J. Pharm. Pharmacol. 1985, 37: 401–404
Received September 17, 1984

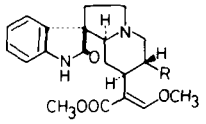
The active principles of the branchlet and hook of *Uncaria sinensis* Oliv. examined with a 5-hydroxytryptamine receptor binding assay

HIROTOSHI KANATANI, HIROSHI KOHDA*, KAZUO YAMASAKI, IZUMI HOTTA†, YOSHIHIRO NAKATA†, TOMIO SEGAWA†, ETSUJI YAMANAKA¶ NORIO AIMI¶ AND SHIN'ICHIRO SAKAI¶

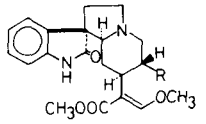
Department of Pharmacognosy, Institute of Pharmaceutical Sciences, Hiroshima University School of Medicine, Hiroshima 734, Japan, †Department of Pharmacology, Institute of Pharmaceutical Sciences, Hiroshima University School of Medicine, Hiroshima 734, Japan and ¶Faculty of Pharmaceutical Sciences, Chiba University, Yayoi-cho, Chiba 260, Japan

Of the alkaloids obtained from *Uncaria sinensis* Oliv., geissoschizine methyl ether, corynantheine and dihydrocorynantheine decreased specific [^{3}H]5-HT binding to membrane preparations from rat brain and from in-vitro experiments on guinea-pig ileum, these alkaloids were found to be partial agonists for 5-HT receptors. Therefore, they might be useful in the treatment of diseases resulting from disorders of 5-HT metabolism.

We earlier reported the development of a screening method for crude drug extracts using a 5-hydroxytryptamine (5-HT) binding assay and the results of this showed that some indole alkaloid-containing plants had strong inhibitory effects on 5-HT binding to membrane preparations from rat brain (Kanatani et al 1984). Among the crude drugs tested, the branchlet and hook of Uncaria was selected for further study because of its high inhibition on second screening. In Chinese (Kampo) medicine, this crude drug has long been used as a spasmolytic, an analgesic, a sedative and for the treatment of headache in hypertension, dizziness, cerebral arteriosclerosis and convulsions. The principle of the drug is mainly in the hooks (with some stems) of *Uncaria rhynchophylla* Miq. (Japanese origin) or *U. sinensis* Oliv. (Chinese origin). Many indole alkaloids have been isolated from *U. rhynchophylla*, e.g. rhynchophylline, isorhynchophylline, hirsuteine, hirsutine, dihydrocorynantheine, corynantheine, corynoxeine, isocorynoxeine (Haginiwa et al 1973), akuammigine, geissochizine methyl ether (Aimi et al 1977). However little is known of the pharmacology of these alkaloids, one of which, rhynchopylline, was proved to act on the central nervous system (Turumi 1958). In small doses it causes a stimulation of the respiratory centre and lowers blood pressure; in large doses it paralyses respiration and produces ataxia. Hirsutine shows a long-lasting depressive effect on the contractions elicited by electrical stimulation of the sciatic nerve or by direct stimulation of the muscle (Harada & Ozaki 1976). It also shows a ganglion blocking action when administered arterially (Harada et al 1974).



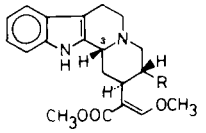
Rhyncophylline (RY)
R = $-CH_2-CH_3$



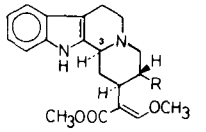
Isorhyncophylline (IR)
R = $-CH_2-CH_3$

Corynoxeine (CX)
R = $-CH=CH_2$

Isocorynoxeine (IC)
R = $-CH=CH_2$



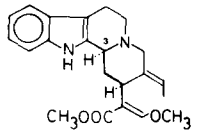
Hirsutine (HI)
R = $-CH_2-CH_3$



Dihydrocorynantheine (DC)
R = $-CH_2-CH_3$

Hirsuteine (HE)
R = $-CH=CH_2$

Corynantheine (CT)
R = $-CH=CH_2$



Geissochizine methyl ether (GM)

We have examined the active principles of the branchlet and hook of Uncaria and studied them pharmacologically.

* Correspondence.

MATERIALS AND METHODS

Plant materials

The hooks of *Uncaria sinensis* Oliv. (Rubiaceae) were obtained from Kojima-Shouten, Osaka, Japan. A part of the plants was deposited in the herbarium at the school of medicine, Hiroshima University.

Uncaria alkaloids

Nine alkaloids used for the pharmacological study were previously isolated from Uncaria plants as already reported (Haginiwa et al 1973; Aimi et al 1977). They were rhynchophylline, isorhynchophylline, corynoxeine, isocorynoxeine, hirsutine, hirsuteine, corynantheine, dihydrocorynantheine and geissoschizine methyl ether (GM).

Assay for specific [^{3}H]5-HT binding

Male Wistar rats ca 150 g were decapitated and the cerebral cortex and hippocampus were homogenized in 10 vol of ice-cold 0·32 M sucrose solution in a Teflon-glass homogenizer and then centrifuged at 700*g* for 10 min. The supernatant was centrifuged again at 50 000*g* for 10 min and the resulting pellet, a crude mitochondrial fraction, was re-suspended in 10 vol of ice-cold 50 mM Tris-HCl buffer solution and dispersed using a Polytron for 10 s. To remove endogenous 5-HT, the fraction was incubated at 37 °C for 10 min, then centrifuged again at 50 000*g* for 10 min and the resulting pellet was used as a membrane fraction.

The assay for specific [^{3}H]5-HT binding for control measurements was performed as follows. To 0·7 ml of membrane fraction in 50 mM Tris-HCl buffer solution, 0·1 ml of [^{3}H]5-HT (15·1 Ci mmol^{-1}, Radiochemical Centre, Amersham) solution (final concentration, 2 nM) and 0·1 ml of methanol or H_2O were added and a further 0·1 ml of 50 mM Tris-HCl buffer containing 10 μM nialamine or 0·1 ml of 5-HT in buffer solution (final concentration, 0·1 mM) was also added. The mixture was then incubated for 10 min at 37 °C. Bound [^{3}H]5-HT was separated from free by filtration on a Whatman GF/B glass fibre under vacuum with two 5 ml washes. Radioactivity trapped on the filter was counted in 8 ml of Univer-Gel (Nakarai Chemicals, Japan). For specific binding of the plant extract, 0·1 ml of extract solution was added to the incubation mixture instead of 0·1 ml of methanol or H_2O.

The inhibition percentage of plant extract for 5-HT binding was calculated using the formula: (B/A) × 100, where A and B are the specific binding values for control and extract, respectively. Saturation parameters were determined from regression lines in a Scatchard plot (Scatchard 1949) and Hill coefficients from Hill analysis (Hill 1909). Protein was determined by the method of Lowry et al (1951).

Effect on guinea-pig ileum

Fresh sections of male guinea-pig (200–300 g) ileum were suspended in Tyrode solution gassed with air and maintained at 37 °C. Muscle contractions were recorded isotonically with a writing lever on a smoked drum. The load applied to the tissue was approximately 1·5 g which allowed the preparation to develop sufficient contraction and tone.

RESULTS

Fractionation and biological activity of each fraction

The Uncaria plants (30 g) were extracted with boiling methanol (30 ml × 3) and the solution was evaporated to dryness. The residue (2·5 g) was suspended in 4% oxalic acid (30 ml) and the suspension was extracted with a mixture of $CHCl_3$ and methanol (9 : 1) (30 ml). Evaporation of the $CHCl_3$ layer to dryness yielded a $CHCl_3$-extract (Un-A, 510 mg). The aqueous layer was made alkaline with ammonia solution and extracted with $CHCl_3$ (30 ml × 4) and then with butan-1-ol (30 ml × 4). Evaporation of each solution to dryness yielded a $CHCl_3$-extract (Un-B, 15 mg) and a butanol-extract (Un-C, 50 mg). The remaining aqueous solution was lyophylized to yield an aqueous extract (Un-D).

Inhibition of [^{3}H]5-HT binding to the rat brain membrane preparation was tested for each fraction and the results were Un-B 85%, Un-C 70%, Un-A 34% and Un-D 32%.

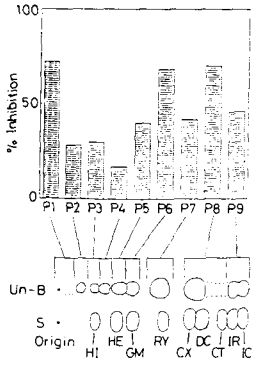


FIG. 1. Preparative TLC of Un-B and the inhibitory effect of each fraction on [^{3}H]5-HT specific binding to membrane preparations from rat brain. Solvent system of TLC: acetone–methanol (20 : 1), Plate: Kieselgel 60 G_{254}, Detection: uv (254 nm). S: standards.

The most active Un-B (mainly crude alkaloids) was further separated into nine fractions by preparative tlc, and the inhibitory activity of each fraction was assayed. As shown in Fig. 1, the activity was concentrated at fractions P-1, P-6 and P-8. When these fractions were compared to known alkaloids previously isolated from Uncaria plants by TLC, the fraction P-6 corresponded to GM, and P-8 corresponded to corynantheine, dihydrocorynantheine and corynoxeine.

Biological activity of pure Uncaria alkaloids

The effect of these alkaloids (GM, corynantheine, dihydrocorynantheine and corynoxeine) and other Uncaria alkaloids (rhyncophylline, isorhyncophylline, isocorynoxeine, hirsutine and hirsuteine) on

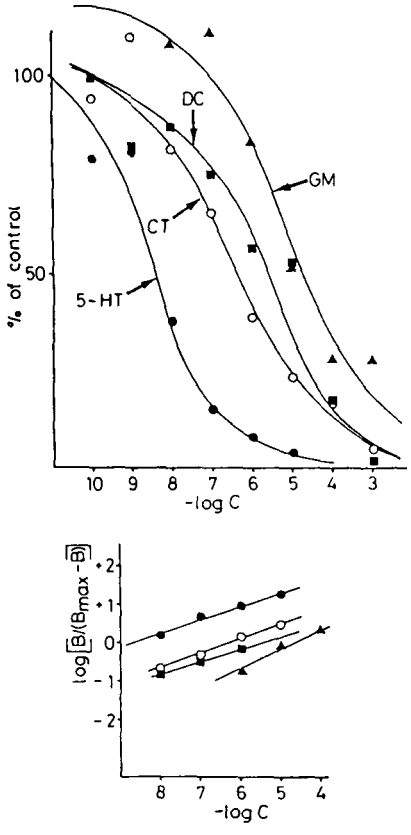


FIG. 2. Inhibitory effects of geissoschizine methyl ether (GM), dihydrocorynantheine (DC) and corynantheine (CT) on [^{3}H]5-HT specific binding to membrane preparation from rat brain. Above: Displacement curves, Below: Hill plots. Each mark, Hill slope and IC50 (M) calculated from Hill plots is: ●—5-HT, 0·38, 2·5 × 10^{-9}; ▲—GM, 0·57, 1·5 × 10^{-5}; ■—DC, 0·36, 2·2 × 10^{-6}; ○—CT, 0·40, 4·0 × 10^{-7}.

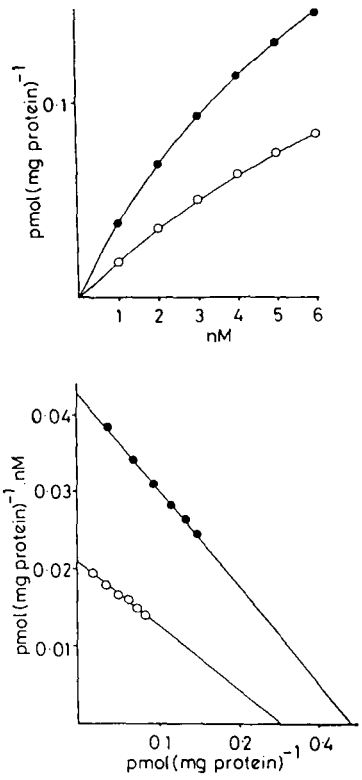


FIG. 3. Inhibitory effect of GM on [^{3}H]5-HT specific binding to membrane preparation from rat brain. Above: Saturation curves, Below: Scatchard plots, ● = control; [^{3}H]5-HT (1–6 nM), ○ = sample; [^{3}H]5-HT (1–6 nM) + GM (1 μM) $^*P < 0{\cdot}05$, $^{**}P < 0{\cdot}01$.
Similar plots were obtained with corynantheine (1 nM) and dihydrocorynantheine (1 nM).

	K_D (nM)	B_{max} (pmol mg prot.$^{-1}$)
Control (●)	7·72	0·33
GM (○)	12·38	0·26*
DC	13·39	0·25*
CT	8·5	0·19**

[^{3}H]5-HT binding was further investigated. Corynoxeine from the active fraction, and another five alkaloids from a less active fraction were ineffective. While alkaloids GM, corynantheine and dihydrocorynantheine from the active fraction were effective in displacing [^{3}H]5-HT binding (Fig. 2). The inhibition curves for these three alkaloids began to plateau at a concentration of about 10^{-3} M. Corynantheine was the most potent as an inhibitor of [^{3}H]5-HT binding, followed by dihydrocorynantheine and GM. Hill coefficients for all alkaloids were less than unity, indicating negative-cooperative binding properties. IC50 values for [^{3}H]5-HT binding are also shown in Fig. 2.

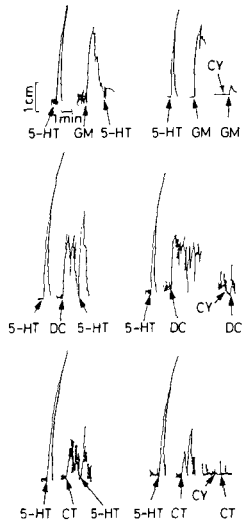


FIG. 4. Effect of geissoschizine methyl ether (GM), dihydrocorynantheine (DC) and corynantheine (CT) on the guinea-pig ileum. The bath was washed out with Tyrode solution after maximal contractions by 5-HT or alkaloids. 5-HT; 50 μM, GM; 50 μM, DC; 50 μM, CT; 50 μM and CY; cyproheptadine (5 μM).

The effects of GM, dihydrocorynantheine and corynantheine on specific [^{3}H]5-HT binding were analysed by Scatchard's method. The results are presented in Fig. 3. All three alkaloids shifted the line from the control to the left in a parallel manner. There was thus no significant difference between the dissociation constant (K_D) for control and alkaloid-treated membranes, but the maximal number of binding sites (B_{max}) decreased significantly.

Responses of the guinea-pig ileum to the alkaloids are shown in Fig. 4. All three alkaloids, at 5×10^{-5} M, contracted the ileum, the effect being one half, or less, of that induced by the same concentration of 5-HT. The response was almost completely abolished by the previous addition of cyproheptadine (5 μM). Furthermore, the alkaloids antagonized the 5-HT induced contraction of the ileum. Therefore, they seem to be a partial agonists for 5-HT receptors.

DISCUSSION

Of the alkaloids obtained from the hook of *Uncaria sinensis*, GM, corynantheine and dihydrocorynantheine, which belong to the 3α-H corynanthe-type, decreased specific [^{3}H]5-HT binding to membrane preparations from rat brain. While oxindole-type alkaloids, such as rhyncophylline, isorhyncophylline, corynoxeine and isocorynoxeine did not show this activity. Of the corynanthe-type alkaloids, however, hirsutine and hirsuteine, which have 3β-H configurations, were also ineffective. The stereochemical structure of effective 3α-H corynanthe-type alkaloids is fairly planar in comparison with the ineffective 3β-H compounds. This difference may lead to a clarification of the structure of the active site of the 5-HT receptor.

From a Scatchard plot, the alkaloids were found to decrease the maximal number of binding sites without modifying the dissociation constant. Furthermore, from the in-vitro experiments on guinea-pig ileum, they were found to be 5-HT antagonists have a partial agonistic action on 5-HT receptors.

Because 5-HT has a wide spectrum of activity some of the clinical effects of *Uncariae ramulus et uncus* may be due to its antagonistic and/or agonistic action on 5-HT receptors. Several compounds thought to be 5-HT antagonists have been used in the prophylactic treatment of migraine and other vascular headaches. The mechanism by which these compounds act remains to be determined but GM, dihydrocorynantheine and corynantheine might have a use in the treatment of migraine or other headaches.

The present results suggest that the receptor binding assay provides an inexpensive, rapid and efficient in-vitro screening method for active constituents expecially when only small quantities are available. Furthermore, the assay enables some deductions to be made about structure-activity relations and molecular mechanisms of the constituents in-vitro.

REFERENCES

Aimi, N., Yamanaka, E., Shinma, N., Fujiu, N., Kurita, J., Sakai, S., Haginawa, J. (1977) Chem. Pharm. Bull. 25: 2067–2071

Haginiwa, J., Sakai, S., Aimi, N., Yamanaka, E., Shinma, N. (1973) Yakugaku Zasshi 93: 448–452

Harada, M., Ozaki, Y., Sato, M. (1974) Chem. Pharm. Bull. 22: 1372–1377

Harada, M., Ozaki, Y. (1976) Ibid. 24: 211–214

Hill, A. V. (1909) J. Physiol. (London) 39: 361–373

Kanatani, H., Kohda, H., Yamasaki, K., Hotta, I., Nakata, Y., Segawa, T. (1984) Shoyakugaku Zasshi 38: 262–271

Lowry, O. H., Rosenbrough, N. J., Farr, A. L., Randall, R. J. (1951) J. Biol. Chem. 193: 265–275

Scatchard, G. (1949) Ann. N.Y. Acad. Sci. 51: 660–672

Turumi, K. (1958) Acta Scholae Medicinalis, Universitatis in Gifu 6: 790–795