CO₂ microdialysis in retrotrapezoid nucleus of the rat increases breathing in wakefulness but not in sleep

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CO₂ microdialysis in retrotrapezoid nucleus of the rat increases breathing in wakefulness but not in sleep. J. Appl. Physiol. 87(3): 910–919, 1999.—Central chemoreceptors are widespread within the brain stem. We suggest that their function at some sites may vary with the state of arousal. In this study, we tested the hypothesis that the function of chemoreceptors in the retrotrapezoid nucleus (RTN) varies with sleep and wakefulness. In unanesthetized rats, we produced focal acidification of the RTN by means of a microdialysis probe (tip containing the semipermeable membrane — 1-mm length, 240-µm diameter, and 45-nl volume). With the use of a dialysate equilibrated with 25% CO₂, the tissue pH change (measured in anesthetized animals) was 1) limited to within 550 µm of the probe and, 2) at the probe tip, was equivalent to that observed with end-tidal PCO₂ of 63 Torr. This focal acidification of the RTN increased ventilation significantly by 24% above baseline, on average, in 13 trials in seven rats only during wakefulness. The effect was entirely due to an increase in tidal volume. During sleep defined by behavioral criteria, ventilation was unaffected, on average, in 10 trials in seven rats. During sleep, the chemoreceptors in the RTN appear to be inactive, or, if active, the respiratory control system either is not responding or is responding with very low gain. Because ventilation is increased during sleep with all central chemoreceptor sites stimulated via systemic CO₂ application, other central chemoreceptor locations must have enhanced effectiveness.

Central chemoreceptors mediate changes in breathing and blood pressure in response to changes in CO₂ and H⁺ within the brain (14, 16–18, 23). Whereas early work located central chemoreceptors at sites just beneath the ventral medullary surface (14, 16), recent experiments in vitro and in vivo have indicated that they have a widespread distribution within the brain stem (4, 5, 13, 17–19). These locations include the regions superficially just beneath the surface of the ventral lateral medulla, the nucleus tractus solitarius, the locus ceruleus, the midline raphe, and the ventral respiratory group. Why are central chemoreceptors located at so many sites? We hypothesize that some chemoreceptor sites vary in their effectiveness, depending on the state of arousal.

In this study, we focused on one part of the chemosensitive region lying just beneath the rostral ventral medullary surface, the retrotrapezoid nucleus (RTN). We asked whether the response to focal acidification of the RTN differs during wakefulness vs. sleep. The RTN is one of several brain stem sites that communicate with the respiratory control network (1, 6, 7, 17, 18, 30). Destruction of the RTN in anesthetized and decerebrate animals reduces resting ventilatory output, often to apnea, and substantially reduces the ventilatory response to systemic CO₂ stimulation (17, 20). Focal CO₂ stimulation of the RTN in anesthetized animals increases ventilatory output by 25–40% of the change observed with stimulation of all central chemoreceptor sites by systemic CO₂ stimulation (13). The RTN appears to provide two sources of input to the respiratory control network: tonic and chemosensory. However, lesions of the RTN in unanesthetized rats (1) and cooling of the rostral ventrolateral medulla (RVLM), including the RTN in unanesthetized goats (9, 21), result in much less dramatic effects. Ventilation (Ve) at rest during wakefulness is unchanged (1) or decreased slightly (9, 21), and the response to CO₂ is decreased much less in comparison to responses to RTN disruption in anesthetized animals (1, 9, 21). These data suggest that the role of the RTN may vary considerably in different arousal states.

In this study, we used a microdialysis probe (CMA/Microdialysis, Acton, MA) to produce a focal acidosis in the RTN of unanesthetized, unrestrained rats. The probe tip with semipermeable membrane is 1 mm in length and 240 µm in diameter, with a volume of 45 nl. The guide tube and dialysis probe are made of a rigid, sturdy material, allowing their use in a chronic animal. In a separate group of anesthetized rats, we measured the distribution of tissue pH changes during dialysis with this probe. Although we have produced a very focal tissue acidosis with rapid and reversible pH changes in the chemoreceptor regions of anesthetized animals by a CO₂ diffusion pipette (13), this glass pipette has limitations in unanesthetized and unrestrained animals.

METHODS

General Preparation

Anesthetized group. Seventeen male Sprague-Dawley rats (300–450 g) were anesthetized with α-chloralose (60 mg/kg) and urethan (550 mg/kg). The trachea, femoral artery, and vein were cannulated. We paralyzed the rats with Gallamine triethiodide (3 mg/kg) and artificially ventilated them with 100% O₂. The arterial blood pressure and end-tidal PCO₂ were monitored, and rectal temperature was maintained at 37.6°C. Bilateral thoracotomies were performed, and a positive end-expiratory pressure of 3–5 cmH₂O was maintained during the experiment. Both vagi were cut, and the ventral medullary surface was surgically exposed. The phrenic nerve was isolated, cut, and placed on a bipolar electrode for recording.
Phrenic nerve activity was amplified (BMA831 amplifiers, Charles Ward), rectified, and integrated. I. Integrated phrenic nerve amplitude (PNA), arterial blood pressure, and end-tidal PCO2 were recorded on a strip-chart recorder (MFE 1400, MFE).

CO2 microdialysis and pH electrodes. A microdialysis probe (CMA/11, CMA/Microdialysis) with 1-mm length of cuprophane membrane and 0.24-mm outside diameter was placed directly into the RTN region from the ventral surface and was connected to a syringe pump for dialysis. A pH electrode coupled to a potentiometer (model EA920, Orion Research) was placed at various distances from the dialysis probe to monitor the tissue pH change. Each rat received one dialysis probe and one pH electrode. The details of the construction of the pH electrode were described previously (5, 13). To allow comparison of tissue pH among animals, the changes in each animal were normalized to the response of the electrode in vivo to the change in end-tidal CO2 from 4 to 9%. All pH electrodes were calibrated in vitro by using standard buffer solutions at pH values of 4, 6, 7, and 10 before and after the experiment.

Conscious group. Fifteen male Sprague-Dawley rats (300–450 g) were anesthetized with ketamine (100 mg/kg im) and xylazine (20 mg/kg ip). The crown of the skull was shaved, and the skin sterilized with benzocaine and alcohol. The head was placed into a Kopf stereotaxic holder, and a dialysis guide cannula (0.38 mm outer diameter) with a dummy was implanted into the medulla. Each rat received one guide tube. The coordinates for probe placement were 2.2 mm caudal and 1.8 mm lateral from lambda and 10.6–10.8 mm below the dorsal surface. The guide cannula was secured with cranial plastic cement, and the wound sutured. The abdominal surface was shaved, the skin was sterilized, an incision was made through the linea alba, and a sterile telemetry temperature probe (TA-F20, Data Sciences, St. Paul, MN) was placed in the abdominal cavity. The incision was closed, and the animal was allowed to recover for 3–4 days. Of the 15 rats, 9 resulted in successful experiments, 7 with guide tubes in the RTN and 2 with guide tubes placed outside of the RTN region.

CO2 dialysis solution. The artificial cerebrospinal fluid (aCSF) was equilibrated with 1) 5%, 2) 25%, 3) 50%, or 4) 100% CO2. The composition of the aCSF was (in mM) 115 sodium, 3.0 potassium, 2.1 magnesium, 2.2 calcium, 131 chloride, and 26 bicarbonate. The pH of the solution was measured to ensure that the equilibration was reliable.

V̇E measurement. The plethysmograph chamber used in these experiments is similar to the setup described by Jacky (12) and Pappenheimer (22). The analog output of the pressure transducer was recorded on a strip-chart recorder (model 1200, Honeywell) and on tape by using a Vetter Digital system (model 3000A). The animal chamber operates at atmospheric pressure, with the inflow and outflow of inspired gases balanced to prevent hyperventilation. Respiratory data were transferred from the Vetter Digital system to the DataPac III software system. With the use of the DataPac III system, a breath-by-breath analysis was performed with the pressure deflections and the respiratory cycle time for each breath being determined over a 20- to 30-s time period. The data were exported to SigmaPlot 4.0 (Jandel Scientific software) and tidal volume per 100 g body wt., frequency, and V̇E per 100 g body wt were calculated for each breath.

The results for V̇E, tidal volume, frequency, V̇O2, and body temperature in the three groups were compared within any group by a one-way ANOVA. Comparisons of responses to 25% CO2 dialysis between sleep and wakefulness were made by means of a two-way ANOVA (SigmaStat, Jandel Scientific software) with post hoc analysis by Tukey or Bonferroni test when indicated.

Experimental Protocol

Experiments with anesthetized rats. After finishing the surgeries, we placed a CO2 dialysis probe directly into the RTN region of each rat and a pH electrode at a single distance from the probe. The precise locations of the dialysis probe and pH electrode were ascertained during postmortem anatomic examination. All animals were first tested for their responsiveness to inspired CO2 after at least 30 min of recovery from probe and pH electrode placement. The baseline end-tidal CO2 was set just above the apneic threshold, usually at 28 Torr. A CO2 response was determined by increasing the inspired CO2 and monitoring PNA, frequency, and tissue pH at end-tidal CO2 values of 28, 42, 56, and 63 Torr. We allowed at least 30 min for the rat to recover from the CO2 stimulation. Dialysis was then performed over a 15- to 30-min period at a speed of 45 l/min by using aCSF equilibrated with 25, 50, or 100% CO2. The effect on PNA, phrenic discharge frequency, tissue pH, and blood pressure was observed and recorded. When dialysis was completed, another systemic CO2 response test was performed. The pH and phrenic amplitude value at 63 Torr end-tidal PCO2 was used as the maximum value for normalizing the pH and phrenic amplitude CO2 dialysis response data. The phrenic response to CO2 dialysis was normalized to percent baseline.

Chronic experiments.

25% CO2 DIALYSIS GROUP. We dialed each rat during both wakefulness and sleep. The dialysis tube and cannula dead space were taken into account along with the dialysis fluid flow...
rate so that $t = 0$ is the estimated time at which the CO$_2$-equilibrated solution reaches the exchange membrane. The rat was judged to be asleep by behavioral criteria: it was curled up, motionless, with eyes closed. We monitored the rat’s behavior carefully during the period of dialysis. Most of the sleep experiments were conducted between 9:00 AM and 3:00 PM, and most of the awake experiments after 3:00 PM when rats were more alert. The rats were initially weighed and then gently held while the dummy cannula was removed and the dialysis probe inserted into the guide cannula. The animals were placed into a plethysmograph chamber (12, 22) and allowed 30–40 min to acclimate. Measurements were taken in room air and with 7% CO$_2$ inhalation during periods of wakefulness and sleep. After the systemic CO$_2$ response, the animals were exposed to room air and allowed to recover for at least 30 min. All dialysis experiments were performed in the room-air-breathing condition. Baseline V$_E$, V$_O_2$, and body temperature measurements were taken. The dialysis pump was run for 20 min at a speed of 45 l/min. The measurements were taken over a 20- to 30-s period at 0, 5, 10, 15, and 20 min during dialysis and at 5- to 20-min intervals after dialysis until V$_E$ returned to, or near to, the control level. The seven rats with correct guide-tube placement received 13 dialysis trials during wakefulness and 10 during sleep. 

50 and 100% CO$_2$ GROUP. We performed 3 trials of 20-min dialysis of aCSF equilibrated with 50% CO$_2$ in two animals during wakefulness and 10 trials of 20-min dialysis of aCSF equilibrated with 100% CO$_2$ in three animals during both wakefulness and sleep.

RESULTS

Measurement of Tissue Spread of pH During Dialysis in the RTN

In Fig. 1, the change in tissue pH during dialysis is expressed as a percentage of the maximum, which is defined in each animal as the change in tissue pH observed with an increase in the end-tidal CO$_2$ from 28 to 63 Torr. We emphasize the change in tissue pH, as it is difficult to make an absolute pH calibration in vivo. When the tissue pH change is measured in vivo during dialysis, a value of 100% represents the same change as observed with an increase in end-tidal P$_{CO_2}$ from 28 to 63 Torr. A value of 200% represents a change twice as large, or from 28 to 98 Torr. A value of 50% represents a change one-half as large, or from 28 to 46 Torr.

Tissue pH was measured by pH electrodes at varying distances from the dialysis probe, which were measured anatomically postmortem. Results are shown for three different dialysis conditions, high (100% CO$_2$ equilibrated with the dialysis solution), medium (50%), and low (25%). With high-CO$_2$ dialysis, the tissue pH change at the probe was approximately that which would occur with an end-tidal P$_{CO_2}$ of 110 Torr, and it decreased with distance such that at ~750 µm from the probe no change was detected. With 50% CO$_2$ in the dialysate, the pH change at the probe was approximately that which would occur with an end-tidal P$_{CO_2}$ of 81 Torr. At 600 µm from the probe, no pH change was detected. With 25% CO$_2$ in the dialysate, the pH change at the probe was approximately that which would occur with an end-tidal P$_{CO_2}$ of 63 Torr, and there was no detectable pH change at ~350 µm from the probe.

In these anesthetized rats, we also measured the amplitude of the integrated phrenic nerve signal and the frequency of phrenic bursts during the systemic CO$_2$ responses and during dialysis in the RTN region. Focal acidification had no effect on frequency and increased phrenic amplitude by ~20% of baseline with 25, 50, or 100% CO$_2$ in the dialysate. This increase was ~25% of the response, with all chemoreceptors stimulated by the change in end-tidal P$_{CO_2}$ from 28 to 63 Torr (data not shown).

Responses to Dialysis With 100 and 50% CO$_2$ in Unanesthetized Rats

In a series of preliminary experiments, we dialyzed the RTN region of unanesthetized rats with 100 or 50% CO$_2$ in the dialysate. We used these high concentrations initially to see if there would be a detectable ventilatory response to focal acidification of the RTN in an unanesthetized rat. With 100% CO$_2$ in the dialysate, we performed 10 trials in three rats with the duration of the dialysis varying from 2 to 15 min. In each case, V$_E$ increased during dialysis and returned to the normal baseline value after dialysis. However, we noted an unexpected finding in these initial experiments. The response seemed to differ if the rat was awake or asleep. In five cases when the rat was resting quietly but not asleep, V$_E$ increased by 10–30% above baseline during dialysis (data not shown). In five other cases, the rat was asleep when dialysis was begun and then awoke during the dialysis. In three of these cases, V$_E$ did not increase during dialysis when the rat was sleeping but then increased dramatically when the rat awoke (see Fig. 2A for 1 example). The peak increase in V$_E$ in these three rats when they awoke during dialysis was 20, 50, and 75% of baseline, respectively. In the remaining two cases with dialysis starting during
and found that \( V_E \) increased in all three by an average of 20% (data not shown). These studies were performed only during wakefulness. The anatomic locations of the dialysis probes for the three rats that received 100% CO\(_2\) dialysis and the two rats that received 50% CO\(_2\) dialysis were in the RTN (Fig. 3). The spread of tissue pH changes measured in the anesthetized rats indicates that regions adjacent or contiguous to the RTN might have been acidified during dialysis with 50 or 100% CO\(_2\).

In the major series of experiments of this report, we decreased the stimulus intensity to 25% CO\(_2\) in the dialysate, thus producing a stimulus at the center that was similar in intensity to that observed with 9% end-tidal CO\(_2\) but focal in nature with the stimulus intensity rapidly decreasing with radial distance from the dialysis probe. We conducted a series of experiments measuring body temperature by telemetry, \( V_O2 \), and \( V_E \) using the whole body plethysmograph during exposure to 7% inspired CO\(_2\), as well as during focal dialysis of the RTN with 25% CO\(_2\) equilibration of the dialysate.

Responses to Dialysis of the RTN Region With 25% CO\(_2\) in Unanesthetized Rats

In all, nine rats were tested successfully, and in seven of these the tip of the dialysis probe was subsequently shown to be in the region of the RTN. The data obtained from these seven animals are given below. The anatomic locations of the seven probes within the RTN region and the two probes that were outside of the RTN region are shown in Fig. 4. The two animals with probes located outside of the RTN region had no ventilatory response to focal acidification (data not shown). The probes located are drawn schematically on the figure to show the size of the region of tissue disruption observed in the sections.

Five rats with probes located in the RTN were tested for their response to inhalation of 7% CO\(_2\) both during sleep and during wakefulness (two rats did not undergo the systemic 7% CO\(_2\) stimulation). Body temperature data, obtained via telemetry from the rat during the time it was in the plethysmograph, are shown in Fig. 5A. Body temperature was significantly lower during sleep (\( P < 0.05 \), two-way ANOVA), and, with 7% CO\(_2\) inhalation, body temperature decreased significantly (\( P < 0.05 \), one-way ANOVA) in both the sleep and the awake tests. There was no significant effect of sleep vs. wakefulness on \( V_O2 \) (Fig. 5B), although \( V_O2 \) did tend to decrease during the exposure to 7% CO\(_2\) (\( P < 0.05 \), one-way ANOVA). \( V_E \) expressed in absolute values (Fig. 6A) was slightly but not significantly lower in room-air breathing during sleep, but with 7% CO\(_2\) inhalation it increased less during sleep than during wakefulness (Fig. 6A), a difference that was significant. However, when \( V_E \) was expressed as a percentage of baseline (Fig. 6B), there was no difference between the response to increased CO\(_2\) during sleep vs. wakefulness. When the \( V_E \) was normalized for \( V_O2 \) during CO\(_2\) inhalation, again there was no significant difference between the responses during sleep and wakefulness, although the values during sleep were lower than during wakeful-
ness (data not shown). The increase in \( V\dot{E} \) with 7% \( \text{CO}_2 \) inhalation was made up of a roughly 50% increase in tidal volume and an 80% increase in frequency. During sleep, tidal volume increased slightly more in absolute terms, but the value before \( \text{CO}_2 \) stimulation was lower, as was \( V\dot{E} \). Frequency showed similar changes in absolute and relative-to-baseline terms.

For dialysis of 25% \( \text{CO}_2 \) into the RTN region, there were, in the seven rats, 13 trials during wakefulness and 10 trials during sleep. As a control, there were 7 trials in five rats with RTN dialysis with the use of a 5% \( \text{CO}_2 \) equilibration. With 5% \( \text{CO}_2 \) dialysis, there was no significant change in body temperature, \( V\dot{O}_2 \), or \( V\dot{E} \). These data are not shown.

The effects of focal acidification of the RTN on body temperature are shown in Fig. 7A. During sleep, body temperature was significantly decreased, but there was no effect of RTN dialysis with 25% \( \text{CO}_2 \) on body temperature. \( V\dot{O}_2 \) (Fig. 7B) was unaffected by sleep or dialysis.

The major findings of the study are shown in Fig. 8. During dialysis, \( V\dot{E} \) expressed in absolute terms (Fig. 8A) or in relative terms (Fig. 8B) increased, on average, only during wakefulness. One-way ANOVA showed a significant increase \((P < 0.001; 17, 19, \text{and } 24\% \text{ increase at } 5, 10, \text{and } 15 \text{ min}) \) in \( V\dot{E} \) in absolute or relative terms only during wakefulness. Two-way ANOVA showed a significant response of \( V\dot{E} \), expressed in absolute terms or as percent baseline \((P < 0.001)\), to \( \text{CO}_2 \) during wakefulness compared with sleep. This average ventilatory response to focal RTN acidification during wakefulness was entirely due to an increase in tidal volume \((P < 0.01); \text{Fig. 9}\). Frequency did not change. Normalizing the \( V\dot{E} \) data for \( V\dot{O}_2 \) did not change the nature of these results. Of the 13 dialysis trials during wakefulness, 9 could be classified as a "response" by using an arbitrary definition of an increase in \( V\dot{E} \) of \( >10\% \). With this definition, there was a response in only 1 of 10 trials during behavioral sleep and in 1 of 7 trials with the 5% \( \text{CO}_2 \) dialysis control.

DISCUSSION
Technical Issues

In this study, we emphasize a chronic, unanesthetized rat model with a preimplanted guide tube used for insertion of a dialysis cannula for subsequent dialysis in the RTN region using aCSF equilibrated with 25% \( \text{CO}_2 \). Body temperature was measured constantly by telemetry, and \( V\dot{O}_2 \) and \( V\dot{E} \) were measured via the whole body plethysmograph. We used the modified version of the plethysmograph (12, 22) with continuous...
flow of fresh inspired gas through the chamber to allow continuous measurement in different states of arousal without interference by the investigator. The use of this model for the application of thyrotropin-releasing hormone to the RTN region has been recently described (7).

There are two concerns with this model: 1) the definition of sleep vs. wakefulness (15), and 2) the intensity and degree of spread of the tissue acidosis produced by the dialyzed CO₂. For these initial studies, we defined sleep by the use of straightforward behavioral criteria. Rats were judged to be asleep when curled up motionless with their eyes closed. All other states were included during wakefulness. We are aware that active sleep (AS) and quiet sleep (QS) are very different states (15) and plan to add electroencephalogram and electromyogram measures to this model to describe more accurately wakefulness and sleep and AS vs. QS. Our behavioral definition is a reasonable beginning for these studies. Rats generally have frequent AS episodes of brief duration such that one would expect that our behaviorally defined sleep periods would consist predominantly of QS (15).

With respect to the tissue acidosis, we measured the brain stem tissue pH at varying distances from the dialysis probe using pH electrodes in anesthetized rats (Fig. 1) (5, 13, 19). Ideally we would do this in the unanesthetized model during sleep and wakefulness, but this is a technically difficult problem. For now, we rely on these data obtained under anesthesia. With dialysis by using aCSF equilibrated with 100% CO₂, the observed pH change is constrained to within 750 µm and, at the probe, is approximately equivalent to that observed with an end-tidal PCO₂ of 110 Torr. The affected tissue volume is 1.4 µl (assuming a spherical volume of pH distribution with a radius of 750); the volume of the RTN region in the rat is ~1.12 µl (assuming a width of 1.4 mm from the pyramidal tract to the lateral aspect of the facial nucleus, a depth of 0.5 mm from the ventral medullary surface to the ventral aspect of the facial nucleus, and a length of 1.6 mm from the rostral to the caudal poles of the facial nucleus) (24, 30). The tissue volume with an acid pH would include structures in the RVLM contiguous to
the RTN, e.g., the facial nucleus, parapyramidal and juxtafacial nucleus, paragigantocellularis lateralis, and perhaps dendrites from subretrofacial and retrofacial neurons (1, 17). Even with this large stimulus, both in terms of intensity and spread, the ventilatory response to this focal acidosis is present only during wakefulness or is substantially greater during wakefulness than during sleep.

With dialysis by using aCSF equilibrated with 25% CO₂, the pH change at the probe is approximately equivalent to that observed with an end-tidal CO₂ of 63 Torr, and it decreases with distance such that, at 550 µm from the probe, there is no detectable change. The volume of affected tissue here is 700 nl. Most, if not all, of the focal acidosis with 25% CO₂ in the dialysate is limited to the RTN region and the facial nucleus. These estimates of the stimulus intensity and spread made in the anesthetized animal are probably greater than that which occurred in the unanesthetized preparation. In the absence of anesthesia, it is likely that the response of cerebral blood flow to the focal acidosis is greater (31), which would decrease the intensity and spread of the acidosis.

**Location of Dialysis Probes**

The location and approximate size of the dialysis probe tips used for 100 and 50% CO₂ dialysis (Fig. 3) and for 25% CO₂ dialysis (Fig. 4) are within the RTN region. Figure 4 also shows the location of the probe tip in two animals that showed no responses to focal tissue acidosis produced by the dialysis during wakefulness. These probe tips (open rectangles) are clearly not within the RTN or even within the estimated region of acidosis produced by the dialysis. The absence of any ventilatory response to dialysis at these sites indicates that brain stem chemoreception is localized: it is not present everywhere.

**Body Temperature and Metabolic Rate Responses to Sleep, 7% CO₂ Inhalation, and Focal RTN Acidification**

We and others (2) found that rat body temperature decreases during sleep, but metabolic rate is un-
changed. With the whole animal exposed to 7% CO₂,
both body temperature and metabolic rate decrease in
both sleep and wakefulness. Others have reported this
decrease in body temperature (10, 25, 28), but meta-
bolic rate is usually unaffected by hypercapnia at this
ambient temperature and CO₂ level (10, 28), although
one other study reported a decrease in metabolic rate
(25). Focal acidification of only the RTN region by
microdialysis has no effect on metabolic rate or body
temperature. The CO₂ effects on metabolism and body
temperature must reflect mechanisms involving other
central chemoreceptor locations or other nonchemore-
ceptor mechanisms.

Blood Flow During Sleep and With CO₂

If cerebral blood flow to the RVLM in hypercapnia
increased more during sleep than during wakefulness,
then part of the absent ventilatory response during
sleep could be attributed to washout of the stimulus.
Total cerebral blood flow remains at or below normal
waking values in QS (31). However, during AS, cerebral
blood flow can increase above values observed during
wakefulness (31), and, in the anesthetized rat, the
baseline and CO₂-stimulated cerebral blood flow are
greater in the RVLM than in the cortex (11, 29). We do
not know what happens to cerebral blood flow to the
RVLM during sleep, but existing data indicate that AS
periods are brief in duration in the rat and account for
only 10–20% of total sleep (15). Also, we observed
smaller or absent ventilatory responses during sleep
vs. wakefulness when we dialyzed the RTN with aCSF
equilibrated with 100% CO₂. It seems unlikely to us
that the absence of any ventilatory response to focal
acidosis of the RTN region in the unanesthetized rat
during behaviorally defined sleep compared with wake-
fullness can be explained by a differential effect of the
stimulus on local cerebral blood flow in these two
states.

The rostral pressor region (8), located near the RTN,
when stimulated can increase blood pressure via sym-
pathetic efferent stimulation. We did not measure blood
pressure in these unanesthetized rat experiments, and
it is possible that in some cases it may have been
stimulated by the focal acidosis. Blood pressure did
increase in some animals with focal RTN CO₂ applica-
tion in anesthesia (13).

Chemoreception in the RTN During Wakefulness,
Sleep, and Anesthesia

Focal acidosis of the RTN region in anesthetized rats
produced by acetazolamide injection (4, 5, 19) or CO₂
diffusion pipette (13) increases phrenic activity by a
large fraction (27–40%) of the response produced by 9%
end-tidal CO₂, a stimulus that affects all central chemo-
receptor sites. In the anesthetized animals used in this
report for evaluation of tissue pH spread, integrated
phrenic nerve activity also increased by a similar
fraction of the response observed with increased end-
tidal CO₂. These results are in contrast to those ob-
tained in the unanesthetized animal. Inhalation of 7%
CO₂ increases V̇E by 170% of baseline. Focal acidosis
of the RTN by the microdialysis probes increases V̇E by
24% of baseline, and this occurs only during wakeful-
ness. Thus the fraction of the total response observed
with focal RTN acidification is 14%, and, in behavior-
ally defined sleep, there is no response to the focal
acidosis.

These findings at first glance seem to be a paradox.
During sleep, focal acidification of the RTN has no
effect, in anesthesia the effect is a large fraction of that
observed with all chemoreceptors exposed to CO₂, and
during wakefulness the effect is significant but is a
relatively small percentage of the effect observed with
all central chemoreceptors stimulated. Anesthesia (with
chloralose-urethan) and sleep clearly differ in their
effects on chemoreception. Our interpretation is that
anesthesia affects other central chemoreceptor loca-
tions more than it does the RTN region; the RTN
becomes an important source of input to the respiratory

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**Fig. 9. Tidal volume (Vᵣ; A) and respiratory frequency (B) in
unanesthetized rats (n = 7) dialyzed with 25% CO₂ in RTN region
during wakefulness (○; n = 13 trials) and behaviorally defined sleep
(□; n = 10 trials). Mean ± SE values are shown. Control room air
values were obtained before and after 20-min period of dialysis. The 4
preexposure control values were combined into single value; 25-min
postexposure value was deleted, as many rats showed an artifactual
increase in Vᵣ because of manipulation of dialysis apparatus when it
was shut off. Vᵣ during focal RTN acidification was significantly
greater during wakefulness when expressed in absolute terms or as
%baseline. There was no response during sleep.**
control system related to CO₂, perhaps the most important. This would explain why, in anesthetized animals, lesions of the RTN region virtually abolish central chemosensitivity and can result in apnea.

In the unanesthetized and awake state, the ventilatory response to focal acidification of the RTN region is but a small fraction of the response to acidification of all central chemoreceptors. All chemoreceptor sites are operational in this state, and the overall response is likely tempered by hypocapnia at other sites. This interpretation is consistent with recent results showing that lesions or cooling of the RTN region in the unanesthetized, awake rat or goat does not have the same dramatic effects on baseline VE or chemosensitivity as thetrized, awake rat or goat does not have the same

interpretation is consistent with recent results showing that lesions or cooling of the RTN region in the unanesthetized rat, stimulate ventilatory output (4, 5). These

regions have not been traditionally identified as important in arousal-related functions of the brain stem. We hypothesize that their role in the control of breathing may be enhanced during sleep.

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