Central chemical control of ventilation in the unanesthetized turtle

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HITZIG, BERNARD M., AND DONALD C. JACKSON. Central chemical control of ventilation in the unanesthetized turtle. Am. J. Physiol. 235(5): R257-R264, 1978 or Am. J. Physiol.: Regulatory Integrative Comp. Physiol. 4(3): R257-R264, 1978.—The cerebral ventricles (lateral-4th) of the fresh water turtle Pseudemys scripta elegans (1-2 kg) were perfused with mock cerebrospinal fluid (CSF) of normal and lower than normal pH. Alteration of the pH, at constant PCO2, was accomplished by altering the difference between the number of completely dissociated cations and anions, the strong ion difference (SID). In a dilute salt solution with no other weak acid buffer the SID is equal to the [HCO3-]. Changing the SID of the mock CSF from control values (mean = 32 meq/l) to 15 meq/l resulted in an increase in the ventilation to metabolism ratio (VE/VO2) of 2-13 times (mean = 7) that of control. Observations revealed no significant difference in ventilation between perfusion with normal pH mock CSF and preperfusion values. The increase in ventilation with the low pH perfusate was found to be similar in animals equilibrated at 20 and 30°C. Decreasing the SID 2 meq/l from control values caused a fourfold increase in the mean VE/VO2 (P < 0.001) indicating that the central chemosensors of Pseudemys display a high degree of sensitivity. The induced hyperventilation persisted for the duration of the perfusion with the low pH mock CSF, in spite of a marked respiratory alkalosis of the arterial blood, demonstrating that the central chemoreceptors are dominant over any peripheral inputs. Since the perfusion was confined to the cerebral ventricles, we conclude that the central chemoreceptors of Pseudemys are in functional contact with the ventricular CSF. This is the first study which demonstrates the existence of central chemical control of ventilation in an ectothermic vertebrate.

central respiratory chemosensors; ectotherm; brain ventricular perfusion; temperature; cerebrospinal fluid

CENTRAL CHEMICAL CONTROL of ventilation has been well established in mammals (3, 4, 13, 14), but there have not been any studies demonstrating central chemosensor control of ventilation in ectothermic vertebrates. It is known, however, that air-breathing ectothermic vertebrates do control the acid-base status of their arterial blood within narrow limits, at any given temperature within their normal range (1, 10, 22, 24). It is also known that a major mechanism used by these animals to control acid-base balance is respiratory gas exchange. What is not known are the nature and location of the central receptors (if any) mediating these responses, or the stimuli necessary to evoke a response.

The technique used in studying central chemosensor control of ventilation in conscious mammals is the perfusion of the ventriculocisternal system with mock cerebrospinal fluid (CSF). This technique, together with more discrete, localized investigations of anesthetized preparations, has implicated the medulla as the probable site for the mammalian central receptors. By analogy with the mammalian system, we therefore set out to identify central chemoreceptor function in an ectothermic vertebrate.

For a variety of reasons, we selected the freshwater turtle, Pseudemys scripta, to be the subject for this study. First, considerable background information concerning the respiratory physiology of this species was available from previous studies in our laboratory (9-12). Of particular significance was our observation (11) that the ventilatory response to inspired CO2 of this animal is pronounced and similar in magnitude to the response of many mammals, suggesting that “mammal-like” central receptors may be present in the turtle. In addition, Heisey (7) found that the brain ventricular system of these turtles is much larger, proportionately, than in that of mammals, and the fourth ventricle was accessible to catheterization. Finally, turtles occupy a central position in the evolution of vertebrates. They are the only direct descendants of the stem-reptiles, or cotylosaurs, which ultimately gave rise to modern reptiles and mammals. Turtles have changed very little since their earliest inception and first appear in the fossil record in the Permian era, some 215 million years ago (25). The demonstration of central chemical control of ventilation in extant turtles would therefore support the concept that central respiratory chemosensors are a phylogenetically old system, whose roots extend back into the remote past of vertebrate evolution.

METHODS

Animals

Semiaquatic freshwater turtles of the species Pseudemys scripta elegans were obtained commercially. The animals were of either sex, weighing from 1 to 2 kg, and were housed in an aquarium with continuously running water and an accessible dry platform. The turtles were fed three times a week with ground beef enriched with vitamins and unsaturated fatty acids. All experiments were performed in the fall and winter.
Catheterization of the Brain Ventricular System

To perfuse the brain ventricular system, chronic catheters were implanted in the animal's lateral and fourth ventricles. An operation was developed that allowed both the perfusion of the animal's ventricular system and the free voluntary movement of the turtle's head in and out of the shell. Unless the turtle could extend and retract its head at will, the buoyancy method (described below) for recording turtle respiratory data could not be used.

At the time of operation the animals were intubated and anesthetized with a mixture of 3% halothane and 25% nitrous oxide in oxygen using a Bird respirator (Mark II). A Fluotec (model 3) anesthesia machine provided halothane. The other gases were delivered at constant percentages by mixing in the bag of the respirator after passing through calibrated flow meters. Anesthesia was maintained throughout the operation with a mixture of 0.5% halothane and 35% nitrous oxide in oxygen.

The animals were placed in a TrentWells stereotaxic apparatus that had been modified for this purpose. An incision was made 4-6 mm from the midline of the skull either to the right or the left of the sagittal crest. The temporal musculature was removed under a dissecting microscope, exposing one of the posterior quadrants of the skull. Whaledent screws were inserted into the parietal and supraoccipital bones to a depth of 2 mm. A dental drill with a no. 5 dental burr was then attached to the stereotaxic probe drive and two holes were made in the skull, exposing the dura. The hole for the fourth ventricle catheter was placed 2 mm lateral to the supraoccipital crest and 4 mm anterior to the distal aspect of the occipital bone in the basal portion of the supraoccipital bone. The hole for the lateral ventricular catheter was placed 5 mm lateral to the midline in the distal curved aspect of the parietal bone some 4 mm below the dorsal surface of the parietal bone. The approach to the lateral ventricle was therefore made from the rear (see Fig. 1).

Tygon microbore tubing (0.030 in. ID) was attached to a 20-gauge stainless steel needle with the hub removed. A hole was cut in the dorsal surface of the needle to enhance flow. The catheters were then filled with sterile saline solution, clamped at their free end, and fixed in a modified electrode holder attached to the probe drive of the stereotaxic apparatus. The needles were quickly lowered 8-10 mm below the dura, being guided into place by the probe drive and the angle of the drilled holes. The catheters were then withdrawn slowly with the now unclamped free end being held approximately 5 cm below the level of the ears. The withdrawal was stopped at the point when constant fluid flow was noted at the free end of the catheter. After resealing the catheters, the needles and catheters were cemented in place to the whaledent screws with dental acrylic.

An incision was made through the skin at the midline on the dorsal surface of the neck and the catheters were run down the incision to the base of the neck. The skin was then closed over the catheters and two holes were drilled in the carapace. The catheters were passed through these holes and taped to the top of the carapace. Two to four days were allowed for recovery. The operated animals could be maintained in a healthy condition for months. Unfortunately, however, the perfusion system remained patent for a maximum of only 3 wk. We were unable to establish the reason for the blockage.

The placement of the needles was verified in eight animals. Saline containing methylene blue was perfused through the ventricular catheters from the lateral to the fourth ventricle at a rate of 40-100 μL/min. After 20 min, the perfusion was stopped and some of the bone surrounding the catheters was removed until visualization of the placement of the needles in the brain could be made. No methylene blue could be observed in the overlying subarachnoid fluid. The rest of the bone overlying the brain was then removed and methylene blue could easily be visualized in the ventricular fluid. We therefore concluded that the perfusion was confined to the ventricular system. Perfusion of the CSF space in conscious goats, however, ran from the lateral ventricle to the cisterna magna (3, 20, 21) reaching the subarachnoid fluid as well as the ventricular CSF, thereby making functional localization of the chemosensitive areas impossible. Since we were perfusing only the brain ventricular system in turtles, mechanistic localization of the central chemosensors became a distinct possibility.

Arterial Catheter

In several experiments, arterial blood samples were taken. A catheter (PE-50) was placed in the right thyroid artery after the method of Jackson et al. (11). The animals were subjected to cold anesthesia by placing them at 4°C overnight. A finger cot was placed over their heads to keep their lungs expanded through-out the procedure. A hole was trephined in the plastron and the right thyroid artery was catheterized. The catheter was then run out of the body cavity at the base of the neck and taped to the carapace. The hole in the plastron was sealed with a tight fitting Lucite plug cemented in place with dental acrylic. The animals were anoxic for approximately 1 h, which is well within their range of tolerance. This procedure was carried out the day before implantation of the ventricular catheters.
Ventilation and Oxygen Consumption

The procedure used to determine ventilation was originally developed by Jackson (9). It utilizes the buoyancy principle in relating changes in lung volume to changes in the animal's weight in water.

The turtle's limbs were restrained by placing adhesive tape completely around the shell. The animal was then placed in a temperature-controlled water bath, and suspended from a counterbalanced Grass model FT .03 strain gauge. The turtle was positioned directly under a ventilated chamber so that it could raise its head out of the water and breathe at will. Any changes in lung volume were sensed by the strain gauge as changes in the animal's buoyancy and were then recorded by a Grass model 79 polygraph. The system was calibrated prior to each experiment by passing a known amount of air into an inverted submerged beaker that was suspended from the strain gauge. By using this method, the animal's tidal volume (ml/breath), mean expired minute volume (ml (BTPS)/kg·min), and respiratory frequency (breaths/min) were determined. Because the turtle was free to move its head out of the water, buoyancy changes unrelated to changes in lung volume occasionally resulted. These artifacts were easily detectable on the record and were discounted.

Oxygen consumption was measured by the open-circuit method. The breathing chamber (volume 0.15 ml) was ventilated with air from a pressurized cylinder at a known rate (280-380 ml/min) as determined by a calibrated rotameter. After leaving the chamber, the gas was then directed through a drying column (Drierite) and into a Beckman model F3 paramagnetic oxygen analyzer for continuous recording of the oxygen content. The oxygen analyzer was calibrated before each experiment by varying the known PO2 of the gas in the analyzer. Frequent checks of the zero position were made throughout the experiment. The analog output of the oxygen analyzer was recorded by a Beckman potentiometric chart recorder. The record obtained for the test period was then integrated by planimetry. Oxygen consumption was computed in terms of ml (BTPS)/kg·min and the mean oxygen consumption. This ratio offers the metabolic rate under normal conditions. In-
was then connected to the catheter entering the lateral ventricle. The fourth ventricular catheter was passed through a hole in the water bath and opened to the air at a level approximately 10 cm below the animal's head and the perfusion was started. The CSF pressure in the turtle's brain was assumed to equal the height of the eflux tube, barring any blockages in the animal's ventricular system. Increased resistance in the system was easily detected due to concomitant decreases in eflux rate. The flow rate through the ventricles (40-150 μl/min) was varied depending upon the experimental situation. Because of the difficulty of the operative procedure there were many failures in the perfusion system. Investigation revealed that the main cause of initial failure was the blockage of the eflux catheter by the choroid plexus. When this occurred the results of the experiment were discounted.

According to Heisey (8) the rate of CSF production for Pseudemys of the same size as those used in these experiments is 1.4 μl/min. Therefore our flow rates were approximately 30-100 times higher than the estimated turtle CSF production. Because these flow rates were high in relation to the animal's own flow rate, a negligible change in the composition of the mock CSF was expected as it passed through the brain ventricles. In order to test whether this assumption was correct, a compartmental analysis was performed. We then compared the values for [CO₂₅] calculated from the derived equation with those which were measured experimentally in the entering and exiting CSF

\[ C_{x(t)} = (C_{x} - C_{x(r)}) \cdot e^{-(t/V)} + C_{x(r)} \]

where \( t \) is time; \( C_{x(t)} \) is the total concentration of a substance \( x \) (meq/l) in the brain ventricular system as a function of time \( (t) \); \( C_{x(r)} \) is the concentration of \( x \) (meq/l) in the entering solution; \( C_{x} \) is the initial concentration of \( x \) in the ventricular system at \( t = 0 \); \( V \) is the flow in μl/min of the entering perfusate; and \( V \) is the ventricular volume in μl.

Two experimental perfusions were performed using mock CSF with a [CO₂₅] of 15 meq/l at a flow rate of 40 μl/min. The average [CO₂₅] in the animal's CSF before perfusion was 31.9 mmol/l. Three measurements were taken at 4, 12, and 23 min after the start of the perfusion (Fig. 2). The deviation of the experimental curve from the calculated curve is within the experimental error of the method for [CO₂₅] measurement. It would then appear that the values of the acid-base determining variables within the ventricular system are dictated by the entering perfusate, with the possible exception of Pco₂.

The brain ventricular CSF volume of animals of the same size as those used in these experiments is approximately 200 μl, as determined by Heisey (7), whereas the total CSF volume (ventricles plus subarachnoid space) is 750 μl (7). In calculating the derived curve of Fig. 2, the 200 μl value for ventricular volume was used. The close agreement between the calculated curve and the experimentally derived curve gives further indication that the perfusion was confined to the ventricular system. Had the subarachnoid space also been perfused, the experimental curve would have decreased at a much slower rate.

**Experimental Protocol**

**Perfusion at different temperatures.** In the first series of experiments, perfusions were carried out on animals equilibrated at either 20 or 30°C. The animals were perfused with mock CSF having a SID of either 32 or 15 meq/l ([HCO₃⁻] - 32 or 15 meq/l). The order of perfusion was varied and in several experiments the flow was stopped to test whether the perfusion per se caused the ventilatory changes.

**Arterial plasma [CO₂₅] and pH were measured in four experiments.** Total plasma CO₂ was determined using the micromanometric method of Van Slyke and Plazin (26). The whole blood pH was immediately measured using a glass microelectrode equilibrated at the experimental temperature (Radiometer no. 5021).

**Sensitivity experiments.** In another series of experiments performed at 20°C, the ventilation-to-metabolism ratio was measured while the animal's ventricular system was being perfused with mock CSF in which the SID was varied by 2 meq/l (30, 32, and 34 meq/l). These experiments were carried out to ascertain the degree of sensitivity of the turtle's central chemoreceptor system.

**Mock Cerebrospinal Fluid**

Table 1 contains the pH's, Pco₂, total CO₂'s, and the SID for the entering artificial CSF solutions used in the various experiments. The Pco₂'s were calculated using the Henderson-Hasselbalch equation and the acid-base parameters provided by Reeves (23) and were found to equal the expected values when bubbling with 3% CO₂ (22 Torr at 20°C, and 29 Torr at 30°C)

A test experiment was performed to determine if the loss of CO₂ through the catheter walls would increase...
TABLE 1. Acid-base variables of various solutions used in ventricular perfusions

<table>
<thead>
<tr>
<th>Temp, °C</th>
<th>SID, meq/l</th>
<th>Pco2, Torr</th>
<th>[H+] meq/l</th>
<th>pH</th>
<th>[HCO3-], meq/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>20°C</td>
<td>+15</td>
<td>22</td>
<td>4.41 x 10^-8</td>
<td>7.356</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>+30</td>
<td>22</td>
<td>2.20 x 10^-8</td>
<td>7.657</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>+32</td>
<td>22</td>
<td>2.07 x 10^-8</td>
<td>7.685</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td>+34</td>
<td>22</td>
<td>1.94 x 10^-8</td>
<td>7.711</td>
<td>34</td>
</tr>
<tr>
<td>30°C</td>
<td>+15</td>
<td>29</td>
<td>5.15 x 10^-8</td>
<td>7.288</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>+32</td>
<td>29</td>
<td>2.41 x 10^-8</td>
<td>7.617</td>
<td>32</td>
</tr>
</tbody>
</table>

* May have varied slightly due to small changes in the barometric pressure.

the pH of the entering mock CSF. Artificial CSF was perfused from the reservoir through the same length of catheter that was used in the ventricular perfusion. The end of the catheter, which normally would enter the brain, was fed directly into the Radiometer pH electrode. Small losses of CO2 (2-3 Torr) were detected in four of six runs; therefore, the Pco2 of the entering mock CSF was probably slightly lower than that of the reservoir.

The pH and total CO2 of the effluent were measured several times during each ventricular perfusion experiment and were found to be approximately equal to the values of the entering perfusate. Therefore, the values of the acid-base variables in the area of the animal's ventricular system, where the flow of the perfusion fluid was greatest, can be assumed to be approximately the same as the entering mock CSF. The acid-base values of the CSF along the walls of the ventricles, where the presence of unstirred layers complicates matters, were impossible to estimate. The acid-base values of brain interstitial fluid, and those of the brain tissue, itself, also could not be determined. More will be said in the discussion section concerning this problem.

RESULTS

Ventricular Perfusion at 20 and 30°C

The \( \dot{V}_E/\dot{V}_O_2 \) was clearly affected by perfusion with the low SID mock CSF (Fig. 3). At 20°C the ventilation-to-metabolism ratio increased from a minimum of 2 to a maximum of 13 times control values. The mean increase in the ratio was approximately seven times that of the control. A one-way analysis of variance revealed the increase to be highly significant \( (P < 0.005) \), indicating that *Pseudemys* possesses central chemosensors in functional contact with the ventricular CSF. The order of perfusion was varied to eliminate sequential bias from the results. Perfusion with the "normal" mock CSF (SID = 32 meq/l) restored the mean ratio to control values \( (P < 0.005) \) (Fig. 3). A similar result was obtained at 30°C, indicating that the sensitivity was the same at both temperatures.

In several experiments, the flow of the perfusate was stopped for at least 0.5 h before measurements were taken. This was done to rule out nonspecific changes due to the effects of the perfusion itself. It can be seen in Fig. 3, that the stop-flow ventilation-to-metabolism ratios fall within the values obtained for the normal mock CSF and the baseline controls.

One experiment was done at 10°C with results very similar to those obtained at 20 and 30°C. The 10°C experiments were later abandoned because two animals that had been successfully operated on, subsequently died during equilibration at this temperature.

Arterial acid-base variables were measured in four experiments. In every case a marked respiratory alkalosis occurred during perfusion with the low (15 meq/l) SID mock CSF. The average \([H+]\) decreased from a control value of \( 1.95 \times 10^{-8} \) (pH = 7.71) to \( 7.59 \times 10^{-8} \) (pH = 8.12) meq/l. The average arterial Pco2 decreased from 22.4 to 6 Torr, and the average total CO2 decreased from 36.9 to 24 mmol/l. Ventilation, however, continued unabated for the duration of the "low" perfusion.

Sensitivity Experiments

The purpose of these experiments was to test the sensitivity of the turtle's central chemosensor system. It can be seen in Fig. 4 that a decrease in the SID of 2 meq/l in the perfusion fluid caused a mean increase in ventilation of approximately four times control values. A one-way analysis of variance showed that this was a highly significant change \( (P < 0.001) \), indicating that *Pseudemys* is very sensitive to small changes in the acid-base variables of its CSF. This change in the SID of the perfusate translates into a pH change in the entering mock CSF of approximately 0.02 U.

Changing the perfusate from the low to the normal SID solution decreased ventilation to slightly above control values. The decrease obtained with this perfusion was also highly significant \( (P < 0.001) \). Increasing the SID of the perfusate to 34 meq/l resulted in no
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**FIG. 4.** Alterations in mean ventilation-to-metabolism ratios at 20°C with changes of 2 meq/l in the SID ([HCO₃⁻]) of perfusate (control, n = 11; 30 meq/l, n = 6; 32 meq/l, n = 7; 34 meq/l, n = 3; stop flow, n = 3). Flow was stopped after perfusion with 32 meq/l solution.

significant change in ventilation. The difference between the ratios obtained during the control period and those obtained during perfusion, with either the normal solution or the 34 meq/l solution, was not significant. These results indicate that a decrease of 2 meq/l in the SID of the perfusate caused large changes in ventilation. The order of perfusion was again altered to prevent any sequential bias in the experiment, and all the experiments were performed at 20°C.

**DISCUSSION**

Central control of ventilation has been previously demonstrated in mammals. Pappenheimer et al. (20) and Fencl et al. (3) performed the only brain ventricular perfusion studies in an unanesthetized animal aimed at delineating central respiratory control. They postulated that the control of ventilation in the endothermic goat was mediated through hydrogen ion changes in the interstitial fluid surrounding the respiratory center neurons. The present study provides the first evidence of central control of ventilation in an ectothermic vertebrate. Perfusion of the brain ventricles of the unanesthetized turtle, with solutions containing various concentrations of acid-base determining variables, resulted in significant changes in ventilation (Figs. 3 and 4).

**Effects of Temperature**

Ventricular perfusions were performed at 20 and 30°C in order to ascertain if chemosensor sensitivity varied with ambient temperature. The results of these experiments (Fig. 3) clearly demonstrate that turtle ventilation displays the same sensitivity to alterations of the acid-base variables of the perfusate at both temperatures. This finding agrees with the results of a study by Jackson et al. (11) that demonstrated that ventilation in *Pseudemys* displayed a similar sensitivity to increases in inspired CO₂ at 10, 20, and 30°C. Several studies in turtles have shown that the [H⁺] and PCO₂ of arterial blood vary directly with temperature. Our results indicate indirectly that the same is true for turtle CSF. It can be seen in Table 1 that when the SID of the perfusate was held constant, while temperature was varied, the pH and PCO₂ of the mock CSF also varied. Ventilation, however, remained constant (Fig. 2) and increased only when the ionic composition (SID) of the mock CSF was altered. It therefore appears that ventilatory changes are directly related to changes in the SID of the mock CSF, not the pH or PCO₂, and that the variable being held constant when temperature changes is the ionic composition of the CSF. Our results are also consistent with the alphastat hypothesis of Reeves (22), which states that the ectotherm is keeping the fractional dissociation of imidazole constant with changing temperatures. At any given temperature, however, changes in the acid-base determining variables would cause changes in the fractional dissociation of imidazole within the ventilation controlling receptors. These receptors would then bring about changes in ventilation to restore the acid-base variables to normal values.

**Comparisons Between the Turtle and Goat**

Pappenheimer et al. (20) reported that the ventilation of the unanesthetized goat increased by 2.5-fold when the CSF [HCO₃⁻] was reduced by 6 mmol/l (from 22 to 16 mmol/l) and by 4-fold when the reduction in [HCO₃⁻] was 15 mmol/l (from 30 to 15 mmol/l). In contrast, the ventilation of our turtles increased sevenfold when the SID of the perfusate was lowered from 32 to 15 meq/l (Fig. 3), whereas a reduction in SID of only 2 meq/l induced a fourfold increase in ventilation (Fig. 4). Clearly, the alterations in ventilation caused by stimulation of central chemosensors are greater in the turtle than in the goat. One possible explanation for this observation may lie in morphological differences between the brains of both animals.

Heisey (6, 7) has described the ventricular system of *Pseudemys* as being more extensive than that of mammals. The turtle brain has ventricles in the paired olfactory and optic lobes, and in the cerebellum, in addition to the ventricles found in the brain of mammals (paired lateral, third, and fourth ventricles). For this reason, a higher percentage of turtle brain tissue is exposed to the CSF than in mammals. Dissection of
the brain of *Pseudemys* revealed that the walls of the brain and brain stem were frequently less than 1 mm thick. Because of the large area of brain tissue exposed to the CSF and the thinness of the brain walls, diffusion of substances from the CSF to a large area of the turtle's brain tissue during ventricular perfusion is highly probable. Therefore many brain-stem structures, which are not readily accessible to the CSF in a ventricular perfusion of the mammalian brain, may be easily reached in the turtle. Because the rate of diffusion depends upon distance, and because the distance from the ventricles to the chemosensitive areas of the turtle's brain is probably less than that of mammals, it follows that the acid-base changes caused by the perfusion would be greater in the turtle than in the mammal.

During the ventricular perfusion of the turtle brain, the acid-base variables (with the possible exception of $PcO_2$) within the ventricles, in the area of greatest flow, are set by the perfusate. The factors dictating the composition of the brain interstitial fluid during a ventricular perfusion are complex. The acid-base status of any group of cells within the brain depends upon the ionic composition of their immediate environment and the tissue $PcO_2$. Tissue $PcO_2$ and interstitial fluid (ISF) ionic composition are functions of the rate of flow of the perfusion fluid, the rate of flow of the arterial blood, the SID's and $PcO_2$'s of the arterial blood and mock CSF, and the rate of CO$_2$ production by the brain tissue. Since most of these variables are unknown, it was impossible in these experiments to estimate the acid-base status of any group of receptor cells located at some point within the brain. Even in a steady state there are too many unknown variables for any accurate estimation of the acid-base status. It is, however, clear from the results of these experiments that changes in the acid-base variables of the perfusate markedly influenced ventilation in *Pseudemys*.

**Arterial Acid-Base Changes and Changes in Ventilation**

The results demonstrate that changes in the acid-base variables of the ventricular perfusate will drive ventilation in the face of a severe respiratory alkalosis in the arterial blood. In every experiment, the hyper-ventilation persisted as long as the perfusion with the low SID solution was continued. Pappenheimer et al. (20) similarly reported that the increase in ventilation of the goat, caused by a reduction of 5 mM in the bicarbonate ion concentration of the perfusate, persisted for the duration of the perfusion, despite a respiratory alkalosis in the arterial blood. The results obtained in the present study demonstrate that central chemical control of ventilation is dominant over peripheral control, and that maintenance of central nervous system acid-base homeostasis is of paramount importance in the turtle.

**Location of the Chemosensors**

One of the unresolved questions in the study of central respiratory chemoreceptors concerns their anatomical location. This uncertainty has, of course, been restricted to mammalian species, on which all previous work has been conducted. Several investigators have reported that the receptors in the cat lie on the ventral surface of the medulla (16, 17, 19), whereas other workers hold that the respiratory center neurons, situated beneath the floor of the fourth ventricle, are the central chemoreceptors (13-15, 20). The turtle provides a useful system for studying this problem since in this animal, unlike mammals, it is possible to restrict perfusion to the ventricles. Our evidence that the perfusion was indeed confined to the ventricles, as described in the methods, is 1) after perfusion with methylene blue, no dye was discovered in the subarachnoid fluid, 2) direct visualization confirmed the fact that the catheters were within the lateral and the fourth ventricles, and 3) using the average ventricular volume (200 $\mu l$) in the equation derived from the compartmental analysis resulted in a calculated curve that almost exactly matched the curve derived experimentally (Fig. 2). For these reasons and because of the magnitude of the ventilatory changes evoked by perfusion with the low SID mock CSF, we conclude that the sensors are in immediate functional contact with the ventricular CSF.

One factor that may be in conflict with the above conclusion is the possibility of diffusion of ions from the ventricular cavity to the neurons on the ventrolateral surface of the medulla. As mentioned previously, the thickness of parts of the turtle's brain stem was frequently found to be slightly less than 1 mm. It is therefore theoretically possible that the ions involved in brain acid-base balance could have diffused from the ventricle to the surface of the medulla and initiated the ventilatory response. The critical factor in the diffusion hypothesis is the latency of the ventilatory response, inasmuch as the time between the initiation of the perfusion and the first definite increase in ventilation would have to agree with the diffusion time.

The latency of the ventilatory response in this study varied from 2 to 15 min, with the mean latency at approximately 5 min. Three of the animals that were perfused with the low SID solution ([HCO$_3^-$] = 15 meq/l) responded within 2.5 min. In the sensitivity experiments where the SID of the perfusion fluid was only slightly lower than normal (2 meq/l), three of the animals responded within 3 min. The average flow rate used during the sensitivity experiments was 100 $\mu l$/min, using the equation derived from the compartmental analysis, the decrease in the SID of the fluid within the ventricles was approximately 1.6 meq/l 3 min after the start of perfusion (the [HCO$_3^-$] went from an average normal value of 32 meq/l to 30.4 meq/l). For the ions involved to diffuse from the ventricular surface to the ventral surface of the brain stem, an adequate concentration gradient would have to be established; also the brain ISF pH in immediate contact with the ventral surface of the medulla would have to be at the lower value. Because the total brain CSF space, according to Heisey (7) is approximately 750 $\mu l$ in these animals, it follows that the time necessary to bring the SID of the subarachnoid CSF to the same concentration...
as that of the ventricles would be two to three times as long as that of the ventricular system alone, even if there were unrestricted mixing. The ependymal surface of the ventricles, and the brain tissue itself, are certainly barriers to unrestricted mixing and would substantially increase the time necessary to equalize the ion concentrations in the ISF, since diffusion is a much slower process than unrestricted mixing.

The above facts indicate that the response time was much quicker than could be expected from that of diffusion to the ventral surface of the medulla. It therefore follows that the central respiratory chemosensors of*Pseudemys* are in immediate functional contact with the CSF, close to the ventricular surface. It is, however, possible that active transport of ions from the ventricles to the surface of the medulla could have occurred since the ependymal cells of the turtle brain extend from the ventricle to the external surface of the brain stem (2). While this possibility exists it seems unlikely due to the short latencies of response.

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