Naturally Occurring Somatostatin and Vasoactive Intestinal Peptide Inhibitors. Isolation of Alkaloids from Two Marine Sponges

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Abstract

The vasoactive intestinal peptide (VIP) and somatostatin (somatotropin release inhibiting factor, SRIF) are important neurotransmitters in a number of basic physiological events. Their disturbances have been reported in many diseases such as cystic fibrosis, impotent man (VIP), Alzheimer's disease, and some tumours (SRIF). Xestospongine B (**1**), sceptrine (**2**), and ageliferine (**3**), three alkaloids isolated from *Xestospongia* sp. and *Agelas novaecaledoniae* are reported as somatostatin and VIP inhibitors. The natural products **1**, **2**, and **3** exhibited a high affinity for somatostatin (IC_{50} = 12 μM, 0.27 μM, and 2.2 μM, respectively), **2** and **3** showed an affinity for VIP (19.8 μM and 19.2 μM, respectively). Due to the interaction between non-peptidic compounds and somatostatin/VIP receptors, these three alkaloids could be promising agents in the research on natural non-peptidic compounds for therapeutical interventions.

Key words

Porifera, *Nepheliospongida, Axinellida*, ageliferine, sceptrine, xestospongine B, somatostatin receptor, vasoactive intestinal peptide receptor.

Abbreviations

VIP:	vasoactive intestinal peptide
SRIF:	somatotropin release inhibiting factor
CNS:	central nervous system
PNS:	peripheral nervous system
[^3H]-QNB:	specific ligand of mAChR
mAChR:	muscarinic acetylcholine receptors

Introduction

The vasoactive intestinal peptide (VIP) is an important neurotransmitter for basic physiological events: it may regulate the cortical blood flow, the energy metabolism and the neuronal activity in the central nervous system (CNS), and has a modulator role in pituitary hormone secretion. VIP has also a wide distribution in the peripheral nervous system (PNS): the main actions are stimulation of secretions (oesophagus, stomach, airway smooth muscle, salivary, water and bicarbonate), control of release of glucagon and insulin, relaxation of vascular and non-vascular smooth muscle, and vasodilatation of the genital system. The deficiency in VIP was reported in Hirschsprung's disease, achalasia of oesophagus, cystic fibrosis, and in impotent man (1). A VIP antagonist inhibited proliferation in non-small cell lung cancer (2). As far as we know, a high affinity between the VIP receptor and a non-peptidic compound has not been described previously.

On the other hand, the somatostatin neurotransmitter (somatotropin release inhibiting factor, SRIF) displayed in some tumour processes (pituitary, pancreas, brain, and breast tumours) (3). Somatostatin presumably plays a role in Alzheimer's disease on account of its low level found as the most consistent biochemical abnormality (4, 5). Recently, the peptidic compounds, octreotide and its analogs, were reported as ligands of somatostatin receptor (6, 7).

Because of the well-known number of biologically active compounds isolated from marine organisms with high affinities for cellular receptors (8), more than 300 marine invertebrates from New Caledonia were screened on 20 receptors using binding methods (9). Nine percent of organisms showed a high affinity to some receptors. *Xestospongia* sp. and *Agelas novaecaledoniae* were then investigated for VIP and somatostatin receptors by a radioreceptor binding assay. This is the first time that non-peptidic compounds from these marine organisms are reported as VIP and somatostatin inhibitors.

Materials and Methods

Bioactive samples study

Animal material: The sponge *Xestospongia* sp. (order Nepheliospongida; family Nepheliospongiidae) was collected at Baie de Prony, New Caledonia at a depth of 10 m, and *Agelas novaecaledoniae* (order Axinellida; family Agelasidae) was dredged on the Norfolk ridge at 200 m deep. They were identified

by Pr. Claude Levi of Museum National d'Histoire Naturelle (Paris). Voucher specimens (R602 and R1413, respectively) are available from Zoothèque at ORSTOM, Nouméa.

Purifications: The bioactive compounds were isolated by bioassay-guided chromatography.

1. *Xestospongia* sp.: Xestospongine B (**1**) was extracted according to the method described by Quirion et al. (10). In short, the freeze-dried sponge (100 g) was extracted at room temperature with EtOAc (2 × 500 ml, 24 h). The organic phase was then extracted with dilute HCl (pH 3). The aqueous phase was basified with aqueous NH_4OH to pH 10 and extracted with EtOAc to give the alkaloid fraction (3.8 g). The ethyl acetate layer was purified by a silica gel (2 × 40 cm) flash chromatography (hexane/Et_2O/MeOH/NH_4OH, 20 : 70 : 10 : 0.5) affording compound **1** (64 mg) and other alkaloids.

2. *Agelas novaecaledoniae*: The freeze-dried sponge (100 g) was extracted with 80% EtOH (3 × 500 ml) according to Païs (personal communication). The solvent was evaporated to dryness. The residue (11 g) was dissolved in water (600 ml), filtered, and passed through a column of Amberlite XAD-7 (250 ml) (2 ml/min). The column was washed with water and then eluted with MeOH/H_2O (1 : 1). The methanol-water fraction (2.35 g) was subjected to a medium pressure silica gel (Merck: 7736) column chromatography eluted with EtOAc/2-butanone/formic acid/H_2O (5 : 3 : 0.5 : 0.5) (11) affording slightly impure sceptrine formate (1.20 g) and ageliferine formate (0.20 g), which were purified using flash chromatography on a C18 column (Waters 5.5–10.5 cm). Pure formate of sceptrine (**2**; 0.45 g) and ageliferine (**3**; 0.12 g) were eluted with 250 ml of H_2O/MeOH (3 : 2).

2 · *formate salt*: FAB-MS: *m/z* = 621 (MH^+); IR, UV, ^{1}H- and ^{13}C-NMR were identical to the published spectra for the acetate (12, 13) except for signals at δ = 8.26 (1H, s) in the ^{1}H-NMR (DMSO-d_6) and δ = 172.29 in the ^{13}C-NMR (H_2O) for HCO_2H. A solution of the formate salt (0.12 g) in MeOH was passed through a column of IRA-410 resin in OH^- form yielding the free base, which was transformed into the hydrochloride form using HCl, and then chromatographed on a C18 column (1 × 10 cm) H_2O/MeOH (3 : 2) yielding the pure hydrochloride salt (0.80 g) which was crystallized from water, mp 117–130 °C dec), $[\alpha]_D$: −22° (*c* 1, MeOH); Lit. (1): mp 115–125 °C (dec), $[\alpha]_D$: −7.4° (*c* 1.2, MeOH); Lit. (3): $[\alpha]_D$: −18° (*c* 4.33, MeOH).

3 · *formate salt*: FAB-MS: *m/z* = 621 (MH^+); IR, UV, ^{1}H- and ^{13}C-NMR were identical to the published spectra (13, 14) except for signals at δ = 8.50 (1H, s) in the ^{1}H-NMR (DMSO-d_6) and δ = 170.89 in the ^{13}C-NMR (H_2O) for HCO_2H. The formate salt was transformed into the hydrochloride salt as described for **2**, $[\alpha]_D$: +7° (*c* 0.7, MeOH); Lit. (2): $[\alpha]_D$ +15.5° (*c* 0.11, MeOH); Lit. (3): $[\alpha]_D$: +4.1° (*c* 0.146, MeOH).

Screening

Extraction (screened extracts): The freeze-dried powder of the two marine sponges (100 g) was extracted with distilled water (4 °C, dark room); the extract was then centrifuged and the freeze-dried supernatant gave extract A.

The residue was extracted with (3 × 500 ml) 80% EtOH at room temperature and the pooled extract was concentrated in vacuo. The residue was diluted with water (1000 ml, 24 h) and partitioned with CH_2Cl_2 (3 × 1000 ml). The aqueous layer was freeze-dried, and the powder dissolved with absolute EtOH to obtain extract B. The dichloromethane fraction gave extract C.

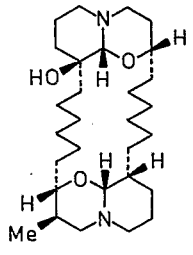
Extracts A and B were further desalted and proteins eliminated through successive RP-8 (Merck: 9324) and silica gel 60H columns (Merck: 7736).

Binding bioassays

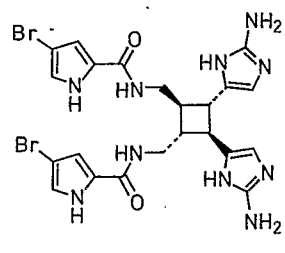
Membranes were obtained from the brain cortex of Sprague-Dawley male rats, homogenized in tris-HCl buffer – 50 nM HCl (pH 7.5), and concentrated by ultracentrifugation (70,000 × g for 10 min). The average concentration in protein was 8–14 mg/ml.

Somatostatin binding: Somatostatin binding receptor assay used [^{125}I]-somatostatin (0.02 nM ± 0.002 nM) as the labelling ligand. The equilibrium binding experiment was done at 25 °C with 0.05 mg/ml membrane preparation in HEPES-KOH buffer 20 nM (pH 7.5), containing 5 mM $MgCl_2$, 0.6% BSA, 0.25 mg/ml bacitracin, and 1 mg/ml aprotinin. Aliquots (0.6 ml) of this preparation were incubated for 60 min. Non-specific binding was determined using 100 nM somatostatin. Binding values of **1**, **2**, and **3** (5 μg/ml) were determined as the deplacement of the specific binding of the labelling ligand. The binding was terminated by filtration through Whatman GF/B glass fibre filters with a cell harvester, followed by three washes with 3 ml of 20 nM HEPES-KOH buffer (pH 7.5). The radioactivity retained on the filters was determined directly with a gamma scintillation counter.

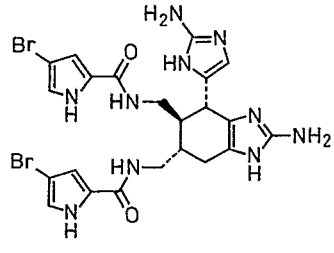
Vasoactive intestinal peptide (VIP) binding: The VIP receptor assay used [^{125}I]-VIP (0.02 nM ± 0.002 nM) as the labelling ligand. The equilibrium binding experiment was done at 37 °C with 0.05 mg/ml membrane preparation in 10 nM PIPES buffer (pH 7.4) containing 2.5 mM $MgCl_2$, 0.1 M mercaptoethanol and 0.2 mg/ml bacitracin. Aliquots (0.6 ml) of this preparation were incubated for 120 min. Non-specific binding was determined using 100 nM VIP. The binding of **2** and **3** was determined as the deplacement of labelling VIP from the specific binding. The binding was terminated by filtration through Whatman GF/B glass fibre filters with a cell harvester, followed by three washes with 4 ml of 10 nM PIPES buffer (pH 7.5). The radioactivity retained on the filters was determined directly with a gamma scintillation counter.



1 Xestospongine B



2 Sceptrine



3 Ageliferine

Table 1 Somatostatin and VIP inhibition from different extracts and pure compounds.

Marine organism	extract/pure compound	concentration (μg/ml)	somatostatin (% inhibition)	VIP (% inhibition)
Xestospongia sp.	ethanolic	20	52	inactive
	dichloromethane	20	41	inactive
	Xestospongine B	5	84	inactive
Agelas novaecaledoniae	ethanolic	20	99	94
	Sceptrine	5	94	89
	Angeliferine	5	93	94

Results and Discussion

Xestospongia sp.: The solvent bioassay-guided partition showed that the alkaloid fraction of the *Xestospongia* alcohol extract was active (Table 1). Five alkaloids were previously isolated and identified from this sponge, four of them belonging to the xestospongine series [araguspongine F, xestospongine B (**1**), xestospongine D, demethylxestospongine B] and xestoamine. These alkaloids (base) were assayed on the somatostatin receptor, and **1** exhibited a good activity with an $IC_{50} \approx 12\,\mu M$ (K_i = 13.4 μM).

This compound, previously isolated from the Australian marine sponge *X. exigua*, has been reported to be a vasodilatative compound, inducing relaxation of blood vessels *in vivo* (15), and to demonstrate also general cytotoxic activity (10). This is the first time, as far as we know, that this compound has been reported to be an inhibitor of somatostatin receptors.

Agelas novaecaledoniae: The compounds **2** and **3** (hydrochloride salts), showed a strong activity on the somatostatin receptor and a medium activity on the VIP receptor (Table 1).

Somatostatin receptor: **2**: $IC_{50} \approx 0.27\,\mu M$ (K_i = 0.30 μM), **3**: $IC_{50} \approx 2.21\,\mu M$ (K_i = 2.47 μM).

Vasoactive intestinal peptide receptor: **2**: $IC_{50} \approx 19.2\,\mu M$ (K_i = 61.8 μM), **3**: $IC_{50} \approx 19.8\,\mu M$ (K_i = 63.8 μM).

The compounds **2** and **3** have already been isolated previously in other *Agelas* species (13, 14): **3** isolated from an Okaniwan *Agelas* sp. was found to be a potent actomyosin ATPase activator (14). This compound also inhibits growth of *Bacillus subtilis*, and is active against *Escherichia coli*, Herpes simplex virus type 1 and Vesicular stomatitis virus; **3** has a moderate barnacle settlement inhibition, showing some potential antifouling activity (13).

The compound **2** isolated from *A. conifera* was found to be an ichtyotoxic principle (16) and, isolated as a dichloride, was reported as the major antimicrobial constituent of *Agelas spectrum* (12), as an antagonist of serotonergic receptor (17), and as a competitive inhibitor of [^3H]-QNB binding to rat brain mAChR (18). The activity of **2** was 143 times more potent on the somatostatin receptor and 2 times more potent on the VIP receptor than the activity displayed on the [^3H]-QNB binding assay.

The non-peptidic compounds **2** and **3** exhibited a high affinity for somatostatin and VIP receptors and **1** for the somatostatin receptor. This information makes it possible to generate a new class of drugs used for therapeutical intervention in VIP and SRIF systems.

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