THE EFFECTS OF COOLING ON AN IDENTIFIED REFLEX PATHWAY IN THE COCKROACH (PERIPLANETA AMERICANA), IN RELATION TO CHILL-COMA

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SUMMARY

1. The effects of cooling were studied in relation to (a) the impairment of locomotion (i.e. chill-coma) and (b) the functioning of various components of the monosynaptic trochanteral hair plate reflex in the cockroach, Periplaneta americana.

2. The mean temperature for onset of chill-coma was 10.5 °C. At this temperature animals were unable to right themselves and visible tremors of the legs and body occurred.

3. Extracellular recordings from metathoracic nerve 5 indicated that cooling from 24 to 15 °C caused a decrease in the background spiking rate of motor neurone Ds. However, cooling from 12 to 9 °C caused a marked increase in the spiking frequency of both Ds and other unidentified neurones. This increase in spiking activity is the probable basis of leg and body tremors that occur during chill-coma.

4. Monosynaptic coupling between hair plate afferent spikes and Ds spikes (following electrical stimulation of the hair plate) was markedly affected by cooling. At 25 °C, hair plate afferent and Ds spikes were always tightly coupled to one another, whereas at 10 °C coupling was very weak. Several lines of evidence suggest that this loss of coupling was the result of failure of central synaptic transmission. The reduced effectiveness of central synaptic transmission during cooling may be a critical factor in the impairment of locomotor ability associated with chill-coma.

INTRODUCTION

Many functional properties of nerve cells (e.g. membrane potential, excitability, conduction velocity, and synaptic transmission) are markedly altered by short- and long-term exposure of the nervous system to temperature extremes (Lagerspetz & Talo, 1967; Talo & Lagerspetz, 1967; Lagerspetz, 1974; Langley, 1979; Harri & Florey, 1979; Zečević & Levitan, 1980). The behavioural consequences of exposing animals to progressively lower temperature is that their reflexive and locomotor

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behaviours are blocked, often in an hierarchal fashion (Roots & Prosser, 1962; Kivivuori, 1980; Staszak & Mutchmor, 1973a).

The reversible cessation of motor activity at low temperatures in insects is called chill-coma (Mellanby, 1939; Colhoun, 1960). In the American cockroach, *Periplaneta americana*, temperatures between 5.9–9.6 °C are sufficient to cause chill-coma (Mutchmor & Richards, 1961). The precise temperature at which chill-coma occurs depends on the temperature to which the animal has been previously acclimated and the length of exposure to the low temperature (for review, see Mutchmor, 1967). The cockroach passes through a hierarchical series of behavioural events and locomotor failures when exposed to sub-coma temperatures (Colhoun, 1960; Staszak & Mutchmor, 1973a). The first in this series of events is the inability of the cockroach to right itself. The series is complete when the cockroach is completely immobile, as indicated by a ventral extension of the maxillary palps. Chill-coma is accompanied by tremors or spasmodic contractions that occur throughout the body.

The physiological basis of chill-coma has remained obscure, although it seems reasonable to assume that the nervous and/or muscular systems are involved. Attempts to correlate onset of chill-coma with the inability of the nervous system to produce nerve impulses have been unsuccessful (Anderson & Mutchmor, 1968; Staszak & Mutchmor, 1973b). In muscle cells of *P. americana*, no excitatory potentials occur below 5 °C in response to neural stimulation (Wareham, Duncan & Bowler, 1974), but this temperature is several degrees below the temperature at which chill-coma occurs.

In the present study, neuromuscular events accompanying chill-coma in *P. americana* have been investigated by recording muscle potentials and monitoring the properties of a selected reflex pathway, the metathoracic trochanteral hair plate reflex (Wong & Pearson, 1976; Pearson, Wong & Fourtner, 1976). This reflex performs an important function in the co-ordinated locomotory activities of the animal and can be monitored over a long period with minimal dissection (Wong & Pearson, 1976; Fourtner, Drewes & Holzmann, 1978; Carr & Fourtner, 1980).

The trochanteral hair plate of the cockroach consists of 50–60 sensilla which lie close to the intersegmental membrane of the coxotrochanteral joint. Displacement of the hair sensilla by a fold in the intersegmental membrane produces tonic and phasic spikes in the hair plate afferent neurones. This activity is conducted centrally along nerve 5 (nomenclature of Pipa & Cook, 1959) toward the metathoracic ganglion. The hair plate neurones are monosynaptically connected to an identifiable motor neurone (neurone Ds; Pearson & Iles, 1971) via a cholinergic synapse (Carr & Fourtner, 1980). The axon of motor neurone Ds leaves the ganglion via nerve 5 and innervates the coxal depressor muscle (muscle 177D, nomenclature of Carbonell, 1947). Excitation of the hair plate afferents by either mechanical or electrical stimulation evokes spikes in neurone Ds. If all afferents are simultaneously excited (e.g. by repetitive electrical stimulation), Ds spikes can be evoked in a 1:1 manner with each stimulus (Carr & Fourtner, 1980). Such spiking activity is then superimposed upon the normal tonic firing pattern of Ds. In the walking animal, deflection of the hair plate sensilla produces afferent spikes which modulate tonic Ds activity, thereby preventing the animal from overstepping (Wong & Pearson, 1976).
Effects of cooling on reflex pathway

METHODS AND MATERIALS

Adult male American cockroaches, *Periplaneta americana*, were maintained in an incubator at constant temperature (28 ± 0.5 °C) for at least one week prior to study. They were fed Ralston Purina dog chow and water *ad lib*.

Electrophysiological studies

Unanaesthetized cockroaches were pinned ventral side up to a wax block. Two pins were placed in both the pronotum and in a posterior abdominal segment. Meso- and metathoracic legs were immobilized in a slightly elevated position. Nerve 5 was exposed by making an incision in the membranous cuticle connecting the coxa and thorax. Since the nerve lies immediately beneath the cuticle at this point, there was little, if any, damage to surrounding tracheae and tissues. The nerve was then carefully lifted over bipolar recording electrodes (silver wire hooks) and covered with a petroleum jelly–paraffin oil mixture. Electromyogram recordings of the coxal depressor muscle (177D) were obtained with fine copper wires implanted in the region of the muscle (Pearson, 1972). Both sets of recording electrodes were connected to differential inputs of oscilloscope amplifiers.

A Grass SD9 stimulator was used to stimulate the trochanteral hair plate. The cuticle surrounding the hair plate was pierced slightly with a stimulating electrode, consisting of an unpainted minuten pin. This procedure permitted the use of low stimulus intensities (1–5 V, 0.05 ms duration) and gave consistent results. Single pulse stimulation was used for all testing.

For determination of conduction velocity in nerve 5, two pairs of bipolar recording hook electrodes were glued side by side, with a distance of 1 mm between the centres. When using these two electrode pairs it was necessary to expose nerve 5 up to the metathoracic ganglion.

Cooling of preparations was done by pumping water from a refrigerated waterbath through copper coils embedded in the wax block to which the animal was pinned. The preparation was surrounded on bottom and sides by the coils. The top of the block was loosely covered by a Petri dish lid to help minimize temperature gradients. Temperature was measured with a tele-thermometer (Yellow Springs Instrument Model 73) and a temperature probe (Yellow Springs Instrument Model 427) in contact with the ventral surface of the undissected contralateral metathoracic coxa. In all experiments, cooling rates were essentially linear (0.5 °C/min). Experiments always began at room temperature (i.e. 24 ± 1.0 °C).

Chill-coma determination in unrestrained animals

For this study, chill-coma was behaviourally defined as the temperature at which the animal could not right itself when turned ventral side up (with a glass probe), because this has been determined to be the first in a series of behavioural events associated with chill-coma in *P. americana* (Staszak & Mutchmor, 1973a). Animals were placed in a 400 ml beaker which was in a cooling chamber (Cole-Parmer, 2851) surrounded by a water-ethanol mixture.
RESULTS

Behavioural and physiological observations during onset of chill-coma

Behavioural observations of 18 unrestrained cockroaches were made as temperature was gradually lowered. Generally, cooling tended to reduce the co-ordinated locomotor activity of animals until they entered chill-coma. Coma was reached at a mean temperature of 10.5 °C (S.D., 0.2). Chill-coma was generally accompanied by visible tremors, or convulsions, of the body and legs.

To investigate the physiological basis of locomotory impairment during chill-coma, electrophysiological recordings were obtained from the coxal depressor muscle during cooling. These recordings showed that muscle potentials, though reduced in amplitude, were still present in preparations cooled to 8–6 °C (i.e. several degrees below the chill-coma temperature).

Further study of the physiological basis of behavioural events associated with chill-coma was done by analysis of cooling effects on the background activity in motor neurone Ds, which innervates the coxal depressor muscle (Pearson & Iles, 1971), and is known to be tonically active at room temperature (Wong & Pearson, 1976). Spikes from Ds could easily be identified in recordings (from nerve 5) by their tonic activity and their 1:1 relationship with coxal depressor muscle potentials (Pearson & Iles, 1971; Wong & Pearson, 1976). The frequency of tonic spiking activity in Ds was determined by 16 observations (one observation = 1 s duration) from each of seven animals.

The tonic firing rate of Ds was markedly affected by cooling. As temperature was
Effects of cooling on reflex pathway

lowered from room temperature to 15 °C, the rate decreased (Fig. 1). However, as cooling continued to chill-coma temperatures, Ds frequency markedly increased for several minutes, reaching a peak frequency which always exceeded 25 spikes/s (e.g. Fig. 2). In each of 5 animals examined below 15 °C, this peak occurred between 12-9 °C. At temperatures below those which induced chill-coma (i.e. less than 9 °C), the rate of tonic firing returned to a low and steady level (approximately 7 spikes/s).

In addition to the marked increase in Ds firing rate seen during chill-coma induction, other unidentified neural units in nerve 5 began spiking or showed increases in background spiking activity during cooling from 12-9 °C. Such spiking activity was always accompanied by large muscle potentials and tremor-like movements of the legs and body. Thus it appeared that the tremors observed during chill-coma induction were due to increases in the spiking frequency of various motor neurones.

Identification of the components of the trochanteral hair plate reflex

Electrical stimulation of the trochanteral hair plate evoked an afferent compound action potential which was conducted centrally in nerve 5 at a mean velocity of 4.7 m/s (Table 1). This value is within the range (3.5-5.0 m/s) found by Wong & Pearson (1976). At room temperature, the onset latency of this compound potential ranged

Fig. 2. Background spiking activity from one cockroach during cooling to chill-coma temperatures (each point represents a mean of 6 measurements ± S.D.). Note the peak frequency of Ds spiking was reached at 9.5 °C. Note the small deviations that occur at or below 9 °C, indicating very regular firing rate of Ds.
Table 1. Data used in the calculation of ganglionic delay in the metathoracic trochanteral reflex of the cockroach

(Afferent and efferent conduction times represent the time necessary for impulse conduction between the recording site and the metathoracic ganglion (1-5 mm). Ganglionic delay was not calculated at 10 °C because of difficulties encountered when measuring afferent conduction velocity at this temperature. Unless otherwise indicated all means were taken from 5 animals.)

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Time from afferent to Ds spike (ms) [mean ± S.E.M.]</th>
<th>Afferent conduction velocity (m/s) [mean ± S.D.]</th>
<th>Efferent conduction velocity (m/s) [mean ± S.D.]</th>
<th>Afferent conduction time (ms)</th>
<th>Efferent conduction time (ms)</th>
<th>Ganglionic delay (ms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>24 ± 1</td>
<td>2.6 ± 0.1</td>
<td>4.7 ± 0.6</td>
<td>3.1 ± 0.6</td>
<td>0.3</td>
<td>0.5</td>
<td>1.8</td>
</tr>
<tr>
<td>20 ± 1</td>
<td>4.2 ± 0.1</td>
<td>3.5 ± 0.8</td>
<td>2.5 ± 0.6</td>
<td>0.4</td>
<td>0.6</td>
<td>2.2</td>
</tr>
<tr>
<td>15 ± 1</td>
<td>7.6 ± 0.1</td>
<td>2.6 ± 1.5</td>
<td>2.1 ± 0.5</td>
<td>0.6</td>
<td>0.7</td>
<td>6.2</td>
</tr>
<tr>
<td>10 ± 1</td>
<td>9.0 ± 0.1</td>
<td>—</td>
<td>1.9 ± 0.5</td>
<td>—</td>
<td>0.8</td>
<td>—</td>
</tr>
</tbody>
</table>

* Eight animals were used.

from 1.0–1.4 ms (Fig. 3A, lower trace). The compound potential was graded in amplitude depending on stimulus intensity; usually 1–2 V pulses (0.05 ms duration) were supramaximal in strength. The afferent potential was consistently followed after 2.0–3.5 ms by spike in motor neurone Ds. This spike was conducted peripherally at a mean velocity of 3.1 m/s (Table 1). Each motor axon spike was followed in a 1:1 fashion by a muscle potential in the coxal depressor muscle (Fig. 3A, upper trace).

**Effects of cooling on coupling of trochanteral hair plate spike to the Ds spike**

Figure 3A–C shows several qualitative effects of cooling on the components of the hair plate. As temperature was lowered below room temperature, the peak amplitude of the afferent and Ds spike potentials gradually decreased. The gradual decrease in the amplitude of the afferent compound spike did not appear to result from decreases in the excitability of individual fibres, since increases in the intensity of electrical stimulation to the hair plate did not restore the potential. Rather the decrease in amplitude appeared to result from a decrease in conduction velocity and/or a decrease in amplitude of the individual spikes. The decreased velocity of the individual spikes would result in a temporal dissociation of the spikes forming the compound spike. The decreased amplitude of the individual spikes is inferred from the results of cooling on the single neurone Ds (Fig. 3).

Another effect of cooling was a marked increase in the time lag between the afferent volley and the Ds spike. As temperature was lowered to approximately 20 °C, most Ds spikes occurred approximately 3.0–4.5 ms after the afferent potential (Fig. 4B). At approximately 15 °C, Ds spikes usually occurred 4.0–9.5 ms after the afferent volley (Fig. 4C). At approximately 10 °C this range was 7.0–9.0 ms (Fig. 4D). At these low temperatures some spikes occurred after 12 ms (Fig. 4D). These spikes probably were not evoked by stimulation, but were due to the relatively high background firing rate of Ds at 10 °C (Fig. 2).

An additional effect of low temperature on the reflex was that hair plate stimulation
Effects of cooling on reflex pathway

Fig. 3. Temperature effects on the trochanteral reflex arc in the metathoracic leg of P. americana. (A) Room temperature (24 °C). The lower trace shows the extracellular recording from nerve 5 in response to a supramaximal stimulus to the trochanteral hair plate. The nerve response consists of a centrally conducted compound action potential derived from the hair plate afferents (bracket) followed, after 2-4 ms, by a peripherally conducted spike in motor neurone Ds (dot). The upper trace is a simultaneous electromyogram recording from the coxal depressor muscle, 177D, and shows the muscle potential (arrow) which follows the Ds spike. (B) At 19 °C, the potentials increase in duration and the time between the afferent spike and the Ds spike increases to 3-4 ms. (C) At 10 °C, the time between the afferent volley and the Ds spike increases to 10-0 ms. The muscle potential is spread out to the point that it is barely visible. At higher sensitivities and lower sweep speeds, the muscle potentials were always clearly evident, however.

became progressively less effective in evoking Ds spikes. At room temperature, only 3% of the stimuli failed to evoke Ds spikes (Fig. 4A). At 21-19 °C, the percentage of Ds failures increased slightly to 7% (Fig. 4B). As temperature was lowered to 16-14 °C, failures increased to 48% (Fig. 4C). At the lowest test temperatures, 11-9 °C, 73% of the stimuli failed to initiate a spike in Ds (Fig. 4D). The exact percentage of failure at 10 °C may actually be higher than 73% because some spikes included in Fig. 4D probably were not evoked but were spontaneous in origin. Thus, the temperature range within which the reflex consistently failed corresponded closely to the temperature range for onset of chill-coma in unrestrained cockroaches.
Effect of cooling on conduction velocity

The mean conduction velocity of the hair plate afferents was 4.7 m/s at room temperature, and was reduced to 2.6 m/s at 15 °C (Table 1). Accurate measurements of afferent conduction velocity below 15 °C were not possible because of the increased duration and temporal dissociation of the compound action potential. The $Q_{10}$ for the afferent conduction velocity between 24–15 °C was 1.7, a value identical to the $Q_{10}$ reported by Chapman & Pankhurst (1967) for afferent (mechanoreceptive) fibres in the meso- and metathoracic legs of *P. americana*. The mean conduction velocity of the motor axon Ds was 3.1 m/s, but as temperature decreased to 10 °C, the conduction velocity decreased to 1.9 m/s (Table 1). This decrease corresponded to a $Q_{10}$ of 2.0.

Changes in ganglionic delay during cooling

Afferent and efferent conduction times along nerve 5 to and from the metathoracic ganglion were calculated from conduction velocities and distances travelled. Delay within the metathoracic ganglion was then calculated by subtracting these afferent and efferent conduction times from the total time elapsed between the occurrence of the afferent and efferent spikes in extracellular recordings from nerve 5. At room tem-
Effects of cooling on reflex pathway

The ganglionic delay (24 °C) was 1.8 ms (Table 1). This value is close to the ganglionic delay of 1.3 ms found by Wong & Pearson (1976). As temperature was lowered, ganglionic delay increased to 6.2 ms at 15 °C. The \( Q_{10} \) for this change in ganglionic delay is 3.0. This value is much greater than the \( Q_{10} \) for conduction velocity indicating that ganglionic delay is much more sensitive to temperature reductions than conduction velocity.

DISCUSSION

In the present study, the physiological properties of the central and peripheral components of a reflex arc (the metathoracic trochanteral hair plate reflex) were examined during gradual cooling to temperatures which induced chill-coma in unrestrained cockroaches. The results indicated that cooling selectively impaired the functioning of the various components of the reflex. These physiological effects correlated well with the occurrence of behavioural signs which characterized chill-coma induction.

One of the first signs of chill-coma is the loss of co-ordinated locomotor abilities, which occurs at about 10.5 °C. At this temperature, the animal is incapable of performing the co-ordinated leg movements required for walking or righting. Based on electrophysiological observations, it appears that this loss of locomotor ability is not the result of an abolition of neuromuscular function. Action potentials in motor neurones persisted at temperatures several degrees lower than chill-coma temperatures. Although the animal initially loses the ability to produce co-ordinated movements, spontaneous leg movements were evident as low as 5.0 °C. Also, myogram recordings at chill-coma temperatures indicated that excitatory muscle potentials, although reduced in amplitude, were still evident and were still coupled to motor axon spiking. If cooling effects on neuromuscular function are not the cause for the loss of co-ordination associated with chill-coma, then an alternative cause may be cooling effects on central nervous system function, in particular integrative processes required for co-ordinated movement. This idea is supported by the observation that cooling produces two marked effects on the monosynaptic coupling between the hair plate afferent neurones and the motor neurone, Ds. First, there was a marked increase in the delay within the metathoracic ganglion; ganglionic delay increased from 1.8 to 6.2 ms as temperature was lowered from 24 to 15 °C. Ganglionic delay was much more strongly affected by cooling \( Q_{10} = 3.0 \) than was conduction rate \( Q_{10} = 1.7-2.0 \), indicating that increased ganglionic delay must be primarily due to an increased time for synaptic transmission between the hair plate afferent and motor neurone, Ds. Second, there was an uncoupling of the monosynaptic connection between hair plate afferents and the motor neurone, Ds.

Cooling could modify central synaptic transmission in several ways. As temperature is lowered, the amount of transmitter released may be reduced, and the time required for release may be increased. These changes could result in a smaller and longer excitatory postsynaptic potential (EPSP) in the postsynaptic neurone. This effect, in turn, could result in a delay in the onset of the postsynaptic spike, as well as a decreased probability for spike initiation. Presynaptic effects of cooling on synaptic
transmission have been noted previously in studies of neuromuscular transmission (Katz & Miledi, 1965; Llinas, Walton & Sugimori, 1978; Charlton & Atwood, 1979). Cooling might also affect postsynaptic events. For example, a reduction in the time course of the postsynaptic membrane response to excitatory transmitter might contribute to the longer time required for ganglionic transmission as well as the increased probability for transmission failure (Jensen, 1972). In addition, the membrane threshold for the initiation of an action potential may increase with cooling. Increases in spike threshold during cooling have been observed in an identified neurone in the locust, Schistocerca nitens (Heitler, Goodman & Rowell, 1977). Without intracellular studies it is difficult to determine which of the above possibilities is most important in delaying and/or uncoupling central synaptic transmission in the hair plate reflex.

This study, as well as other previous studies, has reported that the loss of coordinated movements during chill-coma induction in the cockroach is accompanied by the occurrence of muscle tremors (Colhoun, 1960; Staszak & Mutchmor, 1973a). The physiological basis of these tremors appeared to be a transient and sporadic increase in the firing rate of motor neurones at temperatures between 12 and 9 °C. In the case of the motor neurone Ds, these increases were several times greater than the background rate at temperatures just above this critical temperature range (Fig. 2). The physiological basis for this increase in motor activity is not yet known, but several possibilities exist. One is that at some critical point, cooling somehow transiently increases the intrinsic excitability of the motor neurones, thus leading to sporadic firing and associated muscle tremors. This possibility seems unlikely, however, in view of the fact that at 10 °C hair plate stimulation was no more effective in evoking Ds spikes than at slightly higher temperatures. Another, and more likely, possibility is that the increase in motor activity is the result of some temperature-sensitive modification of either excitatory or inhibitory synaptic inputs onto various motor neurones. For example, during cooling central inhibitory synapses may be selectively inactivated at slightly higher temperatures than excitatory synapses. This inactivation could lead to an abrupt reduction in tonic inhibitory input to motor neurones. Selective blocking of inhibitory synapses by cooling has been noted in other studies. Examples include the inhibitory inputs onto Purkinje cells in the goldfish (Friedlander, Kotchabhaki & Prosser, 1976) and the inhibitory synapses at the crayfish neuromuscular junction (R. L. White, in preparation).

As a general conclusion, this study suggests that one important effect of cooling in P. americana is a blocking of central synaptic transmission which may contribute to the impaired locomotion seen during chill-coma. In addition, the physiological basis of the tremors that accompany chill-coma induction appears to be an abrupt and marked increase in the firing rate of various motor neurones.

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REFERENCES

Effects of cooling on reflex pathway


