PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 157, 472–475 (1978)

Effect of Antipyretics on group A Streptococcal Pyrogenic Exotoxin Fever Production and Ability to Enhance Lethal Endotoxin Shock (40079)

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Like endotoxin, group A streptococcal pyrogenic exotoxins (SPE) produce fever when given iv into rabbits (1–4). Other biological properties associated with the three antigenically distinct SPE types, designated A, B, and C, include: enhancement of the susceptibility of rabbits, mice, and monkeys to lethal endotoxin shock (2, 5, 6); alteration of reticuloendothelial function (7, 8) and the antibody response to sheep erythrocytes in rabbits and mice (9–12). These important biological properties have been reviewed (13).

Administration of pyrogens, such as endotoxin, lipid A, or endogenous pyrogen, either into the hypothalamus or intravenously produces fever which can be inhibited by the antipyretics indomethacin and acetylsalicylate (14–21). These antipyretic agents have been shown to inhibit the synthesis, and therefore, subsequent release of prostaglandins (22). Prostaglandins of the E series, particularly PGE_1 and PGE_2, have been shown to elevate body temperature in rabbits and have been implicated as hypothalamic mediators of fever (14, 20, 21). Other studies have shown that indomethacin, if given before endotoxin, can either abolish or delay characteristics associated with endotoxin shock (23, 24).

The antipyretic effect of cortisone also may involve prostaglandins since cortisone has been shown to interfere with prostaglandin release (25). In addition, cortisone may prevent fever by inhibiting the release of endogenous pyrogen from leukocytes (26–30).

This study was undertaken to determine the effects of the antipyretics indomethacin, acetylsalicylate, and cortisone on the fever response of rabbits to SPE type C and on the ability of SPE type C to enhance susceptibility to lethal endotoxin shock.

Materials and methods. Purified preparations of SPE type C, containing 25% hyaluronic acid as a stabilizing agent, were made as described previously (1, 2, 4). The minimum dose of toxin given iv to produce an average fever response of 0.5° at 4 hr postinjection (MPD-4) was approximately 1.0 μg/kg rabbit body weight. Doses of toxin used were 20 MPD-4/kg (20 μg/kg). Toxin concentrations were determined using the microbiuret protein assay described by Zamenhof (31).

Biological assays. Solutions of SPE were prepared in sterile pyrogen-free phosphate-buffered saline (0.005 *M* phosphate buffer, pH 7.0, plus 0.15 *M* NaCl) for all biological assays. American Dutch rabbits 1.0–1.5 kg were used to determine pyrogenicity and enhanced susceptibility to lethal endotoxin shock (2). Pyrogenicity was measured as described previously (2). The average starting temperatures of the rabbits ranged from 37.6 to 38.1° with mean differences of 0 to 0.3° between groups in an experiment. Enhanced susceptibility to endotoxin shock was measured by giving an iv injection of 25 μg/kg of endotoxin (*Salmonella typhimurium*) 4 hr after SPE injection. The LD_{50} of endotoxin for normal rabbits was 535 μg/kg (1).

Drugs. Indomethacin was generously provided by Merck, Sharp and Dohme (Merck and Co., Inc., West Point, PA) and suspended in phosphate-buffered saline (PBS) at a concentration of 25 mg/ml. Acetylsalicylic acid was purchased from Sigma Chemical Co., St. Louis, MO. The acetylsalicylic acid (8 g in 20 ml PBS) was neutralized by adding 8 *M* sodium hydroxide and the volume adjusted to 40 ml with PBS. Cortisone was obtained from The Upjohn Co., Kalamazoo, MI.

Statistical analyses. The difference in mean fever responses between groups was evaluated at the 4 hr time point or 1 hr after drug injection, using Student's *t* test analysis. The variability in fever responses is indicated by ± SEM. Statistical analyses were performed only at the 4-hr time point or 1 hr after injection of antipyretic agent.

Results. Figure 1 shows the effect on pyr-

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ogenicity of treating rabbits with indomethacin (25 mg/kg body weight) immediately before administration of SPE type C. Whereas control rabbits showed an average fever response of 1.1° at 4 hr, no average fever response was obtained for the indomethacin-treated rabbits ($P < 0.001$). Pretreatment of rabbits with indomethacin, however, did not prevent enhancement of lethal endotoxin shock by SPE type C (Fig. 1). For the groups, either four of five or five of five rabbits died less than 8 hr after treatment with endotoxin.

When indomethacin (25 mg/kg body weight) was given near the time of highest fever response, again the fever was reduced (Fig. 2). A drop in average fever response of 1.1° in less than 1 hr was obtained ($P < 0.005$).

Administration of acetylsalicylate (200 mg/kg) before giving SPE type C (Fig. 3) also effectively prevented the fever response ($P < 0.005$). However, as observed with indomethacin treatment of animals, acetylsalicylate did not protect against enhanced susceptibility to lethal endotoxin shock. Four of five rabbits died in the control group, and five of five rabbits died in the acetylsalicylate-treated group. The fever response induced by

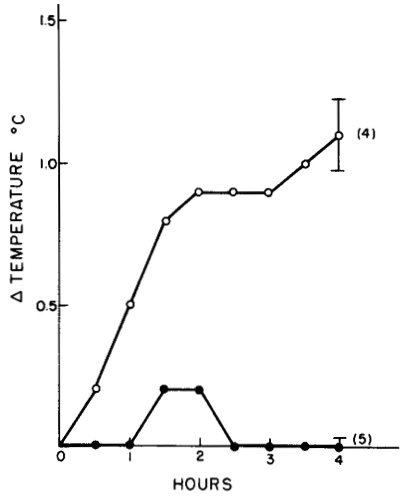

FIG. 1. The effect of indomethacin pretreatment of rabbits (25 mg/kg) on SPE type C fever production and ability to enhance lethal endotoxin shock. Phosphate-buffered saline followed by SPE type C (○); indomethacin followed by SPE type C (●). Doses of SPE type C were 20 μg/kg. *Salmonella typhimurium* endotoxin (25 μg/kg) was given to all rabbits at 4-hr time point. Bars indicate ± 1 SE. Numbers in brackets are number of the five rabbits in each group that died when given endotoxin.

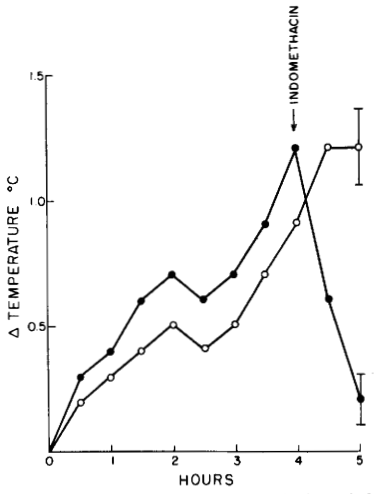

FIG. 2. Alteration of SPE type C induced fever response in rabbits by indomethacin (25 mg/kg). SPE type C followed by phosphate-buffered saline given at 4 hr (○); SPE type C followed by indomethacin given at 4 hr (●). Doses of SPE type C were 20 μg/kg. Bars indicate ± 1 SE.

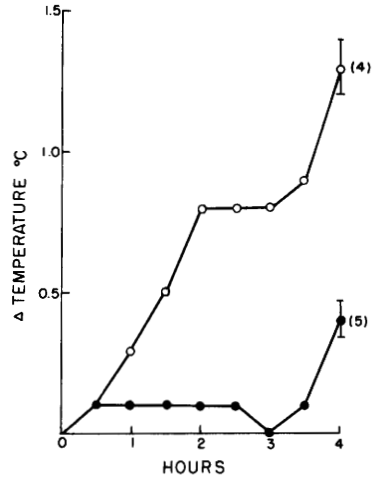

FIG. 3. The effect of acetylsalicylate pretreatment of rabbits (200 mg/kg) on SPE type C fever production and ability to enhance lethal endotoxin shock. Phosphate-buffered saline followed by SPE type C (○); acetylsalicylate followed by SPE type C (●). Doses of SPE type C were 20 μg/kg. *Salmonella typhimurium* endotoxin (25 μg/kg) was given to all rabbits at 4 hr time point. Bars indicate ± 1 SE. Numbers in brackets are number of the five rabbits in each group that died when given endotoxin.

SPE type C was reduced by giving acetylsalicylate (200 mg/kg) near the time of maximum fever response (Fig. 4). A drop in temperature of 0.9° in 1 hr was obtained ($P < 0.005$).

Treatment of rabbits on each of 3 days prior to toxin with 5 mg/kg cortisone and again 2 hr before giving toxin depressed fever production by SPE type C (Fig. 5) ($P < 0.005$). Again, cortisone did not protect the rabbits from enhanced susceptibility to lethal endotoxin shock (Fig. 5). In both groups four of five rabbits died.

Discussion. Several studies have implicated prostaglandin involvement in fever production by endotoxin, Lipid A, or endogenous pyrogen (14–21). The prostaglandin involvement is based on the observations that the antipyretics indomethacin and acetylsalicylate, which inhibit prostaglandin synthetase, inhibit fever production. In the present study, these antipyretics also inhibited fever production by SPE type C, suggesting SPE-induced fever may require prostaglandin synthesis. Continuous synthesis and release of prostaglandin may be necessary to maintain SPE-induced fever since administration of antipyretics during fever also depressed the fever response.

Previously, it has been suggested that cortisone may inhibit fever production by interfering with the release of endogenous pyrogen

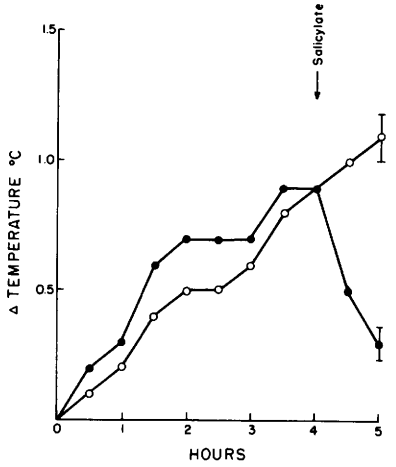

FIG. 4. Alteration of SPE type C induced fever response in rabbits by acetylsalicylate (200 mg/kg). SPE type C (20 μg/kg) followed by phosphate-buffered saline given at 4 hr (○); SPE type C followed by acetylsalicylate given at 4 hr (●). Bars indicate ± 1 SE.

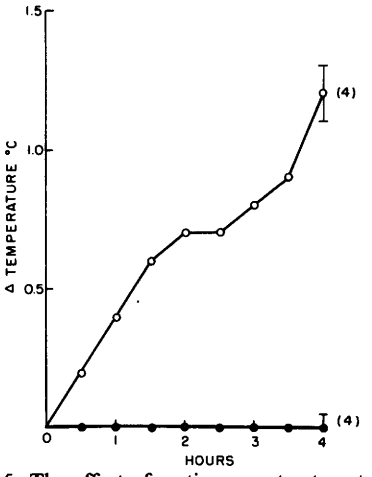

FIG. 5. The effect of cortisone pretreatment of rabbits on SPE type C fever production and ability to enhance lethal endotoxin shock. Rabbits given 20 μg/kg of SPE type C (○); schedule of cortisone pretreatment was: day (−3) 5 mg/rabbit; day (−2) 5 mg/rabbit; day (−1) 5 mg/rabbit; day (0) 5 mg 2 hr before SPE type C. Rabbits pretreated with cortisone and then given SPE type C (●). *Salmonella typhimurium* endotoxin (25 μg/kg) was given to all rabbits at 4 hr time point; numbers in brackets are the numbers of the rabbits in each group that died when given endotoxin. Bars indicate ±1 SE.

from leukocytes (26–28). The fever response of rabbits to endogenous pyrogen is characterized by a 1–2 hr maximum (29, 30), while SPE type C typically produces a gradual rise in fever which peaks near 4 hr. Therefore, SPE may produce fever through a mechanism other than endogenous pyrogen. It is possible that cortisone inhibited fever production by SPE type C through blocking the release of prostaglandin as suggested by a previous study (25) rather than by blocking endogenous pyrogen release. It cannot be discounted, however, that SPE type C acts slowly on leukocytes to induce the release of endogenous pyrogen, resulting in the 4 hr maximum.

A biologically important property of the SPE is their ability to enhance the susceptibility of animals to lethal endotoxin shock (5, 6). The mechanism underlying this property remains unclear. It may, however, be separable from fever production since abolishing fever with antipyretics did not prevent the enhancement phenomenon; control and experimental rabbits died at similar rates and numbers. Further experiments using SPE

type C and endotoxin doses closer to the LD_{50} need to be done to assess fully the role of prostaglandins in the enhancement phenomenon and to assess the ability of antipyretics to modify the enhanced susceptibility to lethal endotoxin shock as previously observed with endotoxin in the absence of SPE (23, 24).

Summary. The fever response of rabbits to group A streptococcal pyrogenic exotoxin (SPE) type C was effectively reduced by pretreatment of the animals with the antipyretics, indomethacin (25 mg/kg), acetylsalicylate (200 mg/kg), and cortisone (5 mg on each of three preceding days and 2 hr before given SPE). Indomethacin and acetylsalicylate also significantly reduced the fever response of rabbits to SPE type C if given near the time of maximum fever response (4 hr after SPE). None of the antipyretic agents protected the rabbits from SPE's capacity to enhance susceptibility to lethal endotoxin shock.

These data suggest that SPE fever production required prostaglandin synthesis, probably PGE_1 or PGE_2, and that the mechanism of fever production by SPE may be different from the mechanism underlying the enhancement of lethal endotoxin shock. Studies are presently being done to elucidate further the mechanisms of fever production and enhancement of lethal endotoxin shock by SPE.

This investigation was supported by Public Health Service research Grant No. AI-06487 and AI-12673 from the National Institute of Allergy and Infectious Disease. Patrick Schlievert was supported by Public Health Service training Grant No. 5T32HL07116 from the National Heart and Lung Institute.

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Received September 1, 1977. P.S.E.B.M. 1977, Vol. 157.