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TEMPERATURE SENSITIVITY OF GRADED SYNAPTIC TRANSMISSION IN THE LOBSTER STOMATOGASTRIC GANGLION

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Summary

We examined the temperature sensitivity of graded chemical synaptic strength within the pyloric circuit of the spiny lobster stomatogastric ganglion. Cooling from 20.4°C to 11.3°C reduced the graded synaptic potential (GSP) amplitude at all six pyloric synapses tested. Cooling appeared to reduce the slope of the linear part of the input–output curve at three of these synapses, and did not significantly alter the threshold for transmitter release at any synapses. Pairs of neurons with a presynaptic pyloric dilator (PD) cell showed reductions in graded synaptic strength at 16.5°C but those with presynaptic lateral pyloric (LP) or ventral dilator (VD) cells did not. A generalized decrease in input resistance is not responsible for the reduced GSP amplitude upon cooling, as determined by input resistance, action potential amplitude and electrical coupling measurements. We conclude that cooling reduces graded chemical strength by a direct synaptic action. Since the PD and VD cells use the same transmitter and act on some of the same postsynaptic cells, their differential sensitivity to cooling further suggests a presynaptic site of action. The temperature range used in our experiments encompasses the range that the animal normally encounters in nature. Thus, the relative importance of graded synaptic interactions in generating the pyloric motor rhythm may vary with transient changes in temperature.

Introduction

Temperature is an important environmental modulator of the behavior of ectothermic animals. Consequently, the effects of altered temperature on neuronal properties and interactions have been extensively studied in ectotherms (reviewed by Florey, 1978; Prosser and Nelson, 1981; Stephens, 1985, 1990). Synaptic junctions are, of course, important targets where changes in temperature act to modify behavior. Most studies of temperature effects on chemical synaptic

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transmission have focused on synapses where action potentials normally trigger the release of transmitter. Many synapses, however, release transmitter as a continuously graded function of presynaptic membrane potential. In invertebrates, for example, graded chemical synaptic transmission is used for transmission of sensory information (reviewed by Bush, 1981; Shaw, 1981; see also Wilkens, 1988), in the production of motor patterns (reviewed by Simmers, 1981; Wilson and Phillips, 1983; Siegler, 1985; Hartline *et al.* 1988; see also Spencer, 1988; DiCaprio, 1989; Toga *et al.* 1990) and for neuromuscular transmission (Davis and Stretton, 1989). The effects of changing temperature on these graded synapses have not been studied.

We have examined the temperature sensitivity of graded chemical synaptic transmission in a neuronal circuit that appears to depend on graded interactions for its normal function. We chose the pyloric motor circuit of the stomatogastric ganglion (STG) which generates rhythmic foregut activity in decapod crustaceans (Claiborne and Ayers, 1987). In the spiny lobster *Panulirus interruptus*, this circuit is a well-defined central pattern generator (CPG) network composed of 14 neurons in six major classes whose synaptic interconnectivity is known in detail (Mulloney, 1987; see Fig. 1). All 14 neurons use action potentials to send signals to distant targets (muscles or neurons in other ganglia) but, within the STG, these spiking neurons depend primarily on graded transmission to generate motor patterns (Raper, 1979; Anderson and Barker, 1981; Graubard *et al.* 1983; Russell and Graubard, 1987; Hartline *et al.* 1988). We report here that the strength of graded chemical synaptic transmission between pyloric circuit neurons is temperature-sensitive over a range that the animal normally encounters in nature. In addition, we provide evidence that temperature effects occur directly at the synaptic junction and cannot be explained by a generalized input resistance change in the pre- and/or postsynaptic cell.

Materials and methods

Pacific spiny lobsters (*Panulirus interruptus* Randall) were purchased from Marinus Inc. (Long Beach, CA) and maintained in marine aquaria at 15°C. The stomatogastric nervous system (Selverston *et al.* 1976) was dissected and placed in a preparation dish filled with *Panulirus* saline of the following composition (in $mmol\,l^{-1}$): NaCl, 479; KCl, 12.8; $CaCl_2$, 13.7; Na_2SO_4, 3.9; $MgSO_4$, 10.0; glucose, 2.0; Tris base, 11.1; maleic acid, 5.1; pH 7.35 (Mulloney and Selverston, 1974). The STG was desheathed, enclosed in a small (1 ml) pool of saline surrounded by Vaseline and constantly perfused at $5\,ml\,min^{-1}$ with oxygenated saline. The saline temperature was controlled with a peltier device and monitored by a small thermistor positioned within a few millimeters of the STG.

Standard intracellular techniques were used for current injection and voltage recordings using KCl-filled ($3\,mol\,l^{-1}$, 10–20 MΩ) microelectrodes. The cell bodies of the pyloric neurons (anterior burster, AB; inferior cardiac, IC; lateral pyloric, LP; pyloric dilator, PD; pyloric, PY; ventral dilator, VD) were identified

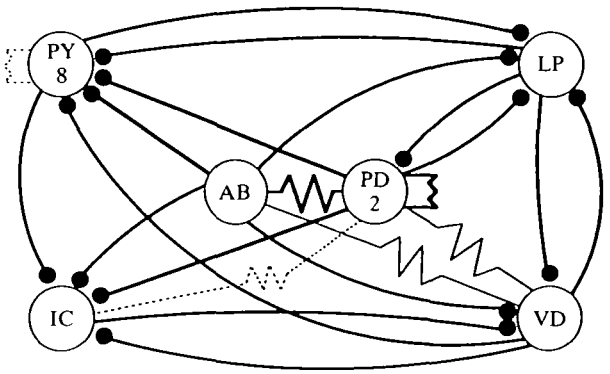

Fig. 1. Summary of the synaptic connections in the pyloric central pattern generator of *Panulirus interruptus* (modified from Mulloney, 1987, and Johnson and Harris-Warrick, 1990). There are eight PY and two PD neurons and one of each other cell type. Resistor symbols indicate electrotonic connections between and among cell types; thick-lined symbols indicate strong connections, thin-lined symbols indicate weaker connections and dashed symbols indicate weak connections. Filled circles indicate inhibitory chemical synapses. PY, pyloric; AB, anterior burster; IC, inferior cardiac; LP, lateral pyloric; VD, ventral dilator.

during rhythmic pyloric activity at 16.5°C by: (1) matching action potentials recorded extracellularly from an appropriate motor nerve root and intracellularly from the soma; (2) the timing of spike activity within the pyloric rhythm; (3) the characteristic shape of membrane potential oscillations and action potential amplitudes; and (4) the synaptic connectivity (Fig. 1). The PY cell population in these experiments was a mixture of early- and late-firing PYs (Hartline *et al.* 1987).

Following cell identification, we replaced the saline in a second Vaseline-walled pool surrounding the input nerve to the STG with 10^{-7} mol l^{-1} tetrodotoxin (TTX). This procedure eliminated all descending modulatory inputs to the STG and thus stopped rhythmic pyloric activity (Russell, 1979; Nagy and Miller, 1987). Possible ascending modulatory inputs from muscle stretch receptors (Katz *et al.* 1989) were eliminated in the initial dissection by removing the appropriate nerves. In some experiments, we isolated pairs of neurons from the rest of the pyloric circuit to ensure that changes in synaptic efficacy were not occurring indirectly (Johnson and Harris-Warrick, 1990). This isolation involved 6-carboxyfluorescein photoinactivation (Miller and Selverston, 1979; Flamm and Harris-Warrick, 1986) and pharmacological blockade (Bidaut, 1980; Eisen and Marder, 1982; Marder and Eisen, 1984).

All experiments were conducted over a temperature range of 20.4–11.3°C, which approximates the natural temperature range (21–12°C) for these animals (G. Martin, Marinus, Inc., unpublished observations). Cooling and heating rates ranged from 0.5 to 1.0° min^{-1} and measurements were taken after at least 5 min equilibration time at each temperature. Each series of measurements was made at three temperatures: 20.4°C (which we will refer to as the high temperature),

16.5°C (mid temperature) and 11.3°C (low temperature). In most experiments, initial measurements were made at the high temperature; some experiments were begun at either the middle or low temperature and showed no qualitative difference in the results. A final measurement was always repeated at the initial test temperature to ensure reversibility. Electrodes were removed from the cell before each temperature change and the cell was re-penetrated after a new temperature had been reached. This procedure was necessary because there was sufficient movement of the STG during temperature changes to dislodge the electrodes and/or damage the cell.

Measurements of graded synaptic strength

The effect of cooling on graded chemical transmission between pyloric circuit neurons was examined after the STG had been superfused with 10^{-7} mol l^{-1} TTX in saline to block spiking synaptic transmission. Graded synaptic strength between a pyloric cell pair at different temperatures was determined from input–output (I/O) curves measured with two presynaptic electrodes (for current injection and voltage recording) and one postsynaptic electrode to record the graded synaptic potential (GSP, Johnson and Harris-Warrick, 1990). I/O curves were constructed from 1 s presynaptic peak polarizations of varying amplitude and sign, plotted against the peak amplitude of the postsynaptic polarization. The stimulation rate was 0.2 Hz; there was no obvious decrement in postsynaptic responses with square-wave presynaptic depolarizations at this frequency. Peak GSP amplitudes were compared at the same levels of presynaptic membrane potential at each synapse (ranging from −35 to −25 mV at different synapses). These levels varied among synapses because the peak GSP amplitude sometimes declined with very large presynaptic depolarizations (not shown in the I/O curves of Fig. 4); amplitude comparisons were made before this decline in GSP amplitude. We do not understand the cause of this decline in GSP amplitude, but weak electrical coupling, which is generally present between pyloric cells (Mulloney, 1987), may contribute (Johnson and Harris-Warrick, 1990). The slope of the I/O curve was obtained from a simple regression line through the data points with measurable GSPs, excluding data points after the peak GSP amplitude had been reached. The threshold for a detectable postsynaptic response was calculated as the x-intercept point of this regression line. Because the sites of the synaptic contacts are electrically distant from our recording site in the cell body, our I/O curve slope and threshold measurements are estimates only; however, they serve here as useful points of comparison among the different temperature conditions.

The input resistance of the cell body was measured with two electrodes, one to pass current and one to record the resulting voltage changes. The input resistance was taken as the slope of the I/V relationship over the linear part of the I/V curve (i.e. hyperpolarized from rest). Antidromic action potentials were elicited by stimulation of the respective peripheral motor nerves with 0.5 ms suprathreshold stimuli at 0.33 Hz. Action potential amplitudes were measured from the resting potential to the peak depolarization. I/O curves for electrical coupling between

pairs of pyloric cells were gathered using the same stimulation and recording protocol as that used for graded chemical transmission. Picrotoxin (PTX) (5×10^{-6} mol l^{-1}) was added to the TTX saline for measurements of PY cell coupling. This appeared to increase PY cell input resistance slightly above that of other pyloric cells (see Fig. 6) and served to make their weak electrical coupling more apparent. Coupling coefficients were determined from the slopes of these I/O curves. To ensure that weak capacitative coupling between the electrodes was not mistaken for weak electrical transmission, we compared weak cellular electrotonic responses with electrode responses after the postsynaptic electrode had been withdrawn to the bath. Statistical comparisions were made with repeated-measures analysis of variance (ANOVA) and subsequent protected *t*-tests.

Results

Effects of temperature on graded chemical synaptic strength

A 1 s depolarizing step delivered to a presynaptic pyloric neuron in saline containing TTX at 20.4 °C elicited a graded synaptic potential (GSP) in its postsynaptic cell. This was characterized by an initial peak hyperpolarization which decayed to a maintained plateau (Fig. 2; see also Graubard *et al.* 1983; Johnson and Harris-Warrick, 1990). The amplitudes of both the peak and the plateau hyperpolarizations and the latency to the peak depended on the amplitude

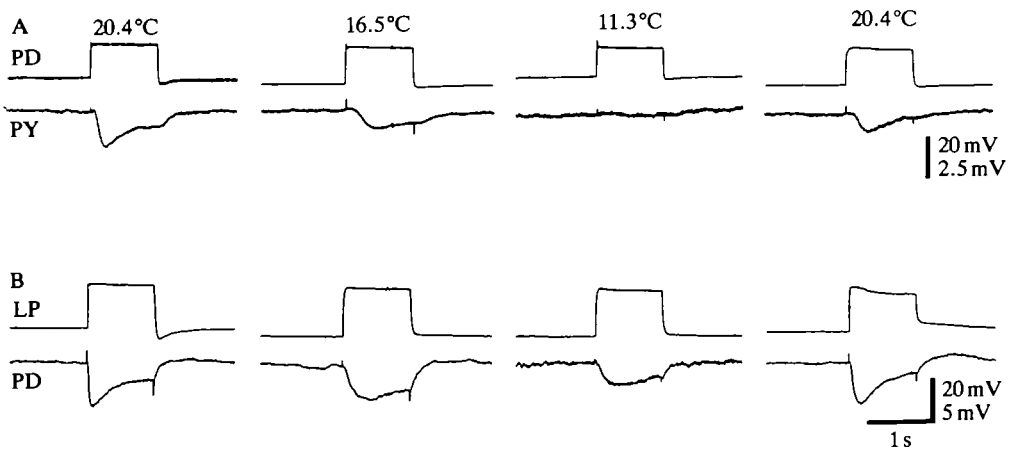

Fig. 2. Cooling-induced reduction of graded synaptic transmission from PD to PY (A) and LP to PD cells (B). Pairs of traces show a 1 s presynaptic depolarization and the postsynaptic response at high (20.4 °C), mid (16.5 °C) and low (11.3 °C) temperature and upon rewarming to the high temperature. In each series of traces, the presynaptic cell was depolarized to the same absolute membrane potential. Resting potentials (mV) at the high temperature were (A) PD −50, PY −51. PY resting potentials did not vary more than 2 mV through the test series. (B) LP −54, PD −50. PD resting potentials did not vary more than 5 mV through the test series.

of the presynaptic depolarization (Graubard *et al.* 1983). With large presynaptic depolarizations (approximately 30 mV from rest), the GSP reached a maximum amplitude; larger presynaptic depolarization often elicited smaller GSPs but could still shorten the GSP rise time. In our experiments, we did not detect transmitter release at rest, as observed in earlier studies (Graubard *et al.* 1983; Johnson and Harris-Warrick, 1990), probably because of the relatively hyperpolarized resting potentials of the presynaptic cells (see Table 2).

Cooling from 20.4 °C (high temperature) to 11.3 °C (low temperature) caused a progressive reversible reduction in the peak GSP amplitude at all the synapses we examined in the pyloric circuit. The different synaptic pairs did not, however, all exhibit the same temperature sensitivity. For example, Fig. 2A shows that, in response to an identical PD depolarization, the peak amplitude of the GSP in a PY cell was reduced to 52 % of the high-temperature value at 16.5 °C (the mid temperature) and was abolished at the low temperature. A second synapse, from LP to PD, (Fig. 2B) was less affected: cooling from high to mid temperature reduced the GSP amplitude to 73 % and this was further reduced to 45 % at the low temperature. In both examples, the reduction in graded synaptic strength was at least partially reversed upon re-warming to the high temperature.

Fig. 3 shows the mean amplitudes of peak GSPs at three temperatures for six different synaptic pairs from the pyloric circuit. Calculated over all pairs, there was a statistically significant effect of temperature on the GSP amplitude ($F_{2,24}$=28.6, P<0.01). However, cell pairs with different presynaptic members showed different sensitivities to progressive cooling. Cell pairs with PD as the presynaptic cell (Fig. 3A) were most sensitive to reduced temperature, with statistically significant decreases in GSP amplitude between high and mid temperatures as well as between mid and low temperatures. Pairs with presynaptic LP cells (Fig. 3B) were less sensitive, with consistent but non-significant re-

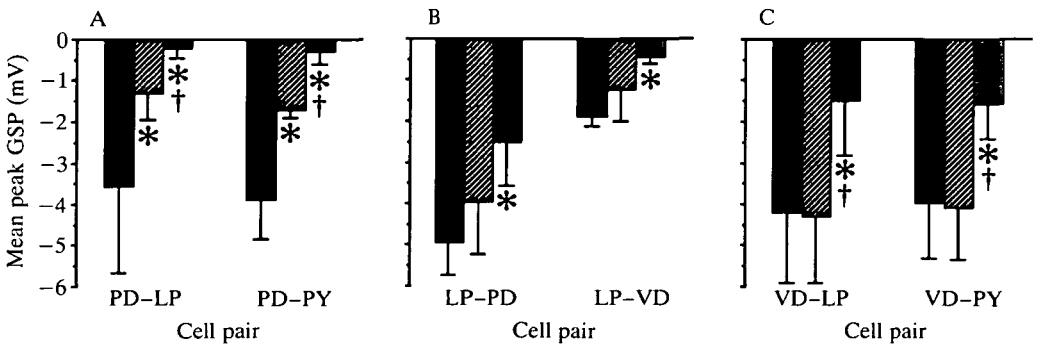

Fig. 3. Cooling-induced reduction of graded synaptic transmission at six different pyloric synapses. For each pair, the mean peak graded synaptic potential (GSP) (±S.E.M., N=3) is plotted at 20.4 °C (filled bars), 16.5 °C (cross-hatched bars), and 11.3 °C (stippled bars). (A) PD synapses. (B) LP synapses. (C) VD synapses. * denotes a measurement significantly different from the value at 20.4 °C (P<0.05). † denotes a measurement significantly different from the value at 16.5 °C (P<0.05).

ductions in GSP amplitude at the mid temperature and significant reductions at the low temperature. Pairs with presynaptic VD cells (Fig. 3C) showed no reduction in GSP amplitude at the mid temperature, but a significant decrease at the low temperature. These differences between presynaptic cells suggest that lowered temperature selectively affects presynaptic function (see Discussion).

Examples of I/O curves from pyloric synapses also demonstrated the effects of cooling on GSP amplitude described above (Fig. 4). In addition, at some synapses, reductions in the slope of the I/O curve appeared to accompany the reduction in GSP amplitude. For example, the I/O curve in Fig. 4 for a PD to PY synapse suggests that a graded decrease in slope occurs as the temperature falls from high, through mid, to low values. Calculated over all synapses, there was a significant effect of cooling on the slope of the I/O curves ($F_{2,24}=13.1$, $P<0.01$, Table 1). The pairs with PD as the presynaptic cell showed significant reductions in the slope of the I/O curve from both high to mid and high to low temperatures. With LP presynaptic to the PD cells, there was a significant mean reduction in the slope of I/O curve from high to low temperature. Slopes of I/O curves at the other synapses were not significantly affected by cooling. There was no significant effect of temperature on the release threshold (Table 1).

Cooling also reversibly slowed the time course of GSPs, and again this effect varied between the different synapses. Cooling-induced increases in the duration of the GSP are seen in both examples of Fig. 2. However, since GSP onset and waveform parameters vary with GSP and presynaptic amplitude, comparisons can only be made with GSPs of similar amplitude and before an amplitude decline is seen on the I/O curve. These conditions are met in the two examples of Fig. 5A,B. At a VD to PY synapse (Fig. 5A) cooling from high to mid temperature delayed the onset of the GSP, slowed the decay to a plateau value and slowed the return to

Table 1. *Effects of temperature on input/output variables of pyloric synapses*

Temperature (°C)		Cell pair					
	Variable	PD–LP	PD–PY	LP–PD	LP–VD	VD–LP	VD–PY
20.4	Slope	0.23±0.01	0.27±0.02	0.33±0.07	0.11±0.06	0.21±0.08	0.28±0.08
	Threshold	−44±2.0	−48±2.0	−48±0.4	−47±0.3	−47±2.8	−47±4.2
16.5	Slope	0.07±0.03*	0.12±0.02*	0.32±0.13	0.09±0.05	0.24±0.09	0.28±0.09
	Threshold	−43±3.9	−49±2.4	−47±3.4	−48±0.5 (−49, −48)	−52±1.5	−48±1.2
11.3	Slope	0.04±0.02*	0.07±0.01*	0.20±0.07*	0.05±0.02	0.15±0.05	0.12±0.06
	Threshold	−38±2.0 (−36, −40)	−44±5.2	−46±1.6	−49±2.3	−45±9.5	−56±1.5 (−54, −57)

Slope and threshold values are expressed as the mean±S.E.M.

$N=3$ for all means except for the thresholds at 16.5°C for LP–VD and at 11.3°C for PD–LP and VD–PY where $N=2$ because cooling abolished the graded synaptic potential in the third experiment. Where $N=2$, both values are given in parentheses.

* These means are significantly different from the mean at the high temperature ($t>2.1$, $P<0.05$).

PD, pyloric dilator; LP, lateral pyloric; PY, pyloric; VD, ventral dilator.

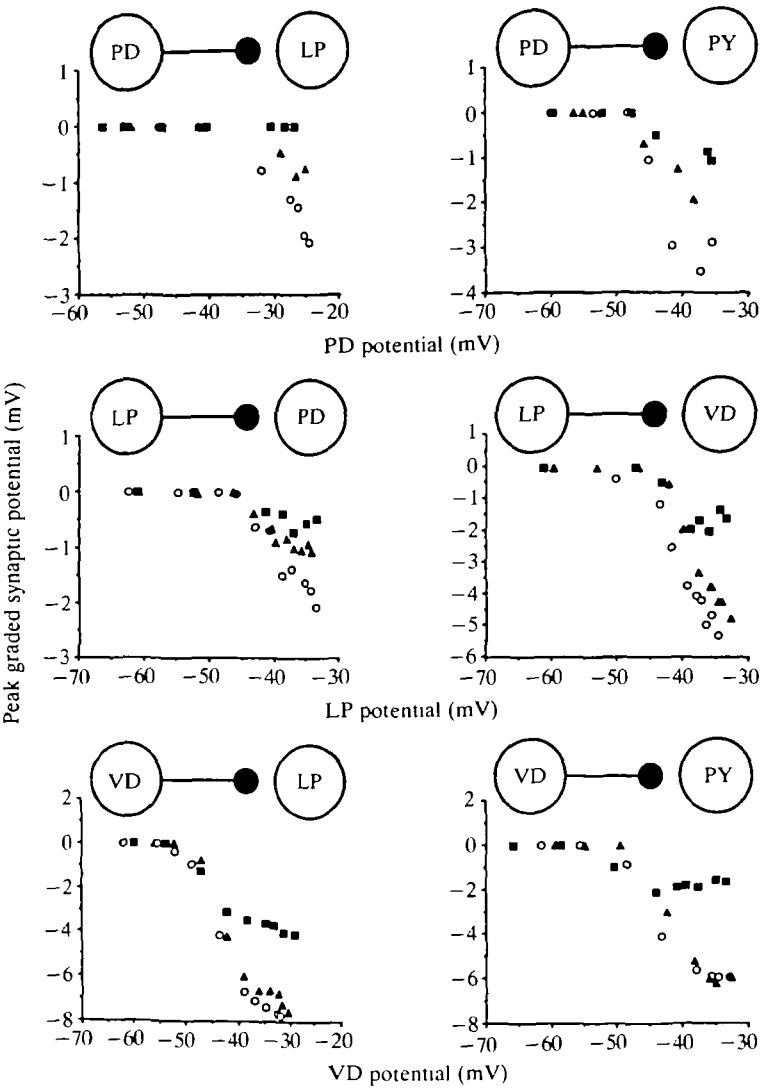

Fig. 4. Effects of temperature on input–output curves (peak graded synaptic potential, GSP, amplitude in postsynaptic cell plotted against presynaptic membrane potential) for graded chemical transmission at six different pyloric synapses. Hyperpolarizations from the presynaptic resting potential are not shown because there was no resting transmitter release at any of these synapses. Open circles indicate measurements at 20.4°C, filled triangles are measurements at 16.5°C and filled squares are measurements at 11.3°C. The AB cell was killed for the PD to PY experiment but not for the PD to LP experiment. However, in other PD to LP experiments where the AB cell was killed, similar reductions in the GSP were seen with cooling. The AB and PD cells were killed for the VD to LP and VD to PY experiments.

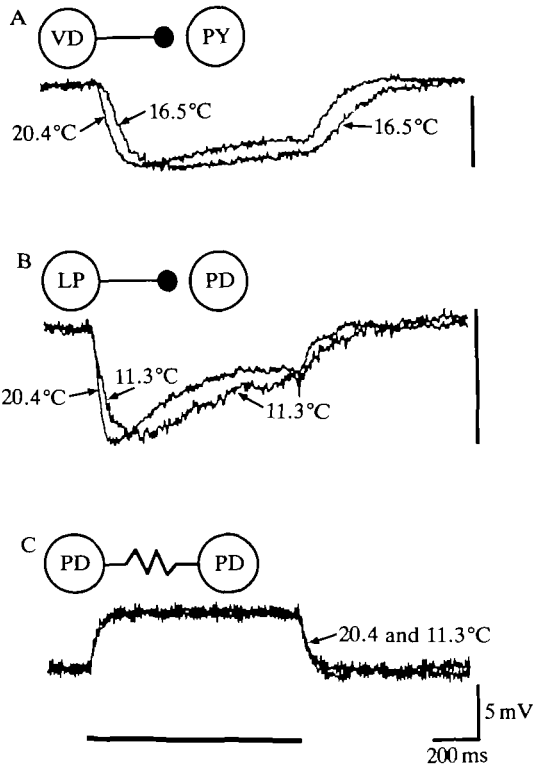

Fig. 5. Effects of cooling on the time course of graded synaptic potentials (GSPs) and electrotonic potentials. Digitized postsynaptic responses to presynaptic stimulation at different temperatures are overlaid. Presynaptic stimulation time for all traces is indicated by the bottom bar. (A) VD–PY GSP at 20.4 and 16.5°C. The AB and PD cells were killed for this experiment. (B) LP–PD GSP at 20.4 and 11.3°C. (C) PD–PD electrotonic postsynaptic potential at 20.4 and 11.3°C.

the baseline after the end of the presynaptic stimulation. At an LP to PD synapse (Fig. 5B), with a faster rise time to peak, cooling from high to low temperature did not change the GSP onset but did increase the time to peak and the decay to plateau. These changes in GSP time course are probably not the result of general changes in membrane properties, since electrotonic potentials between electrically coupled PY or PD cells at high and low temperatures did not show different time courses (Fig. 5C).

Effects of temperature on pyloric cell input resistance

Cell body input resistance

Cooling-induced reductions in GSP amplitude could arise from direct effects at the synapse itself, or through a general reduction in cell input resistance. Since the synapses are located in the neuropil of the STG (King, 1976), a temperature-induced change in the passive spread of current would affect both neuronal

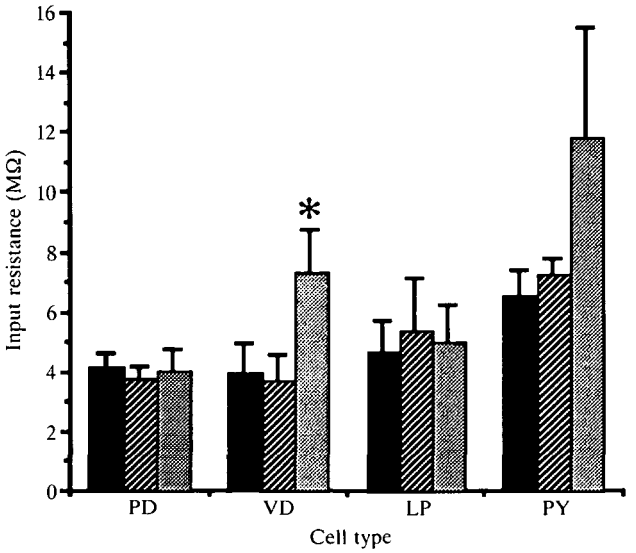

Fig. 6. Effects of temperature on pyloric cell input resistance. The mean (±S.E.M.) input resistance is shown at 20.4°C (filled bars), 16.5°C (cross-hatched bars) and 11.3°C (stippled bars) for 10 PD cells, five VD and LP cells and three PY cells. * denotes a value significantly different from those at 20.4 and 16.5°C ($P<0.05$).

input–output properties and our soma measurements of the amplitude of the pre- and postsynaptic responses. We used the soma input resistance as our first test of a generalized decrease in input resistance caused by cooling. However, there were no significant differences in input resistance with cooling from high to low temperature for the PD, LP and PY cells, and there was, in fact, a significant increase in VD input resistance upon cooling from mid to low temperature (Fig. 6).

Antidromic action potential amplitude

Another measure of input resistance is the action potential amplitude. In STG neurons, the soma is electrically inexcitable, and action potentials propagate passively from a distal point (near the spike-initiation zone) to the soma. Changes in input resistance in this passive pathway would thus alter the action potential amplitude recorded in the soma. Temperature had a significant effect on action potential amplitude ($F_{2,20}=321$, $P<0.01$): cooling significantly and reversibly increased the antidromic action potential amplitude in all the pyloric motor neurons (Fig. 7). The increase in action potential amplitude was accompanied by an increase in action potential duration and a slower conduction velocity (see lower right of Fig. 7). The amplitude of the spontaneously generated action potential also increased with cooling (see Fig. 9).

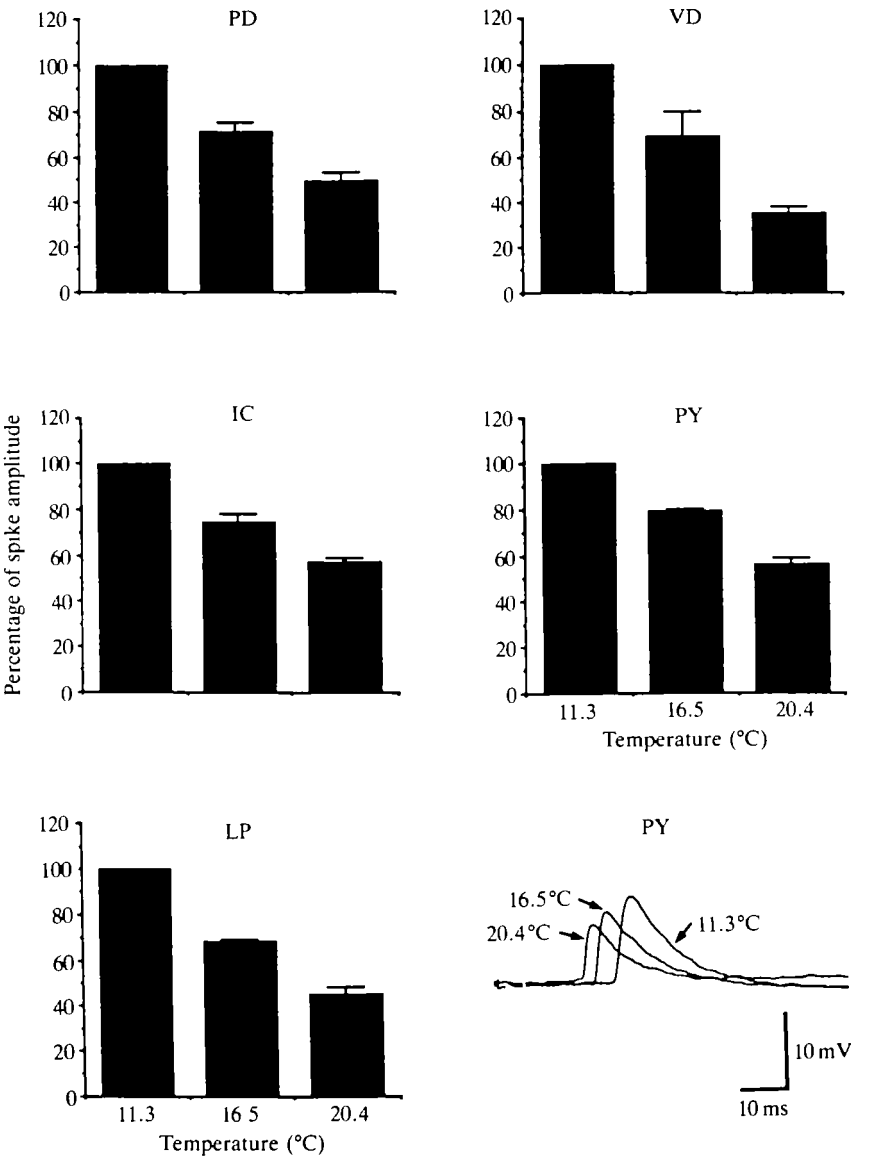

Fig. 7. Effect of temperature on antidromic action potential amplitude recorded from the soma of pyloric motor neurons. Action potential amplitudes at 16.5 and 20°C are compared as the mean (±S.E.M.) percentage of action potential amplitude at 11.3°C. Mean (±S.E.M., N=3) 100% action potential amplitudes (mV) at 11.3°C: PD, 15.5±2.02; VD, 9.2±3.12; IC, 5.1±1.3; PY, 11.2±1.13; and LP, 9.1±5.3. Traces at lower right show antidromic action potentials in a PY cell at the test temperatures. Peripheral nerve stimulation occurs at the beginning of each trace. Action potential amplitudes at all temperatures are significantly different from each other (P<0.01).

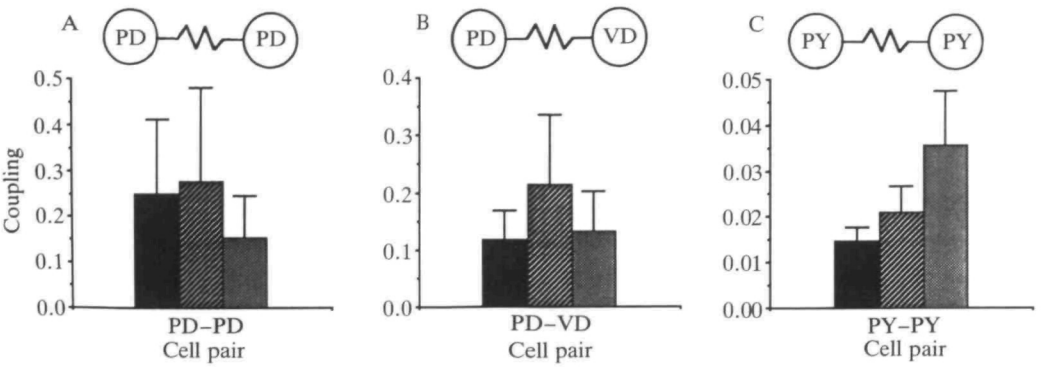

Fig. 8. Effects of temperature on electrical coupling between pyloric motor neurons. Mean (±S.E.M.) coupling at 20.4°C (filled bars), 16.5°C (cross-hatched bars) and 11.3°C (stippled bars) was determined from the slopes of the input–output curves for three PD–PD (A), PD–VD (B) and PY–PY (C) cell pairs.

Electrical coupling

Input resistance measurements from the soma and measurements of antidromic action potential amplitude may not accurately measure input resistance changes limited to the neuropil, where the synaptic contacts are located. For this reason we also examined the effects of altered temperature on electrical coupling between pyloric cells. Cooling-induced reductions in general input resistance in the neuropil would affect both chemical and electrical transmission to like cells in the same manner. Although there was a lot of variability in our measurements (probably caused by the necessity for removal and reimpalement by multiple microelectrodes after each temperature change), there were no significant effects of cooling on electrical coupling between PD cells (Fig. 8A), PD and VD cells (Fig. 8B) or PY cells (Fig. 8C). There was, however, a tendency for increased coupling in PY pairs as the temperature was lowered.

Effects of temperature on resting potentials of pyloric cells

Our measurements of the resting potential were complicated by the necessity to remove the electrodes before each temperature change. However, there were no significant changes in resting potential in any of the cells at the mid or low temperatures when compared to the high temperature (Table 2).

Table 2. *Effects of temperature on resting potentials of pyloric cells*

Temperature (°C)	PD (N=20)	LP (N=12)	VD (N=12)	PY (N=14)	IC (N=3)
20.4	55.1±1.09	57.8±1.45	55.7±2.47	59.2±2.47	63.0±6.66
16.5	55.4±1.31	59.3±1.67	53.8±1.65	57.1±2.53	59.3±4.70
11.3	53.4±1.35	55.2±1.50	55.1±1.85	57.5±2.19	57.7±4.33

Values are mean±S.E.M.
PD, pyloric dilator; LP, lateral pyloric; VD, ventral dilator; PY, pyloric; IC, inferior cardiac.

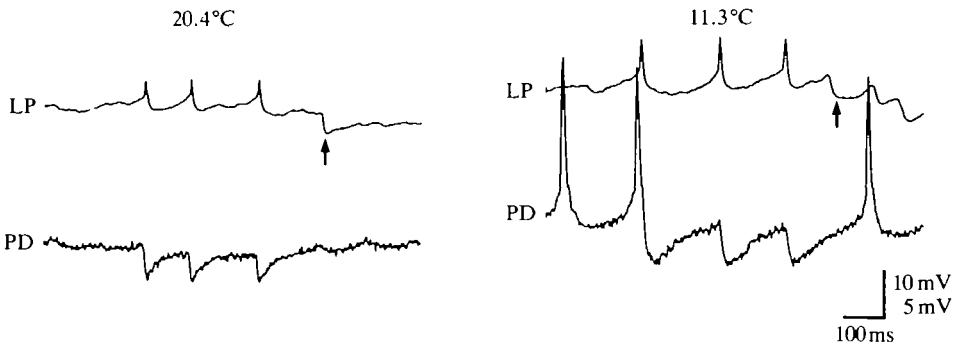

Fig. 9. Effect of temperature on action-potential-evoked IPSPs from LP to PD. Each pair of traces at 20.4 and 11.3 °C shows action potentials in the LP cell and the resulting IPSPs in the PD cell. Top vertical calibration applies to the LP cell and bottom vertical calibration applies to the PD cell. Arrows show IPSPs at the end of the LP burst at both temperatures (from the VD cell). The AB cell was killed for this experiment. Resting potentials at 20.4 °C for the LP and PD cells were −54 and −57 mV, respectively, and at 11.3 °C, −55 and −60 mV, respectively.

Effects of temperature on action-potential-evoked transmission within the stomatogastic ganglion

Spontaneous pyloric cell activity (in the absence of TTX) allowed us to make preliminary observations on the effects of cooling on action-potential-evoked inhibitory postsynaptic potentials (IPSPs). These observations suggest that, unlike the GSP, the amplitude of the action-potential-evoked IPSP is not significantly changed by cooling. Fig. 9 shows the effect of cooling on LP-evoked IPSPs in a PD cell. At the high temperature, a 6 mV action potential in the LP elicited a 3 mV IPSP in the PD; at low temperature, the larger (see above) 12 mV LP action potential elicited a 4 mV IPSP in the PD. In four PD cells, the mean LP-evoked IPSP amplitude (±S.E.M.) was 1.6±0.54 mV at high temperature and 1.9±0.74 mV at low temperature. In addition, the small arrows in Fig. 9 show IPSPs in the LP cell elicited by the VD cell (not shown); these were also maintained with cooling. Average amplitudes of VD-evoked IPSPs in two LP cells were 2.9 mV at high temperature and 3 mV at low temperature. Similar to its effects on the GSP, cooling increased the IPSP duration (Fig. 9). Further studies of spike-evoked transmission are difficult for two reasons. First, the retrograde action potential (evoked by selective stimulation of the axon) does not successfully invade the neuropil and does not evoke transmitter release (Mulloney and Selverston, 1972). Second, orthodromic action potentials evoked by soma depolarization are always accompanied by a GSP (at least at the high temperature).

Discussion

We have shown that graded chemical synaptic strength between neurons of the pyloric circuit of the STG is temperature-sensitive. Cooling from high (20.4 °C) to

low temperature (11.3°C) reduced the GSP amplitude at all pyloric synapses tested. These synapses did, however, display differential sensitivity to cooling from high to mid (16.5°C) temperature: graded synaptic strength was reduced at some synapses but not at others.

Mechanisms by which temperature changes graded synaptic strength

Cooling could reduce graded transmission by a reduction in the synaptic driving force, by a generalized reduction in membrane resistance and/or by directly altering the synaptic transmission process itself. Since the resting potential of the pyloric cells did not significantly change from high to low temperature, reduced synaptic driving force is unlikely to account for graded synaptic strength reductions with cooling.

A cooling-induced reduction in membrane input resistance could reduce graded chemical transmission by reducing the passive spread of current from input sites to output sites in the neuropil (King, 1976). This would change graded synaptic I/O properties by decreasing the peak GSP amplitude and the slope of the I/O curve, and by decreasing our soma measurements of pre- and postsynaptic voltage responses. Three lines of evidence indicate that a generalized decrease in input resistance is not responsible for the reduced GSP amplitude upon cooling. First, as temperature is lowered, both pre- and postsynaptic cells show either an increase or no significant change in soma input resistance. Second, the amplitudes of both antidromic and spontaneous action potentials progressively increase with cooling. This suggests that an input resistance increase occurs somewhere along the pathway that the passively propagated spike follows to the soma. Cooling is known to increase input resistance in a variety of neurons and muscle cells from ectotherms (Prosser and Nelson, 1981; White, 1983; Klein and Prosser, 1985; Adams, 1987), including axons of the spiny lobster (Grossman and Kendig, 1984). An alternative explanation for the increased spike amplitude upon cooling could be that it propagates further towards the soma before failing. Third, there are no significant changes in electrical coupling between like or unlike cells with cooling. In fact, there is a tendency for electrical coupling to increase (PY to PY) rather than to decrease. Using electrical coupling measurements as indicators of input resistance changes is complicated because they reflect a summation of effects on the electrical junctional membranes and on non-junctional membranes. In several species (including crustaceans), electrical junctional conductance is not temperature-sensitive if the cooling is slow (Spray and Bennett, 1985), as it was in these experiments. Thus, junctional conductance changes with cooling probably do not contribute to any coupling changes we observed between pyloric cells. Our results suggest that the input resistance, monitored by measuring soma input resistance, antidromic spike height or electrical coupling, may be increasing at some sites in the cell, but is certainly not decreasing with cooling and therefore cannot account for the reduction in GSP strength.

We conclude that, in the pyloric motor circuit, the strength of the graded synaptic interaction itself is reduced at lower temperatures. We did not directly

address the question of whether the temperature-sensitive steps in transmission are pre- or postsynaptic, but cooling does not appear specifically to affect the postsynaptic response to transmitter in pyloric cells. Graded synaptic strength at PD to LP and PD to PY synapses was reduced at 16.5°C, and sometimes completely abolished at 11.3°C. In contrast, graded synaptic strength at VD to LP and VD to PY synapses changed little at the mid temperature. Only at the low temperature was there a reduction in the GSP at synapses with VD as the presynaptic cell. Since both the PD and VD cells use the same transmitter, acetylcholine (Marder, 1987), one would expect that, if postsynaptic responsiveness was altered by cooling, both VD and PD synapses onto LP and PY cells would be similarly affected. One direct test of this would be to compare neuronal responsiveness to extrinsically applied neurotransmitter. Unfortunately, we could not directly determine the postsynaptic response to applied acetylcholine because muscarinic agonists can also induce bursting potentials in pyloric cells (Nagy *et al.* 1985). Based on the differential sensitivity of the PD and VD synapses to cooling, we suggest that cooling may reduce the graded synaptic strength between pyloric neurons by a presynaptic action. The differential sensitivity to cooling of graded transmission between pyloric cells could be due to a differential sensitivity of calcium entry into the terminals and/or to a differential sensitivity of the release mechanism itself.

Comparison of the effects of temperature on graded and spike-evoked transmission

Similar to our results with graded synaptic transmission in the pyloric circuit, lowered temperature reduces spike-evoked transmission at many synapses in other ectotherms (see reviews by Florey, 1978; Prosser and Nelson, 1981; Stephens, 1985, 1990), including the squid giant synapse (Weight and Erulkar, 1976), motor neuron synapses in insect ganglia (Burrows, 1989), frog (Takeuchi, 1958; Jensen, 1972) and lizard (Adams, 1989), and crustacean neuromuscular junctions (Stephens, 1985). Since the input resistance generally increases with lowered temperature in these preparations, a change in input resistance cannot explain the reduction in synaptic potential amplitude.

As we suggested for the cooling-induced reduction in GSP, decreases in spike-evoked transmission with cooling are also largely explained by presynaptic temperature effects. For example, low temperature reduces quantal content at several neuromuscular junctions (Takeuchi, 1958; White, 1983; Adams, 1989) and decreases spontaneous transmitter release at frog (Fatt and Katz, 1952; Barrett *et al.* 1978) and crab muscle fibers (Parnas *et al.* 1975). Cooling may decrease synaptic potentials by reducing calcium entry into the presynaptic terminal at the squid giant synapse (Charlton and Atwood, 1979; Llinas *et al.* 1987). Like the threshold for transmitter release in our preparation, the threshold depolarization for measuring calcium current at the squid giant synapse is not affected by cooling (Llinas *et al.* 1987). Recently, Delaney and Zucker (1990) found that cooling reduced neurotransmitter release elicited by flash-evoked release of caged calcium

injected into the squid giant synapse. This suggests that, in addition to reducing calcium influx into the squid presynaptic terminal, lowered temperature affects the release processes that follow calcium influx.

In our experiments, cooling increased the latency of the GSP and prolonged its duration, although these effects varied from cell pair to cell pair. These effects of cooling are not due to general changes in the membrane properties of pyloric cells, since cooling did not change the latency and time course of electrical coupling potentials (Fig. 5C). Similar results are found in spike-activated synapses, where the evidence suggests that this arises primarily from an effect of temperature on the release process itself (Katz and Miledi, 1965; Barrett and Stevens, 1972; Llinas *et al.* 1987; Delaney and Zucker, 1990).

Importance of graded synaptic transmission in organizing the pyloric motor pattern

Graded synaptic interactions between neurons of the pyloric circuit are thought to organize the motor patterns produced by this CPG, while the same cells use action potentials to send signals to distant targets (Raper, 1979; Anderson and Barker, 1981; Russell and Graubard, 1987; Hartline *et al.* 1988). The reduction in the graded synaptic strength at temperatures near the lower natural range of 12–21 °C for the spiny lobster implies, however, that the role of graded transmission may vary with temperature. The pyloric motor pattern remains robust at 11.3 °C, although, compared to the rhythm at 20.4 °C, the frequency is slower and firing phase relationships between the pyloric cells are modified (B. R. Johnson, J. H. Peck and R. M. Harris-Warrick, unpublished observations). Perhaps at low temperature, the increase in action potential amplitude and duration maintains spike-evoked synaptic interactions, thus compensating for weaker graded interactions between the pyloric cells. The importance of graded and spike-evoked transmission for pyloric motor pattern generation at different temperatures cannot be determined at present because temperature may affect other neuronal properties that are important for pattern generation. We must also examine the effects of temperature on such properties as endogenous bursting (Johnson *et al.* 1990), plateau generation, firing threshold and post-inhibitory rebound (Getting, 1989) to understand fully the constellation of temperature effects that may produce variable pyloric motor patterns.

We must emphasize that we have only examined the effects of acute temperature changes on graded synaptic transmission. We have not yet studied the compensatory changes that could occur in lobsters maintained for long periods at low temperature. It is clear that many ectotherms, including crustaceans, can acclimate a variety of neural functions to match a wide range of thermal conditions (Langerspetz, 1974; Florey, 1978; Prosser and Nelson, 1981; Stephens, 1985); this includes acclimatory shifts in neuromuscular transmission (Harri and Florey, 1979; Stephens and Atwood, 1982; Blundon, 1989). Seasonal acclimatory changes certainly may occur in graded synaptic transmission such that its functional

significance in organizing the pyloric motor pattern at lowered temperatures would be maintained.

Approximately 99% of animal species are poikilotherms, and must contend with a body temperature at or near environmental levels (Florey, 1978). Despite the importance of temperature for most animals, the neural mechanisms of temperature modification of behavior remain poorly understood. The well-defined pyloric network of the crustacean STG could be a model system to study how temperature acutely affects motor pattern production at different neuronal levels (cellular, synaptic and network) and at what neuronal level(s) compensatory acclimation may occur.

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