Heterologous acclimation: a novel approach to the study of thermal acclimation in the crab Cancer pagurus

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Abstract
Heterologous acclimation: a novel approach to the study of thermal acclimation in the crab Cancer pagurus. Am. J. Physiol. 277 (Regulatory Integrative Comp. Physiol. 46): R24–R30, 1999.—The control of the attainment of acclimation in Cancer pagurus has been studied. Homologous (8 or 22°C) and heterologous acclimation (central nervous system (CNS) and periphery of crabs simultaneously held at 8 or 22°C) were used. The dependence of electrophysiological parameters of dactylopodite closer muscles of walking legs on nerve stimulation was determined between 6 and 26°C. Muscle resting potential (RP) hyperpolarized linearly with increasing measurement temperatures and showed a 69% compensation between 8 and 22°C on homologous acclimation. With the CNS temperature constant at 8°C, the leg muscle RP showed a 72% compensation on heterologous acclimation to 8 and 22°C; when CNS temperature was constant at 22°C, leg muscle RP showed a 48% compensation on heterologous acclimation to 8 and 22°C. In homologous acclimation, the shape of the excitatory junction potential vs. temperature relationship was characteristic of acclimation temperature. In heterologous acclimation, the shape of this plot was related to the temperature experienced by the leg and not by the CNS. Thus acclimation was principally dependent on local tissue temperature and was relatively independent of CNS or hormonal influences.

The ability of ectothermal animals to maintain function in environments that are subject to diurnal and seasonal fluctuations of temperature has been extensively studied. Approaches used have concerned measurements of compensatory or adaptive changes in animals that have experienced natural fluctuations of environmental parameters (acclimatization) or, more usually, studies with a single laboratory-controlled parameter (acclimation). In this way, an extensive catalogue of thermal acclimation and acclimatization responses has been demonstrated (5), particularly for aquatic species. For example, at the level of the organism such compensatory responses have been shown to be adaptive in thermal tolerance (10), thermal preference (3), locomotion (28), and metabolism (14). More recently, attention has focused on determining the physiological, biochemical, and molecular mechanisms that explain those adaptive responses. With respect to metabolism, thermal acclimation can alter metabolite flow through branch points where pathways compete for 1) the same substrate (14), 2) increased mitochondrial enzyme activity (12), and 3) the volume fraction of muscle occupied by mitochondria (6). These changes have been interpreted as compensatory responses to permit enhanced performance at lower environmental temperatures. Acclimation effects have also been demonstrated on the contractile properties of fish muscle (1) and on Ca\(^{2+}\) ATPase activity (38) that clearly would contribute to the observed responses on swimming performance.

The mechanisms involved in bringing about such hierarchical acclimation responses have in recent times focused on three approaches. First, there is the remodeling of the membrane lipid (15). Implicit in this approach is that the structural adjustments in lipid composition change membrane physical state in a manner that compensates for the direct impact of the temperature change. The adaptive significance is to produce a constancy of membrane function (4). Second, changes occur in the concentrations of substrates, cofactors, and enzymes (23). These kinetic responses are again argued to be adaptive in that they compensate for the direct effect of the temperature change on enzyme activities. Third, the differential expression of some protein isoforms has been described to occur during thermal acclimation and acclimatization (21, 39), the implication being that the expression of the isoform most appropriate for the prevailing thermal conditions was adaptive (31). More recently, interest has focused on temperature-induced expression of specific enzymes. Cold-induced expression of a desaturase from carp liver has been reported (35). The thrust of this approach was to relate the temperature-induced expression of the enzyme with changes in membrane lipid saturation to shed light on the cellular mechanisms that report the temperature change.

Implicit in those mechanistic approaches is that acclimation responses occur at the level of the cell. Evidence from fish cell lines shows that isolated cells are capable of acclimation. Responses have been reported in membrane “fluidity” (36), the specific activities of some enzymes (19), microtubule stability (37), and heat resistance (29) consistent with those seen in tissues from whole organisms. However, a possible hormonal requirement was proposed for protein synthesis in cultured fish hepatocytes (24). This raises the interesting question of whether acclimation responses of an organism are a simple summation of individual cellular responses or whether they require an involvement of central control systems (2). Opinion exists that suggests that acclimation may be controlled systemically either directly or indirectly (19, 20, 25).

The proposed study seeks to address this question with a novel technique. Crabs were maintained such that different regions of the same animal were held...
simultaneously at different (heterologous) tempera-
tures. This technique has been used previously in
relatively few studies (7, 26). These earlier studies used
fish, and the metabolic responses investigated sug-
gested that the central nervous system (CNS) played a
hierarchical role. Those experiments with fish were
difficult to interpret for two reasons: first, because the
fish were divided anterior-posterior and second, be-
cause of the variation in the mass of tissue exposed to
the different temperatures (7). In the present study,
crabs were maintained at the heterologous tempera-
tures with an offset lateral separation of the body. This
allowed a direct comparison to be made of the muscles,
from the legs of the same crab, that had experienced
different thermal histories. Furthermore, it enabled
these comparisons to be related to the temperature
experienced by the CNS and eye-stalk hormonal sys-
tem. The neuromuscular system was chosen for study
because it has been shown to be responsive to thermal
acclimation in a variety of decapod crustaceans (13, 16,
33). The efficacy of the heterologous temperature para-
digm was established by comparison with information
obtained with similar electrophysiological measure-
ments on crab leg muscles in which the whole body was
uniformly held for acclimation at 8 or 22°C (homologous
acclimation).

MATERIALS AND METHODS

Condition for homologous acclimation. Cancer pagurus
were fed after capture and held in filtered seawater at 8°C.
Crabs were then held at 8 ± 0.5°C for a further 17 days before
electrophysiological testing. Other crabs were transferred to
increased temperature in steps, over a period of 17 days, to
22°C. Crabs were held at that temperature for a further 2 wk
before use (32).

Immobilized controls. Crabs were placed in the heterolo-
gous apparatus (Fig. 1), and both compartments either were
maintained at 8 ± 0.5°C for 2 wk or were exposed to
increasing temperatures up to 22°C and then maintained for
a further 2 wk at 22 ± 0.5°C. These controls, compared with
free-moving homologous acclimated crabs, identified any
effects of immobilization on the selected neurophysiological
parameters.

Heterologous acclimation. The same temperatures were
selected for heterologous acclimation as those used in the
control homologous free and immobilized acclimation experi-
ments, i.e., 8 and 22°C.

Crabs of appropriate size (10-cm-width carapace) were
chilled to facilitate handling. The crab was then fitted into the
apparatus so that the rubber partition diaphragms separated
the body of the animal longitudinally into right and left sides.
The diaphragm was positioned off-center so that it was
between a cheliped and the mouth parts (Fig. 1); this arrange-
ment isolated a three-quarter body section, including the
CNS, both eye-stalks, and ipsilateral legs at one acclimation
temperature, whereas the contralateral quarter was isolated
at the other acclimation temperature. The animal was then
damped securely in the apparatus.

Two heterologous temperature protocols were arranged as
follows: 1) the larger body portion at 8°C and the smaller part
at 22°C, a condition designated 8CN/8Leg and 8CN/22Leg;
and 2) the larger body portion at 22°C and the smaller part at
8°C, a condition designated 22CN/22Leg and 22CN/8Leg,
where CN refers to central temperature and Leg refers to leg
temperature.

Comparisons could then be made of the extent of the
acclimation responses between legs of opposite sides in the
same animals.

The conductivity of the seawater was monitored and main-
tained to ensure that crabs did not experience changes in
osmotic conditions. Crabs were not fed during the 2-wk
acclimation period. In a series of control measurements of
tissue temperatures with implanted thermocouples in crabs
incubated under heterologous temperature conditions, the
CNS temperature was 19.0 ± 0.16 or 9.9 ± 0.096°C when the
external temperatures were 21.8 ± 0.06 or 8.3 ± 0.15°C,
respectively. In effect, the difference in CNS temperatures
achieved was 9.1°C, which was a smaller difference than was
found between the water baths (13.5 ± 0.17°C) in these
control experiments. These acclimation temperature differ-
ences were also smaller than those used for homologous
temperature acclimation, which was 14°C (8 ± 0.5 vs. 22 ±
0.5°C). The leg muscle temperatures were the same as bath

Fig. 1. Heterologous temperature apparatus. Inner
compartment was heated to 22 ± 0.5°C, and outer
compartment was cooled to 8 ± 0.5°C. Position of
diaphragm partitioning crab is shown as a dashed
line.
temperature and so were the same as for the homologous temperature-acclimated crabs.

Dissection and electrophysiology. After acclimation, a walking leg was autotomized, and the tonic motor axon to the dactylopodite closer muscle was identified within the meropodite article. Dactylopodite closer muscle fibers were then revealed in the propodite article by removal of the overlying carapace. Electrophysiological recordings of resting potential (RP), single excitatory junction potential (EJP), and facilitation were made from dactylopodite closer muscle fibers (types I and II, Ref. 27) with intracellular microelectrodes with conventional recording techniques. The single tonic axon to the closer muscle was selected by nerve splitting and stimulation threshold. Care was taken to ensure that the common inhibitor axon innervating the closer muscle was not stimulated. The experimental temperature range (6–26°C) was controlled by a Peltier junction. In most experiments, the temperature characteristics of measured parameters were determined at selected intervals during a ramp change (0.4 °C/min) in experimental temperature. Sixteen records were taken from each closer muscle fiber at each selected interval (−1–2 °C) within the experimental temperature range and were averaged and analyzed later with the SCAN software package (J. Dempster, Univ. of Strathclyde). Tissues were bathed in crab saline [composition (in mM): 470 NaCl, 20 CaCl2, 10 MgCl2·6H2O, 8 KCl, 10 HEPES acid, pH 7.4 (32)].

RESULTS

Muscle membrane RP. Figure 2 shows the data for RP measurements for dactylopodite closer muscle fibers of Cancer pagurus homologously maintained at temperatures of 8 and 22°C. RP hyperpolarized with increasing experimental temperature in both free and immobilized animals. In both groups, leg muscle RP from cold-maintained animals was significantly hyperpolarized over the whole experimental temperature range compared with those from warm-maintained crabs. In 8°C-maintained crabs, immobilization did not result in significant difference in the RP dependency on temperature; the slope of the regression was −1.29 ± 0.16 and −1.08 ± 0.06 mV/°C for the free (n = 14) and immobilized (n = 16) crabs, respectively. However, immobilization had an effect in the 22°C-maintained crabs; the slope of the regression fell from −1.05 ± 0.061 to −0.76 ± 0.078 mV/°C in the free (n = 16) compared with the immobilized (n = 10) crabs, respectively (P < 0.05). The efficacy of the acclimation response was 69% for the free compared with 104.3% for the immobilized group.

Figures 3 and 4 show the effect of temperature on the muscle membrane RP of heterologously maintained crabs. Muscle membranes hyperpolarized in a linear fashion with increasing experimental temperature. In the first experiment in which the CNS was maintained at 8°C (Fig. 3), a clear acclimation shift of leg muscle RP occurred between the 8CN/8Leg and 8CN/22Leg conditions. The muscle membranes from the legs of the ipsilateral side (8°C) were significantly hyperpolarized compared with the legs on the contralateral side (22°C), and the efficacy of the acclimation was 72%. Figure 4 shows similar data for 22CN/22Leg and 22CN/8Leg conditions. In these experiments also, maintenance of the CNS at the higher temperature did not prevent the acclimation response of leg muscle RP as shown by the displacement of the RP vs. temperature relationship, although the efficacy of the acclimation was reduced to 48%. The slopes of the regression relationship for RP vs. temperature were −0.98 ± 0.07 (8CN/8Leg), −0.92 ± 0.08 (8CN/22Leg), −1.01 ± 0.07 (22CN/8Leg), and −0.92 ± 0.04 (22CN/22Leg) mV/°C and were not significantly different.

EJP amplitude. Figure 5 shows the amplitudes of walking leg EJP obtained over the experimental range of temperatures for both free and immobilized crabs.
homologously held at 8°C. In these animals, the EJP amplitude was largest at an average of 1.50 mV over the range 6–13°C and was reduced progressively in amplitude to 25% of this value at 25°C. The amplitude of the EJP from immobilized crabs showed a similar pattern of change with experimental temperature (Fig. 5) as that of the unrestrained crabs, but over most of the temperature range (6–17°C) the EJPs obtained from immobilized animals were significantly larger. A different pattern of response of EJP amplitudes to acute temperature change was obtained from the legs of 22°C homologously maintained crabs. Relatively little change in EJP amplitude occurred between 11 and 24°C. No significant differences in EJP amplitudes were observed between free and immobilized crabs acclimated at 22°C. Clear differences in EJP amplitude vs. temperature relationships were observed for different acclimation temperatures. In both free and immobilized crabs, EJP amplitudes were larger for 8°C-maintained compared with 22°C-maintained crabs below acute experimental temperatures of 19–20°C but smaller above these values (Fig. 5).

The dependency of EJP amplitude on temperature for heterologously maintained crabs is shown in Figs. 6 and 7. Examination of Figs. 6 and 7 shows that a similar pattern of change in EJP amplitude vs. temperature was obtained from both legs irrespective of whether the temperature of the CNS was maintained at 8 or 22°C. Thus EJP amplitudes, as in the homologously maintained crabs, were larger for 8CN/8Leg and 22CN/8Leg over the temperature range 5–19°C compared with EJP amplitudes of 8CN/22Leg and 22CN/22Leg. Above experimental temperatures of 19°C, EJP amplitudes of 8CN/22Leg and 22CN/22Leg were maintained to a higher temperature than those from 8CN/8Leg and 22CN/8Leg. These differences were qualitatively independent of CNS acclimation temperatures. The acclimation responses of the 8CN/8Leg and 22CN/8Leg were similar to those obtained from 8°C homologously maintained crabs (Fig. 5) in that the highest values were obtained between 6 and 15°C. EJPs progressively declined with increased experimental temperature. The
pattern of response obtained from 8CN/22Leg and 22CN/22Leg of the two different heterologous temperature conditions was similar and was in agreement with the data from 22°C homologously maintained crabs.

Facilitation and latent period. The acute temperature dependency of facilitation of the EJP and its latent period from nerve stimulation in free and immobilized 8- and 22°C-maintained crabs was also measured. However, no significant differences were found between any of the groups of crabs maintained at 8 and 22°C. Nor were significant differences found in EJP facilitation and its latent period from nerve stimulation in free and immobilized 8- and 22°C-maintained crabs. The acute temperature change, the shift of this relationship with long-term temperature change appears to be adaptive. In unrestrained crabs, the RP vs. temperature relationship is displaced to higher temperatures on maintenance at 22°C, a shift that partially compensates for the hyperpolarizing effect of an acute increase in temperature. The extent of this compensation in Cancer pagurus leg muscle RP was 69% in crabs maintained at 8 or 22°C compared with 72% in heterologously maintained crabs in which the central temperature was ~10°C. The fact that peripheral acclimation responses of leg muscle RP occur to a similar extent in heterologous temperature conditions demonstrates that compensatory peripheral responses to temperature can proceed independent of the central temperature. With central components maintained at higher temperatures (~19°C), the acclimation response between 22CN/22Leg and 22CN/8Leg muscle RP was only 48%; that is, the compensatory effect was reduced. This difference in the extent of compensation suggests some modulatory role in the attainment of peripheral tissue acclimation when CNS-hormonal systems were maintained at the higher acclimation temperature. Immobilization did not affect the RP vs. temperature relationship in 22°C-maintained controls only. This immobilization effect was not evident in heterologous maintained crabs because the slopes of the relationships were not different from those obtained for free crabs.

In walking leg muscle fibers of 8°C-maintained crabs, EJP amplitudes decreased with increasing acute experimental temperature, whereas those recorded from 22°C-maintained walking leg muscles were relatively constant over much of the same temperature range. EJP amplitudes were consistently larger in legs maintained...
at 8°C over the acute experimental temperature range between 6 and 19°C. The pattern of responses described is not essentially different whether the crabs were maintained under homologous or heterologous temperature conditions. Decreasing EJP amplitudes with increasing experimental temperature have been reported from Procambarus darkii maintained at 10°C (8) and for 12°C-acclimated Astacus leptodactylus (13). Increased EJP amplitudes at warmer experimental temperatures have been reported in 25°C-acclimated Astacus leptodactylus (13), 21°C-acclimated Pachygrapsus crassipes (34), and Ocypode ceratophthalma, which generated maximal EJP amplitudes over 22–28°C when acclimatized to 26–27.5°C (9).

Muscle tension with neuronal stimulation declines with warming in crustacean neuromuscular preparations (8, 13). Stephens (33) suggested that this might result from the combined effects of 1) RP hyperpolarization moving the membrane potential away from the excitation-contraction threshold, 2) reduction of the EJP amplitude, and 3) decline in the EJP time course. Changes observed in Cancer pagarus muscle RP and EJP amplitudes in response to maintenance at warmer temperatures would largely offset these effects. Unlike EJP amplitudes in Pachygrapsus crassipes (34), however, facilitation did not show acclimation responses with temperature that might contribute to maintenance of muscle function. However, in Cancer pagarus we have shown that facilitation only increased at temperature extremes, a change that was not responsive to thermal history.

The differences in EJP amplitudes in response to direct temperature changes recorded in the present study on Cancer pagarus were dependent on the local muscle acclimation temperature and were relatively unaffected by temperature experienced by the CNS-hormonal systems. Immobilization resulted in an increase in EJP amplitude most evident at lower experimental temperatures in animals immobilized and acclimated to 8°C (Fig. 5). In immobilized animals maintained at 22°C, EJP amplitudes were not increased at the lower range of experimental temperatures. The different patterns of response in EJP amplitudes to temperature, shown in the 8 and 22°C homologously maintained preparations, were also evident in both heterologous temperature conditions. Here the pattern of the response was determined by the leg temperature and not the CNS temperature.

The evidence for a central influence in thermal acclimation is scant and in some cases inconclusive. A governing CNS influence could not be ruled out from work on trout, but no central influence affected trout critical thermal maxima (7). The acclimatization status in fiddler crabs (Uca pugilator) has been reported to be influenced by hormones; eye-stalk extracts from 25°C-acclimated crabs decreased respiration when injected into 15°C-acclimated animals, whereas extracts from 15°C-acclimated crabs significantly increased respiration in 25°C-acclimated crabs (30). This was interpreted as the response to two antagonistically acting hormones on crab metabolic rates. A central hormonal factor that overrides local thermal effects on catfish hepatocytes has also been observed (25). Furthermore, different levels of spinal cord tonic discharges were correlated with persistent changes in muscle metabolism, suggesting that peripheral temperature acclimation was based on adaptive changes in the nervous system (20).

Perspectives

The heterologous temperature paradigm adopted can be used to identify the contributions made by the CNS-hormonal systems to acclimatory responses. This is important because seasonal acclimatization (as opposed to laboratory acclimation) to temperature is likely to be accompanied by responses to changes in a suite of other environmental factors. Some of these factors are likely to be transduced through the CNS alone and not have a direct influence on other tissues (e.g., photoperiod). Thus the heterologous thermal procedure will enable an assessment of the part played by these other factors in the complex, integrated processes of seasonal acclimatization to environmental change.

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