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Inhibition of Dopamine Receptors by Endogenous Amines: Binding to Striatal Receptors and Pharmacological Effects on Locomotor Activity

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Abstract—Endogenous amine 1-benzyl-1,2,3,4-tetrahydroisoquinoline (1BnTIQ) derivatives are synthesized, and their activity for dopaminergic systems are evaluated in vitro and in vivo by receptor binding assay and pharmacological tests. It is proposed that 1BnTIQ derivatives can act as endogenous dopaminergic antagonists. © 2000 Elsevier Science Ltd. All rights reserved.

1,2,3,4-Tetrahydroisoquinoline (TIQ) derivatives exist not only in plants but also in several tissues in mammals.[1] Several TIQ derivatives have been proposed to relate with the pathogenesis of Parkinson's disease.[2] 1-Benzyl-1,2,3,4-tetrahydroisoquinoline (1BnTIQ, **1**), 1-(3′,4′-dihydroxybenzyl)-1,2,3,4-tetrahydroisoquinoline (3′,4′DHBnTIQ, **2**) and 1-benzyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline (6,7DHBnTIQ, **3**) are endogenous amines and the former two compounds can induce parkinsonism to animals.[2c–e] They contain a dopamine and/or phenethylamine moiety in their structure, and they are structurally similar to apomorphine, a well-known dopaminergic ligand [3] (Fig. 1). We considered that these 1BnTIQ derivatives could be endogenous aporphine analogues. Since many compounds containing TIQ skeleton are known as agonists and antagonists of dopamine receptors,[3] 1BnTIQ derivatives also can be dopaminergic ligands. Previously, it was reported about the interaction of Parkinsonism-inducible TIQ derivatives and dopaminergic systems that some of the TIQ derivatives can be taken up into dopaminergic neurons via dopamine transporter,[2f,4] 1BnTIQ derivatives (**1** and **2**) can kill the cultured-mesencephalic neurons (our unpublished data), and 6,7-dihydroxy-1,2-dimethyl-1,2,3,4-tetrahydroisoquinoline (*N*-methyl-salsolinol) can induce apoptosis to dopaminergic neuroblastoma cells.[2e] But it has not been reported that whether Parkinsonism-inducible TIQ derivative can be a ligand of dopamine receptors, and if so, whether this interaction is important for its ability to induce Parkinsonism. In this paper, we examine the binding affinity of endogenous 1BnTIQ derivatives (**1**–**3**) for dopamine receptors, and discuss the structural requirements for these compounds to affect dopamine receptors and the relationship to the pharmacological effects.

Compounds **1** and **2** were synthesized by modifying the method of Gray et al. (1989).[5a] *N*-(β-Phenylethyl)phenylacetamide or *N*-(β-phenylethyl)-3,4-dimethoxyphenylacetamide was synthesized from β-phenylethylamine and phenylacetyl chloride or 3,4-dimethoxyphenylacetylchloride. The product was refluxed with P_2O_5 in anhydrous toluene, affording 3,4-dihydroisoquinoline derivatives. The resultant was refluxed with $NaBH_4$ in ethanol, affording 1-benzyl-1,2,3,4-tetrahydroisoquinoline (1Bn TIQ, **1**) or 1-(3′,4′-dimethoxybenzyl)-1,2,3,4-tetrahydroisoquinoline. Compound **1** was treated with HCl, and recrystallized from ethanol-diethylether, affording colorless crystals (**1** HCl, mp 165–166 °C). 1-(3′,4′-Dimethoxybenzyl)-1,2,3,4-tetrahydroisoquinoline hydrochloride was demethylated with 47% hydrobromic acid. The precipitates obtained after cooling were recrystallized from ethanol-diethylether, affording 1-(3′,4′-dihydroxybenzyl)-1,2,3,4-tetrahydroisoquinoline hydrobromide (**2** HBr, mp 208–211 °C). Compound **3** was synthesized from dopamine and phenylacetaldehyde by Pictet–Spengler condensation.[5b] Structures were confirmed by ^{1}H NMR spectrometry and elemental analysis.

Binding affinity of 1BnTIQ derivatives with dopamine receptors were determined by measuring their ability to displace ^{3}H-labeled specific ligand from rat striatal

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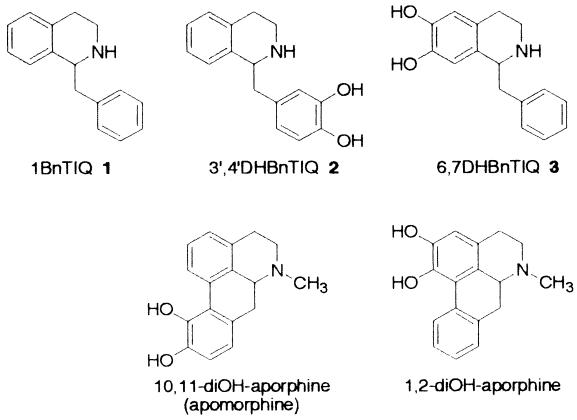

Figure 1. Structures of 1BnTIQ, 3′,4′DHBnTIQ, 6,7DHBnTIQ and aporphine derivatives.

synaptosomes.[6] [^{3}H]-SCH23390 (1.5 nM), [^{3}H]-YM 09151-2 (0.5 nM), and [^{3}H]-GBR12935 (1.0 nM) were used as ^{3}H-labeled ligands for dopamine receptors (D1 and D2 subtype), and dopamine transporter, respectively (Fig. 2). Nonspecific binding was determined as that obtained from assay in the presence of 10 or 100 μM unlabeled ligands (SCH23390, sulpiride, and GBR12909), and was subtracted, respectively. 1BnTIQ derivatives can bind to dopamine receptors with K_i values of 10^{-7}–10^{-4} M (Table 1). The order of binding affinity was **3** > **1** ⩾ **2**, and all three compounds prefer D2 than D1. Both **2** and **3** are dihydroxylated derivatives of **1** and structurally resemble (Fig. 1), but only **3** had higher affinity than **1** and **2**.

The structure–activity relationship studies using dopamine and dopamine-containing dopaminergic ligand indicate

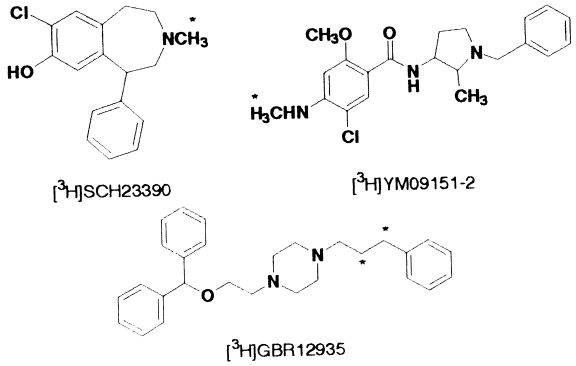

Figure 2. Structures of the ^{3}H-labeled compounds used in binding assay.

Table 1. Binding affinities of 1BnTIQ derivatives for dopamine receptors. K_i values are shown in μM[a]

	D1	D2	Dopamine transporter
1	248.80	3.57	36.62
2	174.84	8.79	21.49
3	9.27	0.64	57.55

[a]K_i values were determined by using the relationship, $K_i = IC_{50}/(1 + [L]/K_D)$, where [L] is concentration of the radioligands used in the assay, and K_D is dissociation constant. $K_D = 1.3$, 0.2 and 5.0 nM, respectively.

that there are two major binding sites in the dopamine receptor, which interact with *N* and catechol, especially m-OH, of ligand molecules.[3,7] Appropriate orientation of *N* and OH are required for high-affinity binding. Although the affinity of 1BnTIQ derivatives are weaker than that of the known dopaminergic ligands such as aporphines, we speculate that **3** meets this requirement, that is, 6,7-diOH group contributes to increase the binding affinity with dopamine receptors, although 3′,4′-diOH group had little effect. Since **2** does not have 6,7di-OH, its affinity is equipotent to **1** and is lower than **3**.

6,7-Dihydroxy-1,2,3,4-tetrahydroisoquinoline (norsalsolinol), which is structurally similar to **3** but lacks the benzyl moiety at the 1-position, had weaker affinity than **3** (data not shown), suggesting that 1-benzyl moiety seems to contribute to the binding with receptors by hydrophobic interaction.

We conclude that OH (catechol) in TIQ moiety, not in 1-benzyl moiety, *N* atom and benzyl functions are important for the binding of 1BnTIQ derivatives to dopamine receptors. These functions in **3** are preferably located so that it can strongly bind to the dopamine receptors.

The binding affinity of 1BnTIQ derivatives to the dopamine transporter was also measured (Table 1). All of them can bind to dopamine transporter with K_i values of 10^{-5}–10^{-4} M. Compound **2** had slightly higher affinity than compound **3**. This is consistent with our previous finding that **2** and **3** are substrates of the dopamine transporter and the affinity is **2** > **3**.[2f]

The dopamine receptors (D1 and D2) and dopamine transporter would recognize 1BnTIQ derivatives in different manner. Dopamine transporter prefers **2** as substrate, while D1 and D2 prefer **3**. It is considered that both dopamine receptors and transporter recognize dopamine moiety of DHBnTIQ, but the favorable conformation of dopamine moiety should be different. DHBnTIQ bind to dopamine transporter with the extended conformation of dopamine, but bind to dopamine receptors with compact conformation of dopamine, probably because it is better for hydrophobic interaction by 1-benzyl moiety.

In order to examine the effect of DHBnTIQs (**2**, **3**) in vivo, an open field test was carried out. Each DHBnTIQ (100–250 mg/kg) was administered intraperitoneally to male C57BL mice (7 weeks), and locomotor activity was measured by means of the open field test, 5, 15, 25, 35 min after administration. The modified Hall's method and apparatus were used for open field test.[8] The apparatus consisted of a bucket (28 cm diameter and 15 cm deep), the bottom of which was divided into 19 areas, with a light (60 W) at 50 cm above the bottom. A mouse was placed in the center of the field, and its ambulation and rearing were measured for 2 min as parameters of locomotor activity. Both DHBnTIQs dose-dependently reduced ambulation and rearing scores, and **3** showed larger effect than **2** (Fig. 3). After the open field test was completed, the induction of catalepsy was evaluated. The mouse was placed with forelimbs on a wire 5 cm

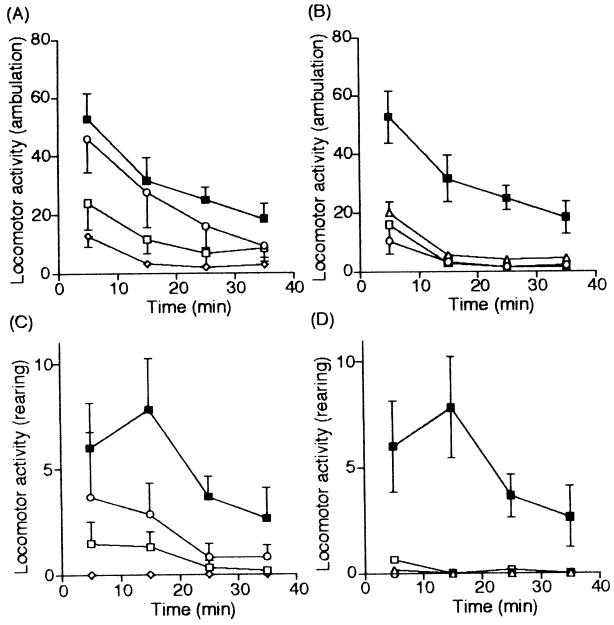

Figure 3. Effect of DHBnTIQ derivatives on locomotor activity. Results of the open field test of **2**-treated mice (ambulation (A), rearing (C)) and **3**-treated mice (ambulation (B), rearing (D)) are shown. Doses: △, 100 mg/kg; ○, 150 mg/kg; □, 200 mg/kg; ◇, 250 mg/kg; ■, control (saline treated). Each plot represents the mean ± SEM (mean + SEM or −SEM in some cases) of 6 experiments.

above the floor, and if it retained that unnatural posture for over 3 min, it was evaluated as cataleptic. Compound **3** treated mice (2:6 of 150 mg/kg and 3:6 of 200 mg/kg treated group) exhibited catalepsy. Both DHBnTIQs can reduce locomotion in mice and **3** is more effective than **2**.

Locomotive activity is regulated by dopamine receptors, especially D2, and D2 antagonists can reduce locomotor activities and induce catalepsy.[9] DHBnTIQs are ligands for dopamine receptors (Table 1), and from the behavioral point of view, they can reduce locomotion (Fig. 3), therefore they may be the antagonists of dopamine receptors. Compound **3** could block the D2 receptor so efficiently (Table 1) that it induced catalepsy at high doses.

In our previous report, we showed that **2** could induce parkinsonism in mice, and proposed its mechanism of action that **2** is accumulated in dopaminergic neurons by dopamine transporter and thereafter it inhibits the mitochondrial respiration, which lead to cell death and parkinsonism.[2f,10] Compound **3** showed no chronic effect,[2f] in spite of its relatively potent activity to inhibit dopamine receptors (Table 1) and to reduce locomotor activity acutely in mice (Fig. 3). If inhibition of dopamine receptors is associated with parkinsonism, **3** should have more potent activity to induce parkinsonism than **2**. We think that dopamine receptor antagonism can lead to acute reduction of locomotor activity, but does not contribute to the pathogenesis of Parkinson's disease. The acute and chronic toxicity of DHBnTIQs may be associated with different biochemical activity of these compounds. Accumulation in dopaminergic neurons by dopamine transporter seems important for the chronic effect (Parkinsonism), whereas the inhibition of dopamine receptors causes the acute effect (reduction of locomotor activity) of DHBnTIQs.

In conclusion, 1BnTIQ and DHBnTIQs, especially 6,7DHBnTIQ **3**, show affinity for dopamine receptors, and DHBnTIQs can induce a remarkable reduction of locomotor activity in vivo. Because they are endogenous amines, we speculate that they act as physiological modulators of the dopaminergic systems.

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