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The neuropeptide proctolin acts directly on *Limulus* cardiac muscle to increase the amplitude of contraction

JACK A. BENSON, ROBERT E. SULLIVAN, WINSOR H. WATSON III and GEORGE J. AUGUSTINE JR.

(J.A.B.) Friedrich Miescher-Institut, P.O. Box 273, CH-4002 Basel, Switzerland, (R.E.S.), Békésy Laboratory of Neurobiology, University of Hawaii, Honolulu, Hawaii 96822, (W.H.W.) Department of Zoology, University of New Hampshire, Durham, N.H. 03824 and (G.J.A.) Department of Zoology, University of Maryland, College Park, Md. 20742 (U.S.A.)

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The pentapeptide proctolin increases the amplitude of contraction but not heart beat frequency of the isolated heart of *Limulus polyphemus*. It acts directly on the heart muscle and has no effects on the neurones of the cardiac ganglion or on the cardiac neuromuscular EJPs. A peptide with molecular weight, enzymatic susceptibilities and physiological effects similar to those of proctolin occurs in the *Limulus* cardiac ganglion. It is suggested that proctolin, or a family of proctolin-like peptides, may modulate muscle contraction in more than one subphylum of the Arthropoda.

Proctolin is a pentapeptide (Arg–Tyr–Leu–Pro–Thr) first extracted from the viscera of the cockroach, *Periplaneta americana*[6,19], which appears to act directly on the hindgut muscles of *Periplaneta*[7] and other insect species[11]. It is known to induce myogenic contractions in insect extensor muscles[12,18] and may have a neurohormonal effect on insect cardiac muscle[14]. Recently, Sullivan[20] has shown that one of the two cardio-excitatory peptides released from decapod crustacean pericardial organs[4,5,10] is either proctolin itself or a close analogue. In this paper, we show that proctolin has an excitatory action on the neurogenic[8] heart of *Limulus polyphemus*, increasing the amplitude of contraction, but not the beat frequency, of the isolated heart. Proctolin acts directly on the cardiac muscle, rather than on the neurones of the cardiac ganglion or on the amplitude of the EJPs at the cardiac neuromuscular junction. From the cardiac ganglion, we have extracted an active fraction which displays an apparent molecular weight and an enzymatic susceptibility very similar to those of proctolin. The action of this fraction on isolated *Limulus* heart is indistinguishable from that of synthetic proctolin. These observations suggest that proctolin, or a family of closely related peptides, plays a physiological role in the modulation of muscle contraction in more than one subphylum of the Arthropoda.

Perfusion of isolated, intact heart-ganglion preparations with increasing concentrations of proctolin resulted in dose-dependent increases in the amplitude of heart

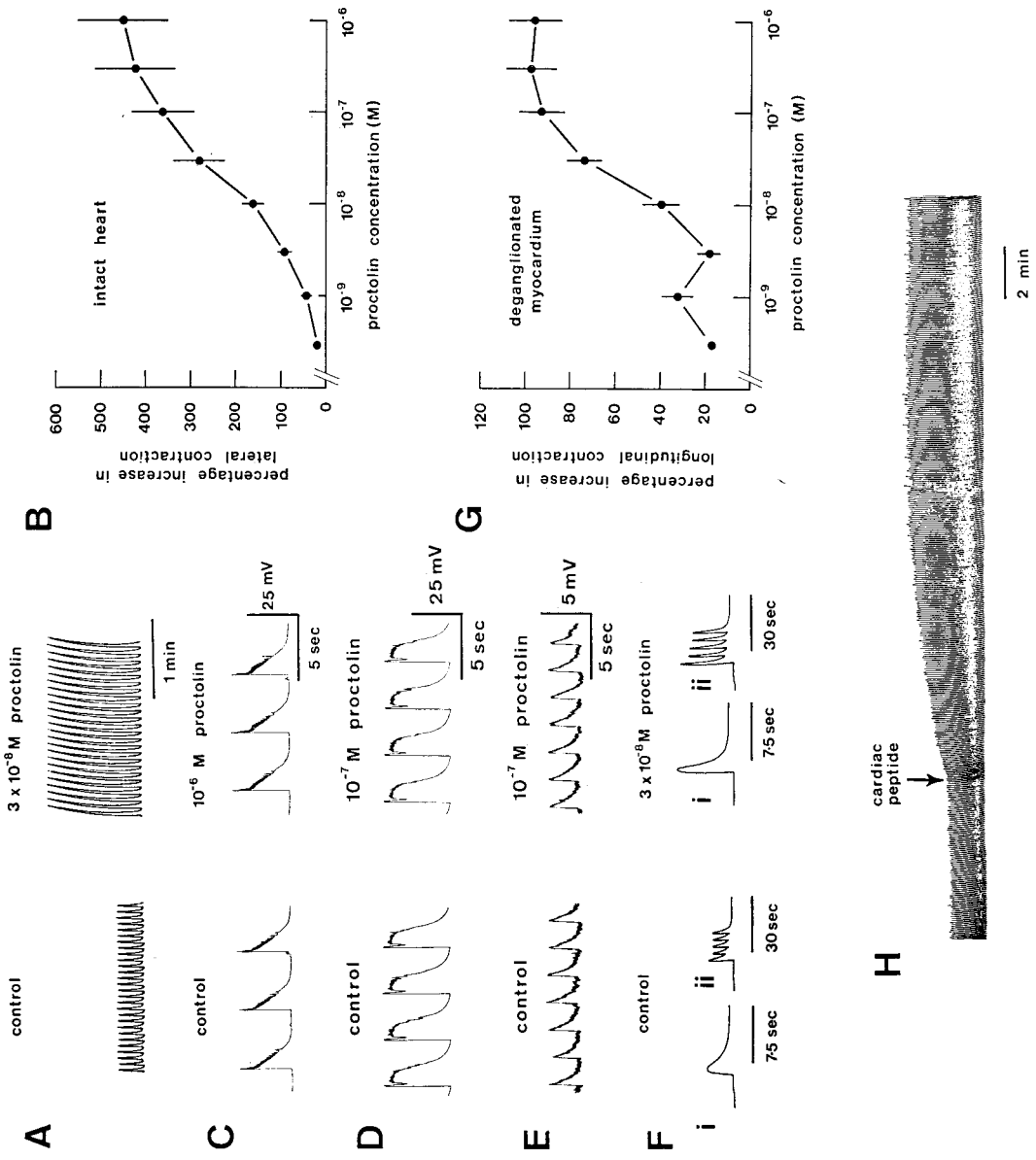

Fig. 1. Responses to proctolin and cardiac ganglion extract of *Limulus* intact heart, isolated cardiac ganglion, and deganglionated myocardium. A: chart recordings from a force transducer showing steady-state lateral contractions in a spontaneously beating, continuously perfused, intact *Limulus* heart-cardiac ganglion preparation in normal sea water and after 20 min in 3×10^{-8} M proctolin. The vertical scale is tension in arbitrary units the same for both records. B: cumulative dose–response curve for responses of 6 intact hearts to increasing concentrations of proctolin. Points are means of the steady-state increase in amplitude of contraction after 20 min in proctolin (records as in A) and bars represent S.E.s. The abscissa is a log scale. C: intracellular microelectrode recordings from a large motoneurone in an isolated, rhythmically active cardiac ganglion exposed to normal sea water followed by 10^{-6} M proctolin. D: intracellular microelectrode recording of summating, compound EJPs from

contraction but with no change in the frequency of heart beat (Fig. 1A). The increase in contraction amplitude began immediately after proctolin application and reached a plateau in 10–20 min, depending on concentration. There was apparently no desensitization in the continued presence of proctolin: the force of contraction stayed at the plateau level as long as proctolin remained in the perfusing sea water. Fig. 1A shows a typical observation, and the data from 6 different hearts are summarized in a cumulative dose–response curve (Fig. 1B). The threshold concentration was between 10^{-10} and 3×10^{-10} M, with a half-maximal effect at about 10^{-8} M. Above 3×10^{-7} M, the effect of increased concentrations of proctolin was smaller, with saturation occurring at about 10^{-6} M. The effect could be reversed by rinsing in sea water, requiring about 90 min for recovery from 3×10^{-8} M proctolin.

The site of action of proctolin on the *Limulus* heart may be one or more of the following: the cardiac ganglion which generates the rhythmic neuronal activity controlling heart beat[8]; the neuromuscular junction between the motor axon from the ganglion and the cardiac muscle; the heart muscle fibres themselves.

The effect of proctolin on the neuronal activity of the cardiac ganglion was investigated using ganglia dissected away from the myocardium. These maintained their rhythmic electrical activity in vitro for many hours. The ganglion consists of a network of many hundreds of small pacemaker neurones and larger follower neurones which are the origin of the cardiac motor axons. GABA and serotonin[1,15,16], as well as other biogenic amines[2,3], are known to alter the amplitude and frequency of *Limulus* heart contractions by acting on the cardiac ganglion neurones. However, when axonal traffic in the ganglion was sampled via an extracellular electrode, treatment with 10^{-6} M proctolin had no effect on the frequency of action potential bursts produced by the ganglion, and caused no discernable change in the duration and composition of individual bursts. This lack of effect was tested further by recording intracellularly the complex electrical activity[17] of individual follower neurones (Fig. 1C). Our observations indicate that 10^{-6} M proctolin leaves the individual follower neurone activity completely unaltered (Fig. 1C), and we conclude that proctolin does not act via the cardiac ganglion.

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a muscle fibre in an intact, spontaneously beating heart exposed to normal sea water followed by 10^{-7} M proctolin. E: intracellular microelectrode recording of single-axon-mediated EJPs from a muscle fibre in a deganglionated myocardium exposed to normal sea water followed by 10^{-7} M proctolin. F: chart recordings from a force transducer showing longitudinal contraction in a directly stimulated deganglionated myocardium in normal sea water and 3×10^{-8} M proctolin. Direct stimulation was via a series of Ag/AgCl wire electrodes at 30 V, and 10 msec duration. Myocardia were in sea water between stimuli or trains of stimuli, and the recording shown was made after 10 minutes exposure to proctolin. The vertical scale is tension in arbitrary units the same for all records. Fi: single stimulus. Fii: a series of 4 stimuli at normal heart beat frequency (0.25/sec). G: cumulative dose–response curve for responses of 5 deganglionated myocardia to increasing concentrations of proctolin. Points are means of 3 measurements of responses as in F (single) for each heart after 10 min at a particular concentration of proctolin, and bars represent S.E.s. The abscissa is a log scale. H: chart recording from a force transducer showing the effect of the active peptide fraction (mol.wt. approximately 600) extracted from *Limulus* cardiac ganglion by gel-filtration. The amount applied at the arrow was equivalent to the extract from one ganglion. The vertical scale is tension in arbitrary units.

Two preparations were utilized to test the effect of proctolin at the neuromuscular junction. Intracellular recordings from intact heart muscle fibres reveal a potential consisting of a series of summated, multi-axonally-mediated EJPs[13] (Fig. 1D). Proctolin (10^{-7} M) did not alter the amplitude, duration, or frequency of these compound EJPs, nor the resting potential of the muscle fibres (Fig. 1D). The second preparation was the deganglionated myocardium in which a branch nerve containing motor axons was stimulated via a suction electrode, while muscle potential was recorded intracellularly. Stimulation was at frequencies and intensities which ensured non-summating, single-axon-mediated EJPs in the muscle fibre (Fig. 1E). Signal averaging before and after treatment with 10^{-7} M proctolin showed that there was no change in amplitude or rise time of these EJPs. We conclude that proctolin does not act presynaptically and does not change muscle membrane resistance at potentials within 15 mV of resting potential.

The action of proctolin directly on the heart muscle was measured by suspending deganglionated myocardia from a force transducer while stimulating with silver wire electrodes placed against the ventral side[3]. As with the neuronally-induced lateral contractions in the intact heart, the deganglionated myocardium shows a dose-dependent increase in force of contraction with proctolin treatment (Fig. 1F). For practical reasons, the contraction measured in these experiments was longitudinal, and the response was measured after only 10 min in proctolin, so that the percentage increase in force of contraction at each proctolin concentration was smaller. The data from 5 different myocardia are summarized in the cumulative dose–response curve shown in Fig. 1G. Threshold concentration was below 3×10^{-10} M, and the half-maximal effect occurred at 10^{-8} M. The response saturated at about 10^{-7} M, a concentration only slightly lower than for the intact heart. These results indicate that proctolin has a substantial effect on the amplitude of contraction of the heart by acting directly on the cardiac muscle fibres.

The low threshold and the magnitude of the proctolin effect on the heart suggested a physiological role for proctolin in the control of heart beat in *Limulus*. We therefore screened crude extracts of *Limulus* CNS and heart-associated nerves using the cockroach hindgut assay for proctolin[20]. Proctolin-like activity was found in the *Limulus* brain, circumoesophogeal ganglia, and the cardiac ganglion. The activity from 30 cardiac ganglia was separated by gel-filtration into 3 active fractions. One fraction eluted just prior to synthetic proctolin, suggesting a molecular weight slightly greater than 600. Trypsin and chymotrypsin treatment of the crude extract for up to one hour had little or no effect on this fraction, but treatment with leucine aminopeptidase at 37 °C for one hour resulted in a reduction of its activity. These enzymatic susceptibilities are characteristic of proctolin. When this active fraction was applied to intact heart-ganglion preparations, it increased the amplitude of contraction but had little effect on heart beat frequency (Fig. 1H) producing a response indistinguishable from the effects of synthetic proctolin. This initial biochemical and physiological analysis suggests the presence, in the cardiac ganglion of *Limulus*, of a peptide with properties similar to proctolin, but caution is necessary in the interpretation of these bioassays until purification and sequence analysis of the ganglionic peptide is completed.

In this paper we have shown that proctolin increases the amplitude of heart contraction in *Limulus* by directly altering the properties of the cardiac muscle fibres. In insects, it has been suggested that proctolin has a modulatory effect on muscle excitability rather than being a synaptic transmitter, particularly in the hindgut[9], although it does appear to alter the CNS–hyperneural muscle preparation in *Periplaneta* through direct effects on the CNS[14]. In decapod crustacea, a proctolin-like peptide occurs in the pericardial organs[20] which are neurohaemal structures that release their contents into the blood stream as it enters the heart[21]. When applied to the intact heart of the crab *Portunus sanguinolentus*, this pericardial peptide elicits a characteristic inotropic action, but it has little effect on heart beat frequency[20]. Similar effects are seen in *Portunus* with synthetic proctolin[20]. Our analysis of the cardiac ganglion extract from *Limulus* suggests the presence of a peptide resembling proctolin, which affects heart beat in a manner similar to synthetic proctolin. It seems likely that proctolin, or a family of proctolin-like peptides, known to occur in insects and a decapod crustacean, also occurs in *Limulus*, and thus widely among the arthropoda. In these groups, such peptides most likely are acting directly on muscle fibres as modulators of contraction in the heart and elsewhere.

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