of AChE was seen in the striatum of the control group, confirming the involvement of the 2 endogenous neurotoxins in the enzymatic inhibition. After injection into the striatum of rat brain, (*R*)-Sal (40.0 nmol) and (*R*)-NMSal (40.0 nmol) induced distinct reduction in the AChE activity to 73.1% and 65.5% of control, respectively. Furthermore, the reduction in the AChE activity was statistically significant in the striatum of both the (*R*)-Sal group ($P < 0.05$) and the (*R*)-NMSal group ($P < 0.01$) compared with control group. Additionally, these data suggested that the inhibitory effect of (*R*)-NMSal on AChE activity was greater than that of (*R*)-Sal. In a time course assay, no change of AChE activity was observed in the striatum of either (*R*)-Sal–treated or (*R*)-NMSal–treated rat brain (Fig. 4). Hence, a conclusion could be drawn on the basis of experimental results that the inhibition of AChE activity was dependent on the endogenous neurotoxin concentrations but independent of time.

A significant increase in ACh concentrations was found in the striatum of rat brain following treatment with (*R*)-Sal or (*R*)-NMSal (Fig. 5). The concentrations of ACh were increased to 131.7% of control on day 3 and further raised to 173.3% of control on day 7 ($P < 0.05$) after (*R*)-Sal injection into the striatum of rat brain. Such an increase was also observed in the rats treated with (*R*)-NMSal. ACh concentrations in the striatum of (*R*)-NMSal–injected rat brain were increased to 239.8% and 306.8% of control on day 3 ($P < 0.05$) and day 7 ($P < 0.01$), respectively.

We examined the impact of (*R*)-Sal and (*R*)-NMSal on DA and its metabolite concentrations in the rat brain striatum. Striatal concentrations of DA were significantly decreased to 25.2% of control ($P < 0.01$) in the rat brain treated with (*R*)-NMSal (Fig. 5), whereas there was no distinct difference in DA concentrations between the (*R*)-Sal group and control group. Conversely, the treatment with (*R*)-NMSal had no significant effect on the concentrations of DA metabolites, whereas a marked reduction in the concentrations of DOPAC and HVA was observed in the (*R*)-Sal–treated rat brain (Fig. 5). Concentrations of DOPAC were reduced to 48.9% of control on day 3 and further decreased to 24.9% on day 7 after (*R*)-Sal injection into the striatum of rat brain. A similar reduction in HVA concentration induced by (*R*)-Sal was also found on day 3 (48.9%) and day 7 (17.46%). Moreover, the decrease in concentrations of these 2 metabolites was more prominent on day 7 ($P < 0.01$) than day 3 ($P < 0.05$) after treatment with (*R*)-Sal. These findings demonstrate that (*R*)-NMSal had a significant influence on the concentrations of DA in the striatum,

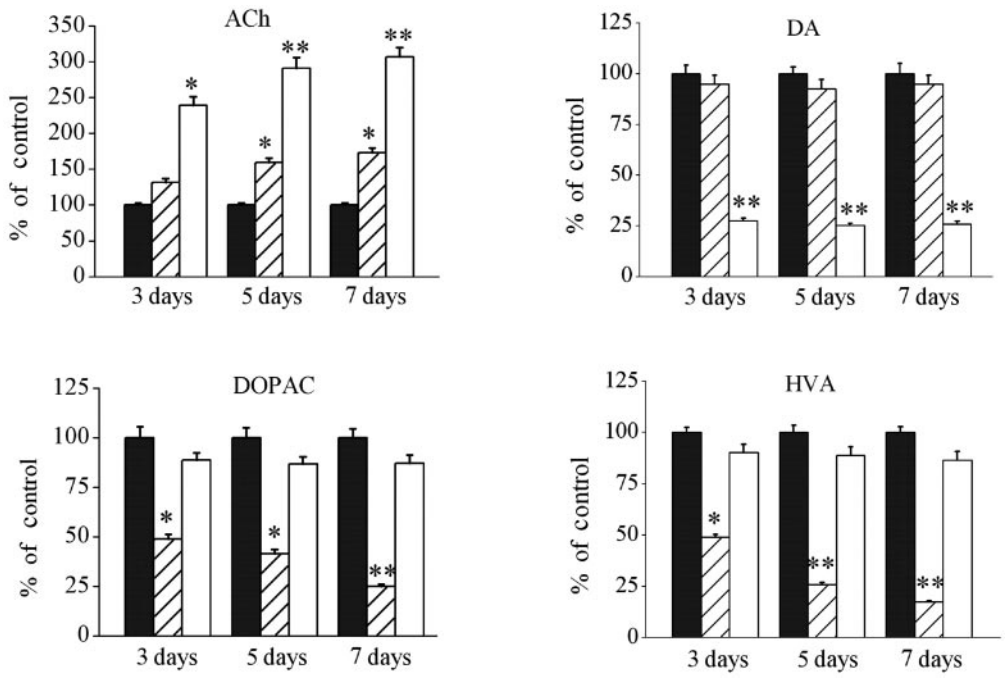

Fig. 5. Effects of (*R*)-Sal and (*R*)-NMSal treatment on the concentrations of ACh, DA, and its metabolites in the rat brain striatum.

At days 3, 5, and 7 after injection of 40.0 nmol (*R*)-Sal or (*R*)-NMSal into the rat brain striatum, concentrations of ACh, DA, DOPAC, and HVA were measured. For comparison, relative concentrations of the 4 compounds between neurotoxin-treated rats and control rats are presented (*$P < 0.05$; **$P < 0.01$; n = 9). Columns and error bars represent the mean and SD of 27 replicate samples collected from 9 rats, triplicate measurement for each sample. The filled, hatched, and hollow columns represent control, (*R*)-Sal, and (*R*)-NMSal groups, respectively. According to the previous report *(25–27)*, the striatal concentrations of ACh, DA, and its metabolites in control rats are as follows [fmol/μL, mean (SD)]: ACh, 5.6 (0.93); DA, 3.46 (0.81); DOPAC, 583 (48); HVA, 270 (32).

whereas (*R*)-Sal may be implicated in the metabolism of DA.

Discussion

Although the focus on the pathogenesis of PD has related to dopaminergic deficits that most of current symptomatic PD therapies attempt to correct, a proper balance between dopaminergic and cholinergic systems is required for the normal function of striatonigral neurons *(28)*. Accordingly, our study concerning the effect of endogenous neurotoxins on the impairment of the cholinergic system is important for understanding the imbalance between DA and ACh. We hypothesized that the dysfunction in PD induced by neurotoxins could be attributed to the imbalance between DA and ACh, as a consequence of excessive ACh concentrations as well as DA deficiency.

In our work, we observed a significant increase in ACh concentrations (Fig. 5) in the rat brain striatum following (*R*)-Sal and (*R*)-NMSal treatment. As mentioned before, the normal function of striatonigral neurons depends on the balance of various neurotransmitters, particularly on the balance between DA and ACh *(29)*, which excite and inhibit striatonigral neurons, respectively. An excess of DA produces an excess of movement, and an excess of ACh produces immobility. Therefore, the results obtained in this investigation support the possibility that the neurotoxins (*R*)-Sal and (*R*)-NMSal could destroy the balance between DA and ACh via the accumulation of an excess of ACh. The abnormalities in the interaction of these 2 neurotransmitters might underlie the dysfunction in PD. Supporting evidence also comes from the experimental result that treatment of rat brain striatum with (*R*)-Sal and (*R*)-NMSal led to concentration-dependent inhibition in AChE activity (Fig. 3), further preventing the hydrolysis of ACh in the striatum. An explanation for the inactivation of AChE is that the quaternary moiety of 1,2-dimethyl-6,7-dihydroxyisoquinolinium ion, a

product from (*R*)-Sal through *N*-methylation and oxidation *(5, 6)*, seemed to bind chiefly through cation-π interaction with π electrons in the aromatic residues on AChE, and thereby modulating the enzyme activity *(30)*. An alternative explanation comes from the previous discovery that free radical formation from arginine *(31)* and organophosphate *(32)* was responsible for the inhibition of AChE activity *(33)*. Thus, endogenous neurotoxins (*R*)-Sal and (*R*)-NMSal exerted their toxic effects on AChE activity probably by free radical production from the oxidation of neurotoxins *(6)*. All these findings provide strong evidence that (*R*)-Sal and (*R*)-NMSal could interfere with the balance between DA and ACh via impairment of the cholinergic system.

The significant changes of DA concentrations, different from those of ACh concentrations, were found in the striatum of (*R*)-NMSal–treated rat brain only (Fig. 5), which may be due to the observation that (*R*)-NMSal deleted the allostery of tyrosine hydroxylase to biopterin and limited catecholamine synthesis *(10)*. These results suggest that in the (*R*)-NMSal–treated rat brain striatum, abnormalities occurred in the both dopaminergic and cholinergic systems, simultaneously contributing to the disruption of balance between DA and ACh and resulting in the development of PD. As to the neurotoxin (*R*)-Sal, it had no distinct effect on the concentrations of DA, but produced a significant increase in the ACh concentrations. These observations demonstrate that the (*R*)-Sal–induced imbalance between DA and ACh may be primarily ascribed to a relatively excessive concentration of ACh, instead of DA depletion (Fig. 6). Regardless of (*R*)-Sal and (*R*)-NMSal, the impairment of cholinergic system occurred via the inhibition of AChE. Nevertheless, it is worth noting that the PD-like syndrome was observed only in the rats treated with (*R*)-NMSal *(9)*. This may be attributed to the cooperative role of dopaminergic and cholinergic systems, which might result in the aggravation of the imbalance between DA and ACh (Fig. 6). The combined impairment of the 2 systems following exposure to (*R*)-NMSal was likely sufficient to induce the parkinsonian syndrome.

Additionally, (*R*)-Sal was found to markedly decrease the striatal concentrations of DOPAC and HVA, suggesting that the inhibition of monoamine oxidase (MAO), an enzyme related to the metabolism of DA, occurred in the rat brain striatum. In contrast, (*R*)-NMSal did not significantly affect the concentrations of DA metabolites in the striatum, which could be explained by the concept that (*R*)-NMSal was a rather weak inhibitor of MAO compared with (*R*)-Sal *(26)*. The results of the present study provide a new possibility that endogenous neurotoxins (*R*)-Sal and (*R*)-NMSal not only affect the dopaminergic system by inhibiting the related enzymes in the synthesis or metabolism of DA, but also impair the cholinergic system by inactivating AChE. Both phenomena would

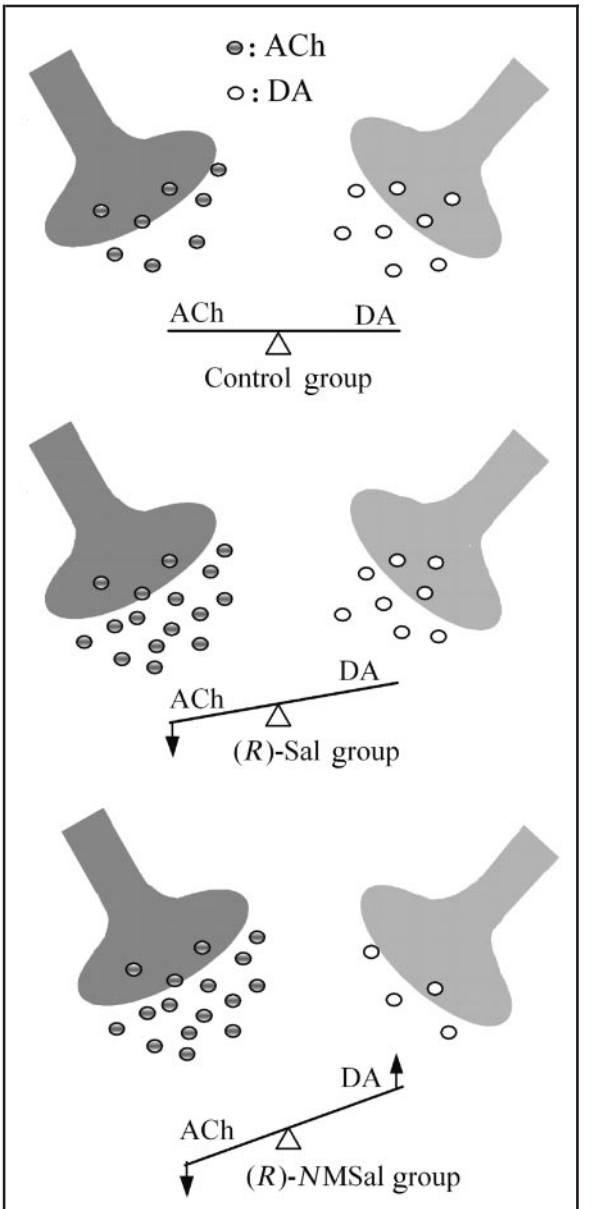

Fig. 6. Effect of endogenous neurotoxins (*R*)-Sal and (*R*)-NMSal on the balance between DA and ACh in rat brain striatum.

On injection of (*R*)-Sal into the rat brain, ACh concentrations rose sharply and the balance between DA and ACh was destroyed. After the treatment with (*R*)-NMSal, ACh concentrations also increased and in addition the reduction of DA concentrations occurred in the striatum. As a result, the balance between DA and ACh was severely disrupted.

lead to the disruption of balance between DA and ACh and contribute to the development of PD.

Although AChE inhibitors, such as rivastigmine *(34)*, have been reported to improve cognitive symptoms in PD patients with dementia, the efficacy of these drugs over a longer period remains under debate. Meanwhile, increasing evidence has shown that onset of PD correlates with reduced AChE activity in muscarinic receptors *(35)* and that organophosphorus exposure is linked to increased risk of PD with diminished AChE activity *(36)*. The results of the present study force us to think in a new light regarding the possibility that the imbalance between DA and ACh, as a result of a cooperative role of dopaminergic and cholinergic systems, might be associated with the pathogenesis of PD.

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