Sleep-promoting effects of endogenous pyrogen (interleukin-1)

JAMES M. KRUEGER, JAMES WALTER, CHARLES A. DINARIELLO, SHELDON M. WOLFF, AND LOUIS CHEDID

Department of Physiology and Biophysics, The Chicago Medical School, North Chicago, Illinois 60064; Department of Medicine, Tufts University School of Medicine, Boston, Massachusetts 02111; and Immunotherapie Experimentale, Institut Pasteur, Paris, France

KRUEGER, JAMES M., JAMES WALTER, CHARLES A. DINARIELLO, SHELDON M. WOLFF, AND LOUIS CHEDID. Sleep-promoting effects of endogenous pyrogen (interleukin-1). Am. J. Physiol. 246 (Regulatory Integrative Comp. Physiol. 15): R994–R999, 1984.—When infused into the lateral cerebral ventricles of rabbits, human endogenous pyrogen (EP) preparations induced dose-dependent increases in slow-wave sleep concomitant with increasing body temperature. Heating EP to 70°C destroyed its sleep-promoting and pyrogenic activity. Anisomycin (an antipyretic) prevented EP from increasing body temperature without affecting its sleep-promoting activity. Intravenous injection of EP induced fever and transient increases in slow-wave sleep but failed to induce prolonged increases in slow-wave sleep. We conclude that the somnogenic activity of EP is not secondary to its pyrogenic activity.

slow wave sleep; rabbits; fever; anisomycin; muramyl peptide

A SUBSTANCE, factor S, that induces slow-wave sleep (SWS) in several species of animals has been shown to be a muramyl peptide (21). Some synthetic muramyl peptides, which have been studied extensively as immunoauguants (8, 12), have also been shown to be somnogenic (22) and pyrogenic (9). They have the additional capacity (like many bacterial agents) of inducing synthesis and release of endogenous pyrogens in vivo and in vitro (9, 27). Thus, muramyl peptide-treated macrophages contain in their supernatants endogenous pyrogen (EP), which produces fever (8). It was, therefore, of interest to determine whether such dialyzed macrophage supernatants or a purified preparation of EP could induce SWS. We found that both preparations induced prolonged increases in rabbit SWS and that excess SWS was observed even when the associated febrile response was blocked with the antipyretic anisomycin.

MATERIALS AND METHODS

The muramyl peptide used in this study, NAc-Mur-L-Ala-d-Gln-OMe or MDP(Gln)-OMe, was designed and synthesized by P. Lefrancier (23). All chemicals were reagent grade. Needles, syringes, glassware, media, and solutions were sterile and pyrogen free.

Preparation of macrophage supernates. Rabbit peritoneal exudate cells (PEC) and human mononuclear cells (PBMC) were prepared as previously described (8). Briefly, PEC were induced by an intraperitoneal injection of sterile mineral oil (50 ml). Rabbits were killed with an intravenous overdose of pentobarbital sodium 72 h later; PEC were harvested from the peritoneal cavity using 300 ml of heparinized (5 U/ml) pyrogen-free saline. Cells were washed twice and resuspended in RPMI 1640 medium (Flow Labs, McLean, VA) supplemented with a combination of penicillin and streptomycin and with N-2-hydroxyethylpiperazine-N'2-ethanesulfonic acid (HEPES) buffer (0.01 M, pH 7.1–7.3). PEC density was 10⁶ cells/ml. PBMC were obtained from blood collected from healthy adult volunteers. The buffy coat was diluted 1:3 with minimal essential medium (MEM) 199 (Institut Pasteur Productions, Paris, France) supplemented with heparin (1:100; Liquemine, Hoffman-LaRoche, Basel, Switzerland). The method of Boyum (4) was used to recover PBMC after centrifugation on Ficoll-Triosil. Mononuclear cells were diluted at 5 x 10⁶ cells/ml in RPMI 1640 medium supplemented with antibiotics and HEPES as described for PEC.

Both PBMC and PEC were incubated for 24 h with 100 μg/ml of MDP (Gln)-OMe. Cell suspensions were then subjected to centrifugation (1,000 g), and supernates were dialyzed overnight against phosphate-buffered saline (PBS; 0.15 M, pH 7.4) to eliminate residual synthetic glycopeptide, because it has a molecular weight of about 500. Control cells were incubated without the glycopeptide, and supernates were treated in the same fashion. MDP(Gln) OMe was used because previous studies showed that although this analogue is not somnogenic (22) or pyrogenic in-vivo, it is capable of activating macrophages in vitro to produce lymphocyte-activating factor (LAF) and endogenous pyrogen (Parant, Riveau, Parant, and Chedid, unpublished observations).

Preparation of purified human EP. Human platelet-pheresis byproducts were separated on Ficoll-Hypaque gradients (11), and the mononuclear cell layer was stimulated with heat-killed Staphylococcus albus (bacterial:leukocyte 10:1) in the presence of 1% fresh bovine serum albumin. The mixture was allowed to settle onto glass bottle surfaces for 1.5 h at 37°C followed by vigorous shaking to remove nonadherent cells. The adherent monocytes were then incubated in serum-free MEM (Biological Associates, Walkerville, MD) for 18 h. The supernate was clarified by centrifugation at 8,000 g and mixed with Sepharose 4B-bound rabbit antihuman EP (11). After 2 h at room temperature, the immunosorbent was
SLEEP-PROMOTING EFFECTS OF ENDOGENOUS PYROGEN

poured into a column and washed with 5 vol PBS (pH 3.0). EP was eluted with citric acid buffer (pH 3.0) and collected into 1% lysine (final concn) and neutralized with NaOH. This material was concentrated and chromatographed over Sephadex G-50, fine (170 × 5.0 cm, 4°C, 0.15 M NaCl, pH 5.9); the 15,000-dalton peak was isolated (10) and concentrated in autoclaved dialysis tubing against dry polyethylene glycol (mol wt 6,000) at 4°C. The concentrated material was then dialyzed against 4,000 vol PBS.

The material we prepared was shown to contain the three isoelectric focusing points of EP (11). Rabbit pyrogen testing revealed 0.1 ml (intravenously) to produce a 1°C monophasic fever, typical of EP, and at dilutions of 10²; this material doubled the pyrhemagglutinin response of murine thymocytes in the LAF assay (28).

Rabbit bioassay. Rabbits were used to assay EP samples for several reasons. 1) Rabbits are frequently used in experimental work on fever (3). 2) Rabbits develop fever after administration of human EP (8); we avoided, therefore, possible species specificity problems associated with transfer of EP from one species to another (3). 3) In rabbits, normal variations in the amount of time spent in SWS from day to day are very small (SD ± 7%) (21); the small standard deviation reduces the number of assays required to demonstrate the presence or absence of sleep-promoting activity. 4) Rabbits (20), unlike rats (20) and cats (16), adapt rapidly to experimental cages, thus an overnight acclimation period is sufficient to allow for valid sleep measurements the next day. 5) We could use the phenomenon of “supranormal” slow waves that are observed after sleep deprivation (21, 26) or administration of sleep-promoting muramyl peptides (22) as an objective criterion for identifying active fractions. 6) Intracerebral ventricular administration of control solutions of artificial cerebrospinal fluid (CSF) does not affect subsequent duration or patterns of sleep in rabbits (20). 7) Rabbits spend less than 5% of their sleep time in rapid-eye-movement sleep (13); thus we simplified the analysis by restricting it to SWS.

Male New Zealand White rabbits weighing 3–4 kg were used. Under pentobarbital sodium anesthesia (Nembutal, Abbott; 15–30 mg/kg body wt) stainless steel screws were implanted over the frontal, parietal, and occipital cortex. Short electroencephalogram (EEG) leads from a plug (ITT-Cannon #MIKO-l-7SH) were soldered to these screws. A cerebral ventricular guide tube was also implanted 4 mm lateral and 1 mm posterior to the bregma as previously described (20). Dental cement (DuAll acrylic) was then applied to insulate the leads and to secure the guide tube and EEG plug to the skull. Following the operation each animal received 150,000 U of procaine penicillin G (Duracillin, Lilly) and a topical application of bacitracin (Lilly) around the incision. One week was allowed for recovery.

Rabbits were brought to the experimental cages for an overnight acclimation period before each experiment; both the housing and experimental rooms were temperature controlled (21 ± 2°C) and on a 12-h light–12-h dark cycle. Intracerebroventricular infusions or intravenous injections were carried out between 8:00 and 10:00 A.M. and were followed by 6 or more h of recording. Six-hour control recordings were obtained from rabbits at the same time of day; these data provide each rabbit with individual control values. Rectal temperatures were measured by a thermistor probe (Yellow Spring Instruments) inserted 10 cm into the rectum. Temperatures were determined immediately after the infusion and 3 and 6 h later. This procedure transiently (5–10 min) disturbed sleep. During control recordings, rectal temperatures were also determined at 3-h intervals. After intravenous injections of EP, rectal temperatures were taken just before the injection, 1–2 h later, and 6 h later. The typical monophasic fever induced by intravenous injection of EP is maximum at about 1 h after injection; rectal temperatures are usually at control values 3 h after injection (10).

EP samples (2–200 µl) were diluted to 0.3 ml with artificial CSF (in mM; 3 KCl, 1.15 CaCl₂, and 0.96 MgCl₂) in pyrogen-free sterile saline (155 mM NaCl, Abbott). Samples were infused over a 45-min period at a rate of 0.7 µl/min icv. In some cases anisomycin (700 µg/rabbit) was also dissolved in either artificial CSF (control) or in the EP-artificial CSF solutions (experimental) for intracerebroventricular infusions. After receiving anisomycin, rabbits were not used again for 2 mo. Cerebral ventricular pressure was monitored to ensure that solutions were infused into the ventricle (20). For systemic injections the vehicle was pyrogen-free saline (Abbott). During and after infusion, EEGs were recorded on Grass polygraphs (model 7D). EEG leads were connected to the polygraph via a rotary commutator (BRS-Tech Serv), which allowed unrestrained movements of rabbits. The EEG signal was also led through a band-pass filter (Buxco, model 1802P), and the 0.5- to 4-Hz component was rectified. The filtered rectified signal was recorded on the polygraph simultaneously with the EEG. The slow-wave rectified signal was also integrated, and integrals were printed on tape every minute. Body movements were recorded from a water-filled tube attached to the EEG cable and connected to a pressure transducer.

EEG records were visually scored to determine duration of SWS. Rabbit sleep is characterized by short SWS episodes (1–10 min), which under control conditions usually occupy 35–45% of the time during 1 h or more of recording (20). The duration of an individual SWS episode was defined as that period of time during which EEG slow waves (0.5–4 Hz) were not interrupted for more than 20 s by the high-frequency low-voltage EEG that is characteristic of an awake animal. Visual assessment of the filtered rectified EEG recording facilitated scoring; the large differences between amplitude of slow waves of a sleeping vs. waking animal are easily distinguishable on the filtered rectified EEG recording. The body movement record was used to distinguish movement artifact on the EEG. Slow-wave amplitudes (mean rectified slow-wave voltages) averaged over 1 min. SWS periods and waking periods were determined for each rabbit using the printed integrals during control and experimental conditions. Under control conditions these values are stable for each animal within about 10% over a period of weeks (20).

In some cases behavioral observations were made at various times after administration of EP samples. In...
particular we sought signs of abnormal autonomic function such as excess nasal and/or lacrimal secretion; these responses are observed after large doses of sleep-promoting muramyl peptides (22). No behavioral abnormalities were noted after EP administration at the doses used.

RESULTS

Effects of human PBMC supernates. Infusion of 10 or 25 μl of solutions obtained from MDP(Gln)-OMe-treated PBMC induced prolonged increases in SWS (Fig. 1). Increases in rectal temperatures above control values were also observed after these infusions. Maximum temperature increases were observed 3 h after the infusion; 6 h after infusion temperatures were still elevated above control values. The excess SWS appeared normal despite the development of fever in the sense that animals continued to sleep episodically, could easily be aroused, and spontaneously awoke from time to time to eat, drink, and groom. In contrast to these results, equal volumes of supernates obtained from control macrophages failed to induce significant increases in either SWS or rectal temperatures. However, if the dose of this preparation was increased to 50 μl, slight increases in SWS and rectal temperatures were observed.

Effects of intravenous administration of rabbit PEC supernates. Intravenous injection of PEC supernates resulted in significant rectal temperature increases char-

![Graph](http://ajpregu.physiology.org/)

**FIG. 1.** Effects of increasing doses of dialyzed macrophage supernates on rabbit slow wave sleep (SWS) and rectal temperatures. **Top ordinate:** rectal temperatures taken 3 h after infusion. At this time febrile responses were greatest. **Bottom ordinate:** percent excess SWS (SWS experimental − SWS control). Percent SWS values for each rabbit were determined during 1 or more 6-h control recordings. Abscissa: microliters of each preparation intracerebroventricularly infused. **Top solid lines:** preparations obtained from MDP(Gln)-OMe-treated human mononuclear cells. **Bottom broken lines** from control macrophages. Each point represents mean ± SE of 4 assays on 4 rabbits.

![Graph](http://ajpregu.physiology.org/)

**FIG. 2.** Time course of slow-wave sleep (SWS) responses following intravenous injection of supernates obtained from rabbit peritoneal exudate cells (PEC). **Top solid line:** effects of intravenous injection (1 ml/kg). **Lower broken line:** effects of intravenous injection of saline (1 ml/kg) in same animals (n = 4). Each point is mean ± SE percent SWS that occurred during 24-min period. Solutions were injected 2–5 min before time 0. After intravenous injection there appeared to be a transient increase in SWS lasting about 96 min. This increase paralleled time course of febrile responses elicited by bolus intravenous injection of endogenous pyrogen (10). After intracerebroventricular infusion of PEC supernates, longer sleep responses (Fig. 3) and febrile responses (6) were observed.

**TABLE 1. Effects of rabbit PEC supernates on rabbit SWS and rectal temperature**

<table>
<thead>
<tr>
<th>Administration</th>
<th>n</th>
<th>Dose, μl/kg</th>
<th>%SWS</th>
<th>Max Increase in Rectal Temp</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iv</td>
<td>3</td>
<td>250</td>
<td>41 ± 6</td>
<td>44 ± 5</td>
</tr>
<tr>
<td>Iv</td>
<td>4</td>
<td>500</td>
<td>41 ± 2</td>
<td>42 ± 1</td>
</tr>
<tr>
<td>Iv</td>
<td>4</td>
<td>1,000</td>
<td>39 ± 3</td>
<td>45 ± 3</td>
</tr>
<tr>
<td>Icv</td>
<td>4</td>
<td>17</td>
<td>42 ± 4</td>
<td>59 ± 1*</td>
</tr>
</tbody>
</table>

Values are means ± SE of 6-h recordings. PEC, peritoneal exudate cells; SWS, slow-wave sleep; Iv, intravenous; Icv, intracerebroventricular. Rectal temperatures taken 1-2 h after iv injection and 3 h after icv infusion. *Significantly different from control, P < 0.05. Each rabbit was used as his own control; paired statistics were used.

characteristically peaking 40–50 min following bolus injection and returning to base-line levels after 3–4 h. Rabbits were also observed to enter a short (40–60 min) period of excess SWS, which coincided with the fever (Fig. 2). Because these periods of excess sleep were of short duration, we were unable to demonstrate significant increases over the 6-h recording period using our routine statistical methods of analysis. However, if we restrict our analysis to the first 96 min, the period in which febrile responses occur (10), significant increases in SWS were observed. During this period percent SWS values were 50 ± 5 (experimental) and 34 ± 2 (control) (P < 0.02). This preparation was also infused intracerebroventricularly; in this case 50 μl were sufficient to induce prolonged increases in SWS and rectal temperatures (Table 1).

Effects of purified human EP. Our results obtained by macrophage supernates suggested that a nondialyzable
TABLE 2. Effects of various doses of purified human endogenous pyrogen on SWS in rabbit

<table>
<thead>
<tr>
<th>Dose, µl</th>
<th>% SWS Postinfusion, h</th>
<th>Rectal Temp, °C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Exptl</td>
</tr>
<tr>
<td>2</td>
<td>38±3</td>
<td>54±1*</td>
</tr>
<tr>
<td>10</td>
<td>38±5</td>
<td>67±2*</td>
</tr>
<tr>
<td>50</td>
<td>35±4</td>
<td>79±5*</td>
</tr>
<tr>
<td>200</td>
<td>35±2</td>
<td>70±7*</td>
</tr>
</tbody>
</table>

Values are means ± SE. SWS, slow-wave sleep. * Significantly different from corresponding control value; Student's t test [df = 3 (or 2) for SWS and 6 (or 4) for temperature] exceeded P = 0.05 level.

substance, produced by macrophages under conditions known to elicit EP, induced excess SWS. To avoid possible non-specific effects of the relatively crude supernate samples we used purified EP for further studies. Four doses of purified EP were tested (Table 2). At the lowest dose (2 µl), excess SWS was observed only during the first 3 postinfusion h. When the dose was increased greater excess SWS was observed and responses persisted throughout the 6-h recording period. In some cases we recorded for 30 h after administration of 200 µl EP. Excess SWS was observed during the first 18 postinfusion h; no rebound in wakefulness was observed in subsequent hours. It is important to mention that during the first hour after infusion excess SWS was observed; following intracerebroventricular infusions of sleep-promoting muramyl peptides excess SWS was not evident until the second postinfusion hour (22). The febrile responses induced by EP were also dose dependent. As described above, the excess SWS appeared normal even though animals were simultaneously febrile.

Purified EP was obtained from macrophages treated with heat-killed S. albus. Thus to control for the possibility of an inadvertent copurification of a heat-stable biologically active bacterial product with EP, samples of purified EP were heated (70°C, 30 min) before intracerebroventricular administration of 10 µl EP is inactivated by this treatment (10, 28). Somnogenic and pyrogenic actions of our purified EP samples were lost after heat treatment; percent SWS values were 41 ± 2 (control) and 41 ± 1 (experimental) (n = 3/group) for the 6 h postinfusion period.

Effects of anisomycin on fever and sleep responses induced by purified EP. Cranston et al. (6) reported that simultaneous intracerebroventricular infusion of anisomycin and EP blocked the expected febrile response. Thus, to determine whether sleep responses persisted in the absence of fever, we infused solutions containing anisomycin and 2 µl of purified EP. Fever responses to EP were completely blocked by anisomycin (Fig. 3), thus confirming the observations of Cranston et al. However, sleep responses elicited by EP were unaffected by the anisomycin treatment. These results are similar to those previously reported that showed that fever after intravenous administration of MDP could be attenuated by pretreatment with an antipyretic without affecting the capacity of MDP (Gln)-OMe to induce sleep (22). Anisomycin by itself did not affect duration of SWS or rectal temperatures during the first 6 h postinfusion; percent SWS was 40 ± 3 (control) and 40 ± 2 (experimental) (n = 4/group). However, the day after anisomycin treatment there were large variations in duration of SWS even though rectal temperatures were normal. Other long-term behavioral effects of anisomycin have been reported (17).

Effects of EP on amplitude of EEG slow waves. Increases in amplitude of EEG slow waves during SWS are observed during the deep sleep that follows sleep deprivation in rabbits (26), rats (1), and humans (2). Similar increases are observed after administration of factor S (21) or other somnogenic muramyl peptides (22). In the present study the amplitude of EEG slow waves during SWS increased 27% above control values in those febrile rabbits that received anisomycin plus 2 µl of purified EP (Table 3). No significant increases in slow-wave amplitudes were observed in those control animals that received only anisomycin. Significant increases in EEG slow-wave voltages during SWS were also observed in the febrile animals that received only purified EP. None of these groups had significant changes in slow-wave amplitudes during waking periods.
Figures have been named in accordance with their biological activities, i.e., endogenous pyrogen for the production of rapid-eye-movement sleep can be correlated with sleep-deprivation: endogenous pyrogen; EEG, electroencephalogram; E, and E, EEG slow-wave voltage during awake periods and slow-wave sleep, respectively.

**DISCUSSION**

The results presented here clearly demonstrate that fever and sleep responses elicited by EP can be separated from one another. After intracerebroventricular administration of EP, fever was blocked by anisomycin but excess SWS was not. It is possible that sleep responses were due to a separate macrophage product copurified with EP, which may independently induce SWS. Traditionally the product(s) of activated macrophages have been named in accordance with their biological activities, i.e., endogenous pyrogen for the production of fever and lymphocyte-activating factor for enhancement of lymphocyte responses to mitogens and antigens. The preparations used in the present experiments contained both EP and LAF activity. There is evidence that those biological activities may be due to a single substance, or closely related family of substances, and are often referred to as interleukin-1 (8, 24). Other studies, however, have shown that EP and LAF activities are not secreted together from macrophages activated by certain analogues of MDP(Gln)-OMe (8); thus the possibility of a separate somnogenic macrophage product remains.

Regardless of the number of macrophage products involved, what is clear from the present studies is that those conditions we used to stimulate macrophages elicited both EP and sleep-promoting activity. That all these may be intimately related is not surprising because many relationships between sleep and temperature regulation have been described. For example, many drugs or neurotransmitters can induce perturbations in both temperature regulation and sleep, and the effects depend on dose, route of administration, and species (25). Under some circumstances increases in body temperature are associated with increases in sleep and/or EEG synchronization (15,30-32). Local changes in brain temperature may also affect sleep; in kangaroo rats increases in SWS or rapid-eye-movement sleep can be elicited by local warming of the hypothalamus (18). In humans deprived of environmental timing cues, the timing and architecture of sleep can be correlated with circadian rhythms of core body temperature; duration of sleep episodes was greatest when subjects entered sleep during peak temperatures (7).

Proposals concerning the role that EP may play in normal sleep and temperature regulation should take into account the fact that normal sleep is usually accompanied by a regulated decrease rather than an increase in body temperature (18). If EP is involved in normal sleep, one might assume that it is delivered by very precise mechanisms, perhaps in concert with other sleep and temperature modulators, to those cells involved in the induction of SWS. Thus in normal sleep the increases in body temperature that we observed after rapid delivery of exogenous EP would not be encountered. It is possible that EP can, under certain physiological conditions, induce excess SWS. Cannon and Kluger (5) reported that increases in EP-like activity accompany increase in circulating leukocytes in postexercise marathon contestants. Shapiro et al. (29) reported large increases in SWS during postmarathon nights. Subsequently, Horne and Staff (19) showed that passive heating, which increased body temperature, also resulted in increased duration of SWS. This could suggest that increases in SWS following exercise result from increases in body temperature rather than EP; however, EP levels were not measured in this study. It is also possible that the subjective feelings of sleepiness that often accompany infectious disease could be due to EP elicited by the infectious agent.

The onset of excess SWS following intracerebroventricular EP administration (Fig. 3) is faster than that following infusion of sleep-promoting muramyl peptides (22). This could suggest that muramyl peptides exert their effects on SWS through a step involving production of EP or another monokine. An alternative possibility is that muramyl peptides and monokines independently affect those neurons involved in the generation of SWS. Evidence for this hypothesis stems from the experiments of Riveau et al. (27), who were unable to detect EP after central administration of very large amounts of MDP(Gln-OMe). However, if small amounts of EP were generated in or near the specific neurons involved in the induction of sleep and/or fever, it could go undetected by current methods of analysis. There is evidence that astrocytes and Cg glioma cells can produce an interleukin-1-like substance (14). We also consider the possibility that EP may contain a muramyl peptide component, because its structure remains unknown.

We thank Drs. J. R. Pappenheimer and M. L. Karnovsky for their counsel and Dr. C. McCormack for reviewing this manuscript. 

This study was supported in part by Office of Naval Research Contracts N00014-82-K-0393, NIH-RR5366, and NIH-AI-15614.

Received 1 August 1983; accepted in final form 13 January 1984.

### References


### Table 3. Effects of human EP on rabbit EEG slow-wave voltages

<table>
<thead>
<tr>
<th>Infusate</th>
<th>n</th>
<th>E, μV</th>
<th>E, μV</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Control</td>
<td>Exptl</td>
</tr>
<tr>
<td>Anisomycin (700μg)</td>
<td>4</td>
<td>20 ± 2</td>
<td>22 ± 3</td>
</tr>
<tr>
<td>EP (2 μl) + anisomycin (700μg)</td>
<td>6</td>
<td>15 ± 1</td>
<td>16 ± 2</td>
</tr>
<tr>
<td>EP (200 μl)</td>
<td>4</td>
<td>19 ± 3</td>
<td>19 ± 3</td>
</tr>
</tbody>
</table>

Values are means ± SE. E, endogenous pyrogen; EEG, electroencephalogram; C, and C, EEG slow-wave voltage during awake periods and slow-wave sleep, respectively. * Significantly different from control, P < 0.05. Individual E, and C, values differed substantially from one rabbit to another; paired statistics were used.
SLEEP-PROMOTING EFFECTS OF ENDOGENOUS PYROGEN


