

COMPARATIVE STUDY OF EFFECTS OF PROSTAGLANDIN E₂ ON cAMP LEVELS IN GONADS OF THE PRAWN *MACROBRACHIUM ROSENBERGII* AND THE CRAYFISH *CHERAX QUADRICARINATUS*

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A B S T R A C T

We compared the effects of prostaglandin E₂ (PGE₂) in vitro, as reflected by cAMP levels, on ovaries and testes of the giant prawn *Macrobrachium rosenbergii* (Decapoda, Caridea) and the red-claw crayfish *Cherax quadricarinatus* (Decapoda, Astacidea). These decapods differ in their reproductive physiology, representing distinct reproductive strategies. In both ovaries and testes of *M. rosenbergii*, PGE₂ (10 µg/ml) significantly stimulated cAMP synthesis, the reaction being augmented by IBMX. In contrast, in both ovaries and testes of *C. quadricarinatus*, PGE₂ had no effect on cAMP levels. In muscular tissue, which served as the control for both species, cAMP levels were unaffected by PGE₂. This comparative study suggests a case of different responses to PGE₂ in the gonads of 2 decapod species that have distinctly different reproductive biologies.

The giant prawn *Macrobrachium rosenbergii* (de Man) (Decapoda, Caridea) and the red-claw crayfish *Cherax quadricarinatus* (von Martens) (Decapoda, Astacidea) exhibit two distinct reproductive strategies, illustrating the great diversity of life cycles that have evolved during the evolution and diversification of the decapods (Schram, 1977, 1982). *Macrobrachium rosenbergii* produces numerous relatively small eggs. Its larval development is dependent on sea water (Ling, 1969). Ovarian maturation is synchronous (O'Donovan *et al.*, 1984), and molt and reproduction are coupled (Ling, 1969). Unlike *Macrobrachium*, *Cherax quadricarinatus* has evolved into a strictly fresh-water species. It lays relatively fewer and larger eggs than *M. rosenbergii* and goes through abbreviated larval development (Jones, 1990). Mature ovaries are characterized by two distinct populations of oocytes of different sizes (Sagi *et al.*, 1996), and molt and reproduction are antagonistic events (Barki *et al.*, 1997).

Recently there has been an increase in the number of studies suggesting a regulatory role for prostaglandins (PGs) in invertebrate reproduction (Loher *et al.*, 1981; Kunigelis and Saleuddin, 1986; Spaziani *et al.*, 1993; Varaskin and Reunova, 1993; Sagi *et al.*, 1995). The regulatory role of PGs in reproductive physiology is well established in mammals, in which these compounds control important reproductive functions such as ovulation (Priddy and Killick, 1993), luteolysis (Bennegard *et al.*, 1991), and parturition (Slater *et al.*, 1994). The effect of PGs is me-

diated through changes in levels of intracellular second messengers, such as cAMP, diacylglycerol, IP₃, and calcium (Negishi *et al.*, 1995). PGs also play a role in the reproduction of different invertebrate orders. In the snail *Helisoma durgi* (Wetherby), PGE₂ stimulates a long-term increase in egg production (Kunigelis and Saleuddin, 1986). In the scallop *Mizuhopecten yessoensis* (Jay), it modulates the rate of proliferation of spermatogonia (Varaskin and Reunova, 1993). In the cricket *Teleogryllus commodus* (Walker), it mediates oviposition (Loher *et al.*, 1981).

A small number of studies have been made on crustaceans. Spaziani *et al.* (1993) reported the presence and gradual increase in ovarian PGE₂ and PGF_{2α} (as well as their precursors) during vitellogenesis in the crayfish *Procambarus paeninsulanus* (Faxon) (see Spaziani *et al.*, 1993; Spaziani and Hirsch, 1997). Further studies by these authors demonstrated that PGF_{2α} induces cAMP-mediated contraction of ovarian tissue in vitro, suggesting a possible involvement of PGF_{2α} in ovulation (Spaziani *et al.*, 1995). Our group demonstrated that PGE₂ significantly stimulates cAMP synthesis in vitro in ovarian tissue of the prawn *M. rosenbergii* (see Sagi *et al.*, 1995).

The remarkable differences in the reproductive physiology of *M. rosenbergii* and *C. quadricarinatus* suggest that different mechanisms of endocrine regulation of reproduction may have evolved in these species. We thus compared the effects of PGE₂ on both male and female reproductive systems of

these two species by measuring changes in cAMP levels in testes and ovaries incubated *in vitro*.

MATERIALS AND METHODS

Animals.—The crayfish and prawns were reared in tanks at $27 \pm 2^\circ\text{C}$. Water quality was controlled by recirculating the water through a gravel biofilter. The animals were fed frozen ground fish and vegetables.

Female *M. rosenbergii* were selected according to the color of the ovary observed through the carapace (Chang and Shih, 1995). Female *C. quadricarinatus* were selected according to the state of development of the ovigerous setae and relative growth of the endopod, representing female sex characteristics (Sagi *et al.*, 1996). For each dissected female, oocyte diameter was determined by means of an objective micrometer from a random sample of 15 fresh oocytes per ovary.

Males of *M. rosenbergii* and *C. quadricarinatus* were selected from the adult population. A sample of the testicular contents was spread on a glass slide and microscopically examined for the presence of mature spermatozoa.

Molt stage was determined for each animal, and all animals selected were at the intermolt stage.

Organ Incubation.—The animals selected were dissected after being anesthetized by submerging them in ice. Gonads and abdominal muscular tissue were removed, weighed, and sectioned into fragments. The fragments were washed and then incubated in Dulbecco's modified Eagle's medium (DMEM, Biological Industries, Beth Haemek, Israel) supplemented with 1% BSA, adjusted to the hemolymph osmolarity of each species, as relevant. The viability of the tissue incubated was previously tested by monitoring the kinetics of incorporation of radiolabeled thymidine and amino acids in a sample of each species. For *C. quadricarinatus*, NaCl (2.494 g/l) and $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ (0.404 g/l) were added to DMEM to give an osmolarity of 428 mOsm. For *M. rosenbergii*, the adjustment was carried out as described elsewhere (Sagi *et al.*, 1991). The incubation medium contained 10 $\mu\text{g/ml}$ of PGE_2 with or without 3-isobutyl-1-methylxanthine (IBMX), a potent inhibitor of phosphodiesterase, the hydrolyzing enzyme of cAMP. For dose-dependent experiments, tissue fragments were incubated using the following concentrations of PGE_2 : 0.05, 0.5, 1, 5, and 10 $\mu\text{g/ml}$. All incubations were carried out for 1 h at 28°C , with slight agitation under an oxygen-enriched atmosphere ($\text{CO}_2:\text{O}_2$, 5%:95%).

cAMP Extraction and Radioimmunoassay.—At the end of the incubation period, tissue fragments were removed from the culture medium and boiled for 10 min in sodium acetate buffer, 50 mM, pH 4. The fragments were homogenized and centrifuged at 970 g for 10 min at 4°C . cAMP in the supernatants was determined by radioimmunoassay, based on the procedure of Frandsen and Krishna (Frandsen and Krishna, 1976). Rabbit antiserum to 2', O-succinyl-cAMP-BSA (BioMaker, diluted 1:1,000), and [^{125}I]-2'-O-succinyl iodotyrosine methylester of cAMP (2,200 Ci/mmol, DuPont NEN, diluted to 0.2 $\mu\text{Ci/ml}$) were added to acetylated samples of the supernatants and to standard cAMP solutions (ranging from 10 pg/ml–2 ng/ml) and incubated overnight at 4°C . Free and bound antigens were separated by the addition of cold (0– 4°C) sodium acetate buffer, 50 mM, pH 6.2 (containing 1% BSA) followed by cold 99.8% ethanol. The tubes were

centrifuged at 670 g for 45 min at 4°C , the supernatants were then discarded, and the dry sediments were counted in a 1275 MiniGamma counter (LKB-Wallac). cAMP levels were expressed as ng cAMP per mg of wet tissue or ng cAMP per μg DNA. Total ovarian DNA content was determined by means of a fluorimetric method (Labarca and Paigen, 1980) to normalize for the increase in yolk protein during the vitellogenic process.

Statistical Analysis.—The significance of differences between the treatments was tested by Mann-Whitney *U* test (a nonparametric alternative to the *t*-test for independent samples). Probabilities below 0.05 were considered significant.

RESULTS

In incubated early vitellogenic ovaries of *M. rosenbergii*, PGE_2 caused a significant ($P \leq 0.01$) increase in cAMP levels (Fig. 1A). The response was augmented by IBMX, which prevented the hydrolysis of cAMP, which resulted in its accumulation ($P \leq 0.01$). In contrast, in incubated early vitellogenic ovarian tissue of *C. quadricarinatus*, PGE_2 had no significant effect on cAMP (Fig. 1B). cAMP levels were significantly increased only by the action of IBMX, via the accumulation of basal cAMP ($P \leq 0.01$). The levels of cAMP in control muscular tissue from both *M. rosenbergii* and *C. quadricarinatus* were not affected significantly by any of the treatments (Fig. 1E, F).

When early vitellogenic ovaries of *M. rosenbergii* were incubated with different concentrations of PGE_2 , a saturation curve was obtained (Fig. 2A). The curve reached a plateau at about 1 $\mu\text{g/ml}$ of PGE_2 , which persisted to 10 $\mu\text{g/ml}$. In contrast, when ovarian tissue of *C. quadricarinatus* was incubated with different concentrations of PGE_2 , cAMP production was not significantly different from the basal ovarian activity (Fig. 2A). The slight increase in cAMP detected at 5 $\mu\text{g/ml}$ of PGE_2 was not significant. In control muscular tissue of both *M. rosenbergii* and *C. quadricarinatus*, cAMP levels remained unchanged at all doses of PGE_2 tested (Fig. 2C).

In ovaries of *M. rosenbergii* at different vitellogenic stages, PGE_2 significantly ($P \leq 0.04$) increased cAMP levels in each stage, with only one exception, as shown in Fig. 3A. In contrast, in ovaries of *C. quadricarinatus*, PGE_2 had no significant effect on cAMP levels in any of the vitellogenic stages examined (Fig. 3B).

In incubated testes of *M. rosenbergii*, PGE_2 caused a significant ($P \leq 0.01$) increase in cAMP levels (Fig. 1C), the response being augmented by IBMX ($P \leq 0.01$). In contrast,

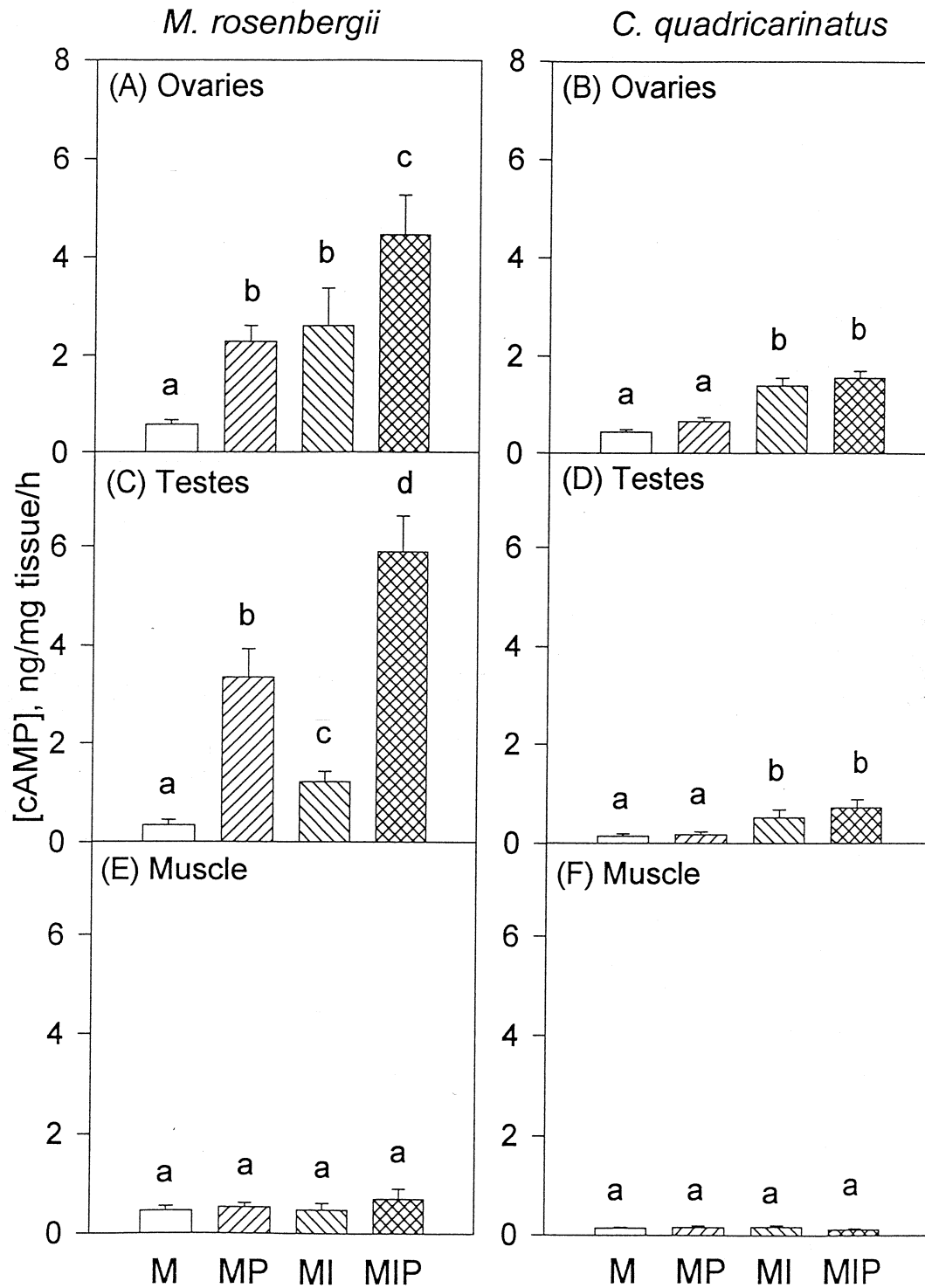


Fig. 1. Effects of PGE₂ and IBMX on cAMP levels in incubated early vitellogenic ovaries of *Macrobrachium rosenbergii* (A), testes (C), and abdominal muscle (E), and early vitellogenic ovaries of *Cherax quadricarinatus* (B), testes (D), and abdominal muscle (F). M = culture medium; MP = culture medium with 10 µg/ml PGE₂; MI = culture medium with 2 mM IBMX; MIP = culture medium with IBMX and PGE₂. Error bars represent SE. Different letters represent significant difference in each type of incubated tissue.

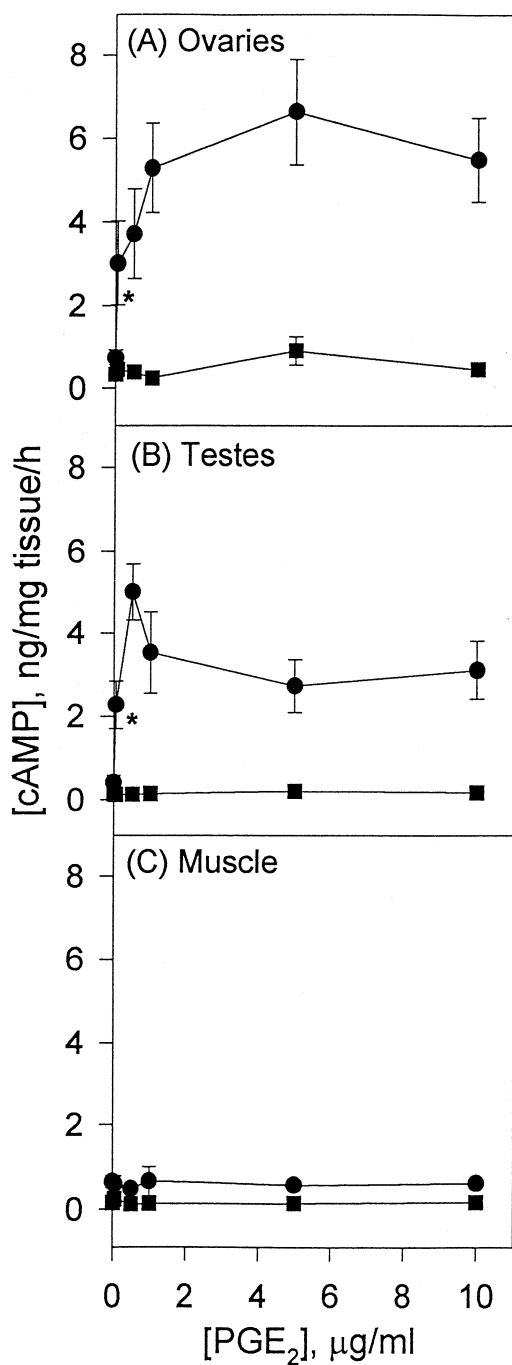


Fig. 2. Effects of different concentrations of PGE₂ on cAMP levels in incubated early vitellogenic ovaries (A), testes (B), and abdominal muscle (C) of *Macrobrachium rosenbergii* (●) and *Cherax quadricarinatus* (■). Error bars represent SE. * Represents significant difference at $P \leq 0.01$ between control and treatment from that concentration.

in incubated testicular tissue of *C. quadricarinatus*, PGE₂ had no significant effect on cAMP (Fig. 1D). cAMP levels were increased significantly ($P \leq 0.01$) only in the presence of IBMX. In control muscular tissue from both species, cAMP was not significantly affected by any treatment (Fig. 1E, F).

When testes of *M. rosenbergii* were incubated with different concentrations of PGE₂, the increase in cAMP levels was evident up to 0.5 μg/ml of PGE₂ (Fig. 2B). Less pronounced, but still significant, effects on cAMP were observed at higher concentrations of PGE₂. In contrast, cAMP production in testes of *C. quadricarinatus* incubated with different concentrations of PGE₂ was not significantly different from the basal activity (Fig. 2B). In control muscular tissue of both species, cAMP levels remained unchanged at all doses of PGE₂ tested (Fig. 2C).

DISCUSSION

The results of our study demonstrate different responses of the reproductive tissue of the two model decapod crustacean species to PGE₂. Whereas both ovarian and testicular tissues of *M. rosenbergii* responded strongly to PGE₂ by significant elevation in the levels of the intracellular second messenger cAMP, gonadal cAMP levels in *C. quadricarinatus* were not elevated in response to PGE₂. This difference may be part of the distinct evolutionary development of the two species.

In mammalian target cells, PGE₂ exerts its effects by stimulating the enzyme adenylyl cyclase, followed by an increase in cAMP levels (Negishi *et al.*, 1995). PGE₂ exerts a significant effect on the reproductive tissues of *M. rosenbergii*, as manifested by a marked increase in the levels of cAMP. This suggests that these tissues may respond similarly to PGE₂. There is, however, very little information in the literature about the biochemical mechanisms of action of PGs on invertebrate cells (Sonetti *et al.*, 1987; Parrish *et al.*, 1992; Spaziani *et al.*, 1995).

PGE₂ has been previously detected in ovaries of *M. rosenbergii* (Sagi *et al.*, 1995). The strong effect of PGE₂ on ovarian cAMP levels during the entire vitellogenic process suggests its possible involvement in the regulation of ovarian metabolic processes. However, Spaziani and colleagues showed that the rate of synthesis and the concentrations of PGE₂ and PGF_{2α} in the ovaries of the crayfish

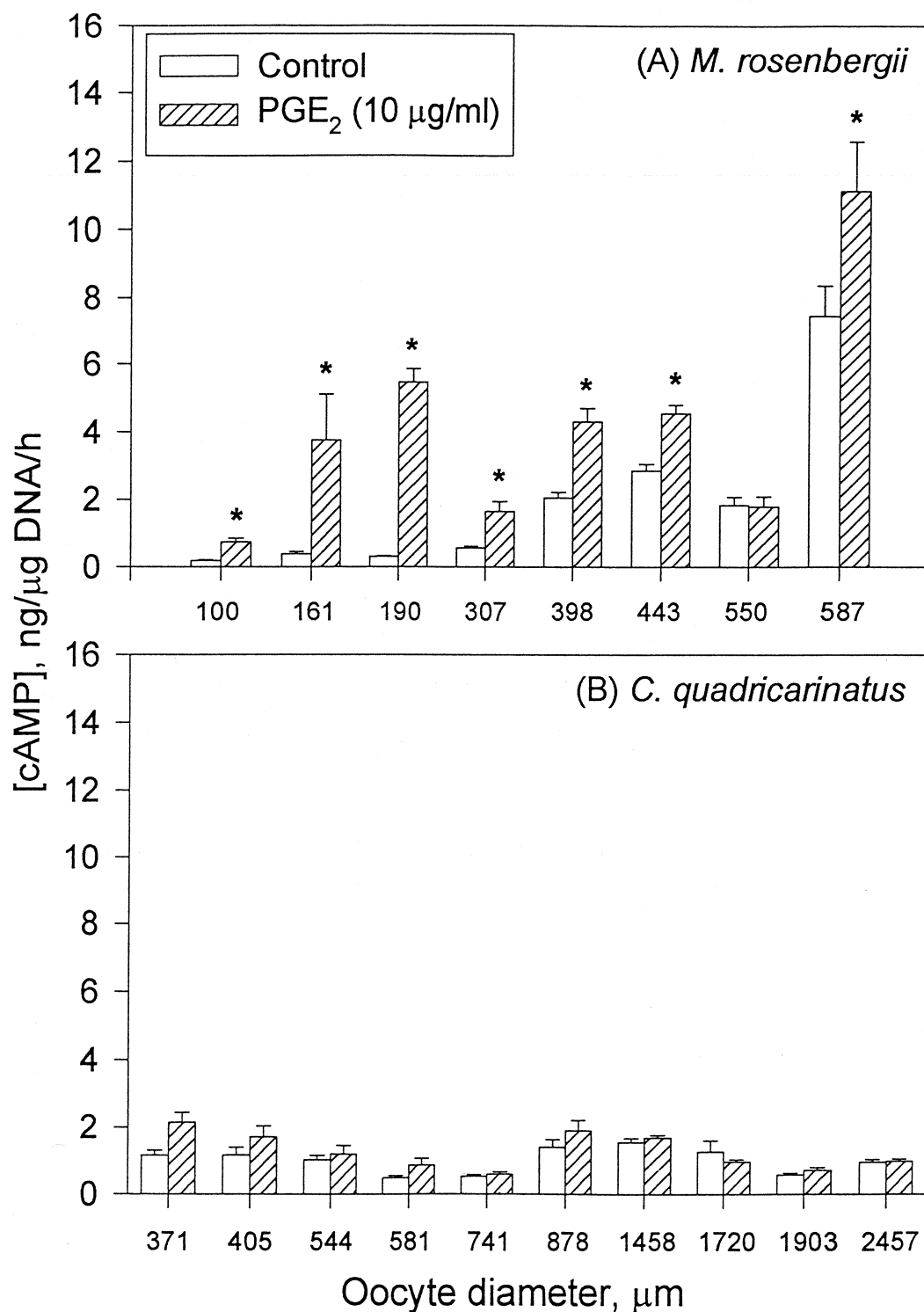


Fig. 3. Effects of PGE₂ on cAMP levels in incubated ovaries of *Macrobrachium rosenbergii* (A) and *Cherax quadricarinatus* (B) in different vitellogenic stages. Error bars represent SE. * Represents significant difference at $P \leq 0.04$ between treatment and control.

P. paeninsulanus increased only toward the final stages of yolk production (Spaziani *et al.*, 1993), and that $\text{PGF}_{2\alpha}$, and not PGE_2 , caused a dose-dependent increase in cAMP levels and contraction of ovarian tissue in vitro (Spaziani *et al.*, 1995). Despite their use of early vitellogenic ovaries, these authors suggested a role for $\text{PGF}_{2\alpha}$ in ovulation. In another arthropod, the silkworm *Hyalophora cecropia* (Linnaeus), cAMP induced in vitro termination of vitellogenin uptake by vitellogenic follicles (Wang and Telfer, 1996). Thus, we cannot exclude the possibility that PGE_2 receptors, which seem to be present in ovaries of *M. rosenbergii* in all stages of ovarian development, might become exposed to a sufficient concentration of their ligands only at the end of vitellogenesis, for example, to terminate the accumulation of yolk or to induce ovulation.

The discovery of an effect of PGE_2 on testicular cAMP in *M. rosenbergii* is the first report of this phenomenon in a male crustacean. The role of PGE_2 in testes of *M. rosenbergii* is not yet known, but in another invertebrate, the scallop *Mizuhopecten yessoensis*, PGE_2 was suggested to modulate the rate of proliferation of spermatogonia (Varaskin and Renova, 1993).

In both ovaries (at all vitellogenic stages examined) and testes of *C. quadricarinatus*, no effect of PGE_2 on cAMP was observed. We have already discussed the possibility of the evolution of different mechanisms that control reproduction in *Macrobrachium* and *Cherax*. Different prostaglandins or other hormones, such as other eicosanoids (Stanley-Samuels, 1994; Stanley-Samuels and Pedibhotla, 1996), may be active in different decapods, e.g., $\text{PGF}_{2\alpha}$ rather than PGE_2 , affect ovarian cAMP in the crayfish *P. paeninsulanus* (see Spaziani *et al.*, 1995). Another possibility is that PGE_2 does affect gonadal tissue of *Cherax*, but acts on the mammalian-like EP_1 -type rather than the EP_2 - or EP_4 -type receptors. In this case, Ca^{2+} , rather than cAMP levels, would be increased (Negishi *et al.*, 1995). Thus, measurements of cAMP would not reflect a possible tissue response. Further studies are needed to elucidate the role of prostaglandins in decapod crustaceans.

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