Alteration of Sleep in Rabbits by *Staphylococcus aureus* Infection

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Abundant evidence suggests that sleep might be altered during infectious disease, although the relationship between sleep and infectious disease has never been examined systematically. To address this issue, we determined the effects of *Staphylococcus aureus* infection on rabbit sleep. Rabbits inoculated intravenously with *S. aureus* demonstrated the expected physiological changes consistent with a state of infectious disease (e.g., lymphopenia, neutrophilia, and fever), as well as time-dependent changes in sleep patterns. The sleep changes were characterized initially by increases in (i) the time spent in slow-wave sleep, (ii) the electroencephalographic slow-wave amplitudes during slow-wave sleep, and (iii) the duration of individual bouts of slow-wave sleep. At 20 to 36 h after inoculation, sleep responses fell to levels below corresponding control values for 6 to 12 h. At 6 to 10 h after inoculation, rapid-eye-movement sleep was suppressed and remained at low levels throughout the remainder of the 48-h recording period. These effects of bacterial infection on sleep were attenuated by antibiotic (cephalothin) therapy. Inoculation with killed bacteria produced similar changes in sleep and other physiological parameters, although significantly higher numbers of organisms were required to produce equivalent responses. We postulate that changes in sleep may represent an adaptive response of the host to infectious disease.

Alterations in sleep patterns during the course of infectious disease have never been systematically examined. The lack of experimental investigation into this area is somewhat surprising, given the common observation that many people experience feelings of increased "sleepiness" during infectious disease and that bed rest is frequently prescribed by physicians as an aid in recuperation (15). Several substances that are associated with bacterial infections or with the body's response to infection have been found to induce sleep when administered to experimental animals. For example, muramyl peptides (MPs) (the monomeric components of bacterial cell wall peptidoglycan), lipopolysaccharide (a component of endotoxin found in gram-negative bacteria), and several lymphokines, including interleukin-1 (IL-1), are all potent somnogens known to increase the amount of time spent in slow-wave sleep (SWS), the amplitude of electroencephalographic (EEG) slow waves, and the duration of individual bouts of SWS (reviewed in reference 22). These considerations led us to investigate the impact of bacterial infection on sleep in rabbits. We report here that sleep is altered during infectious disease and can be modulated by appropriate therapeutic intervention.

MATERIALS AND METHODS

Adult male New Zealand White rabbits (*Pasteurella* and coccidia free) weighing 4 to 5 kg were surgically implanted with EEG recording electrodes and brain thermistors as previously described (24) and were allowed to recover for several weeks prior to use in the experiments. Rabbits were housed individually on a 12 h-12 h light-dark cycle in a temperature-controlled room (21 ± 2°C). In all experiments, two rabbits were tested simultaneously. Prior to the experiments, they were moved to experimental cages and allowed an overnight period of adaptation. Baseline sleep patterns were recorded for 24 h before the animals received any experimental treatment. The animals were then inoculated intravenously (i.v.) with *Staphylococcus aureus* at 8:00 a.m. (time zero on figures) as described below, and recording was continued for an additional 48 h. Blood samples and colonic temperatures were taken at 6- or 12-h intervals throughout this period. In one experiment, both rabbits received *S. aureus* inoculations, but one also received the antibiotic cephalothin (40 mg/kg intramuscularly [i.m.]) and the other also received an appropriate volume of saline. These latter injections were repeated every 12 h. During recording periods, the animals were able to move freely about their cages and had continuous access to food and water.

EEGs and brain temperatures were recorded via a rotary commutator (BRS-Tech Serv). The movement of animals was monitored by using a Grass acceleration transducer connected to the EEG cable. The EEG signal was electronically filtered, and the delta-wave component (0.5 to 4.0 Hz) was rectified by using an EEG analyzer (Buxco Electronics, Sharon, Conn.) (25). These recordings were displayed on a Grass polygraph. The average EEG delta-wave amplitude was also recorded in digital form for each 1-min interval.

Periods of SWS are associated with an increased amplitude of low-frequency (delta) EEG waves and with the absence of body movement. On this basis, the EEG tracing, the filtered and rectified EEG delta-wave signal, and the movement record were visually examined over the first 6 h of the baseline recording period to determine the threshold amplitude of the delta waves associated with SWS for each animal. The data for each animal were then scored in 1-min intervals for the entire experiment; an animal was considered to be in a state of SWS whenever the average delta-wave amplitude for any interval exceeded the SWS threshold amplitude in the absence of movement by the animal. At other times, the animal was either awake or in rapid-eye-movement sleep (REMS). REMS was identified in some animals by visual assessment of the polygraph record, based on the criteria of a low-voltage EEG tracing, a rise in brain temperature, and the sporadic occurrence of phasic body movement (24). Data were analyzed throughout the recording period and summarized for every 2-h interval. Sleep

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parameters evaluated included the percentage of time spent in SWS, the average EEG slow-wave amplitude during SWS, the average length of a bout of SWS, and the number of minutes spent in REMS during each 2-h interval.

For the preparation of bacterial inocula, *S. aureus* (ATCC 29213) was purchased as a lyophilized culture on Coltis-loops. Prewarmed blood agar plates were inoculated and incubated overnight at 37°C, and colonies were then transferred to sterile phosphate-buffered saline (pH 7.4) to achieve a concentration of approximately $2 \times 10^9$ CFU/ml, estimated by using a Klett-Summerson photoelectric colorimeter. The number of CFU per milliliter of inoculum was later verified by plating serial dilutions of the bacterial suspension on blood agar plates and counting colonies after 24 h of incubation at 37°C. In experiments with heat-killed bacteria, suspensions were autoclaved prior to animal inoculation and later confirmed to be free of live bacteria by incubation on blood agar plates. Rabbits were inoculated i.v. in the marginal ear vein with 0.1 to 2.0 ml of the bacterial suspension. Each rabbit was inoculated with *S. aureus* only once and was sacrificed at the end of the recording period.

Blood samples (1 to 2 ml) were collected from the central ear artery and immediately transferred to VACUTAINER tubes containing EDTA. Total leukocyte (WBC) counts were measured by using a model 2N cell counter (Coulter Electronics, Inc., Hialeah, Fla.). Differential WBC counts were made by counting 100 WBCs from blood smears stained with Wright stain; final WBC counts were corrected for nucleated erythrocytes (nRBCs), if present. Plasma cortisol levels were measured by using a radioactive immunoassay kit (Kallestad Labs, Austin, Tex.).

Data were analyzed by using a two-way analysis of variance for repeated measures, and individual means were compared by using Fisher's least-significant-difference test for a priori comparisons. A significance level of $P < 0.03$ was used.

**RESULTS**

Effects of *S. aureus* infection on sleep, body temperature, and hematological parameters. Following i.v. inoculation of rabbits ($n = 16$) with $10^7$ to $10^8$ CFU [mean dose, $(6.1 \pm 1.1) \times 10^7$ CFU] of viable *S. aureus*, the time spent in SWS increased during the period 6 to 18 h after inoculation (Fig. 1A). The enhanced SWS was associated with increases in EEG slow-wave amplitude during SWS (4 to 8 h after inoculation; Fig. 1B) and in the length of individual bouts of SWS (6 to 16 h after inoculation; Fig. 1C). These alterations in sleep were followed by a period during which the time spent in SWS (26 to 34 h after inoculation; Fig. 1A), EEG slow-wave amplitudes (20 to 36 h after inoculation; Fig. 1B), and length of SWS bouts (24 to 36 h after inoculation; Fig.
Neutrophilia, lymphopenia, and elevated plasma cortisol levels are commonly associated with inflammatory, infectious, or stressful conditions in animals, and circulating nRBCs can be associated clinically with septicemia (9, 17); thus, these hematological parameters were examined after either viable or heat-killed S. aureus inoculation. Both viable organisms and the higher dose of killed organisms produced marked neutrophilia from 6 to 48 h after inoculation; the lower dose of killed organisms produced significant neutrophilia only at 12 h after inoculation (Fig. 4A). All three inocula resulted in marked lymphopenia 6 h after inoculation; this effect persisted until 12, 36, and 48 h after inoculation with the low dose of killed organisms, the high dose of killed organisms, and the viable organisms, respectively (Fig. 4B). The number of nRBCs present in peripheral blood also increased from 12 to 48 h after inoculation with viable organisms; this effect was not observed following inoculation with killed organisms (Fig. 4C). Inoculation with either viable organisms or the high dose of killed organisms resulted in significant increases in plasma cortisol levels from 6 to 24 h after inoculation (Table 1). Postmortem blood cultures from animals that received viable S. aureus revealed circulating gram-positive bacteria in 10 of 14 animals tested.

Effects of cephalothin administration on S. aureus-induced alterations in sleep, fever and hematological parameters. The bacteriocidal antibiotic cephalothin, which is known to inhibit the growth of the strain of S. aureus used in these experiments (R. Ghera, W. Nierman, and P. Pienta, American Type Culture Collection Catalogue of Bacteria, Phages and rDNA Vectors, 16th ed., p. 166, 1985), was administered to rabbits to evaluate the effects of antibiotic therapy on sleep patterns during infection. One group of rabbits (n = 8) was inoculated with viable S. aureus (5 × 10⁷ CFU) and saline. Significant time-dependent changes in the time spent in SWS, EEG slow-wave amplitudes, and length of SWS bouts were observed in this group (Fig. 5A to C), as with the group described above (Fig. 1). Another group of animals (n = 8) received cephalothin (40 mg/kg i.m. every 12 h) in conjunction with S. aureus inoculation. Cephalothin markedly attenuated the effects of S. aureus inoculation on the time spent in SWS, although significant increases in SWS still occurred from 6 to 16 h after inoculation (Fig. 5A). Cephalothin did not attenuate the initial stimulatory effects of S. aureus inoculation on EEG slow-wave amplitudes, but
FIG. 4. Effects of inoculation with viable or heat-killed S. aureus on hematological parameters. The numbers of neutrophils (A), lymphocytes (B), and nRBCs (C) were determined prior to and every 6 to 12 h after the i.v. inoculation of rabbits with $10^7$ to $10^8$ CFU of viable S. aureus (○; n = 16), $8 \times 10^7$ (CFU of heat-killed S. aureus (▲; n = 8), or $7 \times 10^7$ CFU of heat-killed S. aureus (▲; n = 12). Datum points represent the mean ± standard error of the mean. The numbers of neutrophils and lymphocytes measured in the preinoculation period were 2,557 ± 147 and 5,629 ± 348 per ml of blood, respectively (n = 36). nRBCs represent the number counted per 100 WBCs. *, P < 0.03 relative to time zero.

FIG. 5. Effects of cephalothin on S. aureus-induced changes in sleep. Rabbits inoculated i.v. with $10^7$ to $10^8$ CFU of viable S. aureus also received an i.m. injection of the antibiotic cephalothin (40 mg/kg; ○; n = 8) or the appropriate volume of saline (▲; n = 8), both at the time of S. aureus inoculation and every 12 h thereafter. Additional animals (▲; n = 6) received cephalothin injections without S. aureus inoculation. Shown are the percentage of time spent in SWS (A), EEG slow-wave amplitudes during sleep (B), and the length of individual bouts of SWS (C) in rabbits for 48 h after inoculation. Base-line data for these animals are not shown but were not significantly different from those shown in Fig. 1A to C. For all panels, datum points represent the mean ± standard error of the mean of values obtained from each rabbit during the preceding 2-h period. *, P < 0.03 relative to corresponding base-line values.

TABLE 1. Effects of S. aureus inoculation on plasma cortisol levels

<table>
<thead>
<tr>
<th>S. aureus (CFU, n)</th>
<th>Plasma cortisol level (µg/dl) at indicated h postinoculation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0h</td>
</tr>
<tr>
<td>Viable ($10^7$ to $10^8$, 16)</td>
<td>3.3 ± 0.3</td>
</tr>
<tr>
<td>Killed ($7 \times 10^7$, 12)</td>
<td>3.5 ± 0.5</td>
</tr>
<tr>
<td>Killed ($8 \times 10^7$, 8)</td>
<td>2.6 ± 0.2</td>
</tr>
</tbody>
</table>

* Time zero samples were taken just before inoculation with S. aureus.

b P < 0.01 relative to time zero.
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it did eliminate the subsequent inhibitory effects (Fig. 5B). Both the initial increase and the subsequent decrease in SWS bout length were attenuated by cephalothin (Fig. 5C). Cephalothin also shortened the duration of S. aureus effects on body temperature, neutrophil and lymphocyte numbers, and plasma cortisol levels (Fig. 6A to D). A third group of animals (n = 6) received cephalothin alone; this treatment did not significantly alter any of the parameters examined (Fig. 5 and 6).

Relationship of sleep patterns to severity of infection. Of the 24 rabbits that received S. aureus inoculations in the preceding experiments, 3 died within 24 h after inoculation, and 3 were sacrificed 12 h after inoculation because of a moribund condition characterized by extreme behavioral depression with unresponsiveness to handling and other stimuli, peripheral vasoconstriction as evidenced by cold extremities, and dyspnea; these six rabbits will subsequently be referred to as group 1. The remaining 18 rabbits (group 2) were affected less severely and remained clinically stable for up to 48 h after inoculation. The severity of the clinical response was related to the dose of S. aureus administered [mean doses administered to group 1 and group 2 were (8.6 ± 2.5) × 10^7 and (4.8 ± 0.6) × 10^7 CFU, respectively; P < 0.01]. The sleep patterns of animals that succumbed to the infection (group 1) were compared to those of surviving animals (group 2) during the initial 12 h after inoculation. Animals in group 1 slept substantially less than those in group 2 from 10 to 12 h after inoculation and also had decreased EEG slow-wave amplitudes and length of SWS bouts from 8 to 12 h after inoculation (Table 2). Group 1 rabbits also exhibited a more rapid rise in body temperature, number of nRBCs, and plasma cortisol levels than did group 2 rabbits (Table 3). Changes in lymphocyte numbers were similar between the two groups; however, group 1 animals failed to exhibit a strong neutrophil response (Table 3).

DISCUSSION

These experiments demonstrated that rabbits with S. aureus infections exhibit marked time-dependent changes in sleep patterns. These changes were characterized by initial increases in the time spent in SWS, the amplitude of EEG slow waves during SWS, and the duration of individual bouts of SWS. Subsequent to these effects, the time spent in SWS, the EEG slow-wave amplitude during SWS, and the SWS bout length all decreased below baseline values. Similar biphasic responses occur during “recovery” sleep subsequent to sleep deprivation (3, 31, 37). S. aureus infection also inhibits REMS for up to 42 h after inoculation; similarly, decreases in REMS can be observed during recovery after prolonged periods of sleep deprivation (3, 11, 19). In addition, hematological changes similar to those produced by S. aureus infection have been described following total sleep deprivation (16).

In contrast to the effects of viable S. aureus, inoculation with the same number of heat-killed organisms did not alter sleep patterns in rabbits. However, sleep patterns were altered after the administration of a hundredfold-higher dose of killed organisms. These changes were qualitatively similar to those induced by viable S. aureus but occurred with a much shorter latency and duration. These differences in the time courses of sleep responses following inoculation with killed or viable S. aureus are probably related to differences in host immune responses or in the availability of MPs. Injection of large doses of killed bacteria would immediately expose the animal to both a large antigenic load and a relatively high dose of MPs contained within cell wall peptidoglycan. Indeed, after injection of purified MPs, the time courses of sleep responses are similar to those observed after injection of killed S. aureus (25). In contrast, when live bacteria are administered, cellular division occurs in vivo, and bacterial numbers gradually increase. Inhibition of the in
vivo multiplication of *S. aureus* with antibiotic (cephalothin) treatment attenuates the magnitude and duration of *S. aureus*-induced effects but does not alter the time of onset, thus providing further indirect evidence that differences in sleep responses may be related to the course of the host immune response.

A potential relationship between infectious disease and sleep has previously been suggested on the basis of observations that several immune response modifiers, including MPs and IL-1, are also potent somnogens (22, 23, 27). These substances alter SWS in several species by increasing the time spent in SWS and by enhancing the amplitudes of EEG slow waves during sleep and the duration of individual bouts of SWS (reviewed in references 21 and 22). MPs have also been reported to inhibit REMS, although this effect varies depending on the species tested and the dose administered (21, 29). These effects of MP administration are thus qualitatively similar to the effects we have observed following infection with viable *S. aureus*, a bacterium containing MPs in its cell wall (10).

Although mammalian organisms do not synthesize MPs de novo (20), it has been suggested that MPs may be obtained from exogenous sources and then perhaps chemically modified in vivo (1, 18). Indeed, the passage of muramyl dipeptide from the intestinal lumen into the blood has been described (J. R. Pappenheimer and K. E. Zich, J. Physiol. (London) 371:138P, 1986), and several body tissues contain MPs (26, 39). Mammalian macrophages possess surface receptors for MPs (35, 36), contain the enzymes necessary to cleave MPs from bacterial cell walls (38), and process bacterial cell walls to produce and release somnogenically active substances of low molecular weight (L. Johannsen, J. Wecke, and J. M. Krueger, Soc. Neurosci. Abstr. 13:261, 1987). The processing of bacterial cell walls by macrophages may be a normal daily occurrence as well as an early event in the initiation and amplification of the immune response. It is therefore possible that MPs play a role in mammalian physiology, particularly during periods of bacterial infection, when MP availability would be high because of the presence of abnormally large numbers of infectious organisms.

MPs are known to induce the in vivo production of another putative somnogen, IL-1 (27, 32, 33). Macrophages stimulated by bacteria and bacterial cell wall products are also known to release IL-1 (4, 7). IL-1 mediates many of the acute-phase reactions that accompany infectious disease, and elevated levels of IL-1-like activity have been reported in the circulation of febrile patients with bacterial infections (5). Moreover, levels of IL-1-like activity in both plasma and cerebrospinal fluid increase during sleep (30; F. A. Lue, M. Bail, R. Gorczyński, and H. Moldofsky, Sleep Res. 16:51, 1987) and may also increase during and after sleep deprivation (H. Moldofsky, F. Lue, J. Davidson, J. Jephthah-Ochola, K. Karayanniotis, P. Saskin, and R. Gorczyński, J. Leukoc. Biol. 42:602, 1987). Sleep patterns characterized by increased SWS and decreased REMS as well as increased plasma IL-1-like activity have also been reported in humans following prolonged exercise (6, 34). Such observations suggest that the in vivo release of IL-1 may also play a role in the enhancement of sleep during states of infectious disease.

Relatively little research has been performed on sleep during states of infectious disease, despite the common subjective experiences of lassitude or sleepiness under such conditions. Studies in the literature have not addressed this question in a carefully controlled manner with standard infectious challenges. For example, in human infants, mild upper-respiratory-tract infections, which were associated with rhinitis but not with fever, did not alter sleep state proportions or total sleep time (12), and only 4 of 14 adults with fever caused by a variety of medical conditions had abnormal EEGs and enhanced SWS (28). Our data demonstrate that sleep is sequentially enhanced and suppressed during bacterial disease in rabbits and thereby indicate that the time at which sleep is evaluated relative to the time of infectious challenge is a crucial consideration for the detection of consistent changes in sleep patterns during illness. The severity of the disease process may also influence the type of sleep changes that occur. Abnormal EEG patterns have been associated with a number of experimental infectious encephalitides (2, 13, 14); however, in these studies, the central nervous system inflammation and damage associated with the infectious challenge are likely to have been responsible for the abnormal EEG activity that was observed. Indeed, historically the study of central nervous

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**TABLE 2.** Comparison of sleep patterns in animals that died from or survived inoculation with viable *S. aureus*

<table>
<thead>
<tr>
<th>Time postinoculation (h)</th>
<th>% of time spent in SWS</th>
<th>Slow-wave amplitude (% of control)</th>
<th>SWS bout length (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Group 1</td>
<td>Group 2</td>
<td>Group 1</td>
</tr>
<tr>
<td>2</td>
<td>38 ± 5</td>
<td>36 ± 3</td>
<td>102 ± 1</td>
</tr>
<tr>
<td>4</td>
<td>56 ± 6</td>
<td>54 ± 3</td>
<td>108 ± 2</td>
</tr>
<tr>
<td>6</td>
<td>66 ± 6</td>
<td>59 ± 6</td>
<td>110 ± 7</td>
</tr>
<tr>
<td>8</td>
<td>61 ± 8</td>
<td>70 ± 4</td>
<td>100 ± 4</td>
</tr>
<tr>
<td>10</td>
<td>41 ± 12</td>
<td>75 ± 4a</td>
<td>86 ± 5</td>
</tr>
<tr>
<td>12</td>
<td>34 ± 8</td>
<td>70 ± 5a</td>
<td>81 ± 3</td>
</tr>
</tbody>
</table>

* There were significant differences between animals that died (group 1, n = 6) and animals that survived (group 2, n = 18) (P < 0.01).

**TABLE 3.** Comparison of clinical indices in animals that died from or survived inoculation with viable *S. aureus*

<table>
<thead>
<tr>
<th>Time postinoculation (h)</th>
<th>Temp (°C)</th>
<th>Cortisol (µg/dl)</th>
<th>Neutrophils (% of control)</th>
<th>Lymphocytes (% of control)</th>
<th>nRBCs (no./100 WBCs)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Group 1</td>
<td>Group 2</td>
<td>Group 1</td>
<td>Group 2</td>
<td>Group 1</td>
</tr>
<tr>
<td>0</td>
<td>39.0 ± 0.2</td>
<td>38.9 ± 0.1</td>
<td>3.0 ± 0.1</td>
<td>3.6 ± 0.5</td>
<td>100</td>
</tr>
<tr>
<td>6</td>
<td>40.5 ± 0.3</td>
<td>40.0 ± 0.1a</td>
<td>13.5 ± 1.3</td>
<td>9.4 ± 0.8a</td>
<td>130 ± 22</td>
</tr>
<tr>
<td>12</td>
<td>40.2 ± 0.4</td>
<td>40.7 ± 0.1a</td>
<td>16.6 ± 1.3</td>
<td>13.1 ± 1.2</td>
<td>88 ± 25</td>
</tr>
</tbody>
</table>

* There were significant differences between animals that died (group 1, n = 6) and animals that survived (group 2, n = 18) (P < 0.03).
system lesions produced during viral infections led Economo to describe sleep as an active process mediated by specific brain regions (8). Our results indicate that increased SWS accompanies the early stages of an infectious challenge, with a pattern resembling that observed during the recovery sleep that follows sleep deprivation. Furthermore, our observations of different patterns of sleep in animals that develop neuropathia and successfully respond to the bacterial challenge, as compared with patterns observed in animals that become neuropenic and eventually succumb to the infection, suggest that sleep may provide a prognostic indicator under some conditions and further imply that sleep may serve an adaptive function in combating infectious disease.

ACKNOWLEDGMENTS

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LITERATURE CITED