involved in this activity are induced by introduction of DNA into the cell. However, studies of microinjection of DNA fragments into cells have shown that intermolecular recombination and ligation occur at a very high efficiency (*16*), indicating that this phenomenon may not be specific to transfection. One use of this joining activity is the ability to construct recombinant DNA molecules in vivo. If selection exists for a desired recombinant molecule, the molecule can be constructed in vivo by cotransfection of the appropriate DNA fragments. These findings also indicate that caution must be used in designing transfection experiments. Studies of DNA after transfection will be studies of complex molecules, not linear molecules, because of the high efficiency of ligation and recombination.

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## Inhibition of Gastric Acid Secretion in the Gastric Brooding Frog, *Rheobatrachus silus*

Abstract. *The female gastric brooding frog* Rheobatrachus silus *broods its young in its stomach. A substance that inhibits gastric acid secretion in a toad stomach preparation in vitro appears to be secreted by the developing young. This substance has been identified as prostaglandin* $E_2$. Rheobatrachus silus *may thus have developed a mechanism whereby prostaglandin secreted by the larvae inhibits acid secretion in the stomach of the female until the larvae have completed development and emerged as juvenile frogs by way of the female's mouth.*

In southeast Queensland there is a rare aquatic frog, *Rheobatrachus silus*. The female frog swallows fertilized eggs or early-stage larvae and broods the young in her stomach. The young frogs eventually emerge by way of her mouth (*1*, *2*). During the period of gastric brooding, the gastric musculature undergoes modification and the oxyntic cells show ultrastructural features typical of the absence of acid secretion (*3*). Oral birth of the young frogs occurs at least 8 weeks after they are ingested; the gastric ultrastructure returns to normal a few days later. Investigators have speculated that the young release a substance that inhibits gastric acid secretion. Because the species is near to extinction, our data in this and previous reports (*1*–*3*) are based on studies of only five female frogs collected over 8 years. Our studies have been performed on histological specimens or on secretions obtained during the short life-span of these females and their young in captivity. Here we provide evidence that *R. silus* tadpoles secrete a substance that inhibits the secretion of gastric acid by an isolated amphibian gastric mucosal preparation. High concentrations of this inhibitory substance, which was identified as prostaglandin $E_2$ ($PGE_2$), were present in aquarium water inhabited by young *R. silus* immediately after their premature birth.

In December 1978 a female *R. silus* expelled 24 early-stage tadpoles while being transferred to an aquarium. Twenty-two of these larvae were transferred to an open plastic container of purified, softened tapwater. The tapwater had been filtered through sand to remove particulate matter, through activated carbon to dechlorinate it, and through a fine micropore filter to exclude large bacteria. The water was aerated gently and maintained at 30°C. Tapwater for the related, control species *Limnodynastes tasmaniensis* was treated in the same way.

Copious, fine cords of mucus were released from the mouths of the tadpoles. Because an inhibitor of gastric acid secretion might be associated with the mucus, the aquarium water, which was replaced daily, was collected and stored at −20°C. Before being assayed, this water was concentrated by freeze-drying; 500 ml of water produced about 0.24 g of solid material that was redissolved in 10 ml of Mackenzie's solution (*4*).

We used the spontaneously secreting gastric mucosa of *Bufo marinus* as the principal bioassay for the potential inhibitor of gastric secretion. The toads were pithed, the stomach was removed, and the gastric mucosa was dissected from the muscle coats. Fundic mucosa was mounted as a diaphragm between two halves of a Lucite chamber to provide exposed mucosal and serosal surfaces measuring 2.0 $cm^2$. The serosal surface was bathed in 12 ml of Mackenzie's solution, a buffered salt solution for *Bufo marinus* tissues (*4*). The mucosal surface was bathed in 12 ml of isotonic NaCl. Both solutions were aerated and circulated by gas lifts of 95 percent $O_2$ and 5 percent $CO_2$. The mucosal solution was held at *p*H 6 by titration with 0.05*M* NaOH in a *p*H stat system (Radiometer Copenhagen). This system provided a measure of the rate of $H^+$ secretion which was expressed as microequivalents per square centimeter per hour. The test solution was applied to the serosal side of the mucosal preparation.

The isolated mucosa of *Bufo marinus* secreted acid spontaneously at a rate of $1.92 \pm 0.69$ $\mu Eq/cm^2$-hour (mean ± standard deviation, $N = 49$). The rate was independent of the sex of the toad and remained relatively constant for at least 3 hours. In each of six experiments, the addition of 1.5 ml of the concentrated aquarium water to the gastric mucosal preparation decreased the rate of spontaneous acid secretion. The mean rate (± S.D.) of acid secretion 30 minutes before the addition of the aquarium water was $1.88 \pm 0.54$ $\mu Eq/cm^2$-hour; during the second 30-minute period of exposure the mean rate was $0.28 \pm 0.24$ $\mu Eq/cm^2$-hour (Fig. 1). A similar degree of inhibition was effected by $10^{-6}M$ $PGE_2$, $10^{-6}M$ somatostatin, and $10^{-4}M$ cimetidine. In two experiments, the inhibitory effect of aquarium water was

reversed by replacement of the bathing solution. Aquarium water holding *L. tasmaniensis* tadpoles had no effect on the rate of acid secretion (control rate, 2.07 ± 0.15 μEq/cm$^2$-hour; rate during the second 30-minute period after addition of concentrated aquarium water, 1.86 ± 0.37 μEq/cm$^2$-hour; $N = 6$).

We then performed thin-layer chromatography (TLC) using Kieselgel-60 plates (Merck) and the organic phase of the solvent system consisting of ethyl acetate, isooctane, acetic acid, and water (11:5:2:10 by volume) (*5*). Freeze-dried aquarium water was reconstituted in 10 ml of 96 percent ethanol and 4 percent $H_2O$, and 1- to 2-ml samples were applied to the plates. A variety of pure prostaglandins, including $PGE_2$ $PGF_{2\alpha}$, $PGA_2$ and thromboxane, were run as reference compounds. The chromatograms were developed and then cut into 12 equal sections; each section was extracted with a solution of 2 ml of ethyl acetate and 0.5 ml of $H_2O$. The ethyl acetate phase was evaporated to dryness with $N_2$ and reconstituted with Mackenzie's solution before being bioassayed. When 1- and 2-ml samples of aquarium water concentrate were run, extracts of the thin-layer plates around and just below the area corresponding to $PGE_2$ elicited decreases in acid secretory rates of 52 and 85 percent, respectively. No significant inhibition of acid secretion was achieved by samples taken elsewhere on the plates.

A radioimmunoassay for $PGE_2$ was carried out with an antibody that showed the following cross reactivities: $PGE_2$, 100 percent; $PGA_2$, 93.1 percent; 15-keto-$PGE_2$, 55.8 percent; and 13,14-dihydro-15-keto-$PGE_2$, 19.1 percent. This antibody was used at a final dilution of 1:3500. When assayed at various dilutions, immunoreactive material in the aquarium water gave a mean value of about 2.9 nmole/ml (range, 2.2 to 3.6 nmole/ml; $N = 12$) for $PGE_2$ in the concentrate.

Subsequent analyses by gas chromatography-mass spectrometry (GCMS) with a VG Micromass 305F instrument coupled through an all-glass jet separator to a Carlo Erba gas chromatograph confirmed the presence of $PGE_2$ in the water. For analysis a portion of freeze-dried aquarium water equivalent to 0.5 ml of a 50 times concentrate was taken up in 2.0 ml of tris buffer (0.05*M*, *p*H 7.4), and the internal standard 20 methyl-PGE (1.4 nmole) was added. Two milliliters of an oximating solution (three parts of an aqueous hydroxylamine hydrochloride solution, 7.2*M*, and 20 parts of a pyridinium acetate buffer, *p*H 5.1, 3.5*M*) were added and the mixture heated at 60°C for

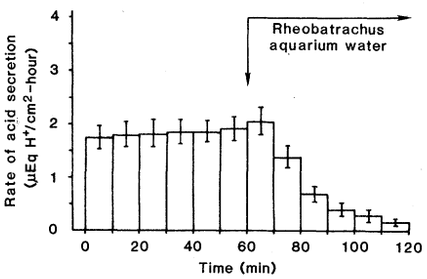


Fig. 1. Inhibition of gastric acid secretion by *R. silus* aquarium water. The serosal surface of a *B. marinus* stomach preparation was exposed to 1.5 ml of concentrated aquarium water in 12 ml of Mackenzie's solution. The data are expressed as the means (± standard error of the mean) for six experiments.

30 minutes. The oximate of $PGE_2$ and other prostaglandins were then extracted into 10 ml of a mixture of ether and ethyl acetate (4:1 by volume), the aqueous phase was discarded, and the organic phase was washed with 2.0 ml of water and taken to dryness under $N_2$.

Prostaglandins present in the extract were then methylated with diazomethane and transferred to a glass tube (50 by 3 mm) with 50 μl of 4*M* imidazole and 50 μl of 2*M* *t*-butyldimethylchlorosilane (BDMS), both of which had been dissolved in dry dimethyl formamide. The tube was sealed and heated at 120°C for 30 minutes. The contents were then placed on a 20 by 5 mm column of Sephadex LH 20 swollen in hexane and ethyl acetate (4:1 by volume). The extract was washed and a portion was injected into the GCMS with an autosolids device. When analyzed in the selected ion monitoring mode, peaks with retention times characteristic of the isomers of $PGE_2$ derivatives were evident in the channel monitoring the mass to charge ratio (*m/e*), the ion resulting from the loss of a butyl radical from the molecular ion of the $PGE_2$ derivative. Comparison of the peak heights with the internal standards indicated 1.42 nmole/ml $PGE_2$ was present in the aquarium water concentrate. No evidence of $PGA_2$, $PGE_1$, $PGE_3$, $PGEM_1$, $PGEM_2$, or $PGI90HE_2$ was found (< 10 p*M*).

Qualitative mass spectrographic analysis of a separate sample of freeze-dried aquarium water, treated to form the methoxime, trimethylsilyl methyl ester of $PGE_1$, confirmed the presence of $PGE_2$; the characteristic ion peaks in the mass spectra corresponded to *m/e* 539 (M); 524 (M − 15); 508.8 (M − 31); 449 (M − 90); 418[M − (31 + 90)]; 295[M − (31 + 127 + 71)]; and 225.

Prostaglandins may have several distinct physiological roles in the gastric mucosa. Prostaglandin synthesized within the mucosa enhances gastric mucosal defense by stimulating the secretion of bicarbonate and mucus (*6*). Endogenous prostaglandin may have a role in regulating mucosal blood flow. Several natural prostaglandins, when administered intravenously or orally, inhibit both basal and stimulated acid secretion in man and a wide variety of other animals. The site of this action of $PGE_2$ appears to be within the parietal cell where it inhibits the ability of histamine to stimulate the production of adenosine 3′,5′-monophosphate (*7*). The amount of $PGE_2$ in aquarium water as measured by GCMS appears to be sufficient to cause significant inhibition of gastric acid secretion. It can be calculated that each brood of tadpoles produced at least 5.7 nmole of $PGE_2$ per day (200 ml of aquarium water per day, freeze-dried to 4 ml, contained 1.42 nmole of $PGE_2$ per milliliter). Since the acid inhibitory dose of $PGE_2$ in vivo is of the order of 142 nmole/kg we interpret the results as indicating that the brood of tadpoles produces sufficient $PGE_2$ to inhibit acid secretion in a female *R. silus* weighing 12 g.

Our results suggest that in *R. silus*, $PGE_2$ inhibits acid secretion in a manner not seen elsewhere in the Animal Kingdom. Inhibition of acid secretion presumably commences when the eggs or early-stage larvae are swallowed, although we have no evidence that sufficient prostaglandin is present in the eggs. Inhibition is maintained by $PGE_2$ emanating from the young, and inhibition ceases as soon as birth takes place.

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