Pyrogen-induced changes in the thermosensitivity of septal and preoptic neurons

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The action of bacterial pyrogens in causing fever has been extensively studied both as a pathologic process (2) and as an adjustment of the thermoregulatory mechanism (11, 19). Studies of the pyrogen-induced responses leading to a rise in central body temperature (16) have shown that fever is produced by a coordinated activation of heat-production and conservation mechanisms, which suggests a central site of action for the pyrogen. This suggestion is supported by the results obtained following direct, microinjection of pyrogens into the brain (5, 13, 17). Maximum sensitivity to the pyrogen and minimum latency of the fever response were seen with injections into the anterior hypothalamus and preoptic area, the region containing thermosensitive neurons that are presumed to be the central thermodetectors (3, 6, 12), activation of which will produce appropriate thermoregulatory responses (7, 10). The experiments reported here were done to test the hypothesis that fever is due to the action of the pyrogen on thermoreponsive neurons of the hypothalamus and preoptic area, and to determine the characteristics of this action.

METHODS

Cats, anesthetized with urethan (0.8-1.4 g/kg, ip), were used. The techniques of single-unit recording and local thermal stimulation have been described fully (6). Briefly, stainless steel microelectrodes, insulated with vinyl lacquer, were used for single-unit recording. Tubular thermodes were placed stereotaxically to bracket the preoptic and septal areas. The temperature of these areas could thus be varied by perfusing the thermode array with warm or cool water. Tissue temperature was monitored by a thermistor probe placed in a contralateral position symmetrical to that of the recording electrode.

The thermal response pattern of a unit was determined by recording the unit's firing rate as brain temperature was slowly varied at a rate of about 0.1 C/min, from about 34 to 42 C. Thermosensitivity, as measured by $Q_{10}$, was calculated from the regression coefficient of log firing rate on temperature.

The pyrogenic agent, Pirumec (Travenol Laboratories) a lipopolysaccharide extracted from pseudomonas cells, was given in doses of 2-3 $\mu$g/kg, iv. The effect of the pyrogen injection on neuronal thermosensitivity was studied by determining the unit's thermal response pattern every 15 min for 2-4 preinjection controls, and continuing thus in the postinjection period until recovery began, or the unit was lost.

In the second series of experiments, the effect of pyrogen on neuronal firing rate at normal core temperature (38 C) was studied. Following determination of the thermal-response pattern, the tissue temperature was clamped at 38 C. Firing rate was recorded continuously at this temperature for a 20- to 40-min control period and for 60-75 min after injection. Following this, the thermal responsiveness of the unit was measured several times at 15-min intervals.

Throughout all experiments, the amplitude and shape of
of the recorded spike were monitored. Spikes which showed marked changes in form were discarded as not being in a steady state during the study. To avoid possible complications due to postinjection refractoriness, only one pyrogen study was performed in any one animal. At the conclusion of the experiment, the location of the unit was marked by electrolytic deposition of iron from the electrode tip and the brain was fixed by perfusion. All localizations were verified histologically.

RESULTS

Twenty units were studied for periods ranging from 45 to 200 min after Piromen injection. A unit was characterized by the slope and shape of its thermal-response curve, barbiturate sensitivity, and localization, as described earlier (6). Two basic thermal-response patterns were found: a smooth, continuous increase in firing rate with heating (Fig. 1); and a response showing a transition from a plateau, an insensitive region, to a markedly thermosensitive zone as preoptic temperature was changed (Figs. 4 and 6). The distributions of cells showing these different responses were also different. Highly thermosensitive (Q_{10} > 2) units of the first type were found only in the preoptic area and ventral septum adjacent to the anterior commissure. This region has been shown to be the site of the central thermodetectors (7, 10). The second type of thermal response was obtained from units with a much wider distribution, including many cells localized to areas outside of the thermosensitive preoptic zone. It was therefore postulated that this second type of neuron was not a primary thermodetector, but rather an interneuron in the regulatory pathways. Units giving this second type of response were very sensitive to barbiturate anesthetics; small doses of barbiturate markedly depressed their firing rates. The presumed thermodetector neurons, on the other hand, were only slightly depressed by barbiturate. The high-barbiturate sensitivity of the presumed interneurons was taken to indicate that their activity was synaptically evoked. The low-anesthetic sensitivity of the thermodetector cells was taken to indicate an inherent or “pacemaker” activity.

Within the interneuron group, two types of responses were seen. Units whose firing rates were high at the warm end of the thermal test range, and whose rates fell to zero as the temperature fell below some threshold value, the warm-sensitive units (Fig. 4), were thought to be interneurons in heat-loss pathways; units whose firing rates rose to some maximum and remained constant there as brain temperature fell, the cool-sensitive units (Fig. 6), were thought to be interneurons in the heat-production and conservation pathways. Normally sensitive, Q_{10}^2, and insensitive, Q_{10}^1, units were also studied.

**High Q_{10} units; the central thermodetectors.** Five neurons of this type were studied. The effect of the pyrogen, in all cases, was to decrease the thermosensitivity of the unit, as shown in Fig. 1. In each graph, log firing rate (impulses/sec) is plotted against temperature (C). The points represent the mean firing rates for intervals of 1C; the lines are the calculated least-squares regressions. Curves A and B are control thermal responses taken at 40 min and 5 min before pyrogen injection, while C–F are determinations made at 15, 30, 60, and 115 min after pyrogen injection. The change in thermosensitivity of this unit, as expressed by calculated Q_{10}, following pyrogen injection is plotted in Fig. 2. Fifteen minutes after injection, the Q_{10}, 8.20, was not significantly different from the mean control value of 9.45 (P > .5). By +30 min, Q_{10} had fallen to 4.56. The minimum thermosensitivity was found at +60 min, when Q_{10} was 3.05; recovery was completed by 100 min postinjection.

The results obtained in all five experiments on units of this type are summarized in Table 1. The 95% confidence intervals were calculated from the standard deviation of the semilog regression coefficient and the value of t for P = .05. Since the regression coefficient is a function of log Q_{10}, the confidence intervals are asymmetrically distributed around the calculated Q_{10}. For the same reason, the confidence intervals for high Q_{10} values are proportionately larger than those for low values. The tabulated postpyrogen data is that obtained 60–75 min after injection, at the time of peak action.

In all cases the unit’s thermosensitivity decreased...
TABLE 1. Effect of pyrogen on high- ($Q_{10} > 2$) and low- ($Q_{10} 1-2$) sensitivity neurons

<table>
<thead>
<tr>
<th>Before Pyrogen</th>
<th>After Pyrogen</th>
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<tbody>
<tr>
<td>$Q_{10}$</td>
<td>95% Confidence Interval</td>
</tr>
<tr>
<td>High-sensitivity units</td>
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</tr>
<tr>
<td>$Q_{10}$</td>
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<td>$Q_{10}$</td>
<td>4.04</td>
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<tr>
<td>$Q_{10}$</td>
<td>11.8</td>
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<tr>
<td>Low-sensitivity units</td>
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</tr>
<tr>
<td>$Q_{10}$</td>
<td>2.12</td>
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<tr>
<td>$Q_{10}$</td>
<td>1.24</td>
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following the pyrogen administration. Note that two of the units became thermally insensitive ($Q_{10} = 1$) at the height of the pyrogen effect. For comparison, the results from two experiments on low-sensitivity neurons are also shown. The thermosensitivity of these units did not change significantly following pyrogen injection (see below).

Two of the high-sensitivity cells studied showed a biphasic response to the pyrogen. A decrease in $Q_{10}$ occurred about 30 min after injection, followed by a return to control levels at +45 min and a second, prolonged depression of sensitivity beginning at +60 min. This temporal pattern is similar to that of the biphasic fever response frequently seen following injection of bacterial pyrogen in unanesthetized animals (1).

As is indicated in Fig. 1, the decrease in thermosensitivity of the unit during pyrogen action was produced by a rotation of the thermal-response curve around the normal core temperature of 38 C, without an overall decrease in activity of the unit. The firing rate at 38 C appeared to be unchangeable while the increase in rate with heating was lessened, as was the decrease caused by cooling. The firing rate of one such unit was recorded continuously from 21 min preinjection to 62 min postinjection, with the brain temperature fixed at 38 C. The results of this experiment are shown in Fig. 3. Plotted on the upper graph is firing rate at 38 C against time. Each point represents the mean firing rate for a 3-min interval. The horizontal bars mark the mean firing rate of the neuron for the 21-min control period and for succeeding 21-min periods following pyrogen injection at zero time (arrow). The vertical bars indicate the standard error of the mean (SE) for each period. A comparison of these means, by analysis of variance, showed no significant difference between them ($P > .25$). Thus, there was no change in the firing rate of the unit at 38 C in the 60-min interval following pyrogen injection. That the pyrogen did act on the cell is shown by the results plotted on the lower graph of Fig. 3, where the mean firing rates at 30 C, as read from the thermal-response curves, are plotted against time. Recovery was complete by 90 min postinjection.

Another unit of this type was studied in a thermal-clamp experiment, with brain temperature fixed at 38 C. The results of this experiment are shown in Fig. 5. Each
point represents the mean firing rate of the unit for a 5-min interval. The bars indicate mean rate and se for the 15-min control period and for succeeding 15-min periods. Following an initial increase, the firing rate decreased below control levels beginning at about 25 min after injection. The increase in rate during the first postinjection period, while statistically significant, was not noted in the other units studied. It is possible, however, that a small change in rate would not be seen during repeated thermal-response tests. This unit was followed for 60 min following injection when the firing rate had fallen to 12.1 ± .40 (mean ± se) from the control level of 15.6 ± 24.

Three cool-sensitive neurons were studied. As shown in Fig. 6, the response of this type of unit to pyrogen was generally the reverse of that described above for the warm-sensitive cells. Following Piromen injection, there was a generalized increase in firing level; both the maximal rate attained with cooling and firing rate at 38 C rose. The response returned to control levels at 75 min after injection. The change in thermosensitivity seen in the warm-sensitive and thermodetector cells was not as marked in this experiment.

Q10 neurons. The responses to pyrogen of five thermally insensitive cells were studied. Two of these experiments were thermal-clamp studies, while in the other three thermosensitivity was tested at regular intervals. Both types of experiment showed that neither the Q10 of the unit nor its firing rate at 38 C was affected by the pyrogen administration.

Three normally thermosensitive (Q10) units were studied. These, too, were unaffected by the pyrogen.

In addition to serving as control experiments, indicating that the observed effects of pyrogen on the thermosensitive cells were not nonspecific actions, these studies were done to test the hypothesis, discussed later, that nonsensitive or normally sensitive neurons act as part of the "set point" mechanism (9) and so should show a change in response following pyrogen administration. This hypothesis is not supported by these results.

**DISCUSSION**

Since the early studies of Liebermeister (14), fever has been viewed as a controlled, upward shift in the operating point of the thermoregulatory system. Implicit in this is the concept that there is no decrease in regulatory capacity during the fever. The results reported here support the view that a shift in set point occurs following pyrogen administration. They also suggest, however, that this shift is accompanied by a decrease in central thermosensitivity.

The set-point shift is clearly indicated by the responses obtained from the warm- and cool-sensitive interneurons. The decreased firing rate of the warm-sensitive cells (Fig. 5), and the increased firing rate of the cool-sensitive ones would tend to drive core temperature up by respectively decreasing heat loss and increasing heat production. The resultant heat retention would continue until the core temperature increased to the point where the firing rates of these units were again at their control levels. A state of thermal balance would thus be re-established until the pyrogen action ceased, and defervescence occurred by a reversal of this sequence.

The observed behavior of the high Q10 units, the presumed central thermodetectors, following pyrogen injection is not, in itself, adequate to explain the shift seen in the interneuron response, since the firing rates of these cells at normal core temperature were unchanged. Thus, no neural drive for shifting the regulatory balance could originate from these neurons. It appears that the observed decrease in Q10 served to allow a rise in core temperature by partially inactivating the central detector system. It has been suggested (9) that the regulatory set temperature is fixed by a neural comparison of the firing rates of units of differing thermosensitivities. As central temperature changes the difference between the firing
rates of these two types of cells would also change, providing an appropriate drive for the effecter mechanisms. It may be that the injected pyrogen, in addition to decreasing the sensitivity of the central detectors, also acted on this second type of unit. While none of \( Q_{10} \) or \( Q_{1/2} \) units studied showed any change in either rate or sensitivity following pyrogen injection, Cabanac et al. (4), working on rabbits, have reported one such cell which did decrease its firing rate following injection of Typhoid vaccine. They did not find any thermoresponsive units which changed sensitivity by a rotation around the neutral temperature, as reported here. All of the thermosensitive cells studied by them changed their firing levels, in a manner similar to that described for the warm- and cool-sensitive interneurons, in this report. Based on the thermal-response curves, and, perhaps, on location in the brain, the units shown in their paper could be classed as interneurons as well. Andersen et al. (1) studying central thermosensitivity in unanesthetized dogs following Piromen injection, found that preoptic heating and cooling would elicit regulatory responses. The pyrexia could be enhanced or antagonized by preoptic cooling or heating, respectively. These experiments only indicate that the preoptic area retained some thermosensitivity, and not whether any change in sensitivity occurred. Based on their data, reported that rabbits made pyrexic with leukocytic challenge to “reset” hypotheses of fever. My own neurophysiological data suggest that the pyrexic animal should become less sensitive, but not insensitive, to central temperature changes.

Rotation of rate vs. temperature curves, as described here for the central thermodetector neurons, also occurs with cold acclimatization in some poikilotherms. Vernberg (18), for example, found that the \( Q_{10} \) of oxygen consumption rate for the salamander, *Plethodon*, decreased with cold acclimatization by a clockwise rotation of the curve. Prosser (15) suggests that such a response may represent a change in the enzymatic pathways which rate limit the particular function studied, since a change in \( Q_{10} \) indicates a change in the activation energy of the rate-limiting chemical process. In the case of neuronal thermosensitivity, it may be that the cell-membrane system which produces a specialized temperature response (either unusually high or unusually low sensitivity) is also the system that is acted upon by the pyrogen.

The acute nature of these experiments precluded the need for sterile technique. It is very likely that products of the tissue damage caused by thermode placements adjacent to the recording sites had some effect on the unit responses; the Piromen-induced changes were thus superimposed on some functional state biased by the earlier introduction of small amounts of possibly pyrogenic material. In all experiments, the basic criterion for a valid response was that it began, with a reasonable latency (20-45 min), following Piromen injection. For this reason also, comparatively large doses of Piromen were used.

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REFERENCES


