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Nitric oxide inhibition abolishes sleep-wake differences in cerebral circulation

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Zoccoli, G., D. A. Grant, J. Wild, and A. M. Walker. Nitric oxide inhibition abolishes sleep-wake differences in cerebral circulation. *Am J Physiol Heart Circ Physiol* 280: H2598–H2606, 2001.—Nitric oxide (NO), being produced by active neurones and also being a cerebral vasodilator, may couple brain activity and blood flow in sleep, particularly during active sleep (AS), which is characterized by widespread neural activation and markedly elevated cerebral blood flow (CBF) compared with quiet wakefulness (QW) and quiet sleep (QS). This study examined CBF and cerebral vascular resistance (CVR) in lambs ($n = 6$) during spontaneous sleep-wake cycles before and after infusion of N^{ω}-nitro-L-arginine (L-NNA), an inhibitor of NO synthase. L-NNA infusion produced increases in CVR and decreases in CBF during all sleep-wake stages, with the greatest changes occurring in AS (ΔCVR, 88 ± 19%; ΔCBF −24 ± 8%). The characteristic CVR and CBF differences among AS, QS, and QW disappeared within 1–3 h of L-NNA infusion, but had reappeared by 24 h despite persisting cerebral vasoconstriction. These experiments show that NO promotes cerebral vasodilatation during sleep as well as wakefulness, particularly during AS. Additionally, NO is the major, although not sole, determinant of the CBF differences that exist between sleep-wake states.

N^{ω}-nitro-L-arginine, cerebral blood flow; cerebral vascular resistance; lamb

NITRIC OXIDE (NO), a powerful vasodilator, was initially identified as an endothelium-derived relaxing factor (15), but is now known to be also produced in the brain by perivascular nerves, glia, and active neurones, thereby having the potential to play widespread roles in control of the cerebral circulation (1, 24). Several studies indicate that NO contributes significantly to the maintenance of resting cerebrovascular tone and plays an important role in the increase in cerebral blood flow (CBF) elicited by elevation of arterial P_{CO_2} (see Ref. 24). Although conflicting results have been reported, NO also contributes to the vasodilatation elicited by systemic hypoxia (21, 41) and by hypotension (28, 47). NO also appears to be an important mediator for the coupling of CBF to neuronal activity and metabolism, a process that is fundamental to CBF regulation (23, 33). Involvement of NO in flow-metabolism coupling is supported by the observation that cortical vasodilatation produced by electrical stimulation of parallel fibers in the cerebellum is attenuated by inhibition of NO synthase (NOS), the enzyme that produces NO from L-arginine (22). Moreover, inhibition of NOS attenuates the cerebral vasodilatation that normally accompanies sensory stimulation (4, 37).

Sleep is markedly heterogeneous in terms of both cerebral neural activity and circulatory performance, with the most prominent circulatory feature being the major increase of CBF of active sleep [AS, also referred to as rapid eye movement (REM) sleep]. All species so far examined experience increases of CBF in AS to levels that substantially exceed those of quiet sleep [QS, also referred to as non-rapid eye movement (NREM) sleep] (see Ref. 14). Perhaps surprisingly, given the lesser consciousness of AS, the CBF levels also exceed those in wakefulness (18, 42, 52). A fall in cerebral vascular resistance (CVR) underpins the elevated level of CBF at the onset of AS, because the accompanying changes in blood pressure are variable among species (14, 17, 18). Commonly superimposed upon the tonic CBF increase of AS are further phasic blood flow surges occurring in association with transient arterial pressure increases (18). Although the exact mechanisms responsible for the tonic increases in CBF during AS are uncertain, this sleep state is characterized by overall increases in neuronal activity (44) and in oxygen and glucose metabolism (see Ref. 14), suggesting that flow-metabolism coupling may be the primary underlying factor.

As yet no published study has examined the role that NO might play in regulating CBF in sleep, despite the many neural, metabolic, and cardiorespiratory differences between sleep states that might modify NO production, or its actions, and so effect changes in the cerebral circulation. We hypothesized that NO has an important vasodilatory role, particularly in the major fall in CVR and the large increase of CBF that characterize AS. In a test of our hypothesis, we sought to determine whether NO synthesis is essential for the

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maintenance of the sleep-state dependent differences in CBF that characterize spontaneous sleep in the young lambs. We employed a new method of recording CBF that is rapidly responding and so well suited to accurately record the markedly variable CBF levels that characterize AS (18).

METHODS

Six newborn lambs (Merino/Border-Leicester cross) were separated from their ewes within 24 h of birth and were housed within a Plexiglas cage. The lambs were taught to feed from a nipple connected to a continuous supply of lamb milk replacer (Veanavite; Shepparton, Australia), and they gained weight normally. Once feeding independently, each lamb was surgically prepared for chronic study. All surgical and experimental procedures were performed in accordance with the guidelines of the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes established by the National Health and Medical Research Council of Australia and were approved by the Monash Medical Centre Committee on Ethics in Animal Experimentation.

Surgery and experimental procedures. Each lamb was anesthetized (halothane 1–2%, nitrous oxide 60%, and oxygen 38–39%) and then instrumented for study with the use of sterile surgical techniques. A transit-time ultrasonic flow probe (2 mm diameter, Transonic Systems; Ithaca, NY) was positioned around the superior sagittal sinus as previously described (17, 18) to record CBF. In brief, a 2 cm × 2 cm section of the skull overlying the intersection of the lambdoid and sagittal sutures was removed to access the superior sagittal sinus. The flow probe was carefully positioned around the superior sagittal sinus, taking care not to damage neural or vascular tissue. A rigid cap of dental acrylic was formed over the probe to stabilize it and to replace the section of skull that had been removed. This technique provides a simple, quantitative, and beat-by-beat measurement of CBF and has been validated for use on the sagittal sinus of the lamb (18).

Nonocclusive catheters (0.86 mm id 1.52 mm od) were inserted into the carotid artery, for blood pressure monitoring (P_{ca}) and blood sampling, and into the jugular vein for drug infusion. A catheter (1.57 mm id, 2.41 mm od) was also positioned under the dura to record intracranial pressure (P_{ic}). To determine behavioral states of wakefulness, QS, and AS, pairs of Teflon-coated stainless steel wires were implanted on the parietal cortex (electrocorticogram, ECoG), at the inner and outer canthus of the left eye (electrooculogram, EOG), and in the dorsal musculature of the neck (nuchal electromyogram, EMG_n) (18). Quiet wakefulness (QW) was identified when the lamb was lying down, when the ECoG displayed a pattern of low-voltage and high-frequency activity, and when eye movements and EMG_n tone were present. In QS, the ECoG displayed a pattern of high-voltage and low-frequency activity, eye movements were absent, and EMG_n tone was reduced compared with that in QW. During AS the ECoG displayed a pattern of low-voltage and high-frequency activity, rapid eye movements were present, and EMG_n tone was absent.

Conditions of study. After a minimum of 72 h postoperative recovery, the lambs were studied on 2 consecutive days. During the study periods, the lamb's cage was partitioned to prevent the lamb from turning around while still allowing freedom to move forward and backward and to stand up and lie down. Food was available to the lambs throughout the study, and room temperature was maintained between 22° and 25°C.

The flow probe was connected to the flowmeter (model T101 Ultrasonic Blood Flowmeter, Transonic Systems), which, along with the electrodes, was connected to an amplifier and signal conditioner (Cyberamp 380, Axon Instruments; Foster City, CA). Electrophysiological signals were filtered with the signal conditioner (0.3–80 Hz, 0.3–80 Hz, 30–80 Hz, for ECoG, EOG, and EMG_n, respectively). Vascular and intracranial catheters were connected to calibrated strain-gauge manometers (Cobe CDX III, Cobe Laboratories; Lakewood, CO), which in turn were connected to the signal conditioner. All pressures were referenced to the midthoracic level when the lamb was lying down. Pressure and flow signals were low-pass filtered at 100 Hz and, along with the electrophysiological signals, they were recorded on a thermal chart recorder (model 7758A, Hewlett-Packard; Waltham, MA) and simultaneously stored on a computer (486 DX/50) at a sampling rate of 200 Hz, using an analog-digital converting board (model 4801A, ADAC; Woburn, MA) and acquisition software (CVSOFT Data Acquisition and Analysis Software, Odessa Computer Systems; Calgary, Canada).

Experimental protocol. On the day of experiment, CBF, P_{ca}, P_{ic}, ECoG, EOG, and EMG_n were recorded for a 3-h baseline period (control) while lambs spontaneously cycled between sleep and wakefulness. The lambs were then given a step-wise infusion of N^{ω}-nitro-L-arginine (L-NNA), a nonselective inhibitor of NOS, as a loading dose (25 mg/kg over 70 min) and then studied for a 3-h period (L-NNA infusion) while L-NNA was infused continuously (20 $mg \cdot kg^{-1} \cdot h^{-1}$, for a total dose of 85 mg/kg). Subsequently, the infusion was stopped, and data were recorded for a further 3-h recovery period (recovery). Finally, to assess the lasting effects of NOS inhibition on the cerebral circulation, we studied the lambs for a final 3-h period on the day following the L-NNA infusion (24 h post-L-NNA).

Data analysis and statistics. After the study was completed, the stored physiological signals were carefully reviewed to detect and reject artifacts and were analyzed second by second using the analysis component of CVSOFT. Averages for blood pressures, blood flow, and heart rate (HR) were calculated for each minute of the control, infusion, 3-h postinfusion, and 24-h postinfusion periods. Cerebral perfusion pressure (CPP) was calculated as the difference between P_{ca} and P_{ic}. CVR was calculated as the ratio of CPP and CBF (CVR = CPP/CBF). Mean values for each sleep-wake state were calculated for each animal in each of the experimental periods, and the data were normalized for each lamb to the mean values recorded during QW in the control period. A total of 77 QS epochs were collected during control, 87 during L-NNA, 25 during 3 h post-L-NNA, and 80 during 24 h post-L-NNA. The corresponding totals for AS were 24, 22, 17, and 29, respectively. Data from a contiguous period of QW were extracted to contrast with each sleep-state value. Nonparametric, repeated measures analysis of variance on ranks (Friedman ANOVA) was used to determine whether differences existed among control, infusion, 3-h, and 24-h postinfusion periods, and repeated for comparisons between sleep-wake states. Nonparametric testing was employed because some data were not normally distributed. Differences that were detected by the ANOVA were isolated using a Student-Newman-Keuls test. Percent changes were calculated by normalizing data to the control values in each behavioral state and contrasted with Friedman ANOVA.

Statistical testing was performed using standard procedures (Excel, Microsoft, http://www.microsoft.com; SigmaStat v2.03, SPSS, http://www.spss.com) with a P value of ≤ 0.05 considered to be statistically significant. To perform ANOVA using a balanced design, estimates for missing val-

ues (2 lambs in the 3-h post-L-NNA infusion period and 2 lambs in the 24-h post-L-NNA infusion period) were calculated for the comparisons between control infusion and postinfusion periods (43). The estimated data were employed solely for the purposes of the ANOVA and are not included in Table 1 nor any figure. Data are means ± SD in the text and table and are means ± SE in the figures, with *n* indicating the number of animals.

RESULTS

Control conditions. Arterial blood gases and pH (means ± SD) in QW were similar to those we have previously recorded in healthy lambs (17, 18) (pH = 7.43 ± 0.03, arterial P_{O_2} = 100 ± 6 mmHg, arterial O_2 saturation = 94 ± 2%, arterial P_{CO_2} = 40 ± 3 mmHg, hemoglobin = 8.4 ± 0.7 g/dl, and base excess = 2 ± 2 mM). As previously found (17, 18), there were no arterial blood gas and pH differences between sleep-wake states in the lambs nor were there significant differences arising from NOS inhibition.

Table 1. *Cardiovascular variables during sleep-wake cycle recorded in newborn lambs before (control), during (infusion), 3 h, and 24 h after L-NNA infusion*

	n	Quiet Wakefulness	Quiet Sleep	Active Sleep
Control	6			
CBF, ml/min		23 ± 3	20 ± 4†	26 ± 2*†
CVR, mmHg·ml^{-1}·min		2.6 ± 0.4	3.0 ± 0.6†	2.3 ± 0.3*†
P_{ca}, mmHg		73 ± 2	72 ± 3	72 ± 4
P_{ic}, mmHg		15 ± 2	15 ± 3	13 ± 3
CPP, mmHg		57 ± 3	57 ± 2	59 ± 4
HR, beats/min		170 ± 20	171 ± 19	167 ± 13
L-NNA infusion	6			
CBF, ml/min		19 ± 4‡	17 ± 4†‡	20 ± 4*‡
CVR, mmHg·ml^{-1}·min		4.1 ± 0.8‡	4.6 ± 1.0‡	4.3 ± 1.1‡
P_{ca}, mmHg		90 ± 7‡	87 ± 8‡	96 ± 14‡
P_{ic}, mmHg		15 ± 3	13 ± 3	12 ± 4
CPP, mmHg		75 ± 5‡	74 ± 5‡	84 ± 13*†‡
HR, beats/min		122 ± 15‡	121 ± 12‡	111 ± 11*†‡
3 h Post L-NNA infusion	4			
CBF, ml/min		18 ± 4‡	17 ± 4†‡	19 ± 3*‡
CVR, mmHg·ml^{-1}·min		3.8 ± 0.7‡	4.2 ± 0.9‡	4.1 ± 0.7‡
P_{ca}, mmHg		79 ± 4‡	78 ± 5‡	86 ± 14‡
P_{ic}, mmHg		13 ± 2	11 ± 3	11 ± 3
CPP, mmHg		66 ± 2‡	67 ± 3‡	75 ± 12‡
HR, beats/min		132 ± 20‡	129 ± 14‡	116 ± 14‡
24 h Post L-NNA infusion	4			
CBF, ml/min		20 ± 5‡	18 ± 5†‡	24 ± 6*†
CVR, mmHg·ml^{-1}·min		3.3 ± 0.9	3.7 ± 0.9†‡	2.9 ± 0.8*†‡
P_{ca}, mmHg		75 ± 2‡	76 ± 2‡	76 ± 2
P_{ic}, mmHg		14 ± 4	13 ± 2	11 ± 3
CPP, mmHg		61 ± 2‡	63 ± 1‡	65 ± 4‡
HR, beats/min		163 ± 10	163 ± 14‡	156 ± 6‡

Values are means ± SD; *n*, number of lambs. L-NNA, N^{ω}-nitro-L-arginine; CBF, cerebral blood flow; CVR, cerebral vascular resistance; P_{ca}, carotid arterial pressure; P_{ic}, intracranial pressure; CPP, cerebral perfusion pressure (P_{ca} minus P_{ic}); HR, heart rate. *$P \leq 0.05$ vs. quiet sleep value; †$P \leq 0.05$ vs. quiet wakefulness value; ‡$P \leq 0.05$ vs. value in the same sleep-wake state during the control period.

Arterial blood pressure and P_{ic} did not differ between any of the sleep-wake states during the control period (Table 1). As a result, CPP did not differ between behavioral states in the control period. There were also no differences of HR between states. However, significant behavioral state differences did exist in CBF and CVR in the control period (Fig. 1, Table 1). CBF was significantly greater in AS than in QS and QW ($P \leq 0.05$) and significantly higher in QW than in QS ($P \leq 0.05$). In addition, CVR was significantly lower in AS than in QS ($P \leq 0.05$) and also significantly lower in QW than in QS ($P \leq 0.05$).

Acute effects of L-NNA infusion. L-NNA infusion significantly increased P_{ca} ($P \leq 0.05$) and CPP ($P \leq 0.05$) in all behavioral states (Table 1, Fig. 2). The hypertension induced by L-NNA infusion was rapid in onset, reached a maximum at the end of the injection of the loading dose of L-NNA, and remained elevated during the L-NNA infusion period (Fig. 3). CPP rose to higher levels in AS than in QS ($P \leq 0.05$) and in QW ($P \leq 0.05$). Accompanying the increase in blood pressure, HR fell significantly ($P \leq 0.05$) in all sleep-wake states, with the lowest level occurring in AS (Table 1). L-NNA infusion was associated with a significant decrease in CBF ($P \leq 0.05$). There was also a significant increase in CVR ($P \leq 0.05$) in each behavioral state (Table 1, Fig. 2) and the sleep-state-dependent differences in CVR recorded during the control period disappeared. With the loss of behavioral state-related differences in CVR, the differences in CBF were largely attenuated, although CBF remained slightly higher in AS than in QS ($P \leq 0.05$), reflecting the higher CPP of AS ($P \leq 0.05$). CBF also remained slightly higher in QW than in QS ($P \leq 0.05$).

During the 3-h post-L-NNA period, a tendency was evident for P_{ca} and CPP to return toward control values (Fig. 2), although in all behavioral states these pressures remained significantly greater than control levels ($P \leq 0.05$ and $P \leq 0.05$, respectively). HR ($P \leq 0.05$) also remained lower than the control value (Table 1). CBF in AS did not differ from the level in QW and only slightly exceeded the level in QS ($P \leq 0.05$). Moreover, as during the L-NNA infusion, the normal sleep-wake differences in CVR that were present in the control period were abolished 3 h post-L-NNA.

Comparison between sleep-wake states of the extent of the circulatory changes following L-NNA administration revealed that AS was the most affected. The greater effect of L-NNA in AS was most marked for CVR, for which the AS value increased by 88 ± 19% (means ± SD) compared with 52 ± 13% in QS ($P \leq 0.05$) and 57 ± 18% in QW ($P \leq 0.05$). Differences were also significantly greater for CBF [−24 ± 8% in AS vs. −14 ± 6% in QS ($P \leq 0.05$) and −17 ± 8% in QW ($P \leq 0.05$)]. Larger changes of P_{ca} in AS (32 ± 14% in AS vs. 21 ± 6% in QS and 24 ± 9% in QW) and of CPP in AS (43 ± 17% in AS vs. 30 ± 6% in QS and 31 ± 9% in QW) were also evident, but failed to achieve significance.

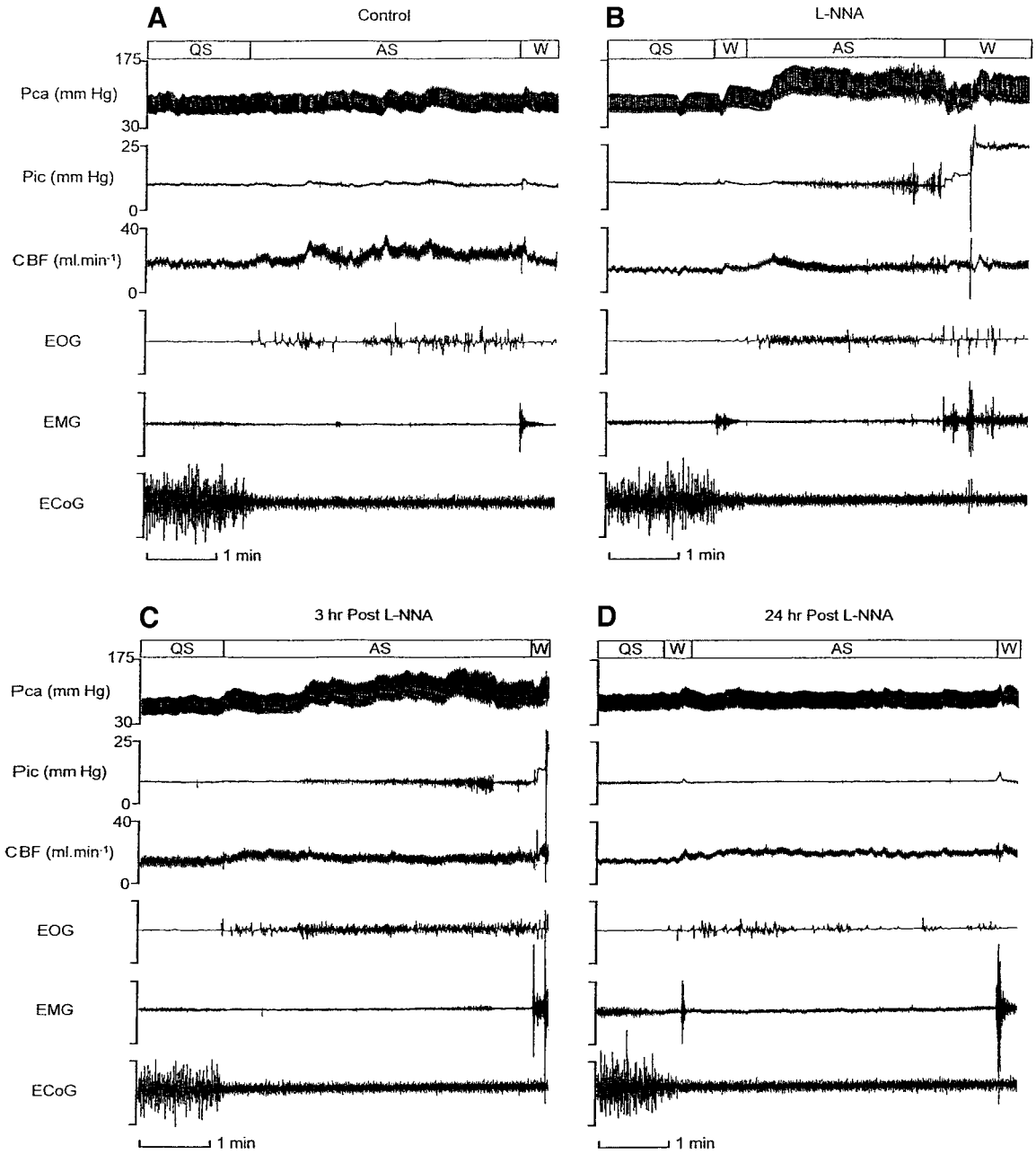

Fig. 1. Physiological recording from a sleeping lamb under control conditions (*A*, Control), during N^{ω}-nitro-L-arginine (L-NNA) infusion (*B*), 3 h after L-NNA infusion (*C*, 3 h Post-L-NNA), and 24 h after L-NNA infusion (*D*, 24 h Post-L-NNA). Control data demonstrate the behavioral state-dependent changes in cerebral blood flow (CBF) that accompanies the transition from quiet sleep (QS) to active sleep (AS), with CBF increasing substantially upon entering AS, then declining on arousal to wakefulness (W). The higher CBF of AS corresponds to a decrease in cerebral vascular resistance (CVR), because arterial blood pressure (P_{ca}) and cerebral perfusion pressure (not shown) remain essentially unchanged between sleep-wake states. L-NNA infusion significantly increased P_{ca} and was also associated with a decrease in CBF and therefore an increase in CVR. Notably, the immediate effect of L-NNA infusion was to eliminate the sleep state-dependent differences in CBF and CVR of the control period (*A*), because CBF no longer increased significantly during AS, despite very large increases of P_{ca} (*B* and *C*). However, the normal increases of CBF and CVR are again evident on the day after L-NNA infusion (*D*). Note the decreased pulsatility of the CBF measurement after L-NNA infusion (*B*-*D*) compared with normal (*A*). The increase in pressure evident at the end of *B* occurred as the animal stood up following the transition to the awake state, therefore increasing the hydrostatic pressure gradient between the catheter tip and the pressure transducer. ECoG, electrocorticogram; EMG_n, electromyogram of the nuchal (neck) muscles; EOG, electrooculogram.

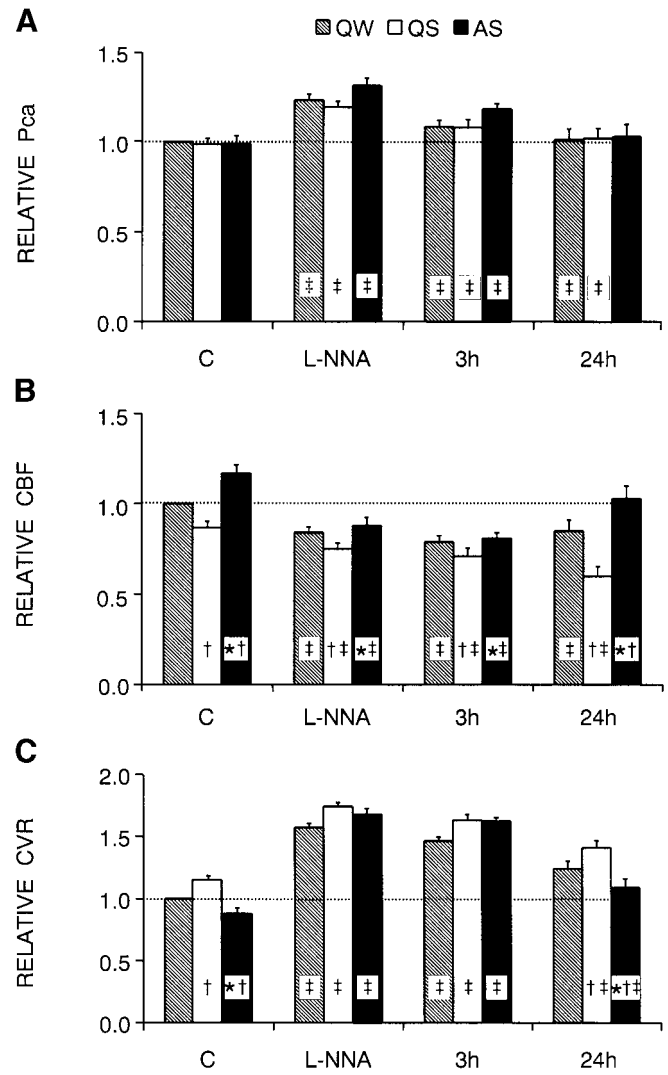

Fig. 2. Average data (means ± SE, $n = 6$) recorded under control conditions (C), during L-NNA infusion (L-NNA), 3 h after L-NNA infusion (3 h), and 24 h after L-NNA infusion (24 h). Note the substantial increases in P_{ca} (*A*), decreases of CBF (*B*), and increases of CVR that occurred during L-NNA infusion and continued for the 3-h period after the infusion. Although P_{ca} had essentially returned to control levels 24 h after the L-NNA infusion, CBF remained significantly depressed (QW and QS) and CVR remained significantly elevated (QS and AS) with respect to the control period. Importantly, the normal sleep state-dependent differences in CBF and CVR that were evident in the control period and eliminated by L-NNA infusion were again evident 24 h following infusion despite persisting depression of CBF and elevation of CVR. *$P \leq 0.05$ vs. QS value; †$P \leq 0.05$ vs. QW value; ‡$P \leq 0.05$ vs. values in the same sleep-wake state during the control period.

Prolonged effects of L-NNA infusion. Twenty-four hours after the L-NNA infusion was completed, major changes remained evident in the cerebral circulation, with the overall level of CBF being depressed and the overall level of CVR being elevated compared with control levels, as shown in Fig. 2. In the period 24-h post-L-NNA infusion, CBF remained significantly lower than the control flow in QS and QW ($P \leq 0.05$, $P \leq 0.05$, respectively), and CVR remained significantly greater than control in QS and AS ($P \leq 0.05$, Table 1). By contrast, both P_{ca} and CPP had largely recovered to control levels, although P_{ca} remained slightly above the control level in QS ($P \leq 0.05$) and CPP remained slightly elevated in all states ($P \leq 0.05$). Significantly, the behavioral state-related differences in these parameters that had been evident in the control period and that were abolished by L-NNA infusion reemerged 24-h post-L-NNA (Fig. 2). Thus CBF in AS had recovered to control levels and was again significantly greater than in QS ($P \leq 0.05$) and also greater than in QW ($P \leq 0.05$); moreover, CBF was again greater in QW compared with QS ($P \leq 0.05$). Likewise, CVR in both AS and QW was again significantly lower than in QS ($P \leq 0.05$).

Sleep patterns. Sleep characteristics in the control period were similar to those previously observed in

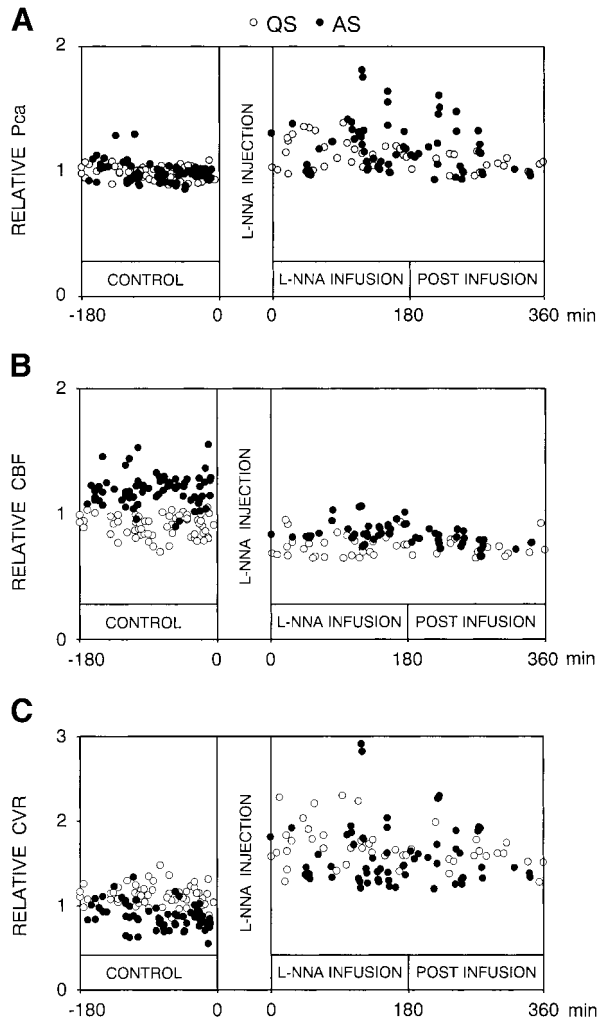

Fig. 3. P_{ca} (*A*), CBF (*B*), and CVR (*C*) recorded from lambs ($n = 6$) in spontaneous sleep-wake states during consecutive experimental periods of *1*) control, 180 min before L-NNA injection (25 mg/kg); *2*) L-NNA, 180 min of L-NNA infusion (20 $mg \cdot kg^{-1} \cdot h^{-1}$ to a total dose of 60 mg/kg); and *3*) postinfusion, a recovery period of 180 min. Values are averaged over 1 min of recording and normalized for each animal to the mean value recorded in QW during the control period. Note that the normal sleep-related difference of CBF (AS > QS), as well as the difference of CVR (AS < QS), is abolished after L-NNA infusion. Also note the significantly increased variability of P_{ca} and CVR that follows L-NNA.

newborn lambs in our laboratory (27). Sleep episodes (epochs per 3 h, means ± SD) were 4.3 ± 2.7 for AS and 12.4 ± 3.6 for QS. Epoch duration (min) averaged 2.9 ± 1.5 in AS and 3.1 ± 1.3 in QS, and total sleep time (% total time) averaged 9.3 ± 6.5 for AS and 20.7 ± 8.6 for QS. After L-NNA administration, lambs continued to exhibit a pattern of cyclic alternation between sleep-wake states, and no feature of AS was affected by L-NNA infusion. For QS, the number of episodes per 3 h was unchanged from control values, but the duration of epochs (min) tended toward lower values during L-NNA infusion (2.3 ± 0.9), 3 h post-L-NNA (1.9 ± 1.0), and 24 h post-L-NNA (2.0 ± 0.2). Time in QS (% total time) fell significantly ($P \leq 0.05$) to about one-half the control level during L-NNA infusion (10.3 ± 7.6), 3 h post-L-NNA (7.8 ± 7.5), and 24 h post-L-NNA (12.9 ± 4.2).

DISCUSSION

Our study is the first to examine the effect of inhibiting NO production upon the cerebral circulation in sleep. It has revealed two major and apparently distinct roles for NO in the cerebral circulation in sleep. First, NO has a primary vasodilating role in setting the overall level of cerebral perfusion in sleep, as it does in wakefulness. Second, NO is the major (though not sole) determinant of the large CBF and CVR differences that exist between sleep-wake states.

Outstanding among the circulatory features of sleep is the increase of CBF with respect to QW that occurs during AS and the decrease that accompanies QS, a pattern that is common to the human and many other species, including the lamb as we confirm in this study (14, 18, 42). The effect of this CBF variation is to produce a hierarchy in the magnitude of blood flow between behavioral states, with AS > QW > QS. Because blood pressure differences between sleep-wake states are small (17), the hierarchy of blood flow is mirrored by a hierarchy of CVR (AS < QW < QS), with the cerebral circulation in AS being significantly dilated compared with other sleep-wake states (17).

Our experimental design employed a new technique that continuously records the blood flow in the superior sagittal sinus. This technique provides a simple quantitative measurement of CBF that is linearly related to arterial inflow (18). Importantly, for behavioral studies where CBF may vary rapidly, this measurement is continuous and responds rapidly (within 1 heartbeat) to variations in cerebral perfusion pressure (18). Cerebral regions contributing to the flow measurement are the entire frontal lobe and the superior portion of the anterior parietal lobe, representing 35% of the total brain mass of the lamb (18). Because all brain regions so far examined exhibit an increased CBF in AS (14), the general pattern of CBF changes that we observed in sleep reflects that occurring throughout the brain. In fact, the CBF increases we report in AS may underestimate the extent of changes occurring in the remainder of the brain, because CBF increases to a greater extent in the cerebellum and brain stem than in the cerebrum (14), from which sagittal sinus flow is derived.

Arterial P_{CO_2} differences made no contribution to the sleep-wake CBF differences, because none were present in these lambs. In adult animals and humans, a slight hypercapnia (2–3 mmHg) develops during non-REM sleep but arterial P_{CO_2} is usually similar in REM and wakefulness in the absence of sleep apnea. When arterial P_{CO_2} changes are found, they account for only a trivial proportion (<5%) of the increase of CBF in REM (14).

We chose the potent NOS inhibitor L-NNA because it offers advantages over other inhibitors in studying the cerebral circulation (10). We have demonstrated using dose-response analysis in fetal lambs that L-NNA reaches a plateau in its effects upon the circulation at a dose of 25 mg/kg, and that it is reversible with infusion of L-arginine (9), because it is in the circulation in other species (12, 31). A higher L-NNA dose of 85 mg/kg was used in this study because the effectiveness of lower doses has been questioned in newborn animals (13).

The immediate effects occurring within 3 h of inhibiting NO production with L-NNA infusion were to produce a prompt increase of CVR and decrease of CBF, effects that were evident in all sleep-wake states. Our observations confirm previous studies in awake animals and humans that have suggested a vasodilatory role for NO in the cerebral circulation under basal conditions (3, 50, and see Ref. 11 for a review) a finding that has not been universal (26, 35). The confirmatory studies reported decrements of CBF between 15 and 45%, a range that incorporates the 17% decrease that we found to occur in the lambs when they were awake. L-NNA infusion was accompanied by a significant increase in arterial blood pressure, just as in anesthetized or awake animals and humans (see Ref. 48 for a review), so that significant increases in CVR underpin CBF decreases. Reported increases of vascular resistance following NOS inhibition in wakefulness range between 50 and 115% (3, 12, 31), a range that includes the increase of 57% that we observed in awake lambs.

That the cerebral vasoconstriction that accompanied L-NNA infusion was found in all behavioral states is the first evidence that NO has an important vasodilating role in sleep as well as in wakefulness. Notably, we found that the effects of NOS inhibition on CVR and CBF, although significant in all behavioral states, were much more pronounced for AS than for the other states, particularly QS. For example, the increase in CVR that occurred during L-NNA infusion in AS (88%) was almost twice that occurring in QS. The effect of the greater vasoconstriction of AS compared with QS, and also that of QW, was to abolish the characteristic hierarchy of CVR between sleep-wake states. The degree of CBF differences among sleep-wake states was also significantly reduced, with a small AS-QS difference remaining only because of the greater arterial pressure response to L-NNA infusion in AS. Thus, in addition to its contribution to setting the overall level of blood flow across behavioral states, NO appears to

have a major role in determining the CVR and CBF differences that exist between sleep-wake states, with the influence being greatest in AS.

That NO has a role in setting basal CBF levels in the awake and sleeping newborn is consistent with previous studies in anesthetized newborns. Similar reductions in basal CBF following NOS inhibition are seen in newborns whether anesthetized (14–18%) (19, 40), awake, or in quiet sleep (14–17%) (this study). These reductions are less than those we observed in active sleep (24%) and are at the lower end of the range of values seen in awake, adult animals and humans (15–45%) (3, 11, 50). Taken together, these considerations suggest that NO, while being important in newborn AS, may be especially important in promoting CBF during REM sleep in the adult. Further support for this suggestion is found in the two- to threefold increase in NOS activity of cerebral vessels that occurs with development from newborn to adult life (39).

We ascribe the early vasoconstrictive effect of L-NNA infusion to the specific loss of a vasodilating influence of NO in the cerebral circulation. In these vasodilating actions, increased production of NO per se may act as the vasodilator. In particular, the large CBF increase of AS is accompanied by overall increases in neuronal activity and in oxygen and glucose metabolism, and several studies have suggested that NO mediates this flow-metabolism coupling (2, 11, 32). Thus it is probable that the increase in CBF during AS is due to increased NO production. Alternatively, NO may be "permissive" of the actions of other vasodilators such as low O_2, CO_2, or excitatory amino acids that are partially dependent on NO (2). More studies are required to distinguish between these alternative actions of NO in sleep.

Depression of CBF following L-NNA infusion was unlikely to be secondary to metabolic depression, because NOS inhibition depresses CBF without concomitant depression of cerebral metabolism (25, 38). Moreover, in our study no major sleep or behavioral changes followed L-NNA infusion to signify global depression of central nervous activity, notwithstanding that the brain was placed at risk of ischemia by the significant depression of CBF relative to demand, particularly in AS. Suppression of sleep following nonselective NOS inhibition with L-NAME has been found in some animal studies (7, 29, 30), an effect that has been ascribed to the arousing effects of the accompanying hypertension. However, sleep reduction following NOS inhibition is not a universal finding (6), and others have found increased sleep an effect that may have been due to the sedative effects of NOS inhibitors reducing anxiety and thereby promoting sleep. In the present study, lambs cycled normally between sleep-wake states following L-NNA infusion, and we saw no significant changes in the composition of AS despite CBF reductions being greatest in this state. Animals also entered QS with the same frequency after L-NNA, although we observed a tendency for QS epochs to be shorter and the total time in QS was significantly reduced. As suggested previously, it is possible that the hypertension accompanying L-NNA infusion explains the shortened QS episodes because hypertension is a powerful stimulus for arousal in lambs (20). That AS was unaffected is also in accord the lesser arousability of lambs in AS.

Many cellular sources of NO may contribute to the vascular relaxation in the brain in sleep that we have identified, because L-NNA is a nonselective antagonist of NO synthesis. NO is produced by a family of three NOS isoforms, composed of neuronal (NOS I), inducible (NOS II), and endothelial (NOS III) isoforms, each a potential source of NO for relaxation of cerebral vessels (1, 11, 45). Although the endothelium is the most likely source of NO relaxing cerebral vessels (11), CBF is also reduced by L-NNA in mice lacking NOS III, supporting a role for other constitutively expressed NOS isoforms in producing tonic vascular relaxation in the brain (34). Because CBF is reduced by selective NOS I inhibition (21), neuronal NOS may be an important source of NO linking functional activity with flow (5, 23), i.e., the "flow-metabolism coupler" (10, 16). Moreover, an important role has been demonstrated for NO, or NO-related compounds, in neurotransmission in vasodilator nerves innervating the cerebral vasculature (46). Additionally, NO is colocalized in sympathetic nerves where it counteracts norepinephrine release and contributes to vasodilatation (51), an action of NO that may be particularly important in the cerebral circulation because the brain has an abundant perivascular sympathetic innervation (8).

The persisting NOS inhibition that remained 24 h after termination of the L-NNA infusion offers insight into the specific mechanisms by which NO might contribute to cerebral vasodilation in sleep-wake cycling. Previous studies in conscious animals identified a long-term (48–72 h) NOS inhibition following L-NNA or L-NAME infusion that was made evident by persistently elevated arterial pressure and by unresponsiveness to NO-releasing stimuli (3, 12). Intriguingly, we saw two persisting effects of L-NNA on the cerebral circulation. First, we observed that CVR remained at high levels and CBF stayed significantly depressed for 24 h after L-NNA infusion. Second, despite the persistence of an overall cerebral vasoconstriction and flow depression, we observed the reemergence of sleep-wake cycles in CVR and CBF that are characteristic of normal sleep. We interpret the persisting vasoconstriction to reflect the loss of a fundamental vasodilating action of NO that is critical in determining the basal level of CVR, but unrelated to the specific state of sleep or wakefulness. This appears to be a unique mechanism dependent on NO because the vasoconstriction and flow depression remained uncompensated throughout the 24 h following NOS inhibition. Our interpretation of the reemergence of sleep-wake differences is that other vasodilating influences that change cyclically during sleep can take over the role of NO that was abolished immediately by L-NNA infusion. Thus, in a manner similar to the presence of pial artery vasodilatory responses to acetylcholine in endothelial and neuronal NOS-deficient mutants (36), there ap-

pears to be a redundancy in the modulating effect of NO on the sleep-wake cycling of CVR and CBF. There are a number of powerful vasodilator agonists whose tissue concentrations change rapidly during cerebral activity and metabolism, and which therefore could mediate the circulatory changes in sleep. Among them, O_2, CO_2, and pH, as well glucose, and lactate, might couple blood flow and metabolism (11, 32). These appear unlikely to represent compensatory agents because the recovery of the sleep-wake differences was slow in our study, at least in excess of 3 h and possibly much longer. Rather, the delayed recovery points to a slower process of compensation. Among the possibilities, upregulation of receptor numbers for receptor-mediated vasodilator agonists such as adenosine, autocoids, and calcitonin gene-related peptide (2, 5, 49) deserve consideration. The prostanoid system deserves particular attention because prostaglandins exert a powerful vasodilating influence in the newborn and adult brain (39).

In summary, we have identified two major vasodilating actions of NO on the cerebral circulation in sleep. The first is to underpin the basal level of cerebral blood over all sleep states; this action appears to uniquely involve NO as cerebral flow depression remains uncompensated with prolonged (24 h) inhibition of NO synthesis. The second is to produce the cyclic variations of CBF that accompany transitions between sleep states; in this action NO seemingly has a major, although not sole role as the cyclic blood flow differences that normally accompany transitions between sleep states reemerge in the face of persisting inhibition of NO synthesis. Because this reemergence is slow, we speculate that the restoring molecule or substance is more likely to be a receptor-mediated transmitter (such as adenosine) than a directly acting effector (such as oxygen or K^+).

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