Comparative Biochemistry and Physiology, Part C 151 (2010) 426–430

Contents lists available at ScienceDirect

Comparative Biochemistry and Physiology, Part C

journal homepage: www.elsevier.com/locate/cbpc

Evaluation of the effects of various chemicals on discharge of and pain caused by jellyfish nematocysts

Laura M. Birsa, Peter G. Verity, Richard F. Lee *

Skidaway Institute of Oceanography, 10 Ocean Science Circle, Savannah, GA 31411, USA

ARTICLE INFO

Article history:
Received 21 October 2009
Received in revised form 20 January 2010
Accepted 20 January 2010
Available online 29 January 2010

Keywords:
Nematocysts
Jellyfish
Lidocaine
Anaesthetic
Tentacles
Nematocytes

ABSTRACT

Jellyfish tentacles in contact with human skin can produce pain swelling and redness. The pain is due to discharge of jellyfish nematocysts and associated toxins and discharge can be caused by a variety of mechanical and chemical stimuli. A series of tests were carried out with chemicals traditionally used to treat jellyfish stings e.g. acetic acid ammonia meat tenderizer baking soda and urea to determine if these chemicals stimulated or inhibited nematocyst discharge and if they brought relief to testers who were exposed to jellyfish tentacles. *Chrysaora quinquecirrha* (sea nettle) *Chiropsalmus quadrumanus* (sea wasp) and *Physalia physalis* (Portuguese man-of-war) were used in the study. It was found that many of the chemicals traditionally used to treat jellyfish stings stimulated nematocyst discharge and did not relieve the pain. However there was immediate relief when a common anesthetic lidocaine was sprayed on the skin of testers in contact with jellyfish tentacles. Initial exposure of tentacle suspensions to lidocaine prevented the nematocyst discharge by subsequent exposure to acetic acid ethanol ammonia or bromelain. Thus lidocaine in addition to acting as an anesthetic on skin in contact with jellyfish tentacles inhibited nematocyst discharge possibly by blocking sodium and/or calcium channels of the nematocytes.

1. Introduction

A common feature of the Phylum Cnidaria are tentacles with stinging cells, i.e. nematocytes or cnidocytes, which contain undischarged nematocysts. The nematocysts are hollow tubules in a saline solution containing neurotoxins. These toxins differ in composition in the different species of jellyfish (Ramasamy et al., 2005a,b; Cheng et al., 2005). The nematocyst neurotoxins can paralyze and often kill the small prey which are the food of jellyfish. Over 30 morphological types of nematocysts have been identified in the various cnidarian species (Östman, 2000). Both mechanical and chemical stimuli cause nematocysts to be discharged suggesting the involvement of both chemoreceptors and mechanoreceptors in the discharge process (Ozacmak et al., 2001). There is some controversy as to what causes the explosion of the nematocytes. Picken and Skaer (1966) suggest a sudden increase in permeability of the capsule wall while Lubbock and Amos (1981) suggest that the explosion is initiated by an increase in the osmotic pressure of the capsular fluid brought about by removal of bound calcium ions. A number of studies have described in some detail the discharge of nematocysts from nematocytes (Picken and Skaer, 1966; Thurm et al., 2004; Tibballs, 2006).

Over the past decade there has been an increase in the reports of jellyfish envenomations in coastal beaches throughout the world. Symptoms of humans exposed to jellyfish tentacles are pain, localized areas of swelling, redness and bleeding (Burnett, 2001; Burnett et al., 1986). Some of the chemicals traditionally used to treat jellyfish stings in humans include dilute acetic acid (vinegar), sodium bicarbonate (baking soda), ammonia, papain or bromelain (meat tenderizer), ethanol and salt water. Many of these traditionally used chemicals are thought to inactivate undischarged nematocysts on the skin so that relief from further stinging is provided. However, nematocyst discharge was observed when several species of jellyfish [sea nettle (*Chrysaora quinquecirrha*), Portuguese man-of-water — (*Physalia* spp.), hydroid (*Lytocarpus philippinus* sp.), and mauve stinger (*Cyanea capillata*)] were exposed to alcohol, acids or urea (Auerbach, 1997; Burnett et al., 1968; Fenner and Fitzpatrick, 1986; Rifkin et al., 1993). Hartwick et al. (1980) noted that alcohols caused massive discharge of nematocysts in the box jellyfish (*Chironex fleckeri*) but that acetic acid inactivated unfired nematocysts in the tentacles. Dilute solutions of local anesthetics, e.g. benzocaine, lidocaine, have been recommended to bring relief from jellyfish stings (Auerbach, 1997).

The present work attempts to answer questions concerning the effects of traditionally used chemicals, e.g. alcohols, acetic acid, on nematocyst discharge, the effect of local anesthetics (lidocaine, benzocaine) on nematocysts discharge and to determine the extent of pain relief that treatment with these chemicals brings to skin in contact with jellyfish tentacles. The three part study was as follows: (1)effects of commonly used treatment chemicals on the discharge of nematocysts from the tentacles of two species of jellyfish, *Physalia physalis* (Atlantic Portuguese man-of-water) and *Chrysaora quinquecirrha* (sea nettle);

* Corresponding author. Tel.: +1 912 5982494; fax: +1 912 5982310.
E-mail address: dick.lee@skio.usg.edu (R.F. Lee).

1532-0456/$ – see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.cbpc.2010.01.007

(2) effect on nematocyst discharge when jellyfish tentacles were initially exposed to lidocaine before the addition of the other chemicals; and (3) determine the relative relief from jellyfish (*Chrysaora quinquecirrha* and *Chiropsalmus quadrumanus*) stings after treatment with various chemicals.

2. Methods and materials

2.1. Collection and preparation of jellyfish

Physalia physalis, *Chrysaora quinquecirrha* and *Chiropsalmus quadrumanus* were collected with nets in coastal waters near Savannah, GA, USA. Jellyfish were transferred to a flowing seawater (salinity: 28 ppt; temperature range: 26–28 °C) tank and pieces of jellyfish tentacles were obtained using dissecting scissors. Tentacles were transferred to seawater in a 50 mL beaker.

2.2. Addition of chemicals to jellyfish tentacles

Tentacles pieces (approximately 500 mm^2) were added to wells (1 mL seawater) in slides. Tentacle nematocytes were examined under a light microscope (Olympus BX60) at 100× magnification. To determine the effects of chemicals on nematocytes, one hundred microliters of each chemical solution was added to wells containing tentacle pieces. Chemicals tested included salt water (28 ppt), acetic acid (5%), ammonia (20%), meat tenderizer (active ingredient: bromelain; 10%), urea (50%), ethanol (70%), sodium bicarbonate (10%) and lidocaine–HCl (4%). In a second series of experiments, lidocaine (4%) was first added to wells with tentacle pieces and after 1 min the other test chemicals (salt water, acetic acid, ammonia, meat tenderizer and urea) were added to wells. A separate well was used for each test chemical. The chemicals used, their concentrations and tests carried out are summarized in Table 1.

2.3. Examination of tentacles after addition of chemicals

Digital photos using a Retiga 1300 digital color camera or Moticam 2300 digital video camera attached to a light microscope were taken of tentacle pieces before and after the addition of each chemical solution. The number of discharged nematocysts in a small section of each slide was counted after each chemical treatment. There was no evidence of nematocyst discharge before the addition of chemicals. This method of quantitation of nematocyst has its limitations because of variability of nematocyst density on tentacles. We suggest that at best it is a semi-quantitative. We tried to cut tentacles at the same position for each study.

2.4. Human skin exposed to jellyfish tentacles and effects of chemical treatments

Two of the authors (R. Lee and P. Verity) exposed the inner forearms of one of their arms to jellyfish [*Chrysaora quinquecirrha* (sea nettle) or *Chiropsalmus quadrumanus* (sea wasp)] tentacles (tentacle area was approximately 25 cm^2). The other arm received no treatment. For the chemical tests, the arm in contact with jellyfish tentacles was treated within 1–2 min by solutions of the various chemicals [lidocaine hydochloride (15%, 10%, 5%, 3%, 1%), benzocaine (5, 10% in ethanol), ethanol (70%), acetic acid (5%), and ammonia (20%)] to one of the arms containing jellyfish tentacles. The experiments were conducted over a 20 day period with only one treatment per day to allow recovery of the arms from the stings received on any one day. Approximately 5 mL of a particular solution was added to the section of skin exposed to the jellyfish tentacle. Observations of skin redness after and before addition of chemicals treated skin were made by outside observers, who were not given any information as to which chemicals were being sprayed on the skin. Notes were taken of the stinging sensation felt as well as external observation of the skin, e.g. swelling, redness, after exposure to jellyfish tentacles and subsequent chemical treatments. A summary of the chemicals used, their concentrations and the tests are summarized in Table 1. Only *C. quinquecirrha* and *C. quadrumanus* were available in the coastal waters of our area during the skin exposure studies.

Table 1
Chemicals used in experiments to assess nematocyst discharge or stinging/pain relief after contact with jellyfish tentacles.

Chemical tested	Soln. conc (%)	Soln conc. of 2nd chemical added	Jellyfish species	Test performed
Ethanol	70	NA	*C. quinquercirrha* *P. physalis*	Nematocyst discharge
Ammonia	20	NA	"	"
Bromelain (meat tenderizer)	10	NA	"	"
Acetic acid	5	NA	"	"
Seawater	100	NA	"	"
Urea	10	NA	"	"
Lidocaine	4	NA	"	"
Lidocaine	4	Acetic acid (5%)	"	"
Lidocaine	4	Ammonia (4%)	"	
Seawater	100	NA	*C. quinquercirrha* *C. quadrumanus*	Pain relief
Deionized water	100	NA	"	"
Ethanol	70	NA	"	"
Ammonia	20	NA	"	"
Bromelain (meat tenderizer)	10	NA	"	"
Acetic acid	5	NA	"	"
Lidocaine	1,3,5,10, 15	NA	"	"
Benzocaine	5,10	NA	"	"

NA = not applicable.

3. Results

3.1. Effects of different chemicals on nematocyte discharge

The addition of solutions of acetic acid, ethanol, ammonia and bromelain (meat tenderizer) to *Physalia physalis* and *Chrysaora quinquecirrha* tentacle suspensions resulted in the immediate discharge of thousands of nematocysts (Fig. 1, Table 1). Quantitation of nematocyst discharge determined that of the four chemicals, meat tenderizer caused the most discharges (112 nematocysts/mm) in *P. physalis* tentacles while it was ammonia (80 nematocysts/mm) in *C. quinquercirrha* (Table 2). Concentrations greater than 1500/mm^2 were found when tentacles of *Chironex fleckeri* were electrically stimulated (Tibballs, 2006). Because of the variable densities of nematocysts along a tentacle, the method used for quantitation of nematocyst discharge is at best semi-quantitative. Thus, we only ascribe significance in discharge numbers when there were orders of magnitude differences between chemical treatments. Little or no nematocyst discharge was observed after the addition of seawater, lidocaine hydrochloride or sodium bicarbonate solutions. Lidocaine inhibited nematocysts discharge by other chemicals, since nematocytes exposed first to lidocaine solutions did not discharge nematocysts after subsequent exposure to acetic acid, ethanol, ammonia or bromelain (Table 2).

3.2. Effects of different chemicals on the stinging and pain felt by testers after their skin came into contact with jellyfish tentacles

A fairly intense stinging pain was experienced immediately after tentacle pieces from *Chiropsalmus quadrumanus* or *Chrysaora*

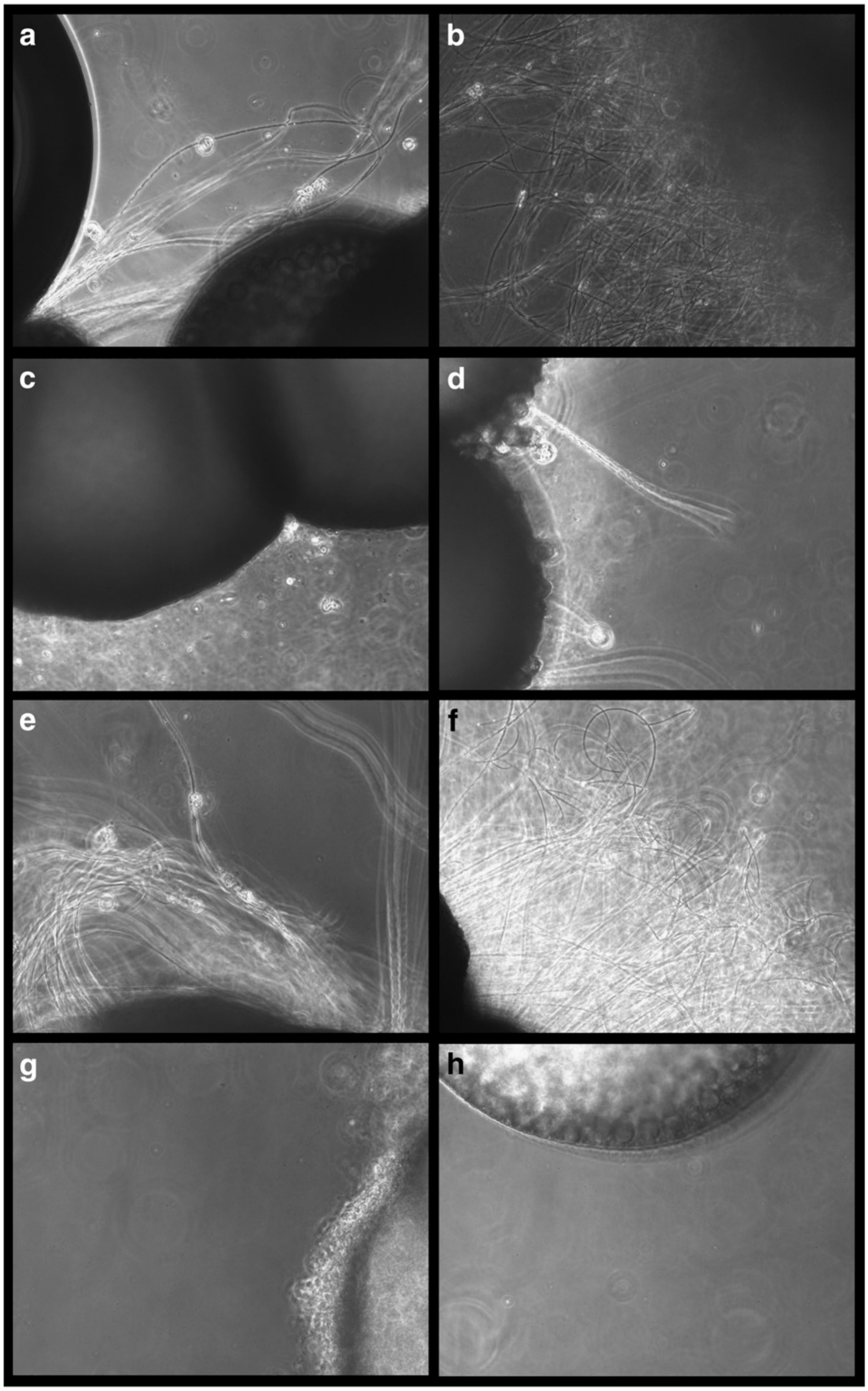

Fig. 1. Photomicrographs of jellyfish tentacles before and after treatment with lidocaine, acetic acid, ammonia, meat tenderizer, sodium bicarbonate and ethanol. Magnification is 100×. a. *P. physalis* tentacle exposed to acetic acid (5%). b. *C. quinquecirrha* tentacle exposed to acetic acid (5%). c. *P. physalis* tentacle exposed to lidocaine (4%) followed in 1 min by acetic acid (5%). d. *P. physalis* tentacle exposed to ethanol (70%). e. *C. quinquecirrha* tentacle exposed to ethanol (70%). f. *P. physalis* tentacle exposed to meat tenderizer (bromelain) (10%). g. *P. physalis* tentacle exposed to lidocaine (4%). h. *P. physalis* tentacle exposed to sodium bicarbonate (10%).

quinquecirrha were applied to the forearms of two testers. The intensity of the sting from *C. quinquecirrha* sting seemed more intense than the sting from *C. quadumanus*. A quite visible area of redness was observed on the skin of both testers within minutes after application of the tentacles from either of the jellyfish species. The intense stinging sensation on the arm remained for approximately 30 min after the tentacle was applied to the arm. The areas of redness remained for up to 24 h on skin that had contact with jellyfish tentacles (Table 2).

When different chemicals were added to skin in contact with tentacles the sensation felt was divided into three categories: (1) treatment caused an immediate relief or at least a reduction in the stinging sensation relative to the untreated arm in contact with a tentacle piece; (2) treatment caused an increased stinging sensation compared to untreated

Table 2
Quantitation of discharged nematocysts after a treatment of suspensions of jellyfish tentacles with various chemicals. For 2 of the studies there was addition of lidocaine to tentacle suspension, followed in 1 min. by addition of acetic acid or ammonia.

Chemical	Number of discharged nematocysts (number/mm ± standard deviation)	
	Chrysaoroa quinquercirrha (sea nettle)	*Physalis physalis* (Portuguese man-of-war)
Ethanol (70%)	66 ± 14	53 ± 26
Ammonia (20%)	80 ± 16	80 ± 5
Meat tenderizer (10%) (Bromelain)	29 ± 10	112 ± 16
Acetic acid (5%)	5 ± 11	100
Seawater	0	0
Urea (10%)	0	0
Lidoocaine (4%)	0	0
Lidocaine (4%) followed by addition of acetic acid	0	0
Lidocaine (4%) followed by addition of ammonia	0	0

arm; (3) treatment did not produce any noticeable difference compared to the untreated arm. Deionized water, meat tenderizer and urea treatments fell into category 3 while ammonia, ethanol and acetic acid were in category 2. Lidocaine hydrochloride solutions fell into category 1 since they reduced the pain associated with the jellyfish stings and reduced the amount of swelling and redness associated with jellyfish exposure. Lidocaine concentrations of 10 and 15% produced immediate relief from the stinging sensation. The 4 and 5% solutions produced relief after approximately 1 min while 1, 2 and 3% solutions required 10 to 20 min before there was any noticeable relief. Benzocaine dissolved in ethanol provided some relief from jellyfish sting but relief from pain took 10 or more min. Evidence of areas of redness was observed after treatment with benzocaine of skin in contact with jellyfish. Little or no areas of redness were observed after adding the higher lidocaine hydrochloride concentrations (4, 5, 10 and 15%) to skin in contact with jellyfish tentacles. After treatment with acetic acid or ethanol more areas of redness were observed on skin in contact with tentacles than untreated skin in contact with tentacles. There was a good correlation between the number of nematocysts discharged and the intensity of the pain felt after a particular chemical treatment (Table 3).

Table 3
Relative relief of jellyfish sting pain intensity after application of various traditionally used chemicals and by the anesthetics, benzocaine or lidocaine. See Methods and materials for concentrations, sources, and experimental protocols.

Chemical	Pain intensity after application of chemical	
	Chiropsalmus quadrumanus (Sea Wasp)	*Chrysaoroa quinquercirrha* (Sea Nettle)
Control (seawater)	0	0
Control (deionized water)	0	0
Lidocaine (5%)	-	-
Lidocaine (10%)	–	–
Lidocaine (15%)	—	—
Benzocaine (5%)	-	N
Benzocaine (10%)	-	N
Ammonia (20%)	+	+
Acetic Acid (5%)	+	+
Ethanol	+	+
Bromelain – Meat tenderizer	0	0

"0" = no apparent change in sting intensity or duration of pain. "+" = exacerbation of pain intensity. "-" = noticeable alleviation of pain intensity and duration. "–" = further reduction in pain alleviation. "—" = maximum observed reduction in pain of jellyfish stings. N = no test with the chemical.

4. Discussion

Chemicals traditionally used to treat skin in contact with jellyfish, i.e. ethanol, meat tenderizer, ammonia, acetic acid, were found to stimulate nematocyst discharge and provided little or no relief from the pain and stinging sensation resulting from jellyfish contact. In fact, several of them, e.g. ethanol, acetic acid, enhanced the stinging pain in skin in contact with jellyfish. Nematocyst discharge did not occur after addition of lidocaine to tentacle suspensions and higher lidocaine concentrations (4 to 15%) provided relief from the pain and sting of jellyfish contact. First adding lidocaine solutions to jellyfish tentacle suspensions prevented nematocyst discharge when acetic acid or ammonia was subsequently added to the tentacles. Alcohols, acids, and bases have been found to stimulate nematocyst discharge of various species of jellyfish (Auerbach, 1997; Burnett et al., 1968; Fenner and Fitzpatrick, 1986; Rifkin et al., 1993).

While vinegar (acetic acid) has long been recommended for the treatment of jellyfish stings there seems to be little solid evidence, including untreated controls, that vinegar provides relief from jellyfish stings. Hartwick et al. (1980) suggested that vinegar provides relief from jellyfish stings by preventing further nematocyst discharge or inactivation of the toxin. However, vinegar has been reported to cause nematocyst discharge of *Physalia* sp., *Pelagia noctiluca*, *Lytocarpus philippinus*, and *Cyanea capillata* (Exton et al., 1989; Fenner and Fitzpatrick, 1986; Fenner et al., 1993; Rifkin et al., 1993). In our work it was found the vinegar caused nematocyst discharge of *Physalia physalis* and *Chrysaora quinquecirrha* and did not bring relief from the stings of *C. quinquecirrha* or *Chiropsalmus quadrumanus*. The Skinsight web site (www.skinsight.com/firstaid/firstAidJellyfishStings.htm) states that you should not apply vinegar to the affected area. A MedPlus site (www.nim.hig.gov/medlineplus/ency/imagepages/8860.htm) recommends washing the affected area with seawater but does not mention a vinegar wash. There is still the possibility that nematocyst discharge by some jellyfish species, species not used in the earlier studies, are inhibited by vinegar.

Lidocaine, an amino amide, appears to provide relief from jellyfish stings by acting both as an anesthetic and by preventing further discharge of nematocysts from tentacles which remain on the skin. Lidocaine acts as an aesthetic by blocking sodium ion channels in nerves that sense pain, thus preventing these nerves from transmitting pain signals to the brain (Binshtok et al., 2007; Hille, 1997). The suggestion has been made that hydrophilic anesthetics, e.g. lidocaine hydrochloride, act on the hydrophilic region of the nerve membrane which is different from the hydrophobic regions of the membrane acted on by hydrophobic anesthetics, e.g. benzocaine (Hille, 1997). The nematocyst thread, which can be up to 1 mm long, can penetrate the skin epidermis to the dermal capillaries (Rifkin et al., 1993; Tibballs, 2006) while lidocaine can penetrate and act down to 5 mm below the skin surface (Wahlgren and Quiding, 2000). Benzocaine while providing some relief from the pain of jellyfish stings was slower acting than lidocaine and also did not prevent the appearance of areas of redness on the skin. The action of lidocaine to inhibit discharge of nematocysts is likely due to blockage of the chemoreceptors and mechanoreceptors that are responsible for the discharge of nematocysts from the nematocytes. This blockage by lidocaine may be due to the action of lidocaine on sodium ion channels in nematocyst membranes. There is evidence that nematocyst discharge is initiated by an increase in the osmotic pressure of nematocyte fluid which is brought about by removal of bound calcium ions (Lubbock and Amos, 1981). Thus, lidocaine may also have some effect on calcium ion channels in nematocyst membranes.

Acknowledgments

The authors gratefully thank the staff at the Marine Education Center and Aquarium (MECA; http://www.arex.ug.edu/aquarium), an educational facility within the Marine Extension Service of the

University of Georgia, for collecting sea wasps. We thank D. Denard and C. Grayson for collecting sea nettles and a Portuguese man-of-war. We thank C. Grayson for much helpful advice during the course of the research on jellyfish.

References

Auerbach, P.S., 1997. Envenomations from jellyfish and related species. J. Emerg. Nurs. 23, 555–565.

Binshtok, A.M., Bean, B.P., Woolf, C.J., 2007. Inhibition of nociceptors by TRPV1-mediated entry of impermeant sodium channel blockers. Nature 449, 607–610.

Burnett, J.W., 2001. Medical aspects of jellyfish envenomation: pathogenesis, case reporting and therapy. Hydrobiology 451, 1–9.

Burnett, J.W., Stone, J.H., Pierce, L.H., Layne, E.D., Cargo, D.G., Sutton, J.S., 1968. Studies on the structure, mechanism of discharge and toxin of sea nettle nematocysts. J. Invest. Dermatol. 51, 330–336.

Burnett, J.W., Calton, G.J., Burnett, H.W., 1986. Jellyfish envenomation syndromes. J. Amer. Acad. Dermatol. 14, 100–106.

Cheng, D., Dattaro, J.A., Yakobi, R., 2005. Jellyfish stings. . http://www.emedicine.com/derm/topic199.htm.

Exton, D.R., Fenner, P.F., Williamson, J., 1989. Cold packs: effective topical analgesia in the treatment of painful stings by *Physalia* and other jellyfish. Med. J. Aust. 151, 625–626.

Fenner, P.J., Fitzpatrick, P.F., 1986. Experiments with the nematocysts of *Cyanea capillata*. Med. J. Aust. 145, 174.

Fenner, P.J., Williamson, J.A., Burnett, J.W., Rifkin, J., 1993. First aid treatment of jellyfish stings in Australia. Response to a newly differentiated species. Med. J. Aust. 158, 498–501.

Hartwick, R., Callanan, V., Williamson, J., 1980. Disarming the box-jellyfish: nematocyst inhibition in *Chironex fleckeri*. Med. J. Aust. 1, 15–20.

Hille, B., 1997. Local anesthetics: hydrophilic and hydrophobic pathways for the drug-receptor reaction. J. Gen Physiol. 69, 497–515.

Lubbock, R., Amos, W.B., 1981. Removal of bound calcium from nematocyst contents causes discharge. Nature 290, 500–501.

Östman, C., 2000. A guideline to nematocyst nomenclature and classification, and some notes on the systematic value of nematocysts. Sci. Mar. 64 (Suppl. 1), 31–46.

Ozacmak, V.H., Thorington, G.U., Fletcher, W.H., Hessinger, D.A., 2001. N-acetylneuraminic acid (NANA) stimulates *in situ* cyclic AMP production in tentacles of sea anemone (*Aiptasia pallida*): possible role in chemosensitization of nematocyst discharge. J. Exp. Biol. 201, 2011–2020.

Picken, L.E.R., Skaer, R.J., 1966. A review of researches on nematocysts. Symp. Zool. Soc. Lond. No. 16, 19–50.

Ramasamy, S., Isbister, G.K., Seymoure, J.E., Hodgson, W.D., 2005a. The in vivo cardiovascular effects of the Irukandjii jellyfish (*Carukia barnesi*) nematocyst venom and a tentacle extract in rats. Toxicol. Lett. 155, 135–141.

Ramasamy, S., Isbister, G.K., Seymour, J.E., Hodgson, W.D., 2005b. Pharmacologically distinct cardiovascular effects of box jellyfish (*Chironex fleckeri*) venom and a tentacle-only extract in rats. Toxicol. Lett. 155, 219–226.

Rifkin, J.F., Fenner, P.J., Williamson, J.A.H., 1993. First acid treatment of the sting from the hydroid *Lytocarpus philippinus*: the structure of, and *in vitro* discharge experiments with its nematocysts. J. Wilderness Med. 4, 252–260.

Thurm, U., Brinkmann, M., Golz, R., Holtmann, M., Oliver, D., Sieger, T., 2004. Mechanoreception and synaptic transmission of hydrozoan nematocytes. Hydrobiology 530 (531), 97–105.

Tibballs, J., 2006. Australian venomous jellyfish, enveonmation syndromes, toxins and therapy. Toxicon 48, 830–855.

Wahlgren, C.-F., Quiding, H., 2000. Depth of cutaneous analgesia fter application of a eutectic mixture of the local anesthetics lidocaine and prilocaine (EMLA cream). J. Am. Acad. Dermatol. 42, 584–588.