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CRUSTACEAN CARDIOEXCITATORY PEPTIDES MAY INHIBIT THE HEART *IN VIVO*

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Summary

Peptide neurohormones exist as functionally similar analogues in a wide variety of invertebrate and vertebrate phyla, and many have been implicated as cardiovascular regulators. In decapod crustaceans, these include the pentapeptide proctolin, crustacean cardioactive peptide (CCAP) and the FMRFamide-related peptides F1 and F2, all of which are found in the pericardial organs located immediately upstream of the heart. Cardioexcitatory activity has been demonstrated by these four peptides in both isolated and semi-isolated arthropod hearts; CCAP, however, has minimal effects on the heart of *Cancer magister*. In the present study, we determined the effects of proctolin, F1 and F2 on the heart of the crab *C. magister* in both *in vitro* (semi-isolated heart) and *in vivo* (whole animal) preparations. In semi-isolated hearts, infusion of each peptide caused cardioexcitation, increasing the rate and stroke volume of the heart. In whole crabs, the peptides were cardioinhibitory; the strongest effects were observed with F1 and F2, which dramatically decreased heart rate, cardiac stroke volume and cardiac output. These results cast doubt on current perceptions of the functional role of cardioactive peptides in the regulation of invertebrate cardiovascular performance *in vivo*.

Key words: *Cancer magister*, heart, stroke volume, cardiac output, neuropeptide, crab, proctolin, FMRF-amide related peptides.

Introduction

In decapod crustaceans, peptide neurohormones include the pentapeptide proctolin (Starrat and Brown, 1975), crustacean cardioactive peptide (CCAP) (Stangier, 1991) and the FMRFamide-related peptides F1 and F2 (Trimmer *et al.* 1987). Cardioexcitatory activity has been demonstrated by these four peptides in both isolated and semi-isolated arthropod hearts (Groome *et al.* 1994; Krajniak, 1991; Mercier and Russenes, 1992; Wilkens and Mercier, 1993). The neurogenic heart of decapod crustaceans consists of a single-chambered ventricle suspended within the pericardial sinus. Hormones released from the pericardial organs, which are located directly upstream of the heart (Alexandrowicz, 1953), or infused into the sinus may affect heart function directly, by acting on either the cardiac ganglion or myocardium, or indirectly *via* the central nervous system (CNS). Nearly all the work on naturally occurring cardiac peptides has been carried out on isolated or semi-isolated heart preparations (Wilkens and McMahon, 1994). These are simple preparations, affording ready access to the heart and allowing easy determination of heart rate, myocardial contractility and cardiac output. Such measurements, however, may not accurately reflect the situation *in vivo* since the isolated hearts were not subject to normal neural and neurohumoral control pathways. In the present study, we determined the effects of proctolin, F1 and F2 on the heart of the crab *Cancer magister* both *in vitro* (in the semi-isolated heart) and *in vivo* (in the whole animal).

Materials and methods

Adult, male *C. magister* Dana (600–850 g) were purchased from commercial fishermen and held in 33±1‰ sea water at 12±1 °C at the Bamfield Marine Station, British Columbia, Canada. Proctolin and F1 were obtained from Bachem Bioscience Inc. and F2 was a gift from Dr J. Mercier.

For intact animal trials, peptides were dissolved in *Cancer* saline (Morris and McMahon, 1989) and diluted so that injectate volumes of 350 μl would achieve circulating concentrations of 10^{-12} to 10^{-6} mol l^{-1} assuming a total haemolymph volume of 26% wet mass of the animal. Hormones were infused into the pericardial sinus *via* a chronically implanted catheter. A pulsed-Doppler flowmeter was used to measure haemolymph flow through all arterial

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systems leaving the heart, allowing determination of cardiac output ($\dot{V}$b), stroke volume (V_S) and heart rate (f_H) (for methods, see Airriess and McMahon, 1994; Airriess *et al.* 1994). In the semi-isolated heart, all arteries were ligated except the sternal artery which was cannulated. The outflow from this artery was fed through an electromagnetic flowmeter to measure $\dot{V}$b and f_H (for methods, see Wilkens and McMahon, 1994). In these preparations, peptide solutions were infused directly into the heart.

Cardiac responses to a saline control and peptide infusion in both semi-isolated and intact hearts were analyzed simultaneously using analysis of variance with repeated measures (ANOVAR) (Potvin *et al.* 1990). Significant ANOVARs ($P<0.05$) were further scrutinised to identify individual treatment effects. All reported P values are based on the correction factors of Huynh and Feldt (1970).

Results

Luminal infusion of proctolin, F1 and F2 caused a positive chronotropic response in the semi-isolated heart of *C. magister* (Fig. 1B–D) (ANOVAR, $P<0.01$). These observations confirm literature reports of cardioacceleration in semi-isolated hearts, but it is interesting to note that even at the highest dosage employed (10^{-6} mol l^{-1}) none of the peptides increased heart rate to values above the control values recorded from intact animals. Positive inotropic responses were also recorded from the semi-isolated heart, indicated by dramatic increases in ventricular pressure in response to each of the peptides (data not shown, similar to Wilkens and Mercier, 1993) as well as by significant increases (ANOVAR, $P<0.01$) in cardiac stroke volume in response to both F1 and F2 (Fig. 1C,D). In a previous report, CCAP had minimal effects on the heart of *C. magister* either *in vivo* or *in vitro* (McGaw *et al.* 1994).

Experiments conducted on intact animals yielded markedly different results. Heart rate was 77±3 min^{-1} (mean ± S.E.M., $N=10$), significantly higher (ANOVAR, $P<0.01$) than that recorded from semi-intact hearts (57±3 min^{-1}, $N=7$), possibly resulting from the absence of neural and neurohormonal inputs in the latter (Wilkens and McMahon, 1992, 1994). Neither stroke volume nor cardiac output differed significantly between the two groups (ANOVAR, $P>0.05$). Infusion of *C. magister* saline (Morris and McMahon, 1989) into the pericardial sinus of whole animals caused no significant change in heart rate, stroke volume or cardiac output (ANOVAR, $P>0.05$; Fig. 1A).

Infusion of peptides into intact crabs, however, caused marked responses which were radically different from those recorded from the isolated preparations listed above. The threshold for responses in both intact and semi-isolated preparations occurred at 10^{-9} mol l^{-1}. The differences were most notable at the highest hormone concentrations and the most dramatic differences occurred in response to F1 and F2 (Fig. 1B–D). In contrast to the excitation observed in semi-isolated hearts following peptide infusion, treatment with proctolin (Fig. 1B) caused no significant change in heart rate (ANOVAR, $P>0.05$), and both F1 and F2 induced marked bradycardia (ANOVAR, $P<0.01$; Fig. 1C,D). This inhibition was of long duration (3 h) and often punctuated by periods of cardiac arrest. Stroke volume and cardiac output did not change significantly in response to proctolin infusion in the present study (ANOVAR, $P>0.05$; Fig. 1B) but increases in both, together with a decrease in heart rate, have previously been shown to follow proctolin infusion in intact *C. magister* (McGaw *et al.* 1994). Few other *in vivo* studies have been reported, but both proctolin and F1 failed to increase the heart rate of lobsters (*Homarus americanus*) after infusion into the pericardial sinus (McMahon, 1992), and proctolin caused transient bradycardia followed by moderate tachycardia in *Carcinus maenas* (Wilkens *et al.* 1985). F1 and F2 both caused significant (ANOVAR, $P<0.01$), long-lasting inhibition of stroke volume and cardiac output (Fig. 1C,D). Control levels of these variables were not regained for 1–6 h following peptide infusion.

Discussion

The natural effects of these FMRFamide-like peptide hormones *in vivo* are, therefore, strongly cardioinhibitory rather than excitatory as previously concluded from *in vitro* studies (Groome *et al.* 1994; Krajniak, 1991; Mercier and Russenes, 1992; Wilkens and McMahon, 1992; Wilkens and Mercier, 1993). The only other report of cardioinhibition by FMRFamide-related peptides involved two hindgut peptides, SchistoFLRFamide and leucomyosupressin, which have negative chronotropic and inotropic effects on semi-isolated crayfish hearts (Mercier and Russenes, 1992); however, their effects in intact crustaceans have yet to be studied.

The reason for the discrepancy between *in vitro* and *in vivo* cardiac responses is uncertain, but may result from direct action of the peptides on the CNS, leading either to a reduction of the excitatory neural drive to the cardiac ganglion or to stimulation of the cardioinhibitory pathway (Wilkens and Walker, 1992). The peptides might also act on other neurohaemal organs, causing release of cardioinhibitory substances. Kobierski *et al.* (1987) reported haemolymph levels of FMRFamide-like peptides of 10^{-10} mol l^{-1} in *H. americanus* but, at present, precise circulating concentrations of these hormones and the receptors they affect in crabs are unknown. The observation that maximum stimulation of the semi-isolated heart only restored heart rate to levels characteristic of intact crabs under resting conditions indicates that heart rate may normally be influenced by excitatory stimulation from the CNS, *via* the cardioregulatory nerves and/or neurohormonal modulators.

Many invertebrate peptides have functionally similar analogues in vertebrates (Fingerman *et al.* 1993; Raffa, 1988). Precise determination of their function in simple systems is important to a full understanding of peptide action in organisms of higher order. Many recent publications have focused on the cardioregulatory roles of peptide neurohormones (for a review, see Wilkens and McMahon, 1992) but, in almost all cases, the

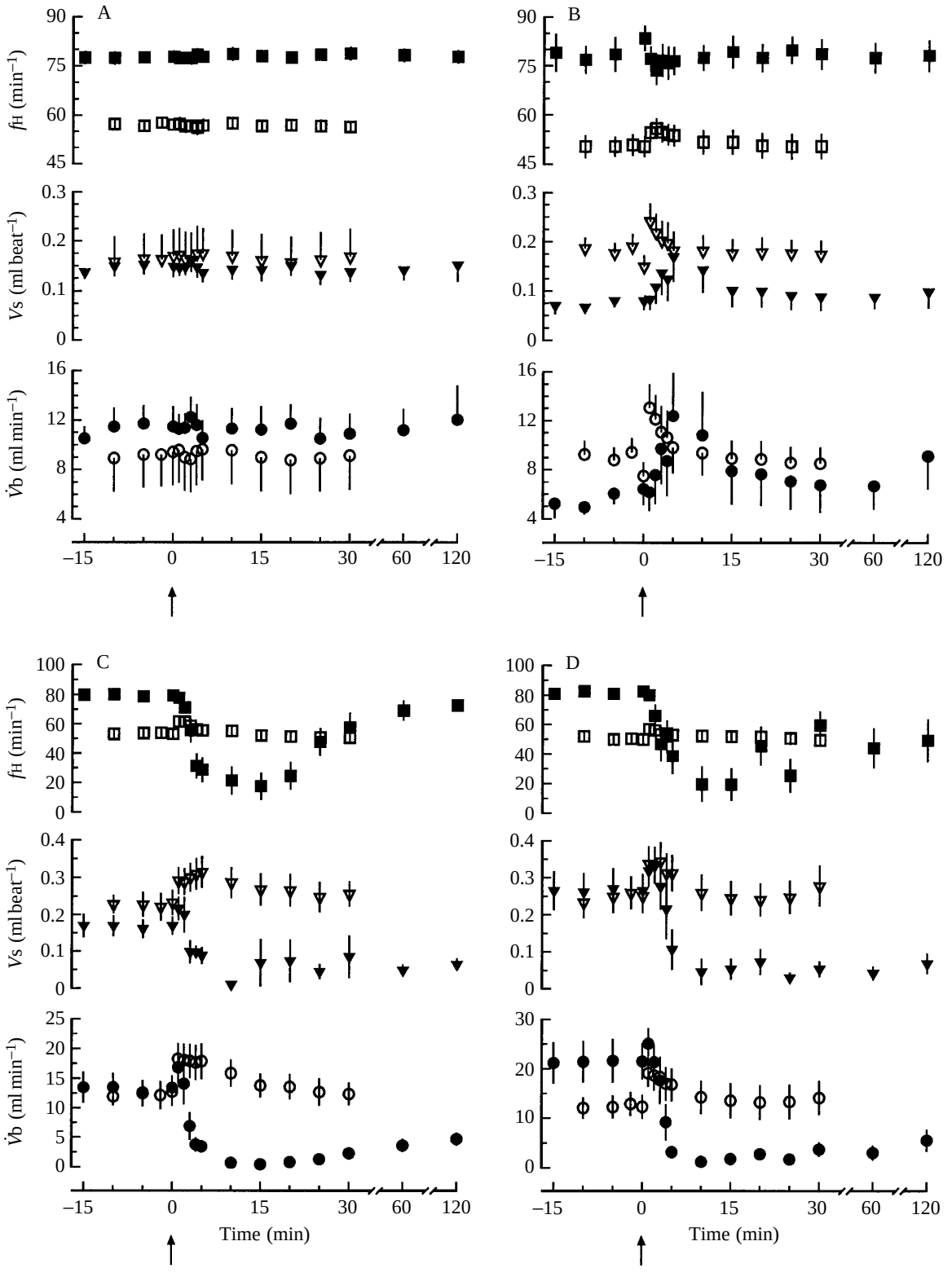

Fig. 1 Heart rate (f_H), stroke volume (V_S) and cardiac output ($\dot{V}_b$) of semi-isolated (open symbols, N=7) and intact (filled symbols, N=10) hearts of *Cancer magister*. Infusion of either physiological saline or peptide neurohormones (at the most effective concentration tested, 10^{-6} mol l^{-1}) occurred at 0 min (arrows). Data shown as mean ±1 S.E.M. (A) Infusion of saline; (B) infusion of proctolin; (C) infusion of F1; (D) infusion of F2.

effects of these peptides were investigated using only isolated or semi-isolated heart preparations. The present study indicates that the cardiovascular effects of many peptides *in vivo* may differ markedly from those determined *in vitro*. Interpretations

based solely on isolated heart preparations may thus be misleading or erroneous. Reliance on frequency measurements as an indicator of cardioactivity is particularly problematic, since heart rate and stroke volume often change independently (Airriess and McMahon, 1994; McGaw *et al.* 1994; McMahon and Burnett, 1991).

These arguments underscore the need to include whole-animal studies not only for research on the crustacean cardiovascular system but for all aspects of invertebrate pharmacology. *In vitro* studies provide insight into the direct actions of hormones on the organ in question, whereas whole-animal responses, particularly where they differ from *in vitro* effects, illustrate the additional importance of extrinsic modulator systems. Thus, it is essential that the roles of (neuro)hormonal effectors be determined from whole-animal studies in conjunction with *in vitro* results.

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