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Research Paper

Specific binding and characteristics of geissoschizine methyl ether, an indole alkaloid of Uncaria Hook, in the rat brain

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ABSTRACT

Ethnopharmacological relevance: Geissoschizine methyl ether (GM) is an indole alkaloid that is a component of Uncaria Hook, and has been identified as the active component responsible for the anti-aggressive effects of the Uncaria Hook-containing traditional Japanese medicine, yokukansan. Recently, GM was shown to reach the brain by crossing the blood–brain barrier in rats following the oral administration of yokukansan. This finding suggested that there may be specific binding sites for GM in the brain. Here we show evidence that tritium-labeled GM ([^{3}H]GM) binds specifically to several brain areas of rats.

Materials and methods: Male rats were used. [^{3}H]GM was synthesized from a demethylated derivative of GM. Specific binding sites of [^{3}H]GM on brain sections were determined by quantitative autoradiography, and maximum binding densities (B_{max}) and dissociation constants (K_d) were calculated. Several chemical compounds were used to clarify the molecules that recognize [^{3}H]GM in the completion-binding assay. Emulsion microautoradiography was also performed to identify the cells that bind [^{3}H]GM.

Results: Specific binding of [^{3}H]GM was observed in the frontal cortex, including the prefrontal cortical region (e.g., prelimbic cortex (PrL)), hippocampus, caudate putamen, amygdala, central medial thalamic nucleus, dorsal raphe nucleus (DR), and cerebellum. B_{max} ranged between 0.65 and 8.79 pmol/mg tissue, and K_d was between 35.0 and 232.6 nM. Specific binding with relatively high affinity (K_d less than 62 nM) was dense in the frontal cortical region, moderate in the DR, and sparse in the cerebellum. The specific binding of [^{3}H]GM in the PrL was significantly replaced by the serotonin 1A (5-HT$_{1A}$) receptor agonist 8-hydroxy-2-(di-n-propylamino)tetralin (DPAT), 5-HT$_{2A}$ receptor antagonist ketanserin, 5-HT$_{2B}$ receptor agonist BW 723C86, 5-HT$_{2C}$ receptor agonist RO60-0175, adrenergic α_{2A} receptor antagonist yohimbine, L-type Ca^{2+} channel blocker verapamil, and μ-opioid receptor antagonist naloxone. Similar results were obtained in the frontal cortex and DR, but not in the cerebellum. Microautoradiography revealed that [^{3}H]GM signals were distributed throughout the frontal cortex, which included neuron-like large cells.

Conclusion: These results demonstrate that specific binding sites for GM exist in rat brain tissue, and suggest that the pharmacological actions of GM are mainly associated with 5-HT receptors in the frontal cortex and DR. These results provide an insight into the neuropharmacology of GM and GM-containing herbal medicines.

1. Introduction

Geissoschizine methyl ether (GM) is an indole alkaloid that contains β-carboline in its structure (chemical structure shown in Fig. 1). The structure of GM is similar to that of yohimbine, which has affinity for adrenergic α_{2A} receptors, and rauwolscine, which has affinity for serotonin 2B (5-HT$_{2B}$) receptors. Pharmacologically, GM has shown to attenuate spontaneous motor activity (Sakakibara et al., 1999), convulsions (Mimaki et al., 1997), head twitching behavior induced by 5-hydroxy-L-tryptophan plus clorgyline (Pengsuparp et al., 2001), and aggressive behaviors (Nishi et al., 2012) and to ameliorate myelin deficits in the brain of a demyelination mouse model induced by cuprizone (Morita et al., 2014). Uncaria Hook, the original medicinal herb of GM, has traditionally been used as a raw material in mixed herbal medicines for the treatment of several psychotic symptoms associated with psychiatric disorders due to its spasmolytic, sedative, and analgesic actions. One Uncaria Hook-containing mixed medicine, yokukansan, is a traditional Japanese (Kampo) medicine that consists

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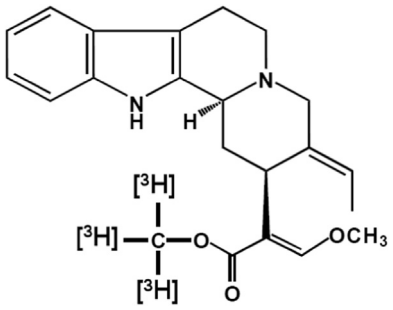

Fig. 1. Chemical structure of tritium-labeled geissoschizine methyl ether ([^{3}H]GM). Three tritium atoms were introduced into the methyl group of the methyl ester in its structure.

of seven medicinal herbs. This medicine has been approved by the Ministry of Health, Labour, and Welfare of Japan for the treatment of neurosis and insomnia as well as night crying and irritability in children. Recently, clinical trials demonstrated that yokukansan improved the behavioral and psychological symptoms of dementia (BPSD), i.e., aggressiveness, agitation, anxiety, hallucinations, sleep disturbance, and psychotic disorders, in several types of dementia, including Alzheimer's disease, without serious adverse effects (Iwasaki et al., 2005), (Monji et al., 2009; Hayashi et al., 2010; Matsuda et al., 2013). Several lines of evidence proposed that serotonergic mechanisms may be responsible for these effects. For example, yokukansan was shown to ameliorate p-chloroamphetamine (serotonergic neurotoxin)-induced aggressive behavior and reduced sociality in rats, and this effect was abolished by the 5-HT$_{1A}$ receptor antagonist WAY-100635, which demonstrated the involvement of 5-HT$_{1A}$ receptors in the effects of yokukansan (Kanno et al., 2009). A similar finding was reported for the anxiolytic action of yokukansan in rats (Yamaguchi et al., 2012). Among a large number of constituents included in yokukansan, GM has been identified as the constituent primarily responsible for the effects of yokukansan. Thus, it was shown to have a partial agonistic action for 5-HT$_{1A}$ receptors, and ameliorated isolation stress-induced aggressive behaviors and reduced sociality in mice by stimulating 5-HT$_{1A}$ receptors (Nishi et al., 2012). GM was also found to ameliorate glutamate-induced neurotoxicity in the pheochromocytoma (PC12) cell line concomitant with an increase in glutathione levels in these cells (Kawakami et al., 2011). On the other hand, a recent basic study proposed that GM could be absorbed into the blood after the oral administration of yokukansan, and then reached the brain by crossing the blood–brain barrier (BBB) (Imamura et al., 2011). Generally, compounds with pharmacological actions exert their effects by interacting with target molecules such as receptors, channels, and enzymes. However, the target molecule(s) of GM in the brain have not yet been identified, and it remains unknown whether GM can specifically bind to brain tissues. Thus, the binding characteristics of GM in the brain need to be clarified in order to explore the pharmacological mechanisms of yokukansan.

The identification and characterization of GM binding sites in the brain will provide an insight into the neuropharmacological actions of GM and GM-containing herbal medicines. Therefore, the present study was designed to identify GM binding sites in the rat brain using quantitative autoradiographical and microautoradiographical techniques with tritium-labeled GM ([^{3}H]GM).

2. Materials and methods

2.1. Isolation of GM

GM was isolated from Uncaria Hook in Tsumura & Co. (Ibaraki, Japan), according to the method described previously (Yuzurihara et al., 2002).

2.2. Synthesis of [^{3}H]GM

[^{3}H]GM was synthesized from a demethylated derivative of GM by RC TRITEC AG Ltd. (Teufen, Switzerland). Briefly, GM was dissolved in 1,4-dioxane and hydrolyzed under alkaline conditions by lithium hydroxide for 2 h at 50 °C to produce demethylated GM, in which the methyl ester in the GM structure was converted to carbonic acid (refer to Fig. 1). The reactant solution was neutralized by adding HCl and then filtered through a Sephadex LH-20 column (GE Healthcare, Buckinghamshire, UK) to remove inorganic salt. The resultant solution was evaporated to dryness, and solid demethylated GM was subsequently obtained.

A total of 3.7 GBq of the [^{3}H]-labeled methyl nosylate solution was evaporated to dryness in a reaction flask. After the addition of solid demethylated GM and 1,8-diazabicyclo[5.4.0]undec-7-ene, the mixture was dissolved in dimethyl formamide and stirred for 4.5 h at room temperature. The corresponding product in the reactant solution was purified by HPLC using a Nucleodur C18 Gravity column (5 μm, 10 × 250 mm; Macherey-Nagel, Oensingen, Switzerland). The desired product was further purified and isolated from the HPLC solvent mixture by solid phase extraction. Thus, the solvent mixture was diluted with water, and loaded on a strata-X cartridge (3 ml, 100 mg; Phenomenex, Torrance, CA, USA), which was eluted with ethanol. The radioactivity and purity of the extracted product in the elutant were analyzed using a TSK gel ODS 80TsQA column (4.6 × 150 mm; Tosoh, Tokyo, Japan) in conjunction with a radio detector (Berthold LB 509; BioSurplus, San Diego, CA, USA). The relative radioactivity of [^{3}H]GM was 3.1 TBq/mmol, and radiochemical purity was 98.5%.

2.3. Animals

Naive seven-week-old male Wistar rats were purchased from Charles River Laboratories Japan (Kanagawa, Japan). They were housed three to five per cage in a temperature (23 ± 3 °C)-, relative humidity (40–70%)-, and light (12 h light/dark schedule; lights on at 7:00 a.m.)-controlled environment and were fed laboratory food and water ad libitum.

This study was carried out in accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the Japanese Association for Laboratory Animal Science. The protocol was approved by the Committee on the Ethics of Animal Experiments of Nemoto Science & Co. and Tsumura & Co. The experiments in the present study were designed to minimize the number of animals used.

2.4. Quantitative autoradiography

Because GM is the natural compound, we referred to our recent study (Mizoguchi et al., 2014) for the method of quantitative autoradiography, in which tritium-labeled 18β-glycyrrhetinic acid, a natural compound derived from glycyrrhiza, was used as the ligand to identify its specific binding sites in the rat brain using quantitative autoradiography. Thus, animals were sacrificed by decapitation, and the brains were quickly removed, immediately frozen in powdered dry ice, and stored at −80 °C. Sections (15 μm) were cut using a freezing microtome in the coronal or sagittal plane, mounted on gelatin-coated glass slides, and stored at −80 °C. The following coordinates relative to the bregma were used for the coronal plane: anteroposterior +4.00 and −3.30, and the sagittal plane: lateral +1.90 and +0.18. On the day of the experiment, sections were thawed, rinsed with 25 mM potassium phosphate buffer, pH 7.8, containing 0.01% ascorbic acid, and incubated with [^{3}H]GM in the same buffer for 4 h at room temperature, followed by 2 × 10 min washes in ice-cold buffer. The sections were then dipped for a few seconds in ice-cold