Changes in body temperature produced by prostaglandins and pyrogens in the chicken

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PITTMAN, Q. J., W. L. VEALE, A. W. COCKERAM, AND K. E. COOPER. Changes in body temperature produced by prostaglandins and pyrogens in the chicken. Am. J. Physiol. 230(5): 1284-1287. 1976.—Bacterial pyrogen from S. abortus equi (SAE) was injected into the wing veins of chickens. Following injection of 0.05-0.5 μg SAE, body temperatures did not change significantly, whereas 2.0 or 10 μg of pyrogen caused falls in body temperature of 0.56 ± 0.10°C and 1.1 ± 0.21°C (mean ± SE, n = 5). The temperature falls were accompanied by a flushing of the comb and an increase in respiratory rate and were not antagonized by 1.0 g of acetylsalicylic acid (ASA) given orally. The injection of SAE (0.1 μg in 1 μl) into the anterior hypothalamus produced fevers averaging 1.24 ± 0.07°C (n = 9) which were antagonized by oral ASA. Injections of SAE at other brainstem loci produced no temperature changes. Seven chickens were also injected with 0.1 pg PGE, in 1.0 μl into the anterior hypothalamus, and they developed fevers averaging 0.90 ± 0.16°C. The results support the concept that prostaglandins may be involved in fever in chickens but suggest that the action of pyrogen injected intravenously may be different from that following its injection directly into the hypothalamus.

fever; microinjection

in mammals, the administration of bacterial pyrogen (endotoxin), both intravenously and directly into the brain, is followed by activation of heat production and conservation mechanisms and fever results (reviewed 3, 4, 6). There is evidence to suggest that pyrogen fever is mediated by the action of prostaglandins within the tissue of the hypothalamus (8, 9, 20, 27). Although thermoregulation in chickens has been studied (11, 13, 22), the sequence of events following administration of pyrogens to these birds has received little attention. Jordan and Hinshaw (16) observed that chickens were extremely resistant to large doses of endotoxin; however, there is evidence of only minimal change in body temperature following intravenous injection of microgram quantities of endotoxin (19). It is known that prostaglandins are natural constituents of chicken brain (14) and that injection of prostaglandin E1 (PGE1) directly into the tissue of the chicken hypothalamus causes fever (21). This is the same area of the brain into which injections of various catecholamines are followed by temperature changes (18).

We have measured body-temperature responses of chickens following intravenous injection of endotoxin and of both endotoxin and PGE, directly into the tissue of the brain.

methods

Body temperature was measured in 15, unrestrained, adult female, white Leghorn chickens with thermistor probes (Y.S.I. type 401) inserted 8 cm into the cloaca and held in place with adhesive tape wrapped around the tail. The potentials from the thermistor bridges were recorded on a pen recorder (Beckman Instruments). Following a minimum 90-min time period for the establishment of a base-line temperature, bacterial pyrogen, dissolved in a volume of 1.0 ml of pyrogen-free, sterile 0.9% NaCl, was injected into a wing vein. The pyrogen used was obtained from Salmonella abortus equi (SAE) after the method of Westphal and Luderitz (30) (Difco Laboratories, Detroit) and administered in dosages of 0.05-10.0 μg. Following injection of pyrogen, body temperature was recorded for a minimum of 180 min. During this time period the behavior of the bird was observed and the color of the comb noted. The birds received more than one injection, but at least 3 days separated each experiment. SAE pyrogen was also injected intravenously into an additional seven chickens, and 15 min later 1.0 g of acetylsalicylic acid (ASA), Acetophen, Charles E. Frosst & Co., Kirkland, Canada) dissolved in distilled water was administered orally. In control experiments in which only ASA was given, birds developed hypothermia averaging 0.41°C. Cloacal temperatures were recorded for a minimum of 90 min before and 180 min after the injection of pyrogen.

To determine levels of salicylate in the plasma, blood was withdrawn from wing veins of four chickens approximately 45 min following ASA (1.0 g) administration. Plasma salicylate levels were estimated by the fluorometric method of Saltzman (25) on a fluorometer (G. K. Turner Assoc., model 110), with an excitation wavelength of 365 nm and an emission wavelength reading at 455 nm.

In 20 other chickens, unilateral stainless-steel guide cannulas (20 gauge) were implanted under sodium pentobarbital (30 mg/kg) anesthesia supplemented if necessary with ether, so that the tips of the cannulas rested above the hypothalamic or other brainstem regions. The coordinates for cannula placements were chosen from the Atlas of Tienhoven and Juhasz (26). At least 5 days were allowed for recovery from surgery before any
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Chicken was used in an experiment. Body temperature was recorded as previously described. For injection, a 27-gauge injection needle was lowered through the guide cannula to a site 2 mm beyond its tip. Injections were made in a volume of 1.0 μl of pyrogen-free, sterile 0.9% NaCl delivered by an infusion pump (Harvard Apparatus Co.) over a period of 1 min. Each chicken received an injection of 0.1 μg SAE pyrogen and a control injection of the saline vehicle alone given in random order at least 3 days apart. In addition, eight chickens were given SAE into the hypothalamus followed by ASA given orally. Seven chickens, each of which had been implanted with a cannula above the hypothalamus, were also injected with 0.1 μg PGE, in 1.0 μl 0.9% NaCl and body temperatures were recorded. Throughout the entire experimental period, ambient temperature was maintained at 21 ± 1°C. At the conclusion of the experiments, the brains of the chickens were perfused with 10% formal saline and the cannula sites were identified histologically.

RESULTS

Table 1 shows the maximum changes in body temperature during the 180-min interval following intravenous injection of SAE pyrogen. The body temperature observed 15 min prior to the injection was chosen as baseline temperature since the temporary restraint during injection often caused transient temperature increases. Falls in body temperature of more than 0.3°C occurred following injections of 2.0 and 10.0 μg of endotoxin, but following injections of 0.05–0.5 μg of pyrogen body temperature showed only a slight drop from preinjection levels. The mean temperature records of the chickens receiving 2.0 or 10.0 μg pyrogen are shown in Fig. 1. The decreases in body temperature, which began about 30 min after the time of injection, were often accompanied by noticeable flushings of the combs and increases in respiratory rates. The chickens sat quietly and often made few movements until after body temperature began to rise. Approximately 90 min after each injection, body temperature rose and returned to normal about 3 h after the injection. During the increase in temperature, the birds often ruffled their feathers and assumed a huddled position, with their heads retracted into the feathers. The combs became pale in color, but during the defervescence they became bright red. Some chickens produced no temperature changes. Figure 2 shows the response of one chicken after injection of saline (upper) and endotoxin (middle). During the rising phase of the fever, the birds would sit quietly in a huddled position, with their heads retracted into the feathers. The combs became pale in color, but during the defervescence they became bright red. Some chickens were given 0.1 μg SAE directly into the hypothalamus, followed 15 min later by 10.0 μg of pyrogen given intravenously and not followed by ASA. Following this amount of ASA given orally plasma salicylate levels averaged 17.61 ± 1.26 (SE) mg/100 ml (n = 4).

Ten of the twenty chickens that were given 0.1 μg SAE pyrogen directly into the tissue of the brain developed fevers averaging 1.24 ± 0.07°C (SE) after latencies of 45–90 min. Microinjections of the saline vehicle into these birds produced no temperature changes. Figure 2 shows the response of one chicken after injection of saline (upper) and endotoxin (middle). During the rising phase of the fever, the birds would sit quietly in a huddled position, with their heads retracted into the feathers. The combs became pale in color, but during the defervescence they became bright red. Some chickens were given 0.1 μg SAE directly into the hypothalamus, followed 15 min later by 1.0 g of ASA administered orally. On these occasions, body temperatures fell by 0.65 ± 0.37°C, (SE, n = 8) below preinjection temperatures.

Ten chickens that were also injected with pyrogen directly into the brain did not show temperature changes after the injections. Histological examination of the brains of these chickens showed that these injections were made into several different brainstem areas, but not into the hypothalamus. The injection sites of the birds that developed fevers following microinjections of endotoxin were grouped in the area of the anterior hypothalamus. The diagrammatic frontal section in Fig. 2 shows the injection site in the bird from which the illustrated responses were obtained.

Seven of the chickens that responded with fever following microinjections of SAE pyrogen were also injected into the same sites with 0.1 μg PGE, and they

<p>| TABLE 1. Body temperature responses of chickens to intravenous SAE |
|-------------------------|------------------|-------------------|</p>
<table>
<thead>
<tr>
<th>n</th>
<th>SAE Dosage, μg</th>
<th>Δ Body Temp, °C</th>
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<tbody>
<tr>
<td>5</td>
<td>0.05</td>
<td>-0.21 ± 0.08</td>
</tr>
<tr>
<td>5</td>
<td>0.1</td>
<td>-0.03 ± 0.05</td>
</tr>
<tr>
<td>5</td>
<td>0.5</td>
<td>0.08 ± 0.14</td>
</tr>
<tr>
<td>5</td>
<td>2</td>
<td>-0.56 ± 0.10</td>
</tr>
<tr>
<td>5</td>
<td>10</td>
<td>-1.1 ± 0.21</td>
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Maximum changes (means ± SE) in body temperature from preinjection levels during the 180-min interval following intravenous injection of SAE pyrogen into adult chickens.

FIG. 1. Curves of means of body temperatures (°C) of adult chickens after intravenous injection of 2.0 μg (○) or 10.0 μg (●) of Salmonella abortus equi pyrogen (SAE pyrogen) at time indicated by arrows. Vertical bars represent 2 SE.
The presence of endotoxin within the hypothalamus is mediated by prostaglandins (8, 20), but it is unlikely that the action of intravenous pyrogen in chickens involves the intracerebral or peripheral synthesis and release of these compounds. Aspirin, an inhibitor of prostaglandin synthesis (27), given orally to chickens did not significantly affect the SAE-induced hypothermia. Although it is possible that the ASA did not reach the brain in sufficient quantity to inhibit prostaglandin synthesis, the amount given was sufficient to lower pyrogen fever in mammals.

Additional evidence that the hypothermia observed following intravenous SAE is not mediated by prostaglandins is that we, and others (21), have observed fever following microinjection of PGE, directly into the hypothalamus. Thus even though a basic difference between mammals and chickens appears to exist with respect to temperature responses to intravenous pyrogen, the effect of PGE, on body temperature is similar to that seen in mammals (28). Thus our results in the chicken provide further support for the concept that prostaglandins may be involved in fever. The mechanism by which PGE, elevates body temperature in chickens is not known, but it may well play a modulating role on a tryptaminergic heat production or conservation pathway which may exist within the chicken hypothalamus. Thus even though a basic difference between mammals and chickens appears to exist with respect to temperature regulation pathways, the fevers observed following the injection of SAE directly into the hypothalamus suggest that the mode of action of the pyrogen injected in this manner is different from its action following intravenous administration. The long latency (45–90 min) before a response is observed suggests the formation of an intermediate pyrogenic molecule, possibly a prostaglandin. This hypothesis is supported by our observations that ASA, which inhibits prostaglandin synthesis, will abolish fever caused by intrahypothalamic injection of SAE. It is possible that the presence of SAE within the hypothalamus may bear little resemblance to the sequence of events accompanying the presence of endotoxin within the circulation. When bacterial pyrogen is injected into the central nervous system of many mammals, the direction of change of body temperature is similar to that observed following intravenous injection (7, 15, 24, 29). However, rats appear to be very resistant to the pyretic effects of intravenous endotoxin and often become hyperthermic (19), yet they respond to endotoxin given intraventricularly with fever (10). Perhaps, in the chicken, the amounts of pyrogen injected either intrave-
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normally or directly into the brain may not have been within the dose range necessary to elicit the appropriate physiological response. Additional experiments to determine the effects of intravenous bacterial pyrogen in young chickens in which the blood-brain barrier is incompletely developed would provide valuable information in this area.

REFERENCES