

Effect Of Marine Organism Extracts On Smooth Muscle Spontaneous Contractility

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ABSTRACT

Sixty-two extracts of marine organisms from the classes Asteroidea and Anthozoa were tested for their effects on rabbit jejunum smooth muscle spontaneous contractility. Most showed a stimulatory effect, with the extracts of *Lophogorgia rigida*, *Muricea austera* and *Muricea appresa* being the most active samples.

INTRODUCTION

The search for active compounds from marine organisms has intensified in recent years. Considering that more than 90% of all living species are found in the ocean and that these have different biosynthetic capabilities (Baker, 1984) compared with species found on land, the probability of finding new active compounds in marine organisms is very high; consequently, the sea offers a rich reserve of interesting, biologically active compounds of potential biomedical value. The variety and diversity of marine organisms surrounding the Peninsula of Baja California (México) motivated us to initiate a systematic study of the effect of ethanol crude extracts of a group of marine organisms on smooth muscle contractility.

MATERIALS AND METHODS

Collection of Marine Organisms

The marine organisms were collected in the Complejo Insular La Partida-Espiritu Santo Island, Cerralvo Island, and Cabo Pulmo B.C.S. (México) (Fig. 1) by scuba diving. The collected samples were cleaned and one of each sample was fixed in EtOH for identification; the rest of the sample was

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kept frozen for extraction. Voucher specimens were retained at the Pharmacognosy Laboratory of the Marine Biological Department of Universidad Autónoma of Baja California Sur (U.A.B.C.S.), México. The identification of the samples was made by Pedro Garza and Federico Sinsel from the Marine Biological Department of UABCS and by Frederick Bayer from the Smithsonian Institute of the National Museum of Natural History in Washington, D.C., USA, according to Brusca (1980), Verrill (1866; 1868) and Matamoros (1984).

Preparation of the extracts

In order to prepare initial extracts for pharmacological testing, one part of the wet material with about five parts of EtOH was macerated for 8 days. Since the purpose of our study was qualitative determination, not quantitative, weights and volumes of extractions were not recorded. The ethanol extracts were rotary evaporated to dryness at no more than 40°C.

Solubilization of the extracts

The ethanol crude extracts were dissolved in a mixture of EtOH:H₂O (1:1), complete with Tyrode solution.

Preparation of segments of jejunum for *in vitro* assays

Male, New Zealand white rabbits (2.5-3.0 kg) were fasted overnight and killed by a blow on the head. Several 5.0 to 6.0 cm segments of the small intestine were rapidly removed and placed in Tyrode solution (pH 7.4) bubbled with 95% O₂ and 5% CO₂ at 37°C. The segments of jejunum were cleaned of all surrounding tissues. They were cut in 1.5 cm long pieces, placed in glass chambers in vertical position with 18 ml of Tyrode solution, and allowed to equilibrate for 30 min or more. The spontaneous smooth muscle contractile activity was recorded on a four channel NARCO BIOSYSTEMS Inc., model 85, Physiograph CPM equipped with a force transducer F-50 previously calibrated. The prepared jejunum were set up with an initial tension of 0.5 g (López *et al.*, 1990).

Scale of activity

The ranges of percentage of stimulation per minute used for the semiquantitative scale were: 24-40% (+1), 40-80% (+2), 80-120% (+3), 120-160% (+4) and > 160% (+5).

Drugs

Acetylcholine (Ach) was bought from Sigma Chemical Company (U.S.A); the drug was dissolved in an appropriate volume of physiological saline solution. Other common reagents were purchased from Merck.

RESULTS

Initially, a systematic selection of 50 ethanol extracts taken from different marine organisms from the classes Asteroidea and Anthozoa was tested on the rabbit jejunum. As shown in Table 1, six of these showed marginal stimulant activity (<+1), five showed little activity (+1), five showed moderate activity (+2), one showed high activity (+3) and four showed very high activity (+4, +5) as stimulators of muscle spontaneous contractility. The extracts of *Muricea austera* (Col. No 325, 308, 345), *Muricea appresa* (Col. No 328) and *Lophogorgia rigida* (Col. No 311) were the most active. The negative results shown in Table 1 did not indicate complete negative activity, because we did not take into consideration the experiments at higher concentration than 75 µg/ml of crude extracts.

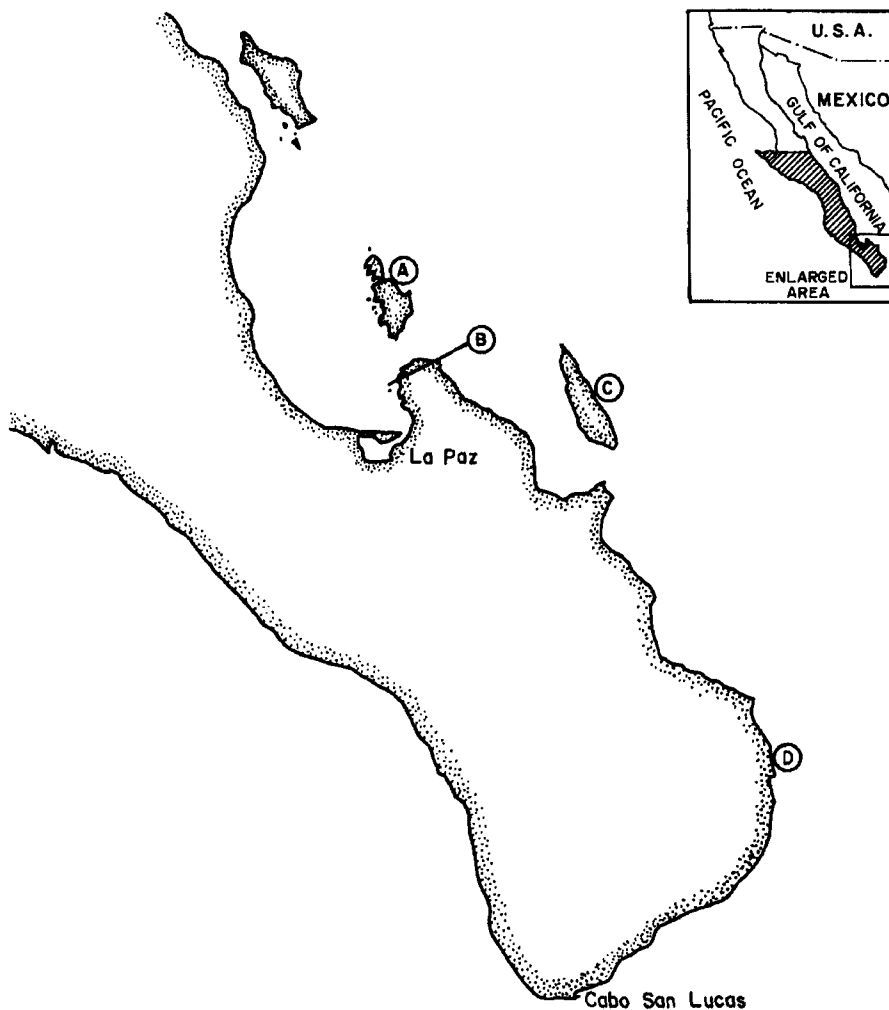


Fig. 1. Map showing the places of collection. A: La Partida - Espiritu Santo Islands, B: Faro San Rafaelito, C: Cerralvo Island and D: Cabo Pulmo, B.C.S.

Fig. 2 shows a comparative record of the effects of different samples at the same concentration; the semiquantitative scale of activity used to indicate the response of the extracts is indicated. A second test was performed on those samples which were active and new extracts were prepared by extracting 50 g of the sample with 200 ml of EtOH. Some of the samples used for this second extraction were kept frozen for 3 years as was the case for *M. appresa* (Col. No 328, 360) and *L. rigida* (Col. No 358). The rest of the samples were recollected [*L. rigida* (Col.

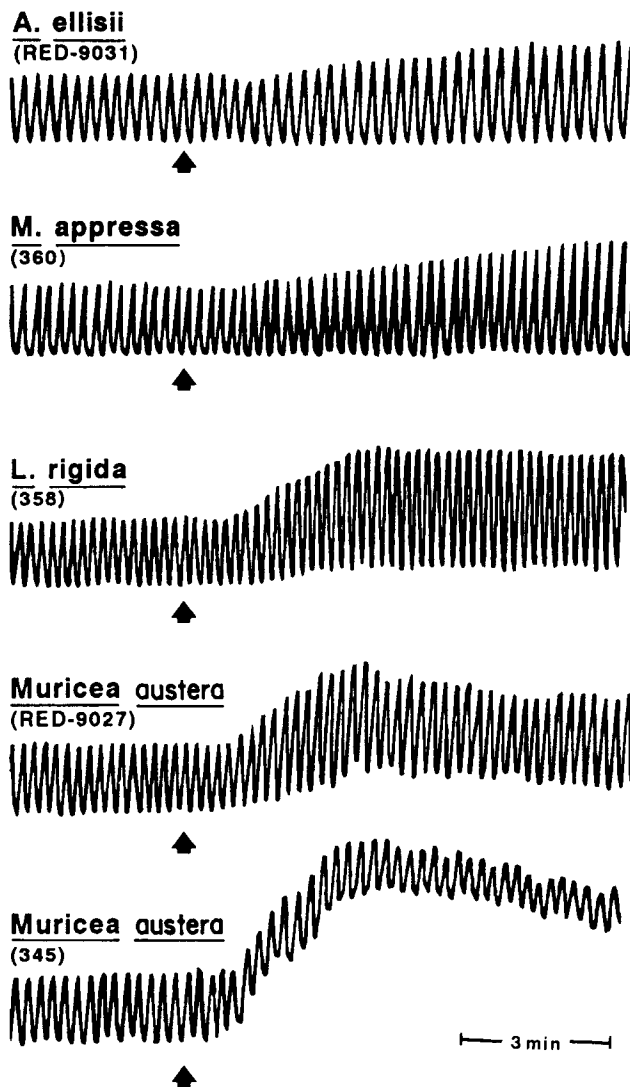


Fig. 2. Stimulation of the contractility of jejunum at different concentrations of ethanol extract of *Muricea austera* (RED 9057). The arrows indicate the point of application of the extract.

No RED9011, RED9036, RED9028), *M. appressa* (Col. No RED9058, RED9042, RED9032) and *A. austera* (Col. No RED9057, RED9027)] and the results of the second test are shown in Table 2. The stimulant effect was dose-dependent (Fig. 3 and 4) and reversible after washing out the tissue with Tyrode's solution until

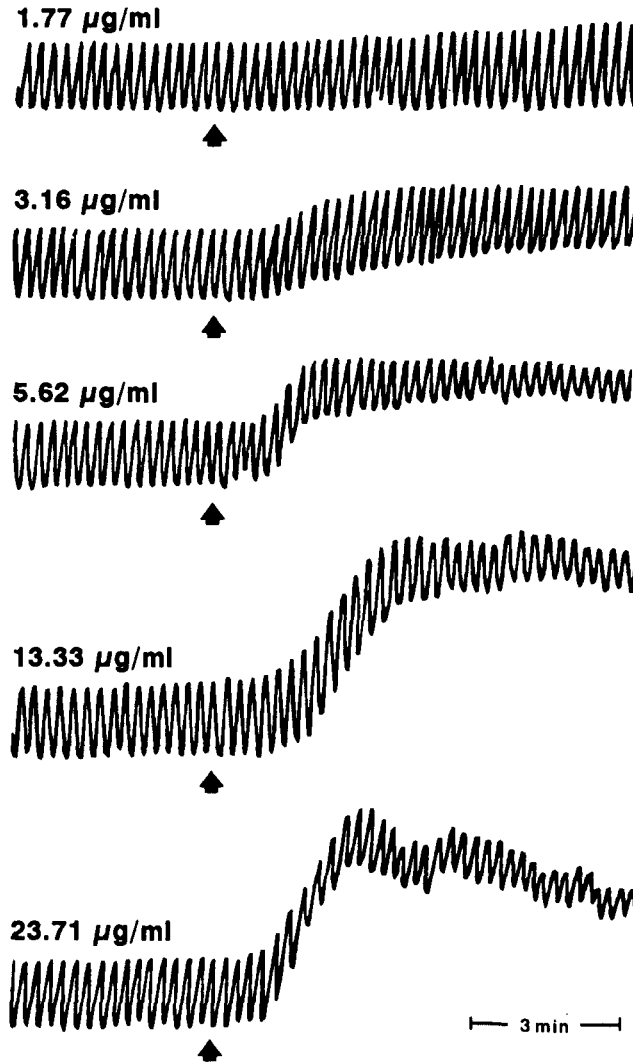


Fig. 3. Effects of *A. ellisii* (+1), *M. appressa* (+2), *L. ridiga* (+3) and *M. auster*a (+4, +5), ethanol extracts on the spontaneous movement of isolated segment of rabbit jejunum. The arrows show the time of application of 23.7 µg/ml of different extracts.

free of extract. We found that a concentration as low as 1.77 µg/ml of *Muricea auster*a extract (Col. No RED9057) significantly increased the amplitude of the contractions of jejunum muscle (Fig. 3).

Table 1. Effect on smooth muscle spontaneous contractile activity.

Col. No.	Scientific Name	Col. Place	Col. Date	Activity ^a
CLASS ASTEROIDEA				
283	<i>Acanthaster ellisi</i>	Partida Island	19 May 87	+2
296	<i>Amphiaster insignis</i>			-
292	<i>Astrometis sertulifera</i>	Partida Island	20 May 87	-
285	<i>Heliaster kubinijii</i>			-
284	<i>Linckia</i> sp.			-
280	<i>Nidorella armata</i>			+1
281	<i>Oreaster occidentalis</i>	Partida Island	19 May 87	-
278	<i>Pharia pyramidata</i>			-
279	<i>Phataria unifacialis</i>			-
CLASS ANTHOZOA				
315	<i>Eugorgia</i> sp.1	Espíritu Santo Island	9 Jun 87	+1
306		Partida Island	20 May 87	+2
316	<i>Eugorgia</i> sp. 2	Espíritu Santo Island	9 Jun 87	+1
327		Faro San Rafaelito	10 Jun 87	+1
323		Espíritu Santo Island	9 Jun 87	+2
311		La Ballena Islote	20 May 87	+3
343, 344, 358, 359	<i>Lophogorgia rigida</i>	Cerralvo Island	14 Aug 87 14 Aug 87 15 Aug 87 15 Aug 87	- - - -
307		Partida Island	20 May 87	+2
325		Faro San Rafaelito	10 Jun 87	+4
308		Partida Island	20 Jun 87	+4
301	<i>Muricea austera</i>	Partida Island	20 May 87	-

Table 1. Continued.

345		Cerralvo Island	15 Aug 87	+ 5
361		Partida Island	27 Aug 87	-
302, 309	<i>Pacifigorgia adamsi</i>	Partida Island	20 May 87	- -
354		Cerralvo Island	15 Aug 87	-
355, 356	<i>Pacifigorgia exilis</i>	Cerralvo Island	15 Aug 87	- -
331, 335	<i>Pacifigorgia</i> sp. 1	Cerralvo Island	14 Aug 87	- -
342 347	<i>Pacifigorgia</i> sp. 2	Cerralvo Island	14 Aug 87	<+ -
297	<i>Muricea</i> sp. 1	Partida Island	20 May 87	<+
303	<i>Muricea appresa</i>	Partida Island	20 May 87	<+
298, 299, 300		Partida Island	20 May 87	<+ - -
321	<i>Muricea appresa</i>	Espíritu Santo Island	9 Jun 87	<+
328, 329		Faro San Rafaelito	10 Jun 87	+4 +1
360		Partida Island	27 Aug 87	-
353		Cerralvo Island	15 Aug 87	-
357	<i>Pacifigorgia</i> sp.3	Cerralvo Island	15 Aug 87	-
341	<i>Pacifigorgia pulchra</i>	Cerralvo Island	14 Aug 87	-
314, 318, 322	<i>Pacifigorgia tenuis</i>	Espíritu Santo Island	9 Jun 87	- <+ -
326		Faro San Rafaelito	10 Jun 87	+2

a: Gradation of results: -, negative; <+, marginal; +, contractile activity positive from 1 to 5 according to the defined scale of activity. (See Materials and Methods).

Table 2. Effect on smooth muscle spontaneous contractile activity.

Col. No.	Scientific Name	Col. Place	Col. Date	Activity ^a
CLASS ASTEROIDEA				
RED 9031	<i>A canthaster ellisi</i>	Espiritu Santo Island	3 Apr 90	+1
CLASS ANTHOZOA				
358	<i>Lophogorgia rigida</i>	Cerralvo Island	15 Aug 87	+3
328	<i>Muricea appresa</i>	Faro San Rafaelito	10 Jun 87	+2
360	<i>Muricea appresa</i>	Espiritu Santo Island	27 Aug 87	+2
RED 9011	<i>Lophogorgia rigida</i>	Cabo Pulmo B.C.S	6 Mar 90	+1.5
RED 9028	<i>Lophogorgia rigida</i>	Espiritu Santo Island	3 Apr 90	-
RED 9036	<i>Lophogorgia rigida</i>	Cerralvo Island	3 May 90	+1
RED 9027	<i>Muricea austera</i>	Espiritu Santo Island	3 Apr 90	+4
RED 9057	<i>Muricea austera</i>	Espiritu Santo Island	3 Apr 90	+4
RED 9032	<i>Muricea appresa</i>	Espiritu Santo Island	4 Apr 90	+3
RED 9042	<i>Muricea appresa</i>	Faro San Rafaelito	8 Aug 90	+3
RED 9058	<i>Muricea appresa</i>	Espiritu Santo Island	8 Aug 90	+5

a: Gradation of results according to the defined scale of activity. (See Materials and Methods).

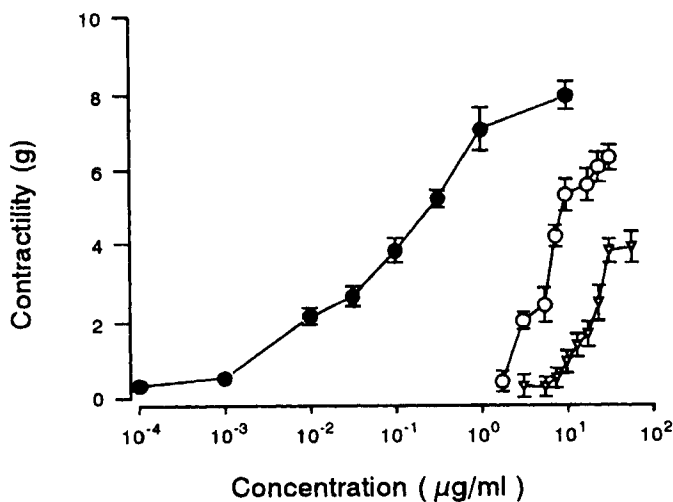


Fig. 4. Dose-Response curve of the stimulant effects for acetylcholine (—●—); *Muricea austera* (Col. No 345) (—○—) and *Lophogorgia rigida* (Col. No 311) (—▽—) ethanol extracts on jejunum smooth muscle spontaneous contractility. Values are means \pm S.E.M. of 10, 3 and 4 experiments for Ach, *Muricea* and *L. rigida*, respectively.

DISCUSSION AND CONCLUSION

Figures 2 and 3 show the stimulant activity of ethanol extracts taken from different marine organisms. The slopes of the dose-response curves (Fig. 4) observed with the extracts of *M. austera* (Col. No 345) and *L. rigida* (Col. No 311) are greater than that seen with the reference compound (acetylcholine), suggesting a distinct behaviour between acetylcholine and the active extracts. Considering all the results reported in the present paper were obtained from crude extracts, we can not propose any specific mode of action, but the study serves as a guide to the selection of marine organisms with stimulant effects on smooth muscle contractility for further work on the isolation and elucidation of the active compounds. Based on these data, further chemical and pharmacological investigations may be suggested for *Lophogorgia rigida*, *Muricea appresa* and *Muricea austera*. For *Muricea austera*, we have isolated esterol present in a crude chloroform extract. The results of this investigation will be communicated in a separate paper.

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