

Gastro-protective effect of capsaicin in *Anguilla anguilla* (Linnaeus, 1758): evidence from an experimental study on gastric bags

Effetto gastro-protettivo della capsaicina in Anguilla anguilla (Linneo, 1758): studio sperimentale su sacchetti gastrici

Maria Gabriella Denaro^{1*}, Gabriella Caruso², Lucrezia Genovese²

¹Dpt. di Fisiologia Generale e Farmacologia, Facoltà di Scienze Matematiche, Fisiche e Naturali, Università di Messina, Salita Sperone, 31 - 98166 S. Agata (ME); ²Istituto per l'Ambiente Marino Costiero (IAMC), Consiglio Nazionale delle Ricerche, Spianata S. Raineri, 86 - 98122 Messina

SUMMARY - An experimental, macroscopical, study was undertaken in european eel (*Anguilla anguilla*) to assess the gastro-protective effect of capsaicin, the active component contained in pepper. Gastric bags were prepared from specimens of *Anguilla anguilla* and used for *in vitro* study of the ability of capsaicin to recover ulcers induced by aspirin or ethanol. Gastric ulcers were experimentally induced and comparison was made with the same experiment repeated in presence of capsaicin at different concentrations (10^{-6} , 10^{-5} , 10^{-4} mol/l). Macroscopical observations showed that capsaicin at low doses (10^{-6} mol/l) was effective in repairing the mucosal layer injured, thus proving *in vitro* its protective effect on the gastrointestinal tract of this fish species. Moreover, the similar physiological behaviour of the protective effect found between our study and other *in vivo* studies performed on rats supported the possibility of using the gastro-intestinal tract of eel as a model substrate for pharmacological and toxicological studies.

RIASSUNTO - Uno studio sperimentale, macroscopico, è stato effettuato in anguilla (*Anguilla anguilla*) per valutare l'effetto gastro-protettivo della capsaicina, un principio attivo contenuto nel peperoncino. Sacchetti gastrici sono stati preparati da esemplari di anguilla ed utilizzati per studiare *in vitro* la capacità della capsaicina di prevenire la formazione di ulcere indotte dall'aspirina o dall'etanolo. Sono state indotte sperimentalmente ulcere gastriche; in parallelo lo stesso esperimento è stato ripetuto in presenza di capsaicina a differenti concentrazioni (10^{-6} , 10^{-5} , 10^{-4} mol/l). L'osservazione macroscopica ha evidenziato come la capsaicina a basse dosi (10^{-6} mol/l) sia efficace nel prevenire l'alterazione dello strato mucosale, dimostrando *in vitro* il suo effetto protettivo sul tratto gastrointestinale di questa specie di pesci. Inoltre, il comportamento simile fra quanto riscontrato *in vitro* ed altri studi condotti *in vivo* su ratti, suggerisce la possibilità di utilizzare il tratto gastrointestinale di anguilla come modello sperimentale per saggi farmacologici e tossicologici.

Key words: *Anguilla anguilla*, Gastric ulcer, Capsaicin, Aspirin, Ethanol.

* Corresponding Author: c/o Dpt. di Fisiologia Generale e Farmacologia, Facoltà di Scienze Matematiche, Fisiche e Naturali, Università di Messina, Salita Sperone, 31 - 96166 S. Agata (ME), Italia; Tel.: 090-6765210; Fax: 090-394030; E-mail: mgdenaro@unime.it

INTRODUCTION

Peptic ulcer represents one of the most spread human diseases in the world; generally, in healthy conditions, mucosal damaging agents and gastric mucosal defences are well balanced (Grossman, 1980). Among these latter, the mucus, covering with a thin layer the surface epithelial cells of the gastrointestinal tract, represent a first line of defence against the acidic environment created by pepsin. Also, mucosal prostaglandins are reported to be involved in modulating gastric mucosal defense mechanisms (Mózsik *et al.*, 1977a; 1977b); their reduction has been considered as a predisposing factor in the genesis of gastric ulcer (Miller, 1983). Other factors which play a role in the mucosal protection include the tight junctions between the surface epithelial cells and the cell turnover, bicarbonate secretion, gastric mucus phospholipids, gastric mucosal blood flow (Miller, 1988).

Different drugs have been tested to experimentally induce gastric ulcer and to study their pathogenesis (Szabò & Goldberg, 1990). For some chemicals (acetic, acetylsalicylic acids, bile salts and ethanol) the capability of breaking the gastric mucosal barrier has been demonstrated (Davenport, 1969). On the other hand, several other compounds such as atropine (Mózsik *et al.*, 1980), antacids and cimetidine (MacKercher *et al.*, 1977), or some coating agents, barrier agents or mucosally active drugs have been used in the treatment of acid peptic disorders (Hollander & Tarnawski, 1987). Particular interest is also addressed to compounds of natural origin, showing significant pharmacological properties for the therapy of peptic ulcer disease, such as compounds derived from peppers. Since ancient times, this fruit (*Capsicum annuum*, belonging to the family of *Solanaceae*) has been known for the therapeutical properties of its active components, particularly concerning its content in capsaicin (8-methyl-N-vanillyl-6-nonenamide). This alkaloid, responsible for the pungent nature of pepper, is detected in this fruit in amounts ranging from 0.01 to 0.22%, while in form of essential oil (capsicol) it is present in concentrations ranging from 1 to 1.5%. This substance induces pyrexia (Szolcsányi, 1982), but a lot of studies have suggested its application in medicine for its therapeutic potential (Szallasi & Blumberg, 1993; Abdel-Salam *et al.*, 1997). Capsaicin is known to have protective effects not only in the stomach but also in the neonatal pre-treatment of chronic colitis induced in the rat by trinitrobenzene sulfonic acid (Evangelista & Meli, 1989). Concerning the mechanism of action of capsaicin, its receptor is a cationic channel which, during its activation, allows a flux of Ca^{2+} and Na^{2+} ions (Winter, 1987; Wood *et al.*, 1988) which induces the depolarisation of neuronal membranes of fibres involved in pain generation; then this sensation is transmitted to the brain via the dorsal ganglion.

Previous research (Faggio *et al.*, 2000) showed that the gastric mucosa of Teleosts exhibits physiological responses similar to those observed in superior Vertebrata; therefore, it is possible to use fish as experimental models in pharmacological and toxicological experimentation.

A study was carried out in order to evaluate the protective effect of capsaicin on gastric ulcers experimentally induced on a Teleost species, *Anguilla anguilla* (European eel), with the objective of verifying whether, even in *in vitro* condition (in absence of any nervous regulation and blood flow) this effect is still present.

MATERIALS AND METHODS

Studied specimens

The specimens under experimentation were European eel (*Anguilla anguilla*) of average age 48 months, showing a mean weight 133 ± 2.3 g and length of 26 cm. They were

maintained in the aquaculture plant of the Istituto per l'Ambiente Marino Costiero (IAMC) of Messina, in circular tanks of PVC (capacity 300 l) under natural photoperiod.

After a period (thirty days) of acclimatisation, during which fish were not feed, eels were sacrificed after treatment with MS 222 (final concentration 10 g/l), their gastrointestinal tract were removed and the stomach isolated (Figure 1). This organ, Y-shaped, includes two regions (cardial and fundal) with gastric glands, and a third one, the pyloric, folded and without glands. After its removal, the stomach underwent to depletion of the muscle and connectival layers in Petri dishes containing physiological Ringer solution, buffered with HCO_3^- , under proper oxygenation, then filled with a not-buffered solution and enclosures were made in order to obtain some "gastric bags".



Figure 1 - Removal of the gastrointestinal tract from *Anguilla anguilla*.
Figura 1 - Prelievo del tratto gastrointestinale di *Anguilla anguilla*.

During a first series of experiments, these bags were incubated for 6 h in a beaker containing Ringer solution buffered with HCO_3^- or with HEPES NaOH. The chemical composition of the solutions used is reported in Table 1; the incubation media (Ringer- HCO_3^- or Ringer-HEPES buffers) were also added with histamine 10^{-4} mol/l, a substance which stimulates the acid secretion by the gastric mucosa of eel (Trischitta *et al.*, 1988).

The pH of all the solutions was 8.0 ± 0.1 . Tissue oxygenation was ensured by the addition to the solutions of a gas mixture containing 99 ml/l O_2 + 1 ml/l CO_2 , for Ringer- HCO_3^- buffer solution, and containing 100 ml/l O_2 , for Ringer-HEPES solution.

During the second series of experiments, the formation of ulcers (both acid and not-acid) on the gastric mucosa was experimentally induced by treatment with ethanol 10% or aspirin (10^{-3} mol/l) (used as control gastric bags). Each of the compounds tested during the experiments were added to the incubation beaker at the beginning of the experiment, except for ethanol 10%, which was dissolved in the not-buffered solution and tested by direct inoculation inside the gastric bag. Aspirin was dissolved in methanol (final concentration in the incubation medium, 1 g/l).

In order to test the gastro-protective potential of capsaicin, both the experiments were repeated, including as a final step the treatment with this ingredient, dissolved in methanol, and tested at decreasing concentrations (10^{-4} , 10^{-5} , 10^{-6} mol/l).

Ions	Ringer-HCO ₃ ⁽¹⁾	Ringer-HEPES ⁽²⁾	Solution without buffer ⁽³⁾
Na ⁺	153	153	153
K ⁺	4	4	4
Cl ⁻	144	144	144.8
Mg ²⁺	1.4	1.4	1.4
Ca ²⁺	2.5	2.5	2.5
HCO ₃ ⁻	20	/	/
H ₂ PO ₄	0.8	0.8	/
Glucose	20	20	20
Gluconate	/	15	20
HEPES	/	4.5	/

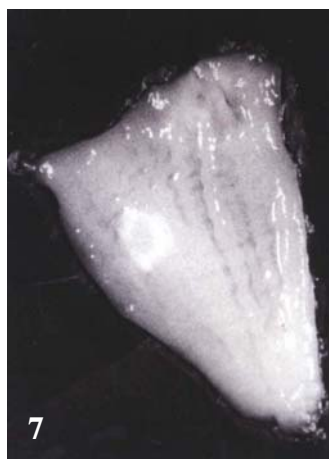
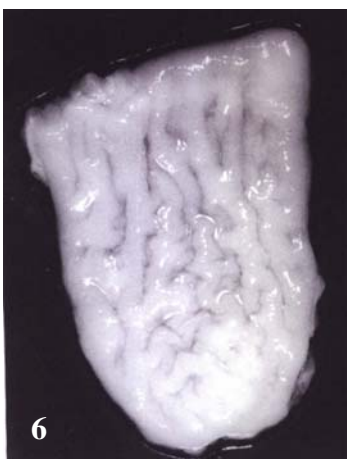
¹T Solutions (1) and (2) were added with 99 ml/l O₂ and 1ml/l CO₂, solution (3) was added with 100 ml/l O₂

Table 1 - Composition of the solutions (concentration in mmol/l).
 Tabella 1 - Composizione delle soluzioni (concentrazione in mmol/l).

After incubation, the gastric bags were opened and underwent to macroscopic observation, in order to detect the presence of gastric ulcers and their recovery after treatment with capsaicin. Each kind of experiment was repeated 6 times.

Plate 1: Figure 2 - Gastric mucosa of *Anguilla anguilla* in normal conditions; Figure 3 - Gastric mucosa of *Anguilla anguilla* after incubation for 6h in Ringer-Hepes and histamine (10⁻⁴ mol/l); Figure 4 - Gastric mucosa of *Anguilla anguilla* after incubation for 6h in Ringer-Hepes, histamine (10⁻⁴ mol/l) and capsaicin (10⁻⁶ mol/l); Figure 5 - Gastric mucosa of *Anguilla anguilla* after incubation for 6h in Ringer-HCO₃⁻ histamine (10⁻⁴ mol/l) and ethanol. The gastric bag is supported by a plastic sheet due to the tissue damage induced by ethanol; Figure 6 - Gastric mucosa of *Anguilla anguilla* after incubation for 6h in Ringer-HCO₃⁻ histamine (10⁻⁴ mol/l), ethanol and capsaicin (10⁻⁶ mol/l); Figure 7 - Gastric mucosa of *Anguilla anguilla* after incubation for 6 h in Ringer-HCO₃⁻ histamine (10⁻⁴ mol/l) and aspirin (10⁻³ mol/l); Figure 8 - Gastric mucosa of *Anguilla anguilla* after incubation for 6h in Ringer-HCO₃⁻ histamine (10⁻⁴ mol/l), aspirin (10⁻³ mol/l) and capsaicin (10⁻⁶ mol/l).

Tavola 1: Figura 2 - Mucosa gastrica di *Anguilla anguilla* in condizioni fisiologiche normali; Figura 3 - Mucosa gastrica di *Anguilla anguilla* dopo incubazione per 6h in Ringer-Hepes e istamina (10⁻⁴ mol/l); Figura 4 - Mucosa gastrica di *Anguilla anguilla* dopo incubazione per 6 h in Ringer-Hepes, istamina (10⁻⁴ mol/l) e capsaicina (10⁻⁶ mol/l); Figura 5 - Mucosa gastrica di *Anguilla anguilla* dopo incubazione per 6h in Ringer-HCO₃⁻ istamina (10⁻⁴ mol/l) ed etanolo. Il sacchetto gastrico è adagiato su un supporto di plastica a causa del danno tissutale prodotto dall'etanolo; Figura 6 - Mucosa gastrica di *Anguilla anguilla* dopo incubazione per 6h in Ringer-HCO₃⁻ istamina (10⁻⁴ mol/l), etanolo e capsaicina (10⁻⁶ mol/l); Figura 7 - Mucosa gastrica di *Anguilla anguilla* dopo incubazione per 6h in Ringer-HCO₃⁻ istamina (10⁻⁴ mol/l) e aspirina (10⁻³ mol/l); Figura 8 - Mucosa gastrica di *Anguilla anguilla* dopo incubazione per 6h in Ringer-HCO₃⁻ istamina (10⁻⁴ mol/l), aspirina (10⁻³ mol/l) e capsaicina (10⁻⁶ mol/l).



RESULTS

Figure 2 shows the aspect of the stomach of eel in normal conditions, with mucosal folding and clear border (boundaries, margins) of the gastric tissue. After incubation in presence of HCO_3^- , the gastric tissue did not show any change (not shown in Figure), while in Ringer-HEPES a wide erosion of the surface mucosal layer was observed, with a flattening of the folded surface (Figure 3). Under this condition, the effects of capsaicin addition were different according to its concentration. In fact, following the incubation with capsaicin at a 10^{-6} mol/l concentration, the gastric mucosa remained undamaged, with clear borderlines and folds (Figure 4), while at higher doses (10^{-5} and 10^{-4} mol/l) this protective effect was mostly undetectable.

The treatment with ethanol 10% resulted in a wide erosion of the gastric mucosa, with deep ulcers (Figure 5). Following the treatment with capsaicin, particularly at the 10^{-6} mol/l concentration a regeneration of the mucosal layer was observed (Figure 6), and ulcers disappeared. The same effect, but not so evident, was recorded after the addition of capsaicin at the highest concentrations (10^{-5} and 10^{-4} mol/l).

The addition of aspirin (10^{-3} mol/l) caused erosion and ulcers in the mucosal layer (Figure 7). Capsaicin determined a protective effect similar to that one previously described, with a greater efficacy at the lowest concentration (10^{-6} mol/l) (Figure 8).

DISCUSSION AND CONCLUSIONS

Results of the experiments performed provided evidence that capsaicin, even “in vitro” studies, such as those performed in our laboratory, played a protective role on the gastric mucosa. Following the treatment with this compound, in fact, it was possible to prevent the erosion of the gastric mucosa caused by the incubation of the gastric bag in solutions containing histamine, while lacking of bicarbonate. This latter is known for its buffer and protective properties on gastric mucosa; therefore, the lacking of bicarbonate and the presence of histamine are equally important for gastric tissue integrity (Trischitta *et al.*, 1988).

In rat, Alföldi *et al.* (1987) have also shown that capsaicin is able to reduce gastric secretion induced by histamine.

The protective effect of capsaicin was mostly detected at the lowest concentration (10^{-6} mol/l), confirming what found in *in vivo* experiments carried out in mammals (Mózsik *et al.*, 1997c). A similar, gastro-protective, effect of capsaicin was also observed after ethanol treatment of the gastric bags, causing a deep tissue damage and ulceration; this was also in agreement with other studies (Holzer & Lippe, 1988; Esplugues & Whittle, 1990; Gyères & Barna, 2002) performed “in vivo” in rats treated with the same experimental procedures. The mucosal microvasculature has been reported to be not only an initial site of damage after ethanol application, but also the site where prostaglandins likely exert their protective effects (Trier *et al.*, 1987).

Capsaicin was also effective in recovering gastric damage produced by aspirin; this compound is an inhibitor of ciclo-oxigenase, enzyme required for the synthesis of prostaglandins (Faggio *et al.*, 2000), which have a protective action on the gastric mucosa. A similar effect was observed by application of resiniferatoxin, an analogous of capsaicin, in rats showing mucosal damage following aspirin and ethanol (Szolcsányi, 1990; Abdel-Salam *et al.*, 1995); both substances (capsaicin and resiniferatoxin) also increased the blood flow in the gastrointestinal tract of rats (Abdel-Salam *et al.*, 1996). Cimetidine is recognised

to exert a protective effect on aspirin- induced gastric damaged mucosa (MacKercher *et al.*, 1977; Szolcsányi & Mózsik, 1984); this effect was also observed by Kang *et al.* (2004).

In our experiment, capsaicin was able, at low concentrations, to repair the ulcers induced by aspirin, in agreement with what found during *in vivo* experiments by Holzer *et al.* (1989) through intragastric application of capsaicin.

The physiological mechanisms involved in the gastric protection during our *in vitro* experiments can be supposed to be related to the production of bicarbonate or mucus, both acting as a protective barrier inside the gastric bag. Above all consideration, it is interesting to note that the mechanisms involved in the mucosal protection or regeneration remain substantially unchanged along the evolutionary scale. As the response of the eel examined to the capsaicin treatment was similar to the one observed in rat and other superior vertebrates, then it is likely to propose this fish as a model species for animal experimentation in pharmacological and toxicological studies. This consideration is in agreement with previous findings (Trischitta *et al.*, 1988; Faggio *et al.*, 2000), which confirmed the suitability of this fish, also in relation to its wide commercial distribution and long resistance to be kept in aquaria, as an animal model for improving knowledge of physiological mechanisms.

REFERENCES

- Abdel-Salam O.M.E., Bòdis B., Karàdi O. & Mózsik G.Y. (1995). Modification of aspirin and ethanol-induced mucosal damage in rats by intragastric application of resiniferatoxin. *Inflammopharmacology*, 3: 135-147.
- Abdel-Salam O.M.E., Szolcsányi J. & Mózsik G.Y. (1997). Capsaicin and the stomach. A review of experimental and clinical data. *J. Physiol.*, 91: 151-171.
- Abdel-Salam O.M.E., Szolcsányi J., Porszasz R. & Mózsik G.Y. (1996). Effect of capsaicin and resiniferatoxin on gastrointestinal blood flow in rats. *Eur. J. Pharmacol.*, 305: 127-136.
- Alfoldi P., Toth E., Obal F., & Hideg J. (1987). Capsaicin treatment reduces histamine-induced gastric acid secretion in the rat. *Acta Physiol. Hung.*, 69: 509-512.
- Davenport H.W. (1969). Gastric mucosal hemorrhage in dogs. Effect of acid, aspirin, and alcohol. *Gastroenterology*, 58: 439-449.
- Esplugues J.V. & Whittle B.J.R. (1990). Morphine potentiation of ethanol-induced gastric mucosal damage in the rat. Role of local sensory afferent neurons. *Gastroenterology*, 98: 82-89.
- Evangelista S. & Meli A. (1989). Influence of capsaicin sensory fibres on experimentally-induced colitis in rats. *J. Pharmacol.*, 41: 574-576.
- Faggio C., Denaro M.G., Lionetto M.G. & Trischitta F. (2000). Protective effects of prostaglandins in the isolated gastric mucosa of the eel, *Anguilla anguilla*. *J. Compar. Physiol. B*, 170: 357-363.
- Grossman M.I. (1980). Peptic ulcer: the pathophysiological background. *Scand. J. Gastroenterol. Supplement*, 15, 58: 7-16.
- Gyires K. & Barna I. (2002). Differences in gastroprotective processes in 6- to 8- and 14- to 16-week-old rats. *Dig. Dis. Sci.*, 47, 12: 2775-2782.
- Hollander D. & Tarnawski A. (1987). Protective effect of sucralfate on the gastric mucosa mediated by endogenous prostaglandins. In "New Pharmacology of ulcer disease. Experimental and new therapeutic approaches", Szabò S., Mózsik Gy. Eds. Elsevier Publishing: 404-412.

- Holzer P. & Lippe I.T. (1988). Stimulation of afferent nerve endings by intragastric capsaicin protects against ethanol-induced damage of gastric mucosa. *Neuroscience*, 27: 981-987.
- Holzer P., Pabst M.A. & Lippe I.T. (1989). Intragastric capsaicin protects against aspirin-induced lesion formation and bleeding in the rat gastric mucosa. *Gastroenterology*, 96: 1425-1433.
- Kang J.Y., Teng C.H. & Chen F.C. (2004). Effect of capsaicin and cimetidine on the healing of acetic acid induced gastric ulceration in the rat. *Gut*, 53: 229-234.
- MacKercher P.A., Ivey K.J., Baskin W.N. & Krause W.J. (1977). Protective effect of cimetidine on aspirin-induced gastric mucosal damage. *Ann. Intern. Med.*, 87: 676-679.
- Miller T.A. (1983). Protective effect of prostaglandin against gastric mucosal damage: current knowledge and proposed mechanism. *Am. J. Physiol.*, 245: G 601-623.
- Miller T.A. (1988). Gastroduodenal mucosal defense: factors responsible for the ability of the stomach and duodenum to resist injury. *Surgery*, 103, 4: 389-397.
- Mózsik G.Y., Abdel Salam O.M.E. & Szolcsányi J. (1997c). Capsaicin-sensitive afferent nerves in gastric mucosal damage and protection. *Akadémiai Kiadó Ed.*: 124 pp.
- Mózsik G.Y., Lovasz L., Kutor G., Nagy L. & Tarnok F. (1980). Experimental evidence for cytoprotective effect of atropine on the rat gastric mucosa. *Acta Med. Acad. Sci. Hung.*, 37: 401-405.
- Mózsik G.Y., Nagy L. & Király Á. (1977a). Twenty five years of peptic ulcer research in Hungary. From basic sciences to clinical practice (1971-1995). *Akadémiai Kiadó Ed.*: 448 pp.
- Mózsik G.Y., Nagy L., Pár A. & Rainsford K.D. (1977b). Cell injury and protection in gastrointestinal tract. From basic sciences to clinical perspectives. *Kluwer Academic Pub. Ed.*: 336 pp.
- Szabò S. & Goldberg I. (1990). Experimental pathogenesis: drugs and chemical lesions in the gastric mucosa. *Scand. J. Gastroenterol. Supplement*, 174: 1-8.
- Szallasi A. & Blumberg P.M. (1993). Mechanisms and therapeutic potential of vanilloids (capsaicin-like molecules). *Adv. Pharmacol.*, 24: 123-155.
- Szolcsányi J. (1982). Capsaicin type pungent agents producing pyrexia. In "Handbook of experimental pharmacology, pyretics and antipyretics". Milton A.S. Ed. Springer-Verlag Publishing: 437-478.
- Szolcsányi J. (1990). Effect of capsaicin, resiniferatoxin and piperine on ethanol-induced gastric ulcer of the rat. *Acta Physiol. Hung.*, 75 (Suppl.): 267-268.
- Szolcsányi J. & Mózsik G.Y. (1984). Effects of capsaicin on the development of gastric mucosal damage by different necrotizing agents and of gastric cytoprotection by PGI₂ atropine and cimetidine on rats. *Acta Physiol. Hung.*, 64: 287-291.
- Trier J.S., Szabò S. & Allan C.H. (1987). Ethanol-induced damage to mucosal capillaries of rat stomach. Ultra-structural features and effect of prostaglandin F₂ and cysteamine. *Gastroenterology*, 92: 13-22.
- Trischitta F., Denaro M.G., Faggio C., Mandolino M. & Schettino T. (1988). H⁺ and Cl⁻ secretion in the stomach of the teleost fish, *Anguilla anguilla*: stimulation by histamine and carbachol. *J. Compar. Physiol. B*, 168: 1-8.

Winter J. (1987). Characterization of capsaicin sensitive neurons in adult rat dorsal root ganglion culture. *Neurosci. Lett.*, 80: 134-140.

Wood J.N., Winter J., James I.F., Rang H.P., Yeats J. & Bevan S. (1988). Capsaicin induced ion fluxes in dorsal root ganglion neurons in culture. *J. Neurosci.*, 8: 3208-3220.