Fish Balistes capriscus skin extract-induced relaxation in mesenteric arterial bed of rat

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Abstract

The vasorelaxing activity of the aqueous extract of fish Balistes capriscus skin (AEBC) on mesenteric arterial bed (MAB) of rats was studied. The bolus injections of AEBC (bolus of 5.1, 10.2, 20.5, and 41.1 mg) significantly inhibited, in a concentration-dependent manner, the maximal contractile response induced by methoxamine (30 μg/mL) in MAB. The vasodilatation action of AEBC is not mediated through β-adrenoceptors or cyclo-oxigenase, since it was not affected by propranolol (20 μg/mL) or diclofenac sodium (3 μg/mL). The vasodilator response induced by subsequent addition of AEBC Balistes capriscus in bolus was significantly reduced in water infusion for endothelium removal. Treatment with an inhibitor of NO synthase (l-NAME, 10 μg/mL) decreased AEBC effect. The guanylate cyclase inhibitor methylene blue (MB, 100 μg/mL) had no significant effect on AEBC-induced vasodilatation. These results suggest that the vasorelaxing effect of AEBC is mediated by endothelium-dependent (NO/EDRF) and endothelium-independent neurally induced vasorelaxation from nonadrenergic and noncholinergic nerves (NO).

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1. Introduction

Investigation of biological, chemical, and ecological phenomena in the marine world contributes to a better understanding of marine habitats, and benefits the applied and basic research in pharmaceutical marine natural products (Konig and Wright, 1995), while research into the pharmacological properties of marine natural products may lead to promising candidates for new drugs and potentially active agents for clinical application. Several marine natural products are, in fact, currently in pre-clinical and clinical evaluation, while others show promising biological activities in vivo and in vitro assays.

The sesterpenoid mananolide, for example, isolated from the sponge Luffariella variabilis in a program to search for new anti-inflammatory compounds, proved to be a potent inhibitor of phospholipase C (Konig and Wright, 1995).

In the city of Rio Grande, Southern of Brazil, the fish Balistes capriscus (tetradontiform, balistidae) has been used by fishermen in the treatment of respiratory disorders, such as asthma and bronchitis as water infusion of dried and powdered skin.

The present study was carried out to investigate the effects of aqueous extract of Balistes capriscus skin on isolated mesenteric arterial bed (MAB) precontracted with methoxamine (20 μg/mL).

2. Material and methods

2.1. Biological materials

Samples of the fish Balistes capriscus were collected during 1999 and 2000 along the coast of Rio Grande, RS.
(southern Brazil) and identified by Dr. Manoel Haimovich from the Department of Oceanography of Fundação Universidade Federal do Rio Grande (FURG), Rio Grande, Brazil. A voucher specimen has been deposited at the FURG fish collection (acquisition numbers 83.0245 and 83.0295). The animals were frozen at −18 °C until used for the assays.

2.2. Extract

Fish skins were dried/toasted in a conventional oven, at the temperature of approximately 180 °C. Coarsely powdered dried skin material (35 g) was extracted with 120 ml of b-distilled water using a Soxhlet extractor, yielding, after lyophilization, 4.79 g of powder (13.7%). The extract was stored and dissolved in Krebs Ringer bicarbonate immediately before all tests were performed. All results are shown as milligram of lyophilized powder.

2.3. Animals

Male Wistar rats, weighing 419.88 ± 6.25 g (n = 52), were obtained from Animal House of the Fundação Universidade Federal do Rio Grande. The rats were housed in temperature-controlled rooms (20–22 °C), with a 12-h-light/12-h-dark photoperiod and 55 ± 1% relative humidity. Standard laboratory chow (Nuvital, Nuvital Nutrientes, Colombo, Paraná, Brazil) and drinking water were provided ad libitum.

2.4. Perfused mesenteric arterial vascular bed of the rat (MAB)

The MAB was removed from animals under brief ether anesthesia and perfused as described by Mc Gregor (1965), as modified by Silva et al. (1984). The superior mesenteric artery with its nerve plexus was carefully separated from the surrounding tissues at a point 2 cm distal to the aorta. A stainless steel cannula was inserted into the artery. A polygraph (Hewlett-Packard) was used to monitor the flow rate, and was continuously recorded on a polygraph (Hewlett-Packard). Since the flow rate was constant throughout the whole experimental period, any pressure alteration reflected changes in vascular resistance. All preparations were allowed to equilibrate for at least 30 min before the start of the experiments.

2.5. Vasodilatory responses

In order to evaluate the vasoactive effect of the extracts and pervascular nerve stimulation (PNS), the perfusion pressure of MAB was increased by infusing methoxamine (30 μM). When a plateau was reached, the extract and all agonists were administered by bolus injection (50 μM). Drugs and extract-induced relaxation (5.1, 10.2, 20.5, and 41.1 mg) was expressed as a percentage of the pressure induced by methoxamine (30 μM).

In experiments with PNS two electrodes, one placed around the hypodermic needle used to cannulate the superior mesenteric artery, the other resting on the vasculature in a lower part of the vascular bed, were used to create transmural field stimulation (amplitude supra maximal of 60 V and pulse duration of 0.5 ms). Currents were applied at various frequencies and time using an electronic stimulator (HSE-D 7801-Hugstetten, Germany).

In order to block the vasoconstrictive action of pascular sympathetic innervation, the MAB was perfused continuously with guanethidine (5 μM), introduced after a period of equilibration. To evaluate vasodilating response, the tone of mesenteric vascular bed was raised by continuous perfusion with methoxamine (30 μM), which was added to the perfusate, in order to maintain active tone of the mesenteric vasculature. The solution also contained guanethidine (5 μM) to block norepinephrine release. The increased perfusion pressure was allowed to stabilize and the preparation was again subjected to PNS, applied for 30 s.

To study the influence of sensory neurones in the vasodilating responses to the extract or PNS, the preparations were perfused intra- and extra-luminally with capsaicin solution (0.1 μM) for 20 min.

2.6. Drugs

The following drugs were used: acetylcholine chloride, Nω-nitro-l-arginine methyl ester (l-NAME) hydrochloride, methoxamine, papaverine, propranolol, salbutamol, guanethidine, capsacin, and atropine from Sigma Chemical Co. (St. Louis, MO, USA), while sodium diclofenac (Voltaren® injection solution) was supplied by Novartis (São Paulo, Brazil). The stock solution of capsacin was prepared in 70% ethanol; all other compounds were dissolved in distilled water.

2.7. Statistical analysis

Data are reported as mean ± standard error of mean (S.E.M.). Statistical differences between means were evaluated using Student’s t test for unpaired and paired observations. The level of significance was 95%.
3. Results

3.1. Vasodilatation in response to aqueous extract of Balistes capriscus skin (AE\textsubscript{Bc}) in MAB precontracted with methoxamine

The basal perfusion pressure, of the MAB precontracted with methoxamine, was 19.7 ± 0.7 mm Hg (n = 52). AE\textsubscript{Bc} (n = 52) bolus injections (5.1, 10.2, 20.5, and 41.1 mg) significantly inhibited, in a concentration-dependent manner, the maximal contractile response induced by methoxamine (Fig. 1).

3.2. Effect of cyclooxygenase pathway inhibitor sodium diclofenac on changes in vasodilator responses to AE\textsubscript{Bc} in mesenteric arterial bed precontracted by methoxamine

To exclude the involvement of prostaglandins in the extract-induced vasodilatation, the MAB was perfused with diclofenac sodium (3 μM) for 30 min. The treatment of preparations with the cyclooxygenase inhibitor (n = 8) did not modify AE\textsubscript{Bc} responses (Fig. 2).

3.3. Effect of β-adrenergic pathway inhibitor on AE\textsubscript{Bc}-induced vasodilatation in mesenteric arterial bed precontracted with methoxamine

To exclude the involvement of the β-adrenergic pathway on extract-induced vasodilatation, the MAB (n = 6) was perfused with propranolol (20 μM) for 30 min. The treatment of preparations with the inhibitor did not modify AE\textsubscript{Bc} response (Fig. 3). On the other hand, the vasodilatory effect of salbutamol (8.0 nmol), a β-adrenergic agonist, was significantly modified, from 32.59 ± 3.85% to 14.33 ± 4.97% (n = 6) in the presence and absence of propranolol, respectively.
Fig. 4. Effects of atropine (3 µM) on relaxation response to Balistes capriscus skin aqueous extract (AEBc) and acetylcholine (Ach) in methoxamine-precontracted mesenteric arterial bed. Relaxation is expressed as a percentage of the contraction induced by methoxamine. The values are means ± S.E.M. (n = 7). (∗) Denotes value significantly different (P < 0.05) from control.

5.12 10.25 20.5 41.13 Ach (5 nM)
0
25
50
75
Control
Atropine (3 µM)

Fig. 5. Effects of capsaicin (0.1 µM) pretreatment on relaxation response to Balistes capriscus skin aqueous extract (AEBc) and PNS-induced vasodilatation in methoxamine-precontracted mesenteric arterial bed. Relaxation is expressed as a percentage of the contraction induced by methoxamine. The values are means ± S.E.M. (n = 8). (∗) Denotes value significantly different (P < 0.05) from control.

3.4. Effect of muscarinic pathway inhibitor on AEBc and acetylcholine-induced vasodilatation in mesenteric arterial bed precontracted with methoxamine

To study the involvement of muscarinic pathway on extract-induced vasodilatation, the MAB was perfused with atropine for 30 min. As shown in Fig. 4, the extract-induced vasodilatation of the MAB were significantly inhibited (P < 0.05) by treatment with atropine (3 µM). The same procedure decreased the acetylcholine-induced vasodilatation from 58.38 ± 6.82% to 10.0 ± 3.1%.

3.5. Effect of capsaicin treatment on changes in perfusion pressure induced by AEBc and perivascular nerve stimulation in MAB precontracted with methoxamine

Capsaicin (0.1 µM) pretreatment for 20 min prevented the vasodilatation induced by aqueous extract of Balistes capriscus, but only for the bolus of 41.3 mg, from 63.97 ± 6.15% to 44.19 ± 7.0% (Fig. 5). The response of MAB to PNS in the presence of capsaicin was inhibited from 22.28 ± 2.66% to 8.78 ± 2.97% (Fig. 5).

3.6. Effect of endothelium removal on changes in perfusion pressure induced by AEBc and acetylcholine in MAB precontracted with methoxamine

To exclude the involvement of endothelium on extract-induced vasodilatation, the MAB was perfused with distilled water for 10 min for endothelium removal (Criscione et al., 1984). In the intact MAB (n = 8), contracted with methoxamine (30 µM), aqueous extractable fraction caused immediate relaxation. After the endothelium was removed the AEBc-induced vasodilatation was significantly changed (Fig. 6). The same procedure decreased the acetylcholine-induced vasodilatation (5 nM, bolus) from 62.0 ± 3.65% to zero.
relaxation response to *Balistes capriscus* by methoxamine. The values are means ± S.E.M. (n = 8). (×) Denotes value significantly different (*P* < 0.05) from control.

3.7. Effect of endothelium-derived NO-cyclic GMP pathway on vasodilating responses to *AEBc* in mesenteric arterial bed-precontracted with methoxamine

As shown in Fig. 7, the treatment of preparation with an inhibitor of NO synthase L-NAME (10 µM) significantly reduced the *AEBc*-induced vasodilatation.

The effect of guanylate cyclase inhibitor methylene blue (MB, 100 µM) on the vasodilatation induced by *AEBc* was also examined. The treatment of preparations with the inhibitor did not significantly modify the *AEBc* response (Fig. 8).

4. Discussion

The results of the present study demonstrate, for the first time, that the skin of *Balistes capriscus*, used in popular medicine in Southern Brazil for treatment of respiratory disorders, produced a dose-dependent and reversible inhibition of methoxamine-induced vascular tone.

The vasorelaxant action of the extract does not appear to involve activation of *β*-adrenoceptors, since incubation of the preparations with propranolol failed to affect its vasorelaxation action.

The relaxation induced by the extract was not affected by sodium dicyclofenac, which indicates that it is not indirectly mediated by the release of prostanooid metabolites derived from the cyclooxygenase pathway of arachidonic acid.

As is well known, the vasorelaxing action of acetylcholine requires the presence of an intact endothelium. The binding of acetylcholine to muscarinic receptors on endothelial cells triggers the release of a potential vasodilator called endothelium-derived relaxing factor (EDRF), discovered by Furchgott and Zawadzki (1980). The EDRF, as nitric oxide (NO), is synthesized from guanidino groups of L-arginine (Palmer et al., 1987) and has been reported to stimulate the production of cGMP, which induces a vasorelaxation in smooth muscle cells (Winquist et al., 1984).

The treatment of the preparations with atropine, at concentration where Ach-mediated vasorelaxation was antagonized (Muccillo Baisch et al., 1998), prevents a great part of the vasorelaxant action of the extract of *Balistes capriscus* skin in MAB. In addition, when the MAB was perfused with distilled water, the vasorelaxant actions of acetylcholine and of the extract were greatly reduced. As exposure to distilled water destroys the endothelial cells (Crisione et al., 1984), these results confirm that in MAB of rats, both acetylcholine (Muccillo Baisch et al., 1998) and the extract-induced vasodilatation are mediated by the release of endothelium-derived substances. Taken together, such results suggest that the vasorelaxant actions of the extract are mediated jointly by endothelium-dependent cholinergic mechanism.

The vasorelaxant effects of endothelium-dependent substances could be inhibited by several L-arginine analogues such as N-monomethyl-L-arginine (LMNMA) and NO*−*-nitro-L-arginine methyl ester (L-NAME) (Palmer et al., 1987; Rees et al., 1989 and Moore et al., 1990). In the present study the aqueous extract of *Balistes capriscus* dried skin caused endothelium-dependent relaxation, which was reversed by L-NAME, at a concentration which Ach-mediated vasorelaxation was antagonized. This suggests that certain compounds present in the skin extract may cause relaxation by increasing NO or other related compounds.

The endothelium-dependent vasorelaxants, such as acetylcholine, could also release endothelium-derived hyperpolarizing factor (EDHF) from the endothelium, which induces vasorelaxation through membrane hyperpolarization (Adeagbo and Triggle, 1993). The possible existence of a novel endothelium-derived relaxing factor in the endothelium of rat MAB was examined in the study of Kamata et al. (1996). The authors suggested that one or more EDHF must exist, other than NO or EDHF. The novel EDHF may relax
the MAB through production of cAMP, but not cGMP. This suggests that the vasorelaxant effect induced by AE was not inhibited by methylene blue. However, methylene blue is not a potent guanylate cyclase inhibitor, and has also been shown to inhibit NO’s activity (Mayer et al., 1993; Lou et al., 1995).

In the MAB, the relaxation induced by PNS has been determined to be mediated by the activation of capsaicin-sensitive sensory fibers which releases neuropeptides locally, with a potent vasorelaxation action on intestinal blood flow (Manzini and Perretti, 1988). Thus, the present investigation has demonstrated the presence of a nonadrenergic, non-cholinergic vasodilator innervation in rat mesenteric arteries.

In the present study, the vasodilatation induced by aqueous extract from Balistes capriscus skin was reduced in the presence of capsaicin, a pungent ingredient in chili pepper (Capsicum sp.), which has been used to damage selectively the subpopulation of sensory neurons and to deplete neurotransmitter, such as substance P and CGRP (Holzer, 1988). This suggests that the Balistes capriscus skin-induced MAB vasodilatation is mediated by primary sensory nerve fibers. Consequently these data show that in the MAB, the vasodilatation induced by the aqueous extract of Balistes capriscus skin is mediated by endothelium-dependent mechanism mediated by EDHF or NO and endothelium-independent neurotically induced relaxation, associated with NO from a nonadrenergic noncholinergic nerves.

Pharmacological and chemical studies are currently in progress to isolate and characterize the active compounds present in the fish skin, and also to investigate the possible mechanism of action.

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