ISOLATION AND IDENTIFICATION OF TETRAHYDROCOLUMBAMINE AS A DOPAMINE RECEPTOR LIGAND FROM *POLYGALA TENUIFOLIA* WILLD*

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ABSTRACT The isolation and purification of a dopamine receptor ligand, tetrahydrocolumbamine from *Polygala tenuifolia* Willd is described. Tetrahydrocolumbamine was shown to inhibit the binding of [^{3}H]-SCH23390 and [^{3}H]-spiroperidol to rat striatum membranes *in vitro* with IC_{50} values of 0.75 ± 0.08 μmol · L^{-1} and 0.92 ± 0.10 μmol · L^{-1}, respectively. The compound inhibited the binding of [^{3}H]-prazosin (IC_{50} value of 46 μmol · L^{-1}), while it did not change the binding of the ligands, [^{3}H]-QNB and [^{3}H]-muscimol to rat cortex *in vitro*. Scatchard plot analysis showed a mixture of competitive and non-competitive inhibition by the compound on both [^{3}H]-SCH23390 and [^{3}H]-spiroperidol binding to membranes from rat striatum.

Key words *Polygala tenuifolia* Willd; Tetrahydrocolumbamine; Dopamine receptor; Striatum

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Compounds acting on the central nervous system (CNS) have been isolated and identified from plants used for medicinal purposes[1]. *Polygala tenuifolia* Willd is a Chinese traditional plant medicine which is widely used for treatment of insomnia, amnesia and convulsion[2]. It will be interesting to investigate if *Polygala tenuifolia* Willd contains active substances of the brain receptor which could explain its reported usefulness for medical purposes. Furthermore, it is important to obtain new chemical structure which can be used as tools to investigate biochemical characteristics of brain receptors. Therefore, we initiated a purification of receptor active substances from *Polygala tenuifolia* Willd.

MATERIALS AND METHODS

Materials *Polygala tenuifolia* Willd was supplied by the pharmacy affiliated to the Hospital of the

Received 1994－5－10.

*This project was supported by the Velux Foundation, Denmark.

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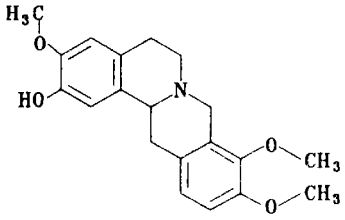
Shaanxi Academy of Traditional Chinese Medicine, China. [^{3}H]-SCH23390 (*N*-methyl-[^{3}H], 2.62 TBq·mmol^{-1}), and [^{3}H]-spiroperidol (benzene ring-[^{3}H], 0.82 TBq·mmol^{-1}) and [^{3}H]-prazosin (7-methoxy-[^{3}H], 2.90 TBq·mmol^{-1}) were from New England Nuclear, Du Pont, USA. All HPLC solvents (HPLC grade) were from Rathburn Chemicals, Scotland.

Isolation of the active compound Batches of dried *Polygala tenuifolia* Willd were extracted with boiling 70% (v/v) ethanol, followed by filtration and flash evaporation. Twenty milligram of the crude extracts was redissolved in 85% water－15% acetonitrile (v/v) and chromatographed on a preparative Bondpak C-18 reverse-phase column (19 mm×150 mm) at a flow rate of 4 ml/min. Aliquots of 1 min fractions were tested for activity in radio-receptor assays (RRA) and the fraction containing [^{3}H]-SCH23390 inhibiting compound were pooled into fraction A (retention time of 54～58 min) and freeze dried. For the second step of HPLC separation, fraction A was dissolved in 0.5 ml of solvent (80% water－20% acetonitrile) and applied to a C-18 ODS reverse-phase column (3.9 mm×150 mm) at a flow rate of 1 ml/min. A substance was collected showing absorption peak at 210 nm with retention times of 8～10 min. As a third HPLC step, fraction A was pooled from the second HPLC step and applied to the same column using the same solvent system as the second HPLC step (80% water－20% acetonitrile, isocratic). The purified compound showing inhibition of [^{3}H]-SCH23390 and [^{3}H]-spiroperidol binding was eluted as single peak at 9 min retention time from the third HPLC step.

Brain membrane preparations Brain tissue from male Wistar rats (weighing 200±20 g) was prepared for binding studies as described[3]. In brief, rat striatum was homogenized in KH_2PO_4 (50 mmol·L^{-1}, pH 7.4) and centrifuged at 30,000×g for 10 min. The pellet was resuspended in KH_2PO_4 at the concentration of 1 mg original tissue/ml. One ml aliquots of membranes were used for [^{3}H]-SCH23390 (2.62 TBq·mmol^{-1}, 0.2 nmol·L^{-1}) binding. Non-specific binding was determined in the presence of flupentixol (1 μmol·L^{-1}). Two ml aliquots of membranes were used for [^{3}H]-spiroperidol (0.82 TBq·mmol^{-1}, 0.2 nmol·L^{-1}) binding. However, here non-specific binding was obtained in the presence of 2-amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene (ADTN) (10 μmol·L^{-1}).

RESULTS AND DISCUSSION

The chemical structure of the purified compound was identified by proton nuclear magnetic resonance (NMR) spectroscopy and by mass spectrometry. When comparing our data with published data of corresponding tetrahydroprotoberberines (THPB), we conclude that the compound is tetrahydrocolumbamine (isocorypalmine).



Chemical structure of tetrahydrocolumbamine

The concentration of isocorypalmine causing 50% inhibition (IC_{50}) of [^{3}H]-SCH23390 (dopamine D_1 subtype receptor, 0.46 nmol • L^{-1}) and [^{3}H]-spiroperidol (dopamine D_2 subtype receptor, 0.55 nmol • L^{-1}) specific binding was determined with a series of concentrations in the binding assay[4]. The IC_{50} values were estimated to be 0.75 ± 0.08 μmol • L^{-1} and 0.92 ± 0.10 μmol • L^{-1} ($\bar{x} \pm s$, n=3) for [^{3}H]-SCH23390 and [^{3}H]-spiroperidol binding respectively. Scatchard plot analysis of [^{3}H]-SCH23390 (Fig 1) and [^{3}H]-spiroperidol binding showed a mixture of competitive and non-competitive inhibition by isocorypalmine, because it decrease both the binding affinity and the number of binding sites (B_{max}).

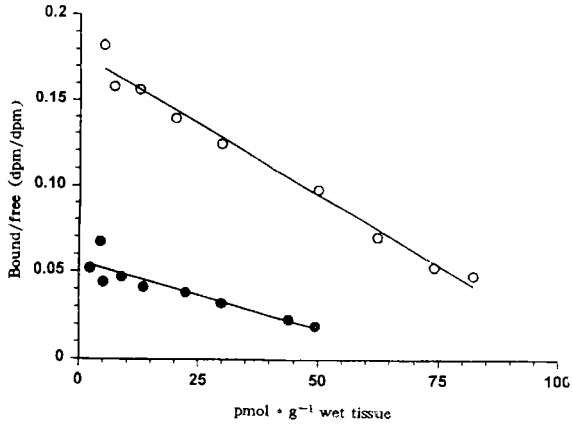


Fig 1 A typical Scatchard plot of [^{3}H]-SCH23390 binding to membranes of rat striatum *in vitro*. Control specific binding (○), addition of tetrahydrocolumbamine 1.5 μmol • L^{-1}(●).

The compound was shown not to inhibit the binding of [^{3}H]-QNB to the muscarinic acetylcholine receptor and of [^{3}H]-muscimol to the $GABA_A$ receptor. However, it inhibited the binding of [^{3}H]-prazosin to the α_1-adrenergic receptor with an IC_{50} value of 46 μmol • L^{-1}. Previously, it has been shown that THPB is a new type of dopamine receptor antagonist based on results of biochemical[5] and behavioral[6] experiments. Isocorypalmine is the first compound isolated from *Polygala tenuifolia* Willd, which shows moderate affinity to striatal D_1 and D_2 receptor subtypes *in vitro*[7]. It is not known if

isocorypalmine purified from *Polygala tenuifolia* Willd shows pharmacological effects *in vivo*. Plants used in ethnopharmacology are a rich source of new compounds that act as CNS receptor ligands. Application of RRA and HPLC is an effective method to find new active compounds from the medicinal plants.

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从远志中分离鉴定出一种多巴胺受体活性化合物

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提要 从中药远志中提出一种多巴胺受体的配基,四氢非州防己胺。此化合物在体外可抑制[^{3}H]SCH23390 和[^{3}H]螺哌隆与大鼠纹状体膜的结合, IC_{50}值分别为 0.75±0.08 μmol·L^{-1}和 0.92±0.10 μmol·L^{-1}。它在体外还能抑制[^{3}H]哌唑嗪和大鼠脑皮质细胞膜结合(IC_{50}值为 46 μmol·L^{-1}),但不能改变[^{3}H]QNB 及[^{3}H]muscimol 对膜的结合。Scatchand plot 分析显示此化合物对[^{3}H]SCH23390 和[^{3}H]螺哌隆与膜结合的抑制作用是通过竞争性与非竞争性混合机制而实现的。

关键词 远志; 四氢非州防己胺; 多巴胺受体; 纹状体