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Fever Produced in the Squirrel Monkey by Human Leukocytic Pyrogen (40463)

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Fever results from the action of leukocytic pyrogen (LP) on central regulatory controls of body temperature. LP is released as the result of interaction between bacteria and other exogenous pyrogens with host phagocytic cells. Although the chemical characterization of LP is not complete, it is likely that the pyrogens produced by different species share chemical similarities. Two distinct human LPs have been described (1), one with a molecular weight of 15,000 and the other of about 40,000. Because pure human LP is generally not available in amounts adequate for tests in human subjects, smaller animal models are preferable for most investigations of the action of human LPs. The rabbit, the animal most used for fever research, has been shown to develop fever in response to both human LPs (1, 2). Although this animal model is convenient and reliable for certain experiments, for studies on the action of human LP on CNS temperature controls subhuman primate models may be more appropriate because of their well-differentiated brains. In previous tests in which large doses of human LP were given iv to Rhesus macaques, febrile responses were relatively small and the magnitude of the response was not related to the dose of LP (3). In addition, these large primates are expensive to purchase and maintain and since they are not available for import at this time, another primate model was sought. Because the squirrel monkey, *Saimiri sciureus*, a small New World primate, showed great sensitivity and reliable febrile responses to peripheral and central administration of bacterial endotoxin in previous research (4) we tested the response of this species to determine if it might provide a more useful primate model for experiments on human LP.

Materials and methods. Four adult male squirrel monkeys weighing 0.6–1.0 kg were used. These animals were tuberculin negative and had no signs of infectious disease. The monkeys were kept in individual cages in a neutral thermal environment (22–24°) and tested at the same time (1000 hr) each day in an environmental chamber controlled at 23 ± 0.5°. Each animal was maintained on Wayne monkey diet and water. Before surgery and testing, the animals were trained to sit quietly in restraining chairs especially designed for the species. An array of six intracerebral guide cannulae was implanted over the preoptic/anterior hypothalamic (PO/AH) region in each monkey (5). On test days the animals were placed in the environmental chamber 1–2 hr before injections were made. Experiments were separated by at least 3 days. Oxygen consumption and skin temperatures were measured (5) during tests of the response to central injection of LP. For comparisons with the response of the rabbit to iv human LP, New Zealand rabbits which had been habituated to restraint were also tested.

Human LP. Human blood was obtained from individual volunteers in acid citrate dextrose. Buffy coat concentrates were made by removing the uppermost 50-ml of packed cells after a 1500 g centrifugation for 3 min. Buffy coats were diluted in 0.85% NaCl and the mononuclear layer was separated on a Ficoll-Hypaque gradient (1). The mononuclear cells were washed, and 20 million cells were resuspended in Hank's balanced salt solution, with 100 U of penicillin G, 8 μg of gentamycin and 2 U heparin/ml. AB serum stored at −70° was added to make a final concentration of 10% (v/v). Heat-killed *Staphylococcus albus* was also added in a bacteria:leukocyte ratio of 20:1. Leukocyte suspensions were shaken gently at 37° for 30 min, centrifuged at 250*g* for 10 min, and resuspended in fresh Hank's balanced salt solution without serum or bacteria at a concentration of 5 million leukocytes/ml. After 18 hr at 37° in a stationary incubator, the

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suspensions were centrifuged at 2200*g* for 30 min and stored at 4° in 0.02% sodium azide.

Crude human LP was concentrated and applied to an immunoadsorbent column containing anti-human LP antibody attached to Sepharose 4B (6). The pyrogen was eluted at pH 3.2 in citric acid buffer, neutralized with 0.1 *N* NaOH, concentrated and chromatographed on Sephadex G-50 (fine) (165 × 5.1 cm) in 0.85% phosphate buffered saline, pH 7.4. Only the 15,000 molecular weight LP was isolated and concentrated. Samples were frozen at −20° until ready for use. SDS polyacrylamide gel electrophoresis, pH 8.6, of this material showed a faint, single band when stained with Coomassie Blue. Protein concentration was less than 1 μg/ml using absorption at 280 nm with bovine serum albumin as a standard.

Injections. Non-pyrogenic isotonic saline was added to human LP to make up 0.10 ml volume for injection into the saphenous vein of the squirrel monkey or into a marginal ear vein in the rabbit. For intra-PO/AH administration of LP a 30 gauge injection cannula was passed through the lumen of a guide cannula to the appropriate depth and 1 μl of pyrogen solution was injected over a 30-sec period using a microliter syringe. Rectal temperatures were recorded at 5 min intervals using thermistor probes (10 cm insertion) and an automatic recorder (Datalogger, United Systems Corporation).

Results. Intravenous injections of LP in squirrel monkeys caused rapidly developing monophasic febrile responses in which the magnitude of the increase was related to the dose of LP (Fig. 1, upper). Control iv injections of saline vehicle had little effect on body temperature (average rise = 0.2°). Latency of fever onset (0.2° increase) after pyrogen was 5–10 min in two monkeys and 10–30 min in the other two. The latter animals generally showed a slight transient decrease in temperature which began immediately after LP was injected. Fever duration was 0.5–3.75 hr and was directly related to the magnitude of the LP dose. Acetaminophen (5 mg/kg, iv) given 30 min before LP reduced (Fig. 2), but did not totally abolish the response to LP (100 μl/kg) in tests on three monkeys.

Intravenous injection of LP in rabbits caused fevers with peaks that were generally lower than those seen in the squirrel monkeys

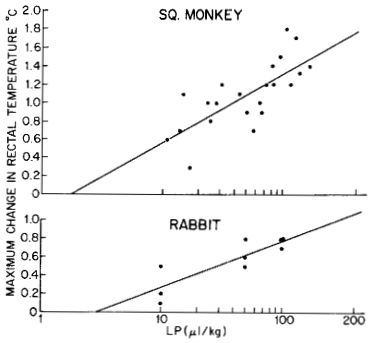

FIG. 1. Intravenous injections of human LP in squirrel monkeys (upper) and rabbits (lower) caused increases in body temperature that were related to the dose of LP. β for squirrel monkey data = 0.745/decade; β for rabbit data = 0.504/decade.

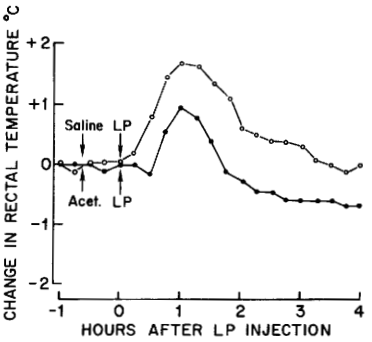

FIG. 2. Effects of pretreatment with acetaminophen (5 mg/kg, iv) on fever produced by human LP (150 μl/kg, iv). Scores are means for three monkeys. Control saline vehicle (0.10 ml) and LP injections were made in the same animals on another day.

(Fig. 1, lower). When fever developed in rabbits, rectal temperature increased within 10–15 min after injection and remained elevated for 0.8–2.0 hr. Control injections of saline vehicle in rabbits did not cause fever.

In 17 experiments single and bilateral injections of human LP into the PO/AH region of the squirrel monkeys caused fevers with peaks of 1.2–2.1° which lasted longer than the 5 hr recording period (Fig. 3). The rapid rise in temperature in the chill phase was preceded by an increase in oxygen consumption, vasoconstriction in the tail, or both. Injections directed at posterior hypothalamic sites did not cause fever.

Discussion. In these experiments squirrel monkeys developed febrile responses to peripheral and central administration of the 15,000 molecular weight LP derived from

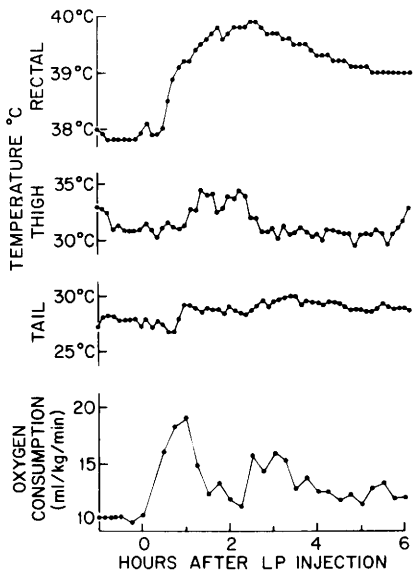

FIG. 3. Effects of bilateral intra-PO/AH injection of human LP (1 μl) on oxygen consumption, skin and rectal temperature in a single monkey.

human monocytes which were similar to those seen in this species after homologous LP (4). Reduction in the febrile response by an antipyretic further indicates that the response was like a normal fever reaction. The results extend the information obtained previously on the febrile response of the squirrel monkey to bacterial endotoxin (4), homologous and cat LP (5) and indicate that this primate is a suitable model for fever research. These monkeys are sensitive to pyrogens that cause fever in man and the results of the present experiments indicate that they are more sensitive to human LP than the rabbit. Unlike some higher subhuman primates (7–11) squirrel monkeys showed reliable dose-related fevers in response to iv pyrogens.

The fevers produced by injecting human LP iv and directly into the brain of the squirrel monkey indicate that central temperature controls of this species are sensitive to heterologous pyrogen. In earlier research on fever produced by inflammatory exudates in rabbits and dogs, LP was considered to be completely species-specific in its pyrogenic activity (12). However, more recent research on reactions of dogs, cats, rabbits, and goats (13), squirrel monkeys (5), and other species, to heterologous LP suggests that there is a common aspect of all LP molecules which is capable of acting on CNS receptor sites to produce fever. The febrile response to heterologous LP is rarely exactly the same in all characteristics to that produced by homologous LP, but the differences may be due to slight differences in the molecular structure of homologous and heterologous LP (13), to differences in access of the LP molecules to the brain, or to differences in peripheral fate of heterologous and homologous LP. Further chemical characterization of pyrogen derived from human cells and tests of the febrile reactions produced by these pyrogens in the squirrel monkey may aid understanding of the pyrogen/central receptor interaction responsible for fever in man.

Summary. Squirrel monkeys developed fever in response to relatively small intravenous and intracerebral doses of highly purified human leukocytic pyrogen (LP). The findings indicate that this species is an appropriate new model for research on human endogenous pyrogen. Cross-reactions of various species to LP suggest a similarity in the molecular structure of all leukocytic pyrogens.

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