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The heart of *Ciona intestinalis*: eicosanoid-generating capacity and the effects of precursor fatty acids and eicosanoids on heart rate

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Summary

Eicosanoids are a group of oxygenated fatty-acid derivatives formed from C20 polyunsaturated fatty acids including arachidonic and eicosapentaenoic acids. In mammals, these compounds have been shown to be key molecules in several physiological processes including regulation of the vascular system. This study determined whether eicosanoids or their precursors are involved in the regulation of heart rate in the sea squirt *Ciona intestinalis*. Eicosanoid generation by both heart and blood cells was measured. The major lipoxygenase products formed were both derivatives of eicosapentaenoic acid, namely 8- and 12-hydroxyeicosapentaenoic acids (8-HEPE and 12-HEPE). Smaller amounts of 8,15-dihydroxyeicosapentaenoic acid (8,15-diHEPE) were also formed. The cyclo-oxygenase product prostaglandin E was also found in small amounts in the heart. Isolated hearts were exposed either to these fatty acid precursors or to 8-HEPE, 12-HEPE or prostaglandin E_3, and the effect on heart rate was recorded. Both eicosapentaenoic and arachidonic acids stimulated the heart rate at concentrations between 50 and 200 $\mu mol\,l^{-1}$. 12-HEPE (5 $\mu mol\,l^{-1}$) and prostaglandin E_3 (50 $\mu mol\,l^{-1}$) caused a modest increase in heart rate, while 8-HEPE had no significant effects at any of the time periods studied ($\leqslant$180 min). Overall, the results show that arachidonic and eicosapentaenoic acids have limited effects on heart rate and only at concentrations unlikely to be routinely liberated *in vivo*. Similarly, the eicosanoids tested had a minor stimulatory activity on heart rate. The potential mechanisms for this stimulation are discussed. Overall, these results suggest that such compounds are of limited importance in regulating the heart and vascular system of sea squirts.

Key words: eicosanoid, heart, vascular system, eicosapentaenoic acid, sea squirt, *Ciona intestinalis*.

Introduction

Eicosanoids are a family of biologically active oxygenated derivatives of C20 polyunsaturated fatty acids (PUFAs). Two main pathways are involved in the production of eicosanoids. The lipoxygenase (LO) pathway transforms PUFAs into lipoxins, leukotrienes and monohydroxy fatty acids, whereas the cyclo-oxygenase pathway produces prostaglandins and thromboxanes. These pathways predominantly use two PUFAs, arachidonic acid (AA, 20:4*n*-6) and eicosapentaenoic acid (EPA, 20:5*n*-3), as a substrate. Eicosanoids, in particular the prostaglandins (PGs), play many key roles in physiological processes including water and solute transport in the kidney, the initiation of parturition and haemostasis (Stanley, 2000).

Two main PGs of significance to the mammalian vascular system are prostacyclin (PGI_2) and thromboxane A_2 (TxA_2). Prostacyclin is synthesized by the vascular endothelium and causes vasodilatation as well as inhibiting platelet aggregation (Bunting et al., 1976). These activities are opposed by TxA_2, which is strongly pro-aggregatory for platelets and causes vasoconstriction (Hamberg et al., 1975). The balance between these two compounds may be of importance in maintenance of the vascular tone and in haemostasis in general (Fitzgerald et al., 1987; Ullrich et al., 2001). As well as these two prostaglandins, other eicosanoids including prostaglandin E_1 (PGE_1) have vasoactive effects such as vasodilation (e.g. Carlson et al., 1969).

An increasing body of literature has revealed not only that eicosanoids are present in every major invertebrate phylum (Stanley and Howard, 1998) but also that they play key roles in a great variety of processes in invertebrates such as reproduction, immunity and ion transport (Stanley, 2000). Our understanding of whether eicosanoids play any role in the vascular system of invertebrates is limited to a single study by Agnisola et al. (1994), who studied the effects of AA on the systemic heart of the octopus *Octopus vulgaris*. They found that perfusion with AA (10^{-7} to $10^{-5}\,mol\,l^{-1}$) caused a concentration-dependent increase in heart rate that had a biphasic effect on inotropism (positive for the lowest concentration, $10^{-7}\,mol\,l^{-1}$, but negative for the two higher concentrations, 10^{-6} and $10^{-5}\,mol\,l^{-1}$). A potent vasoconstrictory effect was also noted, associated with an increase in coronary resistance.

The tunicate heart makes an excellent subject for studying

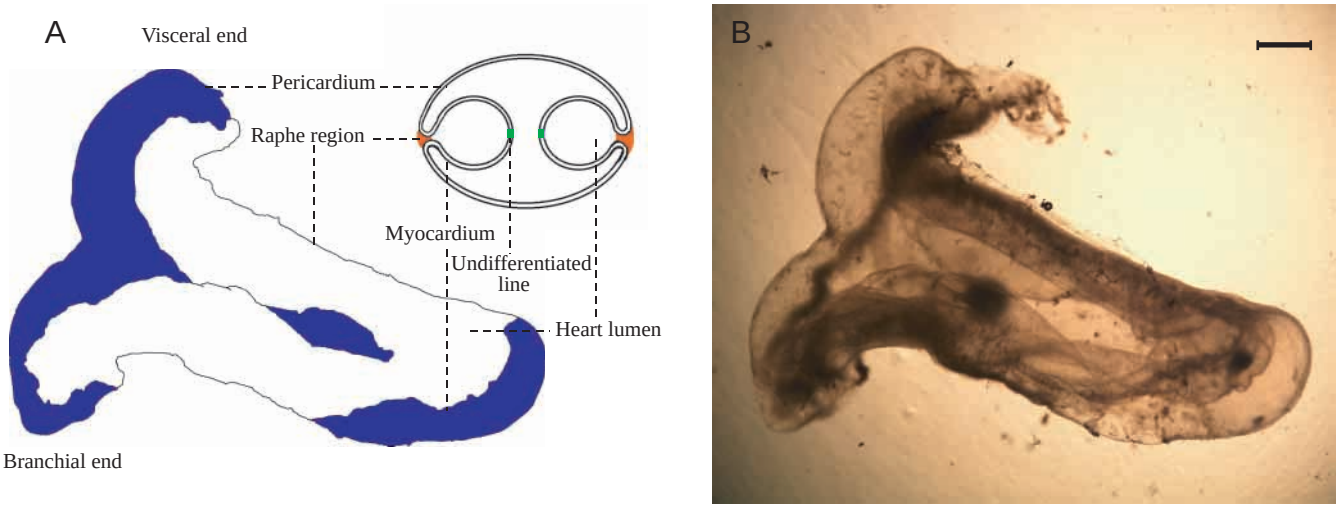

Fig. 1. Schematic diagram (A) and accompanying micrograph (B) showing the structure of a living heart excised from *Ciona intestinalis*. Scale bar, 0.1 cm.

the potential effect of eicosanoids for several reasons. The animals are readily obtainable, and the size and simplicity of organization of the hearts make them easily excisable. In the sea squirt *Ciona intestinalis*, the heart is a simple V-shaped tube enclosed in a fluid-filled, transparent pericardium (see Fig. 1). The heart forms as an invagination of the pericardial wall and remains attached to the pericardium along its length by a raphe (Anderson, 1968). The heart itself is a single layer of myoepithelial cells with an area of non-muscle cells, termed the undifferentiated line, running the length of the heart approximately opposite the raphe (Martynova and Nylund, 1996). The heart and circulatory system are valveless, and blood is pumped around the body by a series of peristaltic waves passing along the heart. There is no innervation of the heart; pacemaker regions, present at either end of the heart, alternate in periods of activity, thus changing the direction of contraction. These heart-rate reversals occur in all tunicate hearts (Goodbody, 1974) and are unique among the chordates (Kriebel, 1968a,b). Heart function, with special reference to reversals, has been extensively studied in tunicates with respect to mechanical properties and the effects of drugs and salinity (e.g. Scudder et al., 1963; Kriebel, 1968a; Anderson, 1968; Goodbody, 1974; Shumway, 1978). Blood cells circulate through the body of tunicates through distinct channels, and in *C. intestinalis* a complete circulation takes approximately 1 min (Skramlik, 1929). Circulation in the test can be extensive in many tunicates, but in *C. intestinalis* it is limited to the peduncle region (Goodbody, 1974).

The evidence concerning the effects of cardioregulatory drugs on the tunicate heart is contradictory, in that some workers have found that acetylcholine, which normally depresses cardiac activity in mammals, has no effect (Scudder et al., 1963), while others have found that it either increases the heart rate (Ebara, 1953) or enhances the dominance of the abvisceral pacemaker (Waterman, 1942, 1943). Similarly, adrenaline has been found to increase the heart rate in tunicates by some workers (Scudder et al., 1963) or to decrease it by others (Keefner and Akers, 1971).

The present study examines the nature of the eicosanoids synthesized by the heart and blood cells of *C. intestinalis* and investigates the potential action of the precursor PUFAs and their eisosanoid derivatives on heart rate.

Materials and methods

Animals

Adult specimens of *Ciona intestinalis* (L.) were collected from Queen's Dock, Swansea, Wales, from August to December 1999 and from October to December 2000. The animals were maintained in an aquarium with constantly flowing sea water for at least 2 weeks prior to experimentation. No attempt was made to feed the animals during this period.

Eicosanoid biosynthesis by heart and blood cells

The heart was excised from individual *C. intestinalis* using a binocular dissecting microscope. After complete removal of the test, the underlying mantle was cut through longitudinally to expose the visceral cavity. The heart was removed by cutting through the hypobranchial, visceral and test blood vessels, ensuring that the pericardium remained intact and that any associated tissue was removed. Blood cells were obtained by cutting through the heart *in situ* immediately after excision.

Five hearts (approximately 0.2 g wet mass) were obtained using the above method. Each heart was cut into small pieces and disrupted in 1 ml of marine saline (0.5 mol l^{-1} NaCl, 12 mmol l^{-1} $CaCl_2.2H_2O$, 11 mmol l^{-1} KCl, 26 mmol l^{-1} $MgCl_2.6H_2O$, 50 mmol l^{-1} Tris; pH 7.4) using a glass homogenizer before the resulting suspensions were pooled. Similarly, blood cells were pooled from approximately five individuals and collected into ice-cold marine anticoagulant (0.45 mol l^{-1} NaCl, 0.1 mol l^{-1} glucose, 30 mmol l^{-1} trisodium citrate, 26 mmol l^{-1} citric acid, 10 mmol l^{-1} EDTA; pH 4.6),

washed twice by centrifugation in marine anticoagulant (1500 ***g***, 10 min, 4 °C) and resuspended in 5 ml of cold marine saline. Both suspensions were incubated with 5 μmol l^{-1} calcium ionophore A23187 (Sigma Chemical Co. Ltd, Poole, UK) for 20 min at 16 °C and subsequently centrifuged (1500 ***g***, 10 min, 4 °C) to remove debris. An internal standard, PGB_2 (200 ng), was added, and the supernatants were extracted using Sep-Pak C_{18} minicolumns as detailed previously (Knight et al., 1999).

Lipoxygenase products were separated by reverse-phase high-performance liquid chromatography (RP-HPLC) using an Ultrasphere C_{18} ODS column (250 mm×4.6 mm; Beckman Coulter, High Wycombe, UK) and a linear gradient changing from 100 % water:methanol:acetonitrile:acetic acid (45:30:25:0.05 by volume, apparent pH 5.7) to 100 % methanol, over 40 min, with a flow rate of 0.6 ml min^{-1}. Material eluting was detected using Waters 991 or 996 diode-array ultraviolet detectors (Waters Chromatography, Cheshire, UK). Peaks were identified by co-chromatography with authentic standards and by reference to previous mass spectrometric analysis (Knight et al., 1999). Quantification of products was by reference to the extraction efficiency of the internal standard (PGB_2) using published molar extinction coefficients.

To quantify the cyclo-oxygenase products, $PGE_{2/3}$, hearts were excised, ionophore-incubated and Sep-Pak-extracted as previously described but without the addition of an internal standard. The eluate was tested with a commercially available PGE enzyme immunoassay (EIA) kit (Amersham Pharmacia Biotech UK Ltd, Little Chalfont, UK) as directed in the protocols provided. The sensitivity and range of the assay was 2.5–320 pg per well, and its published cross-reactivity with other eicosanoids was 25 % with PGE_1, 0.04 % with $PGF_{2\alpha}$, <0.1 % with 6-keto-$PGF_{1\alpha}$ and <0.001 % with AA.

Effect of eicosanoids and precursor fatty acids on heart rate

Hearts were dissected from adult *C. intestinalis* as described above and placed in solid watch-glasses containing 2 ml of sea water at 16 °C. Any associated tissue was carefully removed prior to observation. The heart rate was counted for two periods of 60 s by direct observation using a binocular microscope, and the mean value was used to calculate the heart rate (beats min^{-1}). The heart rate was determined for a period of 30 min at 16 °C prior to the addition of test material. Hearts with erratic or unusual rates were not used further. Arachidonic acid (AA; 0–200 μmol l^{-1}), eicosapentaenoic acid (EPA; 0–200 μmol l^{-1}), 12-(*R/S*)-hydroxyeicosapentaenoic acid (12-HEPE; 0–5 μmol l^{-1}), 8-(*R/S*)-hydroxyeicosa-pentaenoic acid (8-HEPE; 0–10 μmol l^{-1}) and PGE_3 (0–50 μmol l^{-1}) were added to each heart preparation, and heart rate was monitored for up to 6 h. In all cases, in parallel experiments, the effect of the addition of the appropriate amount of the vehicle (ethanol) was also tested. All eicosanoids and precursor fatty acids were obtained from Cayman Chemical Co. (Ann Arbor, USA).

Statistical analyses

Data are presented throughout as mean values ± 1 S.E.M. Significance of differences between conditions was determined using univariate analysis of variance (ANOVA) with Dunnett's post-tests. Significance of differences at each time period was determined with a one-way ANOVA with Dunnett's post-test.

Results

Structure of the heart of Cione intestinalis

As the structure of the heart of tunicates has already been described in detail elsewhere (e.g. Anderson, 1968; Goodbody, 1974), only a brief description is given here. The V-shaped heart is 1–2 cm long and easily excised from surrounding tissues (Fig. 1). The transparency of the pericardium made it possible to observe the waves of contraction passing along the myocardium with the aid of a binocular microscope.

Eicosanoid profiles of the heart and blood cells

Previous studies have examined the eicosanoid-generating capacity of the ovary, branchial basket, intestine and tunic of *C. intestinalis* (Knight et al., 1999). The principal lipoxygenase (LO) products of heart were found to be 8-hydroxyeicosapentaenoic acid (8-HEPE) (peak 4) and 12-HEPE (peak 2) (Figs 2, 3). In all cases, smaller amounts of 8,15-dihydroxyeicosapentaenoic acid (8,15-diHEPE; peak 1) were found (Figs 2, 3). As well as these identified products, two other compounds with conjugated dienes (indicative of monohydroxy fatty acid derivatives) were observed (peaks 3 and 5 in Fig. 2). No further attempt was made to identify the compounds under these peaks. *C. intestinalis* heart was also found to display PGE-immunoreactivity consistent with a level of 6.8±0.5 ng g^{-1} wet mass (mean ± S.E.M., N=4).

Blood cells produced a similar range of products except at lower levels. For example, levels of the principal product observed, 8-HEPE, were only 1.45±0.36 ng 10^{-6} cells (mean ± S.E.M., N=4).

Effects of eicosanoids and their precursor molecules on heart rate

After a brief period of acclimation, the basal heart rate for *C. intestinalis* specimens collected in 1999 was 31.9±0.7 beats min^{-1} (N=113), which was significantly higher than that for those collected in 2000 (20.3±1.3 beats min^{-1}, N=21) (means ± S.E.M., independent Student's t-test, P<0.01). Although there was an apparent slight decrease in the basal heart rate of specimens collected between August and December 1999, this was not statistically significant (Fig. 4). The difference between the year classes, in addition to the normal variation among hearts, made it necessary to express each heart rate as a percentage of the basal reading for that particular heart rather than as beats min^{-1}.

Over a period of 180 min, the heart rate was found to decrease by approximately 34.3 % (Fig. 5). After 24 h, some

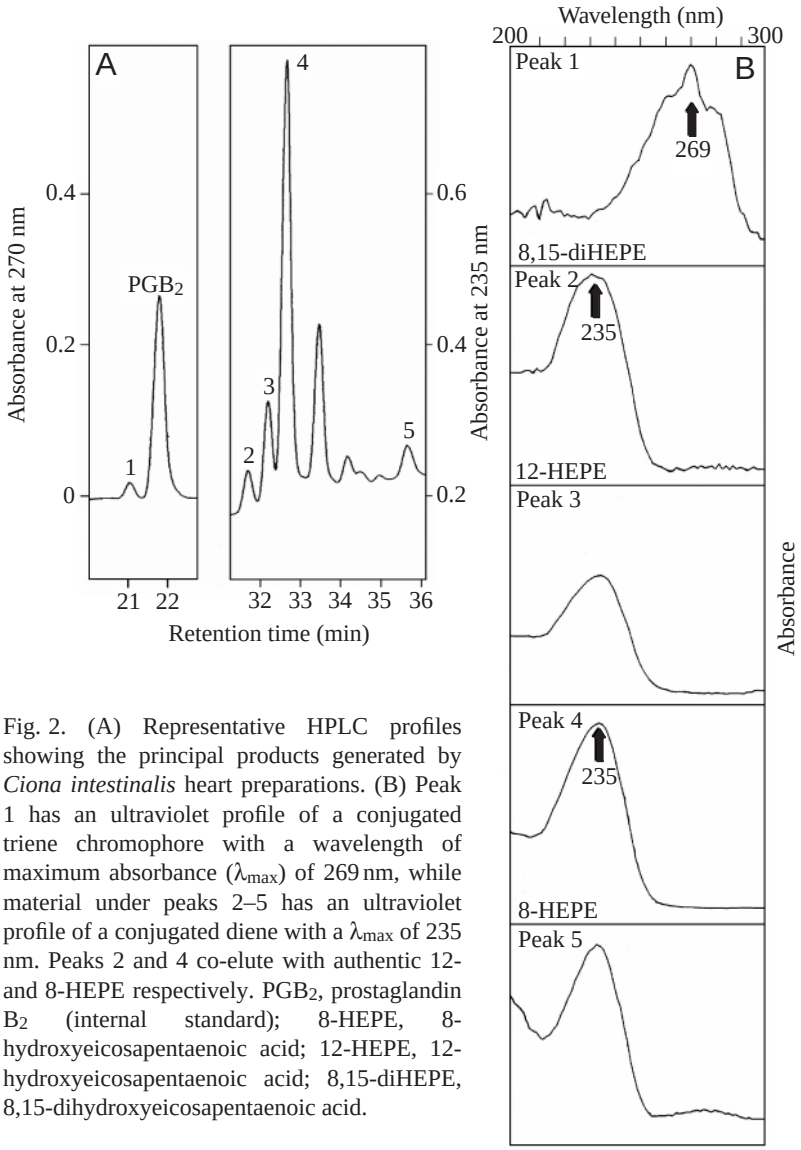

Fig. 2. (A) Representative HPLC profiles showing the principal products generated by *Ciona intestinalis* heart preparations. (B) Peak 1 has an ultraviolet profile of a conjugated triene chromophore with a wavelength of maximum absorbance (λ_{max}) of 269 nm, while material under peaks 2–5 has an ultraviolet profile of a conjugated diene with a λ_{max} of 235 nm. Peaks 2 and 4 co-elute with authentic 12- and 8-HEPE respectively. PGB_2, prostaglandin B_2 (internal standard); 8-HEPE, 8-hydroxyeicosapentaenoic acid; 12-HEPE, 12-hydroxyeicosapentaenoic acid; 8,15-diHEPE, 8,15-dihydroxyeicosapentaenoic acid.

heart preparations were still beating, but erratically and slowly. Addition of the vehicle (10 µl of ethanol) was found to have a significant negative chronotropic effect on heart rate over a period of 180 min. This general retarding effect was shown to be individually significant at two time periods (60 and 120 min; Fig. 5). When the effects of the PUFAs were investigated, it was observed that EPA and AA produced an overall significant stimulatory effect on heart rate at all concentrations used (50–200 µmol l^{-1}; Fig. 6A,B). However, in the case of AA, this was only individually significant at one time point (180 min) and with one concentration (200 µmol l^{-1}) (Dunnett's post-test, P<0.006). With EPA, individual time-related effects were seen at 60 (P<0.004), 120 (P<0.001) and 180 min (P<0.001) with 50 µmol l^{-1} only (Dunnett's post-tests). Overall, PGE_3 at 50 µmol l^{-1}, had a stimulatory effect, but only at one time point (120 min) was it individually significant (Fig. 7). At 10 µmol l^{-1}, PGE_3 was without significant effect at all time periods (results not shown). 12-HEPE at 5 µmol l^{-1} had a statistically significant overall stimulatory effect, but this was not observed at individual time points using a Dunnett's post-test. Unlike PGE_3 and 12-HEPE, 8-HEPE was without significant effect at the concentration tested (5 µmol l^{-1}).

Heart reversals occurred at variable intervals, with successive reversal periods often of different lengths, although the frequency of contractions was fairly consistent for any given animal. There was no clear effect on the frequency of beat reversals following exposure to EPA, AA, 8-HEPE, 12-HEPE or PGE_3 (data not shown). Although not quantified, there were no visible effects of any of the compounds tested on stroke volume.

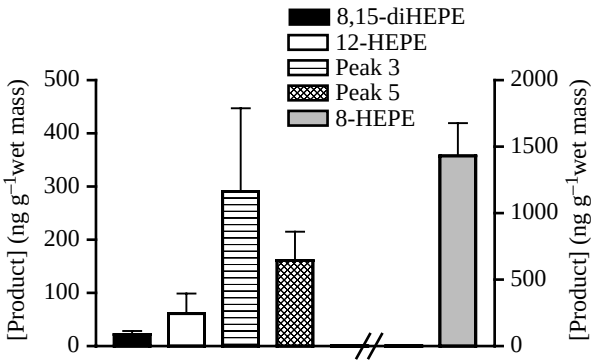

Fig. 3. Quantification of lipoxygenase product generation by heart tissue from *Ciona intestinalis*. Values are means + S.E.M., N=4.

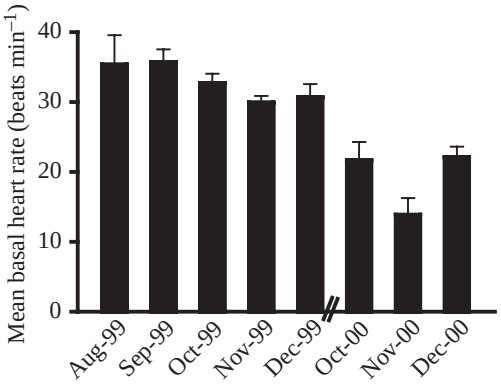

Fig. 4. Effect of season on the heart rate of excised *Ciona intestinalis* hearts. Values are means + S.E.M., N=4–50.

Discussion

The present study has shown that both the heart and blood cells of *C. intestinalis* produce a range of lipoxygenase (LO) products including 8- and 12-HEPEs and 8,15-diHEPE. This product profile is similar to that found for the branchial basket, intestine, ovary and tunic in the same organism (Knight et al., 1999). 12- and 8-LO activity, leading to the generation of 8- and 12-hydroxy fatty acids, has been reported in a diverse range of invertebrates including corals (Bundy et al., 1985), molluscs (Steel et al., 1997), crustaceans (Hampson et al., 1992; Hill and Holland, 1992) and echinoderms (Meijer et al., 1986; Hawkins and Brash, 1987). 12-LO metabolites in the mollusc *Aplysia californica* are involved in neuronal intracellular signalling (Piomelli, 1991), while 8-LO products are powerful inducers of oocyte maturation in starfish (Meijer et al., 1986).

Knight et al. (1999) found that most of the major LO products of *C. intestinalis* were EPA-derived, but they also noted smaller amounts of 8,15-dihydroxyeicosatetraenoic acid (an AA-derived product) and a lipoxin-like material (potentially AA-derived). The finding of only EPA-derived products in this present investigation probably reflects the smaller quantities of heart tissue and blood cells used. Hence, if a larger mass of heart tissue or a greater number of blood cells had been used, the smaller AA-derived products would probably have been detected. Other urochordates, such as the ascidian *Botryllus schlosseri* and the thaliacean *Dolioletta gegenbauri*, are known to contain EPA as the main C20 fatty acid component of cellular phospholipids (Carballeira et al., 1995; Pond and Sargent, 1998). The dominance of *n*-3 over *n*-6 fatty acids in marine phytoplankton is well-documented (e.g. Ackman et al., 1968), and the general abundance of *n*-3 PUFAs in marine lipids is attributed to this (Sargent and Whittle, 1981).

The only cyclo-oxygenase product quantified in the present study was $PGE_{2/3}$. The approach used was enzyme immunoassay which, although highly sensitive (picogram levels), is not totally specific in that other compounds can cross-react with the antibodies used. Hence, the small amounts of PGE-immunoreactive material observed in the heart of *C. intestinalis* could have resulted from other contaminating eicosanoids, and care should therefore be taken in any interpretation of these results. Furthermore, in the present study, no attempt was made to determine whether the heart or blood cells of *C. intestinalis* can generate either $TxA_{2/3}$ or $PGI_{2/3}$, both compounds with vascular activity in mammals (Ullrich et al., 2001). To date, no researchers have conclusively demonstrated the presence of these compounds in any invertebrate. For example, Hampson et al. (1992) reported the generation of low levels of thromboxane B (TxB) (the stable breakdown product of TxA) immunoreactive material by blood cells of the shore crab *Carcinus maenas*, but it could be argued that this resulted from non-specific binding of the antibodies employed leading to false positives.

Other workers have observed considerable variance in

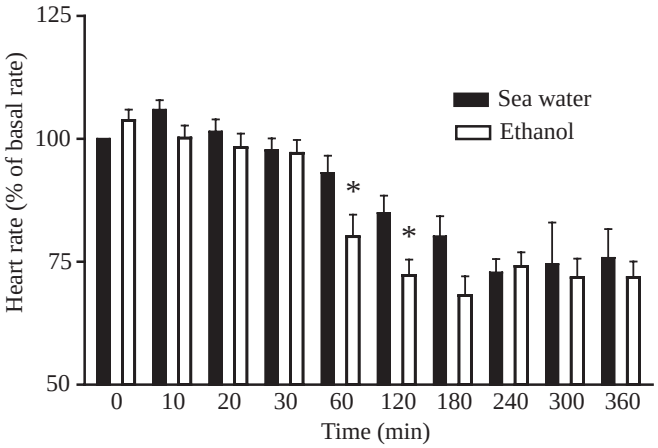

Fig. 5. Effect of incubation time on the heart rate of *Ciona intestinalis* hearts expressed as a percentage of basal rate. Values are means + S.E.M., *N*=3–15. *Significantly different (*P*<0.05) compared with sea water alone.

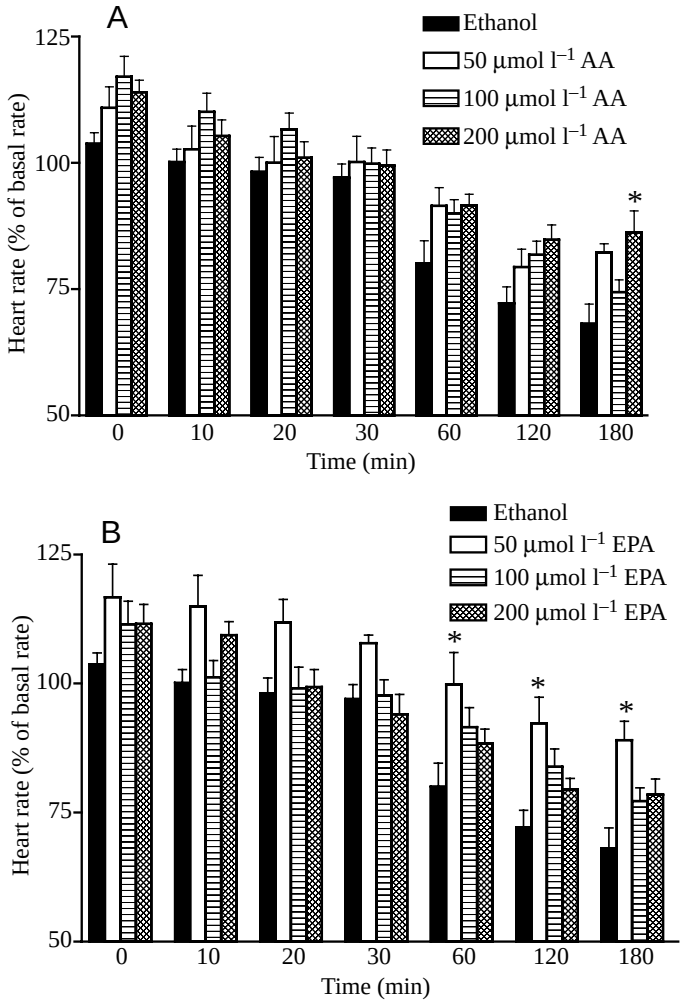

Fig. 6. Effect of (A) arachidonic acid (AA) and (B) eicosapentaenoic acid (EPA) on the heart rate as a percentage of basal rate. Values are means + S.E.M. *N*=6–15. *Significantly different (*P*<0.01) compared with ethanol (vehicle) control.

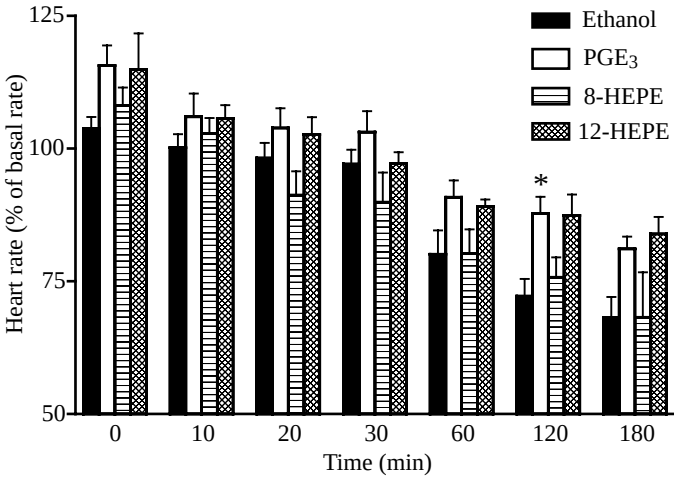

Fig. 7. Effect of 50 μmol l^{-1} prostaglandin E_3 (PGE_3), 5 μmol l^{-1} 8-hydroxyeicosapentaenoic acid (8-HEPE) and 5 μmol l^{-1} 12-HEPE on *Ciona intestinalis* heart rate expressed as a percentage of basal rate. Values are means + S.E.M., *N*=5–15. *Significantly different (*P*=0.007) compared with ethanol (vehicle) control.

heart rate between *C. intestinalis* individuals. Kriebel (1968a) noted that heart rate depended upon the size and temperature of the animal. In the present study, the experimental temperature was constant, but the animals ranged in size and it was observed, in agreement with Kriebel (1968a), that the smaller individuals displayed higher heart rates than the larger specimens. Indeed, the significantly lower heart rates observed for animals obtained in 2000 could be explained because they were collected several months later in the year and they would have reached a greater size before collection. Shumway (1978) and Kriebel (1968a) recorded mean *in vivo* heart rates of 32.9 and 20.7 beats min^{-1}, respectively, for *C. intestinalis* at 10 °C, and Kriebel (1968b) noted that the beat frequency remained the same following excision. Interestingly, the mean heart rates of 31.9 and 20.3 beats min^{-1} observed *in vitro* in the present study, from animals collected in 1999 and 2000 respectively, are similar to the two *in vivo* rates reported (Kriebel, 1968a; Shumway, 1978). The differences in reported rates probably represent different experimental conditions compounded by natural variation, especially that caused by the size differences of the animals tested.

The frequency of heart reversal in tunicates has been widely reported (e.g. Anderson, 1968; Kriebel, 1968a; Shumway, 1978; Jones, 1985). In the present study, it was found that these occurred infrequently and with no clear pattern. Jones (1985) found that heart reversals in *Ascidiella aspersa* were also erratic at the population level but consistent within individuals. Shumway (1978) reported regular beat reversals in *C. intestinalis in vivo* approximately 2 min apart.

It is clear that both AA and EPA had a significant positive chronotropic effect on the heart of *C. intestinalis*, particularly after a 60 min incubation. What is not clear, however, is whether this is a direct effect on membranes in the heart tissues caused by the incorporation of exogenous fatty acids or one involving the generation of eicosanoids. Both cyclo-oxygenase (PGE_3) and LO (12-HEPE) products also have stimulatory effect on sea squirt hearts, suggesting that at least some of the effect of fatty acids may be *via* such a route. In the only other study on the effects of fatty acids on invertebrate hearts, Agnisola et al. (1994) showed that AA was readily converted by *Octopus vulgaris* heart into several different eicosanoids, but the potential role of these compounds in the observed effects of the parent fatty acid on the heart was not directly investigated. Instead, they examined the effect of inhibitors of the biosynthesis of eicosanoids on the heart preparations. They found that indomethacin (a cyclo-oxygenase inhibitor) and nordihydroguaiaretic acid (NDGA; a non-selective LO inhibitor) had some effect. In particular, it was found that indomethacin potentiated the stimulation in coronary resistance induced by AA while NDGA slightly reduced this effect. A possible explanation of these observations was that AA induced the generation of vasoactive eicosanoids (either vasoconstrictive or vasodilatory) and that the presence of indomethacin shunted product generation from cyclo-oxygenase-derived towards vasoconstrictive LO products, with resulting effects. Unfortunately, they failed to carry out key experiments using exogenous eicosanoids to determine whether any had activity so as to test this hypothesis. Furthermore, the inhibitors employed are not totally specific and could have had effects independent of LO or cyclo-oxygenase inhibition.

Several other mechanisms may exist that could explain the effects of EPA and AA on sea squirt hearts independent of eicosanoid generation. For example, in bovine coronary artery, EPA has been shown to induce nitric oxide (NO) production in the endothelial cells, resulting in vasodilation (Omura et al., 2001). While NO has been shown to be a potent vasodilator in mammals (Ignarro, 1990), to our knowledge there are no reports of such activity in any invertebrate. These possibilities deserve further investigation.

Finally, mention should be made about the potential *in vivo* effects of PUFAs and eicosanoids in the regulation of the vascular system of tunicates. It is clear that free PUFAs are highly unlikely to be present *in vivo* at concentrations as high as the maximum (200 μmol l^{-1}) employed in the present study. However, some of the eicosanoids tested in this study were biologically active in the range 5–10 μmol l^{-1}, and such concentrations may be present within the heart on the basis of the amounts of these compounds observed following ionophore challenge (see Fig. 3). The observed stimulatory effects of PUFAs and some eicosanoids, although significant, are relatively small, and it is difficult to envisage how such changes would affect a sessile organism such as *C. intestinalis* in which there may not be a requirement for the vascular system to adjust cardiac output rapidly in response to sudden environmental change. While the circulatory system of *C. intestinalis* has been shown to be closed (Skramlik, 1929), the vessels are not lined by an endothelium and, hence, cannot be

said to be true blood vessels (Goodbody, 1974). Any change in blood pressure or blood flow would therefore have to be caused by the action of the heart itself rather than by vasoconstriction or vasodilation elsewhere in the circulatory system.

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