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Chief Editor José Paula

Coral reefs of Mauritius in a changing global climate

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Aims and scope: The *Western Indian Ocean Journal of Marine Science* provides an avenue for the wide dissemination of high quality research generated in the Western Indian Ocean (WIO) region, in particular on the sustainable use of coastal and marine resources. This is central to the goal of supporting and promoting sustainable coastal development in the region, as well as contributing to the global base of marine science. The journal publishes original research articles dealing with all aspects of marine science and coastal management. Topics include, but are not limited to: theoretical studies, oceanography, marine biology and ecology, fisheries, recovery and restoration processes, legal and institutional frameworks, and interactions/relationships between humans and the coastal and marine environment. In addition, *Western Indian Ocean Journal of Marine Science* features state-of-the-art review articles and short communications. The journal will, from time to time, consist of special issues on major events or important thematic issues. Submitted articles are subjected to standard peer-review prior to publication.

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Word from the Editor

The last couple of years have been a time of change for the Western Indian Ocean Journal of Marine Science. The journal has a new and more modern layout, published online only, and the editorial Board was increased to include more disciplines pertaining to marine sciences. While important challenges still lie ahead, we are steadily advancing our standard to increase visibility and dissemination throughout the global scientific community. The central objective of the journal continues focused on the Western Indian Ocean region and serving its growing scientific community.

We are pleased to start the publication of special issues of the journal, launched here with the publication of manuscripts from the University of Mauritius Research Week 2016. The special issues aim to contribute for advancing marine science in the WIO by focusing on specific themes, geographical areas or assembling contributions from scientific meetings. The editorial processes are exactly the same as for regular issues, with double peer-review, and guest editors are considered.

José Paula
Chief Editor

Editorial Note · Coral reefs of Mauritius in a changing global climate

The University of Mauritius Research Week (UoM RW) has been held on an annual basis since 2007 and was organized for the 9th time from 19-23 September 2016. The Research Week is geared towards dissemination of knowledge generated through research activities at the University and by relevant stakeholders in accordance with the UoM's vision of "*Excellence in Research and Innovation*". In line with national priorities, the UoM organizes this event to provide insightful research outcomes not only for the advancement of academic knowledge, but for the benefit of the community at large, through robust policy recommendations.

Out of the multiple submissions made during the UoM RW 2016, a number of manuscripts in the field of ocean/marine sciences were selected to be published in the Western Indian Ocean Journal of Marine Science (WIOJMS), as a special issue entitled "Coral reefs of Mauritius in a changing global climate". This issue is presented in the context of Mauritius being surrounded by a beautiful but delicate coral reef ecosystem, which provides ample ecosystem services contributing to the national economy, but which is subjected to extreme climatic events. Hence, in this special issue several contributions advancing our scientific understanding for sustainable use and management of marine resources in a globally changing marine environment are articulated. The original article by Mattan-Moorgawa *et al.* investigates the photo-physiology of diseased and non-diseased corals. Coral diseases are becoming more common on reefs worldwide due to both local and global stressors. Ramah *et al.* then present a short communication related to substrate affinity by two giant clam species found on the Mauritian coral reefs. Giant clams are under threat worldwide and information on their substrate affinity and habitat aims at providing insightful information towards their sustainable management. In addition, Nandoo *et al.*, in an effort to optimize nucleic acid extraction protocols from marine gastropods, present an original article based on a comparative study using the gastropod genera *Planaxis*, *Cypraea* and *Drupella*. These marine gastropods are ecologically important for coral reefs, especially the coral-eating *Drupella*. Moreover, given the importance of intertidal molluscs, Kaullysing *et al.* document the density and diversity of the benthic molluscs while comparing sheltered and exposed coastal habitats. Appadoo & Beeltah report on the biology of *Platorchestia* sp. (Crustacea, Amphipoda) at Poste La Fayette, Mauritius. Studies on Amphipod diversity and distribution are important especially since studies on marine biodiversity are scarce around Mauritius. Another original article by Ragoonaden *et al*. analyses the recent acceleration of sea level rise in Mauritius and Rodrigues. Such studies are more important than ever in the light of a globally changing marine environment with small island states faced with issues related to rising sea level. Two field notes, based on field observations, are presented by Bhagooli *et al.*, documenting a variety of coral diseases, and *Stylophora pistillata*-like morphotypes occurring around Mauritius Island, respectively. Kaullysing *et al.* also present a field note on coral-eating gastropods observed around Mauritius.

Apart from the local contributors, international collaborators also contribute two original articles in this special issue. Casareto *et al.* characterize the chemical and biological aspects of a coral reef of Mauritius focusing on benthic carbon and nitrogen fixation. These studies related to benthic productivity are important for understanding sustainability of coral reefs and/or lagoonal fisheries. On the other hand, Tokumoto *et al.* document the first detection of membrane progestin receptor (mPR)-interacting compounds from Mauritian coral reef and lagoonal seawater. They used cutting-edge technology to detect key regulators of reproduction in seawater. These contributions in terms of original articles, short communications, and field notes generate new scientific knowledge that may better inform policy and decision making in the field of coral reef studies and management in Mauritius, while contributing to the understanding of coral reefs in the wider Western Indian Ocean region.

Prof. Sanjeev K. Sobhee
Pro-Vice Chancellor (Academia)
The University of Mauritius

Detecting membrane progestin receptor (mPR)-interacting compounds from coral seawater in Mauritius

Toshinobu Tokumoto[1]*, Shinya Kodani[1], Takehiro Miyazaki[1], Yoshimi Suzuki[1], Beatriz Casareto[1], Theeshan Bahorun[2], Ranjeet Bhagooli[3]

[1] Integrated Bioscience Section, Graduate School of Science and Technology, National University Corporation, Shizuoka University, Ohya 836, Suruga-ku, Shizuoka 422-8529, Japan

[2] ANDI Centre of Excellence for Biomedical and Biomaterials Research MSIRI Building, Réduit 80837, Republic of Mauritius

[3] Department of Marine & Ocean Science, Fisheries & Mariculture, Faculty of Ocean Studies, University of Mauritius, Réduit 80837, Republic of Mauritius

* Corresponding author: tokumoto.toshinobu@shizuoka.ac.jp

Abstract

Progestins are key regulators of reproduction. Although the physiological effects of progesterone are mediated by regulating the expression of genes associated with nuclear progesterone receptors (nPRs), new insights into progesterone activity was provided when membrane progestin receptors (mPRs) were identified. Also, mPRs have been shown to be a novel target for endocrine disrupting chemicals (EDCs). Natural hormonal compounds or artificial chemicals that interact with mPRs were sought as possible novel pharmaceuticals or EDCs.

Chemicals dissolved in coral reef seawater from Albion, Mauritius that interact with mPRs were screened during this study. The relative binding affinity of these compounds to the mPRα *in vitro* was evaluated using a steroid binding assay with crushed cell membranes from stably transfected cells containing the mPRα gene. As a result, binding activity in the compounds obtained from coral reef was detected. The highest binding activity was detected in a lagoon area when compared with that in the reef and beach areas. These results suggest that natural hormonal compounds with affinity for mPRα are produced, and under some specific conditions, accumulated, in seawater of a coral reef area at Albion.

Keywords: coral seawater, natural compound, membrane progestin receptor, progestin

Introduction

Progestins are a class of steroid hormones that act as key-regulating factors controlling reproduction. Natural progestin, 17α,20β-dihydroxy-4-pregnen-3-one (17,20β-DHP), was identified in different fish species as the oocyte maturation-inducing steroid (Nagahama & Adachi, 1985). Synthetic progestins have been produced and are frequently used for medical purposes. Progesterone is a natural progestin in humans (Stanczyk, 2003) produced by the ovary, depending on physiological conditions and gonadotropin levels (Ashley *et al.*, 2009). Although the physiological effects of progesterone are mediated by regulating the expression of genes associated with nuclear progesterone receptors (nPRs) (Boonyaratanakornkit & Edwards, 2004), new insights into the activities of progesterone were provided when membrane progestin receptors (mPRs) were identified (Zhu *et al.*, 2003a; Zhu *et al.*, 2003b). Nowadays, the new concept is accepted that progestins act on mPRs and induce nongenomic actions. mPRs have also been recognized as new targets for endocrine disrupting chemicals (EDCs). However, while both nPRs and mPRs are receptors for progestins, there are no sequence similarity between these two receptors. Thus, the structure of nPRs and mPRs are thought to be completely different. Natural hormonal compounds or artificial chemicals

that interact with mPRs as possible novel pharmaceuticals or EDCs were sought. Certain nongenomic effects of progestins, such as oocyte maturation, are mediated by mPRs on the plasma membrane and induce rapid intracellular changes. This nongenomic progestin-induced activity causes the cells to proceed through meiosis (Tokumoto *et al.*, 2006; Zhu *et al.*, 2003a). The broad distribution of mPRs in different tissues suggests that they perform different functions in a large range of target tissues, such as sperm motility (Ito *et al.*, 2011; Tubbs & Thomas, 2009), T-lymphocytes proliferation (Ndiaye *et al.*, 2012) and sex behavior (Frye *et al.*, 2014).

Thus, in medical terms, mPRs are attractive pharmaceutical targets. For instance, high expression levels of mPR mRNAs have been reported in ovarian and breast cancer cells (Charles *et al.*, 2010; Dressing *et al.*, 2012). It has been proposed that a signaling pathway of breast cancer progression mediated by mPRα occurs in basal phenotype breast cancer (Zuo *et al.*, 2010). mPR homologues have also been identified in yeast as a receptor for an antifungal protein, osmotin, (Narasimhan *et al.*, 2005), so mPR homologues in fungus are possible targets for anti-fungal drugs (Villa *et al.*, 2011). By the finding of agonistic activity of diethylstilbestrol (DES) on fish oocyte maturation, mPRs have also been recognized as new targets for EDCs. Treating oocytes with the EDC, DES induces final oocyte maturation in goldfish and zebrafish (Tokumoto *et al.*, 2004). Data from steroid binding assays have demonstrated that some chemicals, such as DES, DES analogues, Nandrolone and Org2058 show binding affinity for mPRs (Thomas *et al.*, 2007; Tokumoto *et al.*, 2007). Therefore, it is admissible to hypothesize that such artificial or natural compounds instead of steroid hormones have the ability to act like mPR ligands. Screening systems for mPR-interacting chemicals need to be established to help the search for novel pharmaceuticals and to identify new EDCs.

Recent research efforts have been geared towards establishing procedures to screen potential mPR ligands (Tokumoto *et al.*, 2016). A steroid binding assay using crushed cell membranes from stably transfected cells with the mPR gene is well established and applicable (Tokumoto *et al.*, 2007). Cell lines with a transformed cDNA for mPRα and a recombinant luciferase gene named Glosensor have also been established (Nakashima *et al.*, 2015). These cells can be used to monitor the effects of ligands on mPRα based on intracellular cAMP levels. The study using these cell lines indicated that cAMP concentration decreases in response to an mPRα ligand (Nakashima *et al.*, 2015). The results support previous findings suggesting that mPRα is coupled to G proteins activating preferentially inhibitory G proteins (Gi) regulating adenylyl cyclase activity (Thomas, 2008; Thomas *et al.*, 2007; Zhu *et al.*, 2003b).

So far, expressing and purifying a recombinant mPRα protein in *Pichia pastoris* yeast has been successful (Hossain *et al.*, 2015; Oshima *et al.*, 2014). A relatively large amount of mPRα protein with hormone binding activity was purified using established methods. The recombinant protein will be useful to establish a molecular probe to detect mPRα interacting agents.

Though mPRs are recognized in a wide diversity of organisms, from fish to humans (Thomas, 2008; Tokumoto *et al.*, 2012), and progestin binding activity has particularly been revealed in many species (Ito *et al.*, 2011; Josefsberg Ben-Yehoshua *et al.*, 2007; Kazeto *et al.*, 2005; Roy *et al.*, 2017; Thomas *et al.*, 2007; Tokumoto *et al.*, 2006; Zhu *et al.*, 2003a), no studies have investigated which chemical compounds from seawater interact with mPRs. EDCs that interact with mPRα were not detected in samples from polluted areas in Mauritius. Surprisingly, a potent activity in the samples from coral reefs was detected, where the site was originally set as a negative control. It was decided to identify possible new natural hormonal compounds from coral seawater on which to apply novel research tools that specifically interact with mPRα. Sampling from coral seawater was started in Thailand, and Okinawa, Japan. However, no, or only low binding activity was detected in samples from Thailand or Okinawa. It is thought that hormonal active compounds were accumulated in shallow coral reefs at Albion. A sampling point was therefore established at Albion, Mauritius. At first three different areas (coast, lagoon, and surf) at Albion beach and Flic-en-Flac were sampled (Sadally *et al.*, 2015). Here, we describe the detection of natural hormonal compounds from coral seawater under the prevailing conditions in Albion coastal waters.

Materials and Methods

Sample collection and eluting the compounds from the column

Seawater samples were collected in 10-L plastic bottles. The compounds dissolved in the seawater were concentrated in a column (Sep-Pak C18, Plus PS-1 and Oasis HLB; Waters Corp. Milford, MA, USA) after filtration through a glass fiber filter connected to a peristaltic pump that traps particles > 1 µm. Approximately

20–50 L of seawater was applied to a single column. After concentrating the samples, the columns were dried down and transported to Japan.

The columns were washed with ultra-pure water to remove salts. Then, bound compounds were eluted with acetone, and the acetone solution was dried under a stream of nitrogen gas. The resulting powdered compounds were dissolved in ethanol to measure mPR-interacting activity.

Steroid binding assay

Membrane fraction preparation: The cells were washed three times with PBS, scraped into HAED buffer (Hepes, 25 mM; NaCl, 10 mM; EDTA 1 mM; dithiothreitol, 1 mM; pH 7.6 at 4 °C), and sonicated for 15 sec, followed by centrifugation at 1,000 × g for 7 minutes to remove any nuclear and heavy mitochondrial material. The resulting supernatant was further centrifuged at 20,000 × g for 20 minutes to obtain the plasma membrane fraction (Tokumoto *et al.*, 2007).

Membrane binding assays: Steroid binding assays were conducted to measure mPR-interacting activity of compounds in samples by using radio-labeled progestin. [1,2,6,7 ^{3}H]-17α-Hydroxyprogesterone (85 Ci/mmol) was purchased from Amersham Biosciences (Piscataway, NJ, USA) and enzymatically converted to radiolabeled 17,20β-DHP by 3α,20β-hydroxy-steroid dehydrogenase (Sigma, St Louis, MO, USA), as described by Scott *et al.* (1982). Progestin receptor binding was measured in the membrane fraction following procedures established previously (Tokumoto *et al.*, 2007). Four mL of ethanol or samples dissolved in ethanol was added into each tube. Two hundred mL of membrane fraction and 200 mL of [^{3}H]-17,20β-DHP solution were added and incubation was started. A set of three tubes contained 2 nM [^{3}H]-17,20β-DHP (total binding), and another set of three tubes contained samples to measure competitive binding activity. After a 30-min incubation at 4°C, the reaction was stopped by filtration through GF/B filters (Whatman, Maidstone, UK) presoaked in HAED buffer containing 2.5% Tween 80. The filters were washed three times with 5 mL wash buffer (25 mM HEPES, 10 mM NaCl, and 1 mM EDTA; pH 7.4 at 4°C) and bound radioactivity was measured with a scintillation counter. If the samples contained the compounds binding to the progestin binding site of mPRα, radiolabeled 17,20β-DHP was displaced by the compounds. Displacement of the radiolabeled 17,20β-DHP was expressed as a percentage of maximum specific binding of 17,20β-DHP to the membrane fractions.

Luminescence assays

To confirm the results of the membrane binding assay, the activity of samples from seawater was assessed by a newly established assay.

The day before the assay, 8×10^4 cells/well were split into 96-well cell culture plates. Three wells were used for each sample. The culture medium was removed carefully the next day and 35 µL of CO_2-independent medium was added as equilibration medium containing a 2% (v/v) dilution of GloSensor™ cAMP Reagent stock solution and 0.001% Tween 80. Then, the plate was set in a luminescence detector (Luminescensor JNRII; ATTO, Tokyo, Japan). Detection was started immediately and continued for 30 minutes to 2 hours or until a steady-state basal signal was obtained at 25°C. Luminescence intensity was measured in each well every 5 min. Then, the plate was removed from the detector, and the medium was exchanged for CO_2-independent medium without luciferin and Tween 80 (125 µL/well) containing ligands dissolved in ethanol. After 5 minutes, 25 µL of forskolin stock solution (60 µM in CO_2-independent medium) was added, and the plate was returned to the detector. Luminescence intensity in each well was measured every 5 minutes. The maximum value detected at 5 minutes after forskolin stimulation was the end result (Nakashima *et al.*, 2015).

Results

The compounds that showed mPR binding activity were concentrated to identify natural mPR ligands. Seawater was sampled from a coral reef at Albion (Sadally *et al.*, 2015) and the compounds were concentrated and dissolved in seawater using Sep-Pak cartridges, which are commonly used in environmental assessments. Three resin types (Sep-Pak C18, Plus PS-1, and Oasis HLB) were tried in a pilot experiment to select the appropriate one to concentrate the compounds dissolved in coral seawater. Among them, Sep-Pak C18 tended to clog even after filtration with a glass filter to remove particles from the seawater samples. The Sep-Pak Plus PS-1 and Oasis HLB were not stacked and were able to concentrate > 50 L of seawater per cartridge. The Oasis HLB trapped a relatively large quantity of hormonally active compounds compared to the other cartridges. The Oasis HLB is designed to bind hydrophobic and hydrophilic compounds for a higher yield. Thus, the Oasis HLB cartridge was selected for further sampling.

Three different points in Albion coastal waters were checked for the presence of compounds that reacted

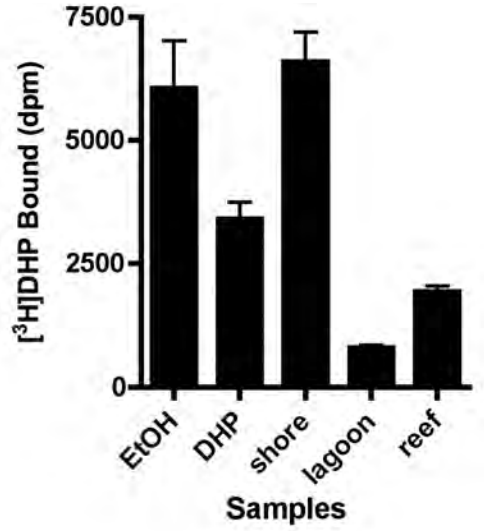

Figure 1. Competitive activity against progestin binding of crude extracts from coral seawater. Compounds dissolved in seawater were concentrated in a column. The samples (shore, lagoon, and reef) were eluted from the column twice with acetone and competitive progestin binding activity of the samples was determined. Competitive activity of the negative and positive control: ethanol as solvent for samples (EtOH); 17,20β-DHP at 1 μM (DHP) respectively, are included.

with the mPRα. The reef was considered the point closest to the edge of the lagoon, the lagoon was the central point between the reef and the beach, and the shore was in the area closest to the beach. Seawater was sampled and added to 10 L plastic bags. The seawater samples were pre-filtered with a glass filter cartridge and then concentrated in the small cartridges. Approximately 50 L of seawater was concentrated in a single cartridge. The cartridges were dried by air from a peristaltic pump and were delivered to a laboratory in Japan for further analysis. The compounds from seawater were eluted from the cartridge with acetone and dried under a stream of nitrogen gas. The dried samples were dissolved in ethanol at a concentration that corresponded to 100,000 times that in seawater. The reactivity of samples against mPRα was analyzed in the steroid binding assay using a membrane fraction prepared from goldfish mPRα-expressing cells. We detected competitive binding activity in samples from the lagoon and reef but not in those from the shore (Fig. 1). The activity was most potent in samples from the lagoon (Fig. 2). Samples from the lagoon showed competitive activity after a 1,000-fold (10^{-3}) dilution. This concentration corresponded to 100 times that in seawater. In contrast, samples from the reef showed competitive binding activity after a 100-fold (10^{-2}) dilution. The concentration corresponded to 1,000 times that in seawater. Samples from the shore had no specific binding activity.

The same results were obtained from the cell-based assay. Luminescence reflected the intracellular concentration of cAMP that changed in response to binding with mPRα in double-gene transfected culture cells with mPRα and recombinant luciferase. The effect of pre-treatment with the compounds on changes in intracellular cAMP concentrations stimulated by forskolin was analysed (Fig. 3A). The goldfish

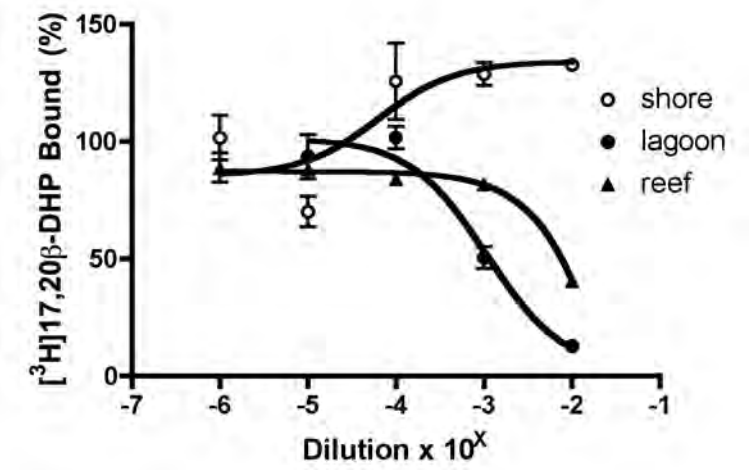

Figure 2. Concentration-dependent competitive progestin binding. A competition assay was conducted with serially diluted samples. The final sample concentrations were diluted 100–1,000,000 times, corresponding to 1,000–0.1 times of that in seawater. Values are relative to that of the 100% ethanol treatment. Nonlinear regression analyses for changes in luminescence were conducted using GraphPad Prism for Macintosh (ver. 6.0C; Graph Pad Software, San Diego, CA, USA).

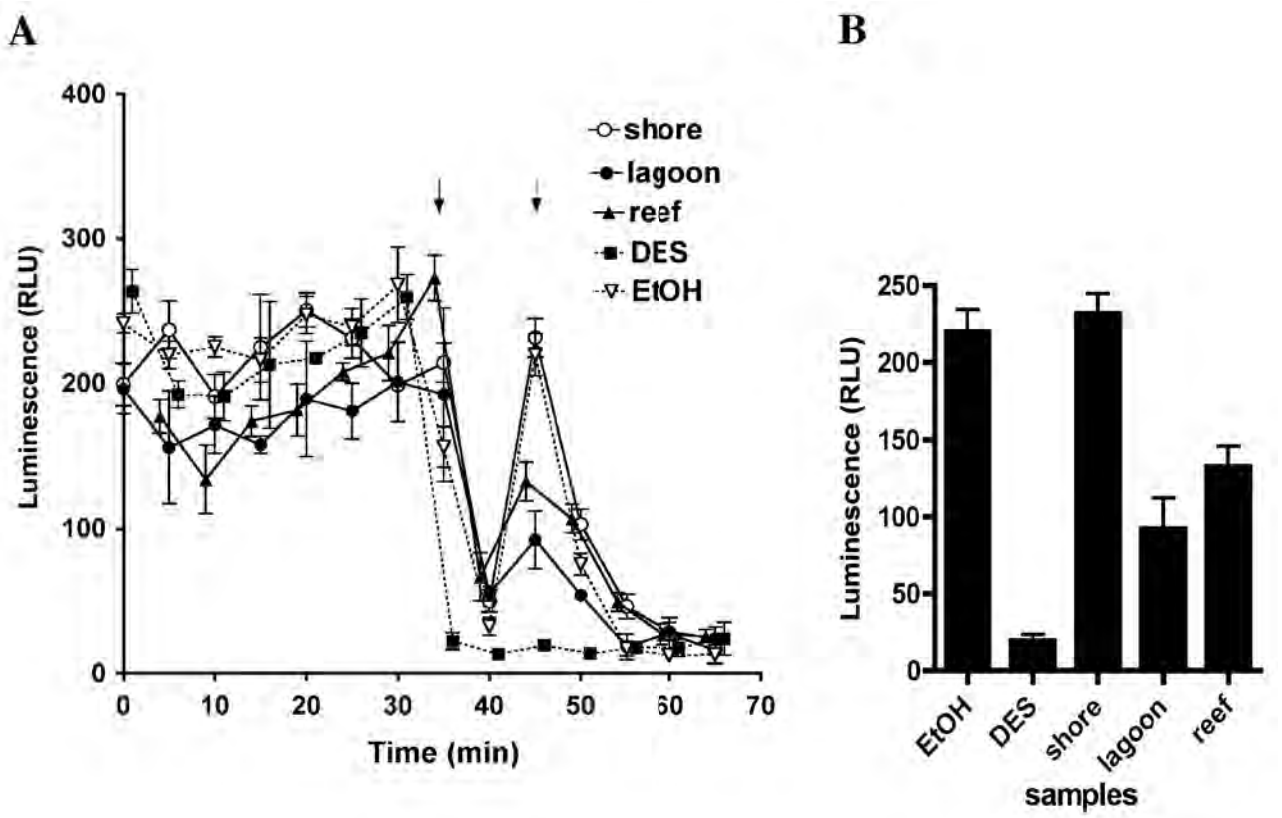

Figure 3. Forskolin-stimulated luminescence is inhibited by compounds from coral seawater. (A) Changes in luminescence after adding the sample compounds 30 minutes later, and forskolin 35 minutes later were chased for 45 minutes (black arrow). Each value is the mean of three separate wells. Vertical bars show standard deviations. (B) Relative luminescence values at 45 minutes represent the value compared with the ethanol (EtOH)-treated cells set to 100%. The tested compounds were from the shore, lagoon, and reef. Each compounds was added at a 1,000-fold diluted final concentration of the extract, corresponding to 100 times that in seawater. Diethylstilbestrol (DES) was added at 10 μM.

mPRα ligand DES decreased intracellular cAMP concentration through a nongenomic action. Samples from the reef and lagoon decreased cAMP levels, and the magnitude of the activity was highest in samples from the lagoon (Fig. 3B).

These results suggest that seawater in the coral reef area contained some steroid active compounds that reacted with mPRα.

Discussion

mPR binding activity of compounds from coral seawater was detected to identify natural receptor ligands besides progestins. Similar results were obtained in a recently established cell-based assay (Nakashima *et al.*, 2015). Along with sampling from Albion, Mauritius, sampling was conducted in Thailand, and Okinawa, Japan. However, no or low binding activity was detected in samples from Thailand or Okinawa. In contrast, relatively high steroid binding activity was detected when the activity of compounds concentrated from seawater around the coral reef at Albion, Mauritius, was tested. We sampled shore, lagoon, and reef areas at Albion (Sadally *et al.*, 2015). Higher binding activity was detected in the lagoon and reef area samples, and maximum binding was detected in samples from the lagoon. These compounds are likely produced by organisms living in the Albion lagoon, assuming that limited exchange of seawater within and outside the Albion reef.

Seasonal changes in the compounds were assessed by comparing samples collected in winter (end of July to early August) and summer (March). Although a relatively large volume of seawater was filtered, the samples collected in the winter showed almost no activity (data not shown). These results suggest that the target compounds occurred in negligible amounts in winter. In contrast, samples from summer (March) showed higher activity.

Corals generally spawn during the summer and steroids have been reported in seawater when coral spawn. Corals spawn simultaneously during 1 day, and steroids have been detected in several to tens of pg/ml,

corresponding to 100 pM, during this period (Twan *et al.*, 2003). Steroids were under the detection limit on days other than mass spawning. Membrane receptor reactivity on other days besides the spawning day were detected during the present study. The steroid concentrations were calculated to be 100 nM in seawater based on activity, which was 1,000-fold higher than the concentrations reported during the spawning season. Thus, it is thought that hormonally active compounds from coral seawater are novel and possess high affinity for the mPRα. These results were confirmed in a cell-based assay (Nakashima *et al.*, 2015). Although this assay enables real-time monitoring of nongenomic steroidal actions through the mPRα, the assay has poor sensitivity; however, the results show that the compounds are highly potent.

mPRs are recognized in a broad diversity of organisms, from fish to humans (Thomas, 2008; Tokumoto *et al.*, 2012), and progestin binding activity has particularly been revealed in many species, such as goldfish, seatrout, zebrafish, frogs, cattle, rats, mice, and humans (Josefsberg Ben-Yehoshua *et al.*, 2007; Smith *et al.*, 2008; Tokumoto *et al.*, 2006; Tubbs & Thomas, 2009). mPRs are expressed in reproductive tissues (ovary, uterus, and testes), kidney, brain, and spinal cord among vertebrates, including fish, mice, and humans (Hanna *et al.*, 2006; Labombarda *et al.*, 2010; Zhu *et al.*, 2003a). The broad distribution of mPRs suggests that these receptors play a role in a wide variety of steroid-related functions. The roles of brain mPRs in the regulation of mammalian sex behavior have also been investigated (Frye *et al.*, 2013; Frye *et al.*, 2014). mPRs participate in breast tumor growth by inhibiting apoptosis in cancer cells (Dressing *et al.*, 2012). It has been proposed that the gene expression level of mPRα is a biomarker for breast cancer survival (Xie *et al.*, 2012). Progesterone activates the pathway to generate cancer stem cells through mPRs in mammary cells (Vares *et al.*, 2015). Furthermore, progesterone is an immune-modulator that may interact with mPRα, mPRβ, and mPRγ to induce rapid non-genomic responses that inhibit proliferation of human T-cells that may attack the fetus (Chien *et al.*, 2009). Progesterone signaling by mPRα is associated with the inflammatory response and parturition, and this association may contribute to the functional withdrawal of progesterone, leading to labour (Lu *et al.*, 2015). As mPRs are potential cellular mediators of various responses to progesterone, some studies have looked for new drugs to treat diseases such as cancers of the reproductive tract and encephalitis.

In this study, steroid-active compounds were detected in seawater collected from a shallow coral reef for the first time at Albion, Mauritius. Seawater samples were fractionated by high-performance liquid chromatography to identify chemicals with mPR activity, and the fractions from some peaks revealed mPR binding activity. Further investigation is warranted to thoroughly determine the binding activity and chemical structure of targeted hormonally-active compounds that interact with the mPR.

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