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IN VIVO MUSCLE PROTEIN SYNTHESIS RATES IN THE AMERICAN LOBSTER *HOMARUS AMERICANUS* DURING THE MOULT CYCLE AND IN RESPONSE TO 20-HYDROXYECDYSONE

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Summary

Simultaneous measurements of *in vivo* rates of protein synthesis (K_s) in claw, leg and abdominal muscles were made in the American lobster *Homarus americanus* at three stages of the moult cycle. K_s values are significantly elevated during the premoult (stage D_2–D_3) and fall during the intermoult (stage C_4) periods in all three muscles. Postmoult (stage A/B) levels are not significantly elevated above intermoult levels. Intermoult levels are between 0.3 and 0.4 % protein synthesized per day. In the premoult animals, the ribosomal activity (milligrams protein synthesized per microgram RNA per day) of the claw, abdominal and leg muscles is elevated three- to fivefold. The claw muscle maintains an elevated ribosomal activity into the postmoult stage whereas, by this stage, that of the other muscle tissues has fallen to intermoult levels. The RNA/protein ratios of the three muscle groups from intermoult, premoult and postmoult animals do not show any significant differences. 18S ribosomal RNA levels fluctuate slightly, with no consistent pattern over the moult cycle. *In vivo* injection of premoult concentrations of 20-hydroxyecdysone (20-HE) into intermoult lobsters results in elevated K_s values and ribosomal activity for the muscles after 3 days. RNA/protein ratios remain constant in the muscles in response to injections of 20-HE *in vivo*. *In vitro* preparations of leg muscle treated with 20-HE did not show similar elevated rates of protein synthesis.

Key words: protein synthesis, lobster, *Homarus americanus,* moult cycle, 20-hydroxyecdysone, ribosomal mRNA.

Introduction

Adult crustacean growth is an intermittent process centred around ecdysis, when the old exoskeleton is shed and body volume increases owing to increased uptake of water. During ecdysis in some species of crustaceans, water uptake into the haemolymph can increase fivefold, enlarging the space available for new tissue growth (Mykles, 1980). During the moult cycle, a series of physiological and cellular events in preparation for ecdysis (premoult; stages D_1–D_4), during recovery from ecdysis (postmoult; stages A and B) and at intermediate stages (intermoult; stage C_4) occur throughout the body of the animal. Determining the stages when specific muscle tissues grow during these cyclical events and identifying the key regulatory factors have been the subject of our previous investigations (El Haj *et al.* 1984, 1992; Houlihan and El Haj, 1985; El Haj and Houlihan, 1987; Harrison and El Haj, 1994).

Morphological measurements during lengthening of the muscle fibres in the legs of the American lobster *Homarus americanus* have shown that increases in fibre length occur over ecdysis and during the immediate postmoult period (El Haj *et al.* 1984). In the lobster extensor muscle, there is a shift over the moult cycle from a greater percentage of large myofibrils in premoult fibres to predominantly smaller myofibrils in the postmoult fibres. An increase in myofibrillar surface area in postmoult animals is due to an increase in the size of myofibrils as a result of the addition of thick and thin filaments, which has been suggested to be due to longitudinal myofibril splitting, as in vertebrate muscle (El Haj *et al.* 1984).

In contrast to leg muscles, claw muscles have been shown in some crustaceans to undergo a massive atrophy during premoult to allow the remaining claw muscle to be pulled through the narrow basi-ischium joint during ecdysis (Skinner, 1966; Mykles and Skinner, 1990). Protein synthesis rates, measured using pulsed amino acid incorporation studies in the Bermuda land crab *Gecarcinus lateralis*, are elevated during the premoult (stage D_0–D_4) and postmoult (2–6 days post-ecdysis) periods (Skinner, 1965). Mykles and Skinner (1982*a*,*b*, 1990) suggest that, during postmoult, an elevation in rates of protein synthesis may result in the addition of sarcomeric protein, whereas the premoult increase may account for an increase in the production of degradative enzymes. In contrast to claw muscles, leg muscles do not

undergo atrophy prior to ecdysis, as they are sufficiently narrow to pass through the joints at the base of the legs (Mykles and Skinner, 1982*b*). As a result, the growth pattern over the moult cycle in leg muscle may be slightly different from the growth pattern in claw muscles. Results from our investigations into muscle growth in the lobster have shown 10–20 % protein degradation in the claw during premoult and no protein degradation in the leg and the abdominal muscles. Muscle growth may vary within muscle groups over the moult cycle, which presents an interesting model of differential regulation.

The factors responsible for regulating muscle growth in Crustacea have not yet been determined, although proposals for hormonal and mechanical stimulation have been made (Mykles and Skinner, 1990; El Haj *et al.* 1992; Harrison and El Haj, 1994). In the lobster, passive stretch has been shown to promote muscle lengthening (Houlihan and El Haj, 1985), addition of sarcomeres and up-regulation of actin mRNA levels (Harrison and El Haj, 1994). This process, however, is very slow, with elevation in mRNA levels occurring 1–2 weeks and fibre lengthening up to 3 weeks after the stretch is applied. Elevations in levels of ecdysteroid moulting hormones, in particular 20-hydroxyecdysone (20-HE), are involved in controlling many processes over the lobster moult cycle (Chang, 1989). In *H. americanus*, during premoult, haemolymph ecdysteroid titres increase markedly through stages D_1 and D_2 and then fall in stages D_3–D_4, immediately prior to ecdysis (Snyder and Chang, 1991). Recent studies have demonstrated the presence of ecdysteroid receptors in premoult muscle from *H. americanus* using antibodies to the *Drosophila* ecdysteroid receptor (El Haj *et al.* 1994). By injecting premoult concentrations of 20-HE, intermoult lobsters can be induced to enter premoult and begin to synthesize a new exoskeleton (Ranga Rao *et al.* 1973).

Measurements of *in vivo* protein synthesis rates in the leg muscles of *Carcinus maenas* have previously been carried out using an adaptation of a radiolabelled amino acid flooding dose technique devised by Garlick *et al.* (1980) (El Haj and Houlihan, 1987; Houlihan *et al.* 1990). In the present study, we used this method to measure and compare *in vivo* rates of protein synthesis in *H. americanus* in three muscle groups (claw, leg and abdomen) within the same individual to investigate differential rates of protein synthesis in different muscle groups over the moult cycle. The effects of injection of premoult concentrations of exogenous 20-HE on muscle synthesis *in vivo* and *in vitro* were also monitored in this investigation.

Materials and methods

Juvenile lobsters, *Homarus americanus*, were maintained in circulating sea water as described in Conklin and Chang (1991). Animals were checked twice daily to obtain accurate determinations of premoult (stage D_3–D_4), immediate postmoult (stage A/B within 24 h after ecdysis) and intermoult status. Premoult animals were confirmed by examination of pleopods and by the degree of cuticle digestion on the dorsal carapace and chelipeds (Cheng and Chang, 1991).

The *in vivo* free pool technique used was as described by El Haj and Houlihan (1987) for *Carcinus maenas*. A single dose of L-[2,3,4,5,6-^{3}H]phenylalanine (specific activity 100–130 Ci mmol^{-1}) (Amersham, UK) at 50 μCi ml^{-1} (1.85 MBq ml^{-1}) in a lobster saline containing 150 mmol l^{-1} phenylalanine at a dose of 1.0 ml 100 g^{-1} body mass was injected into the sinus at the base of a walking leg. A time course of isotope uptake after incubation was initially measured (N=12). After initial injection, animals were left for 1, 2 or 3 h before dissection. All animals were maintained in aerated sea water at 15–18 ˚C during this period. Haemolymph samples were taken from the base of the third walking leg at 1, 2 or 3 h after injection. 50 μl samples were added to an aquasol scintillant (Optiphase, HiSAfeIII, LKB, Pharmacia, UK) in a Beckman LS1701 scintillation counter and expressed as disints min^{-1} nmol^{-1} phenylalanine. Subsequently, for further experiments, all animals were left for 1 h following injection prior to dissection of the muscle tissue.

The muscles from the crusher claw, abdomen and merus of the walking leg (both extensor and flexor muscles) were dissected out and frozen in liquid N_2 for storage at −70 ˚C until subsequent analysis. Samples were analysed using a modified protocol from El Haj *et al.* (1984) and Goldspink *et al.* (1987). Approximately 250 mg of the muscle sample was homogenized, centrifuged at 10 000 ***g*** for 10 min and the resulting pellet washed in ice-cold 2 % perchloric acid (PCA). After the initial homogenization, samples were taken for total protein analysis according to Bradford (1976) and the supernatant after centrifugation was kept for analysis of the specific activity of the free pool. The pellet was solubilized in 0.3 mol l^{-1} NaOH, after washing twice in ice-cold 2 % PCA, and incubated at 37 ˚C for 1 h. DNA and proteins were precipitated by adding ice-cold 12 % PCA to the solubilized sample. The precipitate was further washed with 2 % PCA and the combined acid-soluble fractions were removed for estimation of total RNA concentration by ultraviolet absorption at 232 and 260 nm, as described by Ashford and Pain (1986). Comparisons of values obtained in this way were made with total RNA measurements using the guanidium isothiocyanate extraction technique described in Harrison and El Haj (1994). Protein pellets after RNA determinations were hydrolysed in 6 mol l^{-1} HCl at 110 ˚C for 24 h. The hydrolysate was evaporated to dryness and taken up in citrate buffer, pH 6.3. Phenylalanine was then converted to β-phenethylamine and quantified using a fluorometric assay procedure according to Garlick *et al.* (1980). All measurements of radioactivity were made as described above for the haemolymph samples.

The fractional rate of protein synthesis (K_s, the percentage of the protein mass synthesized per day) was calculated from:

$$K_s = (S_b/S_a)(100/t)\,, \qquad (1)$$

where S_b is the specific activity of protein-bound phenylalanine (disints min^{-1} nmol^{-1}), S_a is the specific activity of free L-

phenylalanine (disints min^{-1} $nmol^{-1}$) and t is the duration of the incorporation in days.

The protein synthesis rate per unit of RNA (ribosomal activity; K_{RNA}; μg protein μg^{-1} RNA day^{-1}) was calculated according to the following equation:

$$K_{RNA} = 10K_s/([RNA]/[protein])\,. \quad (2)$$

In vivo *experiments*

A premoult concentration (10^{-6} mol l^{-1}) of 20-HE was injected into intermoult *H. americanus* through the sinus at the base of the walking leg. Animals were left for 3 days in the holding conditions described above. They were then subjected to *in vivo* injections of ^{3}H-labelled phenylalanine for protein labelling experiments as described above. If the animals were subsequently injected with an additional dose of 20-HE and left for a further 3 days, they produced a new exoskeleton and commenced an unsuccessful ecdysis as described previously (Ranga Rao *et al.* 1973).

In vitro *experiments*

Walking legs of *H. americanus* were removed by autotomy. Removal of cuticle along the side of the length of the merus exposed the carpopodite extensor and flexor muscles. The whole leg was then incubated for 36 h in a medium based on that previously described by Brody and Chang (1989). In particular, tissue culture medium M199 was used with addition of 40 mg l^{-1} proline, 190 mg l^{-1} $MgCl_2$ and 22 g l^{-1} NaCl to adjust the osmotic conditions and the concentrations of amino acids to give values similar to those found in lobster haemolymph. Bovine serum albumin (BSA, 1 %), penicillin (10^6 i.u. ml^{-1}), streptomycin (100 μg ml^{-1}) and fungizone (20 μg ml^{-1}) were added to the culture medium.

Extensor and flexor muscles could be incubated for up to 48 h without loss of viability. Tests were carried out to demonstrate that the muscles were still capable of responding to electrical stimulation by contraction (data not shown). A vital dye (0.1 % Procion Yellow) was also used, which will not enter cells with viable intact membranes, and muscle membranes were found to remain intact for up to 48 h (data not shown).

Increasing concentrations of 20-HE (10^{-10} to 10^{-6} mol l^{-1}) were added to the medium with [^{3}H]phenylalanine (2.5 μCi ml^{-1}). Equivalent diluted vehicle was added to the control cultures. When the incubations had been completed, the muscles were dissected free, washed in lobster saline and placed in 2 % PCA as described earlier. After solubilization in 0.3 mol l^{-1} NaOH, samples were taken for protein assay according to the method of Bradford (1976) and scintillation spectrometry was performed as described above.

RNA extraction and northern blotting

Total RNA was extracted from equivalent sample masses of claw muscle (crusher claw) of young adult lobsters at various time points through the 45-day moult cycle using the acid guanidium thiocyanate–phenol–chloroform technique as described in Harrison and El Haj (1994). An equivalent volume of each total RNA sample was treated as described in Harrison and El Haj (1994) with some modifications; in particular, samples were subjected to electrophoresis on a 1.2 % agarose gel and transferred onto nylon membranes (Boehringer Mannheim, UK) *via* capillary blotting. The cDNA for rat 18S ribosomal RNA was labelled with [^{32}P]dCTP (Amersham International, UK) to a specific activity of 10^9 disints min^{-1} μg^{-1} using a random primer labelling kit (Pharmacia, UK). Prehybridization and hybridization times were 3 h and overnight, respectively, followed by two stringency washes of 30 min each in 4×SSC (standard saline citrate), 0.5 % sodium dodecylsulphate (SDS), two 15 min periods in 2×SSC, 0.1 % SDS and one 20 min period in 1×SSC, 0.1 % SDS all at 65 °C. After washing, the nylon membranes were exposed to X-ray film (Hyperfilm-MP, Amersham) in the presence of intensifying screens and left at room temperature for 3 h prior to developing.

Statistics

All data are expressed as the mean ± S.E.M. Comparisons of the means from treatments and corresponding controls were carried out using Student's *t*-tests with 95 % confidence limits. Multiple comparisons of the means were carried out using analysis of variance (ANOVA).

Results

Table 1 shows the time course of [^{3}H]phenylalanine uptake *in vivo* into muscle tissues of intermoult *H. americanus*. The level of [^{3}H]phenylalanine in the haemolymph decreases with time of incubation from 2.5×10^5 disints min^{-1} ml^{-1} haemolymph at 1 h to 1.7×10^5 disints min^{-1} ml^{-1} haemolymph at 3 h post-injection.

Table 1. *Rate of protein synthesis* (K_s) *in three muscle tissues (abdomen, claw and leg) measured 1, 2 and 3 h following an* in vivo *injection of [^{3}H]phenylalanine in lobster saline*

Muscle	Time (h)	S_a (disints min^{-1} $nmol^{-1}$ phenylalanine)	K_s (% day^{-1})
Leg	1	1203±133	0.333±0.130
	2	1181±56	0.239±0.068
	3	1876±400	0.163±0.046
Abdomen	1	1798±401	0.224±0.042
	2	1751±107	0.313±0.078
	3	1935±470	0.196±0.055
Claw	1	3652±308	0.382±0.040
	2	2664±366	0.244±0.024
	3	2764±409	0.124±0.007

Values are means ± S.E.M., N=4.

Free pool specific activity (S_a) represents the ratio of labelled to unlabelled phenylalanine in the cytoplasm of the muscle cells from abdomen, claw and leg.

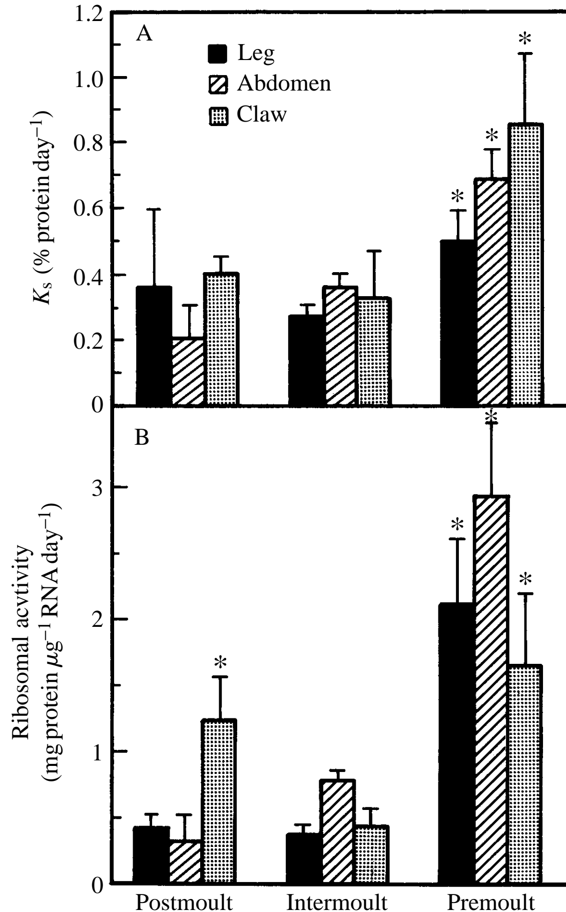

Fig. 1. (A) Rate of protein synthesis per day (K_S) in three muscle groups (leg, abdomen and claw) in the lobster *Homarus americanus* at three stages of the moult cycle (N=4). (B) Amount of protein synthesized per day as a proportion of the total pool of RNA (K_{RNA} or a measure of ribosomal activity) in three muscle groups (leg, abdomen and claw) at three stages of the moult cycle. Values represent means + S.E.M. An asterisk indicates a significant difference from the value for the intermoult stages (P<0.05).

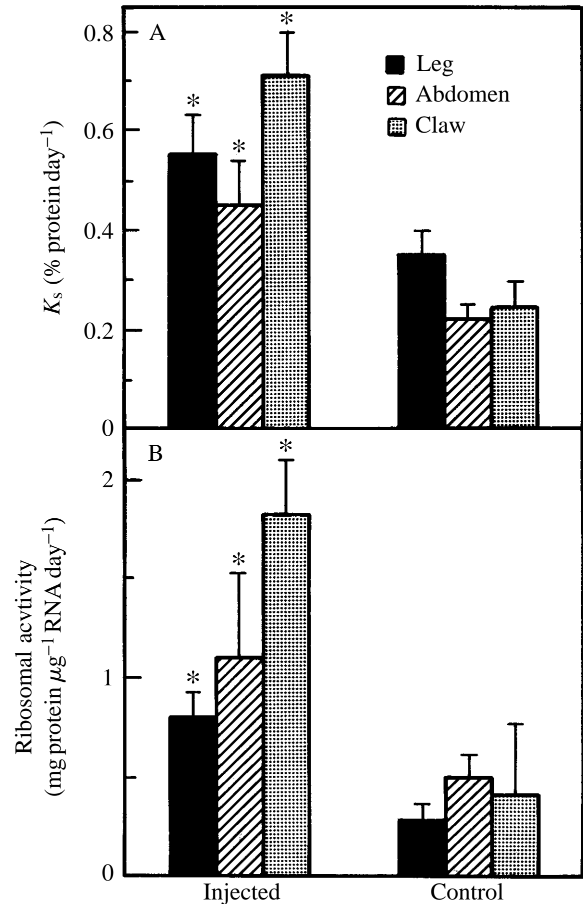

Fig. 2. (A) Rate of protein synthesis per day (K_S) 3 days following an injection of a premoult concentration of 20-hydroxyecdysone (20-HE) (10^{-6} mol l^{-1}) into three muscle groups in the lobster *H. americanus*. Controls were injected with saline (N=6). (B) Ribosomal activity (see Fig. 1B) in three muscle groups in the lobster 3 days after injection of 20-HE. Values represent means + S.E.M. An asterisk indicates a significant difference from the value for the control group (P<0.01).

In claw muscle, the ratio of labelled to unlabelled phenylalanine falls significantly (P<0.05) during the 3 h period, from 3652 to 2764 disints min^{-1} nmol^{-1} phenylalanine. In the abdomen and the leg, however, the specific activity of the free pool remains relatively constant over time and there are no significant differences between 1 h and 3 h values (P>0.05). With the significant fall in free pool label concentration in the claw, there is a decline (not significant) in the rates of protein synthesis (K_S) calculated after 2 and 3 h of incubation, which may be due to the relatively reduced uptake of labelled phenylalanine into protein as the specific activity of the free pool declines. After 1 h, the K_S values for all three tissues range from 0.2 to 0.4 % of the total protein synthesized per day. Throughout the rest of the experiments, the animals were dissected 1 h after injection, which was a similar period to that used previously for similar experiments on other crustaceans held at similar temperatures (El Haj and Houlihan, 1987; Houlihan *et al.* 1990).

Fig. 1A shows the rates of protein synthesis (K_S) over the moult cycle in premoult, intermoult and postmoult animals *in vivo*. K_S values vary to some extent between claw, leg and abdominal muscle, which will reflect the proportions of different types of muscle within the muscle groups and possibly variation in perfusion rates between the three muscle groups. All three muscle groups have significantly elevated K_S values during the premoult period compared with the intermoult period. Immediate postmoult values are slightly elevated in claw and leg muscle but the differences are not significant. When the K_S values are expressed in terms of ribosomal activity, the differences between premoult values and intermoult values are further emphasised (Fig. 1B). Interestingly the ribosomal activity of the claw muscle shows an elevated protein production during the postmoult period as well.

Fig. 2A shows that there were significantly elevated levels of protein synthesis in terms of K_S and ribosomal activity in the claw, abdominal and leg muscle groups 3 days after injection of 20-HE *in vivo*.

When the amount of RNA present is expressed as a function

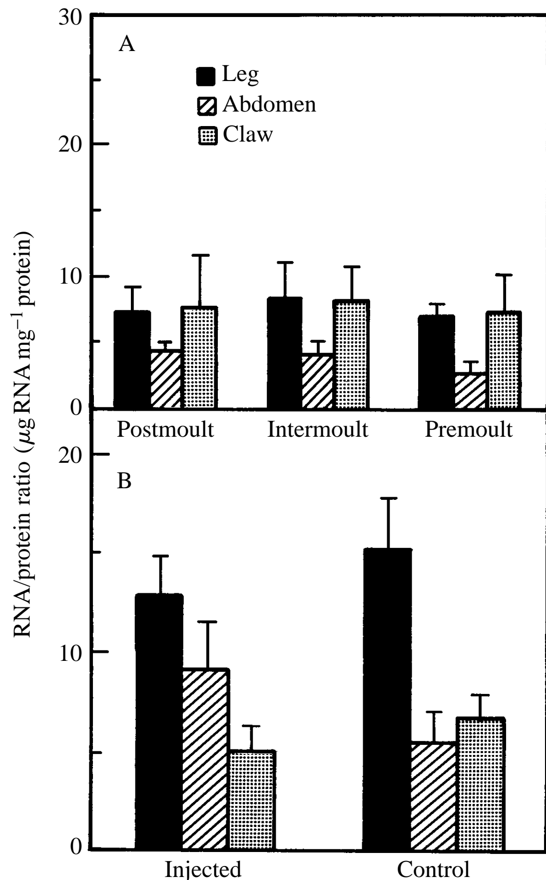

Fig. 3. (A) Ratio of RNA to protein levels in three muscle groups (leg, abdomen and claw) in the lobster *H. americanus* at three stages of the moult cycle (*N*=4). (B) Ratio of RNA to protein levels in the same muscle groups from animals injected with 20-HE 3 days previously or injected with saline (controls) (*N*=6). Values represent means + S.E.M.

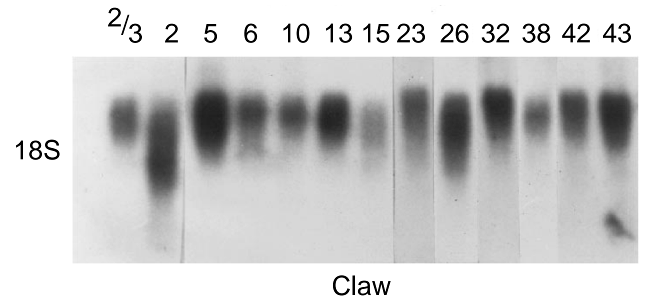

Fig. 4. Northern blot of extracted RNA from claw muscle from lobsters at various intervals through the moult cycle. Hybridization was carried out with a cDNA for rat 18S ribosomal RNA (see Materials and methods). The numbers indicate days postmoult in lobsters that moult approximately every 45 days. Each lane represents a different animal.

Table 2. *Effect of* in vitro *20-hydroxyecdysone treatment on the rate of protein synthesis in the walking leg muscle of* Homarus americanus

20-HE concentration ($mol\,l^{-1}$)	Rate of protein synthesis (disints min^{-1} mg^{-1} protein day^{-1})
0 (control)	319±41 (189–494)
10^{-10}	402±49 (233–627)
10^{-8}	331±39 (136–507)
10^{-6}	430±53 (260–645)

Values are mean ± S.E.M. with the range given in parentheses; *N*=8 combined leg extensor and flexor muscles per treatment from four individuals.

20-HE, 20-hydroxyecdysone.

of protein levels in the three muscle groups, there is no significant change in total RNA levels over the moult cycle or in response to injection of 20-HE (Fig. 3A,B). Fig. 4 shows the levels of 18S ribosomal RNA in claw muscle samples at intervals over the moult cycle in young adult lobsters which moult periodically over a 40- to 50-day cycle. The abundance of ribosomal RNA fluctuates slightly, with no indication of large elevations during the premoult or postmoult periods when corresponding K_s levels are high.

Results from *in vitro* incubations of leg muscles did not show any changes in protein synthesis rates in response to increasing doses of 20-HE. Table 2 shows the effects of 20-HE injection at concentrations of 0.015–1.5 $\mu g\,ml^{-1}$ (10^{-6} to $10^{-10}\,mol\,l^{-1}$) incubated with extensor/flexor muscles using *in vitro* preparations. Uptake of radiolabel into protein in relation to total protein content (in milligrams) was constant in the four treatments.

Discussion

K_s values decline with time after injection of labelled amino acid. This may be due to a reduction in the specific activity of the free pool of [^{3}H]phenylalanine as a result of the declining specific activity in the haemolymph with time after injection. Time course experiments with *Carcinus maenas* kept at similar temperatures (approximately 15 °C) also showed a decline in the free pool [^{3}H]phenylalanine concentration with time (El Haj and Houlihan, 1987). The time used for labelling in *C. maenas* was 1 h, and a similar labelling time was chosen for *H. americanus*. This ensured that there was sufficient uptake of label into the protein in the muscle for detection while the free pool levels were also still elevated. The K_s values for the three muscles in *H. americanus* were slightly lower at approximately 0.3 % total protein day^{-1} than those reported by El Haj and Houlihan (1987) and Houlihan *et al.* (1990) for *C. maenas* (approximately 0.5–1.0 % day^{-1} in muscle tissue from fed crabs held at 15 °C). Using this *in vivo* technique, mammalian muscle has been shown to have approximately 10-fold higher synthesis rates (Goldspink *et al.* 1987) at 37 °C than those measured in the lobster. Fish slow muscle at a similar temperature has a similar range of K_s values to those of crustaceans (approximately 0.5 %; Houlihan, 1991).

The rate of synthesis of a particular muscle will vary with the proportion of fibre types, as has been demonstrated

previously for fish and crustaceans (Procrnjic *et al.* 1983; El Haj and Houlihan, 1987). In *H. americanus*, the similar K_s values for the different muscle groups may be due to the mixed population of fast and slow muscle fibre types in these combined extensor and flexor muscles. Although the claw crusher muscle is predominantly composed of slow fibres, the abdominal and leg combined extensor and flexor muscles are composed of a mixed population of slow and fast fibres (Jahromi and Atwood, 1971; Ogonowski and Lang, 1979).

Rates of protein synthesis also vary over the moult cycle as has been shown previously for other species of crustaceans *in vivo* (e.g. the land crab *Gecarcinus lateralis*; Skinner, 1965, 1966) and *in vitro* (e.g. the green shore crab *C. maenas*, El Haj and Houlihan, 1987) at similar stages in the moult cycle to those investigated in this study. Using the *in vivo* flooding dose technique in *H. americanus*, we have been able to compare *in vivo* rates of protein synthesis over the moult cycle in three different muscle groups in the same individual. All three muscle groups show elevated rates of protein synthesis during the premoult (stage D_2–D_3) period. Immediately following the moult (stage A/B), K_s has fallen nearly to intermoult levels.

It has previously been suggested that differential regulation of protein synthesis may occur in muscle tissues from moulting crustaceans and that this may be related to ecdysteroid receptor activity (El Haj *et al.* 1992, 1994). In the land crab claw muscle, protein levels decrease by up to 40 % during premoult and there is a marked reduction in fibre cross-sectional area (Mykles and Skinner, 1990). For *H. americanus*, an aquatic crustacean, our measurements of dry/wet mass ratios over the moult cycle (data not included) suggest that this atrophy may not be as pronounced as in *G. lateralis*. Lengthening in the leg and abdominal muscles indicates that these muscle tissues increase in mass in preparation for the moult. Our results from this study showing equivalent rates of protein synthesis in all three muscle groups over the moult cycle suggest that differential regulation of protein synthesis may not be a mechanism for the differential responses of muscle groups to moult-inducing stimuli. In the claw muscle, an elevation in the rate of synthesis of proteins when the muscle mass is decreasing may correspond to an increase in the production of degradative enzymes with a net reduction in muscle mass.

When the rates of protein synthesis are expressed as a function of the total RNA content in the three muscle groups, giving an estimate of ribosomal activity, the rate is elevated during the premoult period. However, RNA/protein ratios remain constant over the moult cycle. Using a heterologous rat 18S ribosomal cDNA probe, levels of ribosomal RNA in *H. americanus* were found to vary slightly over the moult cycle; however, there was no marked elevation in levels during either the pre- or postmoult period. This indicates that elevations in protein synthesis rates in the lobster are not likely to be due to increases in ribosomal numbers but rather to the activity of the ribosomes. However, Skinner (1968) investigated ribosomal content over the moult cycle in *G. lateralis* and found that ribosomal RNA levels remained elevated through ecdysis and for at least 2 weeks thereafter.

Protein synthesis rates and ribosomal activity were elevated in the three muscle groups within 3 days after injecting a premoult concentration of 20-HE into intermoult lobsters. The RNA/protein ratio remained constant after 20-HE injection, which implies that the elevated rate of protein synthesis is not due to an increase in the ribosomal content of the muscle. Our studies agree with previous work on other crustacean tissues showing an effect of 20-HE *in vitro*. Injections of 20-HE into intermoult crayfish (*Orconectes virilis*) result in a twofold elevation in uptake of labelled amino acids *in vitro* into the midgut gland after 20 h (Gorell and Gilbert, 1971).

In lobster muscle, 20-HE may act at the level of protein synthesis by increasing ribosomal processing of mRNA pools or directly on gene transcription through a steroid response region. 20-HE may act through a receptor to promote increased levels of sarcomeric synthesis directly or through the production of intermediary factors which then initiate muscle growth. An ecdysteroid receptor has been located in the muscle and eyestalk tissue of *H. americanus* using an antibody to the *Drosophila* ecdysteroid receptor (El Haj *et al.* 1994). Using immunocytochemistry, such studies revealed that there may be differential activation of the receptor at various stages of the moult cycle, but further work is needed to clarify this.

In this study, attempts to mimic the effects of 20-HE on muscle protein synthesis *in vitro* were unsuccessful. Using a range of premoult concentrations of 20-HE, there was no effect on protein synthesis rates in the leg muscle. The incubation time was 36 h; however, in subsequent experiments in which the incubation time was extended to 48 h, there was also no effect (data not shown). *In vitro* incubations of other crustacean tissues with 20-HE have shown conflicting results. In the land crab *G. lateralis*, twofold elevations in protein synthesis rates in the midgut gland and the epidermis were measured in response to 20-HE application of a similar concentration and duration of treatment to those in this study (Paulson and Skinner, 1991). Preliminary studies on a *C. maenas* extensor muscle preparation using radiolabelled uridine uptake have demonstrated a dose-dependent response of RNA synthesis rates to 20-HE treatment (Whiteley *et al.* 1992). In contrast, previous *in vitro* studies in the crayfish *O. virilis* have failed to find a response. Gorell and Gilbert (1971) were not able to stimulate the midgut gland using 20-HE treatment to elevate rates of RNA or protein synthesis. It may be that there are some species differences between crabs and lobsters, but these differences may also be due to the level of receptor activity in intermoult animals and the ability to stage these animals accurately.

Our *in vitro* results for the lobster may suggest a requirement for additional factors, such as growth factors produced by other tissues, to play an intermediary role in the action of 20-HE. Various factors have been suggested to have such a role as mediators of growth although, as yet, none has been characterised. Earlier studies on the lobster *Homarus americanus* have demonstrated insulin-like immunoreactivity in the muscle, and a potential role for this in growth modulation was suggested (Sanders, 1983), but further work is needed to

address this question. It is also possible that a combination of active receptors and additional factors is necessary to modulate ecdysteroid action on muscle *in vivo*.

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