Intracellular pH and buffering power of rat brain

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Roos, Albert. Intracellular pH and buffering power of rat brain. Am. J. Physiol. 221(1): 176-181. 1971.—Intracellular pH and bicarbonate concentration and cortical CO₂ concentration were derived from the distribution of 5,5-dimethylloxazolidine-2,4-dione (DMO) between arterial plasma water and brain water in 106 rats anesthetized with pentobarbital and 24 rats anesthetized with nitrous oxide-oxygen. Arterial CO₂ tensions ranged between 8.5 and 133 mm Hg. Regression equations were derived to express the relations between the measured or derived cerebral acid-base parameters, and arterial or cerebral tissue CO₂ tension. Excellent agreement was found with DMO measurements on the cat brain previously published from our laboratory. The type of anesthesia had no effect on acid-base relations. Cerebral CO₂ content derived from DMO distribution agreed closely over a wide range of CO₂ tensions with direct CO₂ measurements made by previous workers.

brain CO₂, intracellular pH of brain cells

IT IS WELL KNOWN that the acid-base relations of the central nervous system have a profound effect on many of its functions. In most of the studies concerned with evaluating these relations, cerebrospinal fluid was sampled; its composition was assumed to be a reflection of the external milieu of the brain. Only recently has the ionic composition, including acid-base relations, of the cerebral interstitial fluid come under direct scrutiny (3, 10); evidence is accumulating that the fluid, in the steady state, indeed closely resembles the fluid which surrounds the organ.

The intracellular acidity and buffering power of the central neurons and glial elements are still a matter of uncertainty. This information must be obtained by indirect means, since direct intracellular measurement of H ion concentration of the brain cells is not yet possible. The CO₂ method, which had given useful results in other tissues, has been applied to the brain for this purpose. By determining total CO₂ content of a brain sample obtained at a particular CO₂ tension, and making assumptions about size and acidity of the extracellular compartment, the intracellular fraction of CO₂ can be calculated. When this fraction is taken to be partitioned solely between physically dissolved CO₂ and bicarbonate, intracellular pH (pHi) and bicarbonate concentration can be derived (these studies have been reviewed in ref 15).

Not only is the derivation of intracellular CO₂ content sensitive to the assumed extracellular parameters, but the measurement of cerebral CO₂ content offers technical difficulties which only recently seem to have been resolved (4, 7, 12). The nonvolatile nature of the weak acid 5,5-di-methyloxazolidine-2,4-dione (DMO), introduced for the purpose of intracellular pH measurements (20), offers an important technical advantage over CO₂. The principle of its application is similar to that of CO₂. In a previous paper (15), I reported the results obtained with this compound applied to cat brain. At cerebral CO₂ tension of 40, I found pH to be about 7.15, with no significant differences between white and grey matter, brainstem, cerebellum, and cerebral cortex.

In the present paper the acid-base relations of the rat brain will be analyzed, again by means of the distribution of DMO, with the purpose of answering these questions: 1) are there significant species differences between cat and rat; 2) what are the acid-base relations at extremely high and low CO₂ tensions; 3) are the acid-base relations affected by the type of anesthesia; 4) what is the accuracy of the DMO method compared with direct measurement of cerebral CO₂ content? In addition, a comparison with the acid-base relations of skeletal muscle has been made; this will be discussed in the second paper where the muscle data from the same rats are reported.

METHODS

Male Sprague-Dawley rats weighing 250–400 g were used in two groups of studies which differed by the type of anesthesia.

a) In 106 experiments the rats were anesthetized with intraperitoneal pentobarbital (40 mg/kg). The kidneys were exposed by bilateral flank incisions and ligated at the hilum. The trachea was cannulated, and total paralysis was induced by intraperitoneal injection of 20–30 mg of gallamine (0.2–0.3 ml). The animals were then artificially ventilated with a Harvard rodent respirator for periods of 50–120 min. This is sufficiently long for steady-state distribution of DMO between blood and tissue to be accomplished (15). Ventilation was kept constant at tidal volume of 4.5–9 ml and rate of 33–100/min. Hypocapnia, normocapnia, and moderate hypercapnia (arterial Pco₂ 5.5–9.5 mm Hg) were accomplished by ventilation with 100% oxygen. To obtain CO₂ levels between 72 and 94 mm Hg, respiratory dead space was increased from less than 0.5 ml to 2 ml. For very high arterial CO₂ tensions (125–183 mm Hg) the animals were ventilated with 15% CO₂-85% O₂ without added dead space. Rectal temperature monitored with an electric thermometer was kept at 37°C by intermittent use of an electric heating pad.

In some experiments an additional intraperitoneal dose of 5 mg of gallamine was given about 1.5 hr after the first,
and occasionally 4 mg of pentobarbital. Mean arterial pressure was monitored from a femoral arterial catheter with a small mercury manometer (volume displacement 6 mm³/cm). Only experiments in which the pressure exceeded 95 mm Hg arc included in the results. In the great majority it ranged between 100 and 150 and was quite stable. No consistent correlation between blood pressure and arterial PCO₂ could be established.

b) Twenty-four rats were anesthetized with a mixture of fluothane and nitrous oxide-oxygen. Each animal was enclosed in a 2-liter glass cylinder through which a flow of 70% N₂O - 30% O₂ at a rate of 3.5 liters/min was maintained. Fluothane was added at an initial concentration of 0.5%; this was raised to 1.5% over a period of a few minutes. When the rat was unconscious (about 8 min after start), he was removed from the cylinder and tracheotomy started, and the animal connected to the respiratory pump and ventilated with 70% N₂O without fluothane. Total period of fluothane breathing did not exceed 20 min. At CO₂ tensions between 81 and 183, N₂O concentration was reduced to 60% in order to maintain arterial PCO₂ in excess of 100 mm Hg. While at CO₂ tensions over 20, mean arterial blood pressure remained stable above 120 mm Hg; at lower tensions four of the eight animals had labile blood pressure, which could be maintained at levels over 100 mm Hg only by periodic cutaneous stimulation.

Either before or immediately after start of artificial ventilation, 0.05 mc of ¹⁴C-labeled DMO (SA 7.8-8 mc/mmol) dissolved in 0.4 ml of saline was injected into a leg vein. In a few experiments the injection was made intraperitoneally; in a few others 25 mg of carrier DMO were added to the injection fluid. Since the data from these rats were in no way different from those of the main group, the results have been pooled. Arterial blood samples (0.2 ml) were collected anaerobically from the catheter into heparinized capillary tubes and analyzed at 37°C for pH, Pco₂, and Po, with the Instrumentation Laboratory model 113 analyzer as described previously (15). From two to four blood samples were collected at intervals to 15-40 min, the first sample no sooner than 30 min after beginning of artificial ventilation, the last immediately before death. Only those studies were included in which the final two or three pH values checked to 0.03 unit; the agreement was closer. An exception must be made for four of the eight extreme hypocapnic studies (arterial PCO₂ < 10), where a steady downward drift in pH of 0.06-0.08 units was observed in the course of 22-46 min. These four experiments have nevertheless been included in the results. In the arterial PCO₂ range of 8-15 mm Hg the final two or three blood samples varied by not more than 1 mm Hg; in the 30-50 range the maximal difference was 2, in the 70-90 range 4, in the 125-183 range 6 mm Hg. Po₂ was 300-400 mm Hg in normocapnia and hypocapnia, 200-300 in hypercapnia. Single values for blood pH and Pco₂ were assigned to each rat by averaging the last two or three measurements. After the last blood sample had been measured, 0.4-0.5 ml of arterial blood was collected in a small heparinized tube and overlaid with mineral oil. The plasma was separated, weighed, diluted with 2 ml H₂O, and stored.

Immediately after the last sample the rat was decapitated, the skull opened, and three 30- to 100-mg samples of the cerebral hemispheres were taken. The lightly bloated samples were weighed to the nearest 0.01 mg, and again after drying for 24 hr (room temperature, 2 cm Hg). After addition of 4 ml H₂O, they were ground, and 0.5 ml of the suspension was pipetted into vials containing 15 ml of the scintillation mixture used previously, and counted (15). Quenching of the suspension was identical with that of water. Diluted plasma (0.5 ml) was simultaneously counted.

Calculations. Corrected counts per minute were expressed per gram of brain water or plasma water. Plasma water content was taken as 0.950 g/ml, plasma density as 1.023 (1). The results of the three brain samples from each study were averaged, since they never differed by more than 5%. Intracellular pH was calculated in the usual way (20), employing for the weight ratio extracellular water/intracellular water (We/Wi) both 0.045 and 0.20. Two separate calculations were carried out at each of the two values for We/Wi.

a) The composition of extracellular water was taken to be identical with that of mean capillary plasma water. Mean capillary pH was derived from measured arterial composition and mean capillary Pco₂, 1 mm Hg less than tissue Pco₂. The latter, which has been found to be equal to CSF Pco₂, was obtained from the data of Pottén and Siesjö (13). Total DMO concentrations in capillary and arterial plasma water are, of course, identical in the steady state.

b) Extracellular water was taken to have the composition of the bulk cerebrospinal fluid. The pH of CSF was calculated from the published data on Pco₂ and total CO₂ content of cerebrospinal fluid, both as functions of arterial Pco₂ (7, 13), which had been obtained on rats under conditions nearly identical with those of my studies. The pK₁' and molar solubility, S, of H₂CO₃ in CSF at 37°C were taken from Mitchell et al. (8). Table 1 lists the CSF parameters and total CO₂ content nearly identical with those of my studies. The pK₁' and molar solubility, S, of H₂CO₃ in CSF at 37°C were taken from Mitchell et al. (8).

<table>
<thead>
<tr>
<th>TABLE 1. Parameters of cerebrospinal fluid</th>
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<td>Arterial Pco₂, mm Hg</td>
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<tr>
<td>Pco₂, mm Hg (14.0)</td>
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<td>Total CO₂, umoles/kg</td>
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<td>pH (1.60)</td>
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<td>pK₁' of H₂CO₃</td>
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lated pH$_i$ to the estimated parameters. The effect of the size of the extracellular compartment has been discussed before (15): a larger compartment leads to a lower derived pH$_i$, more so at low than high P$_{co_2}$. This can be seen again in Fig. 3. If extracellular fluid is assumed to be a capillary ultrafiltrate, changes in its pH affect computed pH$_i$ by about the same amount. On the other hand, when the brain cells are assumed to be bathed in cerebrospinal fluid, the effect of a change in the fluid's estimated pH on derived pH$_i$ is surprisingly small. Thus, at Wc/Wi = 0.20 the sensitivity of pH$_i$ to CSF pH is somewhat greater, but is still only 0.03 to 0.04 per 0.1 unit change in CSF pH$_i$.

From pH$_i$ and tissue P$_{co_2}$ (= CSF P$_{co_2}$ (13)), intracellular bicarbonate concentration (mEq/kg intracellular water) was calculated (S of H$_2$CO$_3$ in brain water = 0.03136 (17), pK$_1'$ = 6.12 (17)). Finally, total cerebral CO$_2$ content (nmol/kg brain) was obtained as the sum of total extracellular (CO$_2$ (either capillary plasma water or CSF), intracellular bicarbonate, and intracellular dissolved CO$_2$ (S = 0.0291 (18)). Cerebral water content in this calculation was taken as 0.784, a value which pertains to cerebral cortex; Wc/Wi as 0.045. The derived total CO$_2$ content is practically independent of the assumed size of the extracellular compartment. No correction was made for blood content nor for Donnan distribution across capillary walls.

RESULTS AND DISCUSSION

Figures 1 and 2 give the direct measurements. Figure 1 shows that at arterial P$_{co_2}$ = 40 arterial pH was 7.51, according to regression, or 7.48 if the average of the 10 rats at P$_{co_2}$ 38–42 is taken. These values agree well with those obtained by Pontén and Siesjö (14) on 21 unanesthetized rats breathing room air (P$_{co_2}$ 38.5, pH 7.47). If a system such as blood or blood plus interstitial fluid is closed except with regard to CO$_2$, the relation between the logarithm of its P$_{co_2}$ and pH will be nearly linear (19). The marked deviation from linearity shown in Fig. 1 is compatible with the entry of fixed acid at low P$_{co_2}$. It is well known that such a process involving the tissue release of lactic and other acids takes place in a number of species including the rat (5). As an additional factor the removal of some cations from the extracellular space at low P$_{co_2}$ cannot be ruled out. If the "ideal" log P$_{co_2}$ – pH relation for the closed extracellular space would have a slope corresponding to the

![Figure 1](http://example.com/figure1.png)

**Fig. 1.** Semilog plot of arterial pH against arterial CO$_2$ tension. Unless specified, curves and equations in this and subsequent figures represent regressions applied to data from 106 rats anesthetized with nitrous oxide-oxygen; intermediate curve: regression of pentobarbital studies; for both Wc/Wi was taken as .045. Lower curve: regression of pentobarbital studies at Wc/Wi = .20; individual data not shown. Extracellular fluid = cerebrospinal fluid.

![Figure 2](http://example.com/figure2.png)

**Fig. 2.** Semilog plot of relation between ratio of DMO concentration in brain water over that in arterial plasma water, and arterial CO$_2$ tension.

![Figure 3](http://example.com/figure3.png)

**Fig. 3.** Semilog plot of relation between intracellular pH and cerebral tissue CO$_2$ tension. Upper curve: regression applied to rats anesthetized with nitrous oxide-oxygen; intermediate curve: regression of pentobarbital studies; for both Wc/Wi was taken as .045. Lower curve: regression of pentobarbital studies at Wc/Wi = .20; individual data not shown. Extracellular fluid = cerebrospinal fluid.
regression slope at $P_{CO_2} = 45$ shown in Fig. 1, namely 0.778, this would mean that plasma pH at arterial $P_{CO_2} = 10$ had been depressed by 0.17 unit (from 7.97 to 7.80).

Figure 3 shows that intracellular pH at tissue $P_{CO_2} = 40$ mm Hg falls between 7.13 and 7.05. Intracellular buffering capacity, $d(\log P_{CO_2})/d(pHi)$, of the brain of rats anesthetized with pentobarbital (60 mg/kg) or phenobarbital (100 mg/kg) to be only 1.4 (12), while with nitrous oxide-oxygen it was in excess of 2.0 and varied with $CO_2$ tension (7). The authors thought that this difference might at least in part be explained by the barbiturates' suppression of the increase in brain lactic and pyruvic acid production with hypocapnia. However, cerebral levels of these acids in hypocapnea were apparently equally raised with the different types of anesthesia (3), although in normocapnea a reduction in acid concentration did occur with large doses of phenobarbital (175–200 mg/kg) (4). It should be pointed out that in our studies the anesthetic dose was considerably less than that used by these previous workers.

The slope, $d(pHi)/d(pHa)$, derived from Fig. 4, is 0.431 at $We/Wi = 0.045$, 0.369 at 0.20.

Figure 5 indicates that the slope, $d(\log HCO_3^-)/d(\log P_{CO_2})$, at tissue $CO_2$ tension = 46 mm Hg (corresponding to arterial $P_{CO_2} = 40$) is 0.635 ($We/Wi = 0.045$) or 0.690 ($We/Wi = 0.20$). In comparison the corresponding regression slopes of true plasma (human) in vivo and in vitro are only 0.17 and 0.30, respectively (2).

The logarithmic plot of total cortical $CO_2$ against tissue $P_{CO_2}$, which is nearly linear, has a slope of 0.662 at $P_{CO_2} = 46$ (Fig. 6).

Figure 7 shows the agreement between the present results and those obtained on 25 cats reported previously (15). The cat data have been recalculated on the basis of assumed equality of $P_{CO_2}$ and pH in interstitial and directly measured cerebrospinal fluid, and of undissociated DMO concentrations in mean capillary plasma water and interstitial fluid. The slightly higher pH in the cat at low $CO_2$ tensions might be due to the cat brain's smaller tendency to accumulate lactic and pyruvic acids with hypocapnia (6, 7).

The results obtained in my group of 24 rats anesthetized with nitrous oxide-oxygen have been compared with those of a group of 35 rats studied by Kjällquist and co-workers (7). These animals, similarly anesthetized with nitrous oxide-oxygen, were exposed to various $CO_2$ tensions for nearly the same length of time (45 min) as those studied by me (70 min); the brain was then frozen in vivo, and the total $CO_2$ concentration of the supratentorial skull content directly measured. For comparison, I have converted my DMO distribution data into the same parameter. Water content of supratentorial mass was taken as 0.784, the value found by the Swedish workers. Since uncertainty exists...
about the relative quantity of bulk CSF in the mass, two sets of calculations were carried out, assuming either no bulk fluid or 9%, almost certainly an exaggerated figure based on dog data (16). In both cases interstitial fluid was taken as equal to CSF, the composition of which was derived from measurements by the same workers (7) on some of the same rats (see Table 1). In addition, a third calculation was carried out on the assumptions of interstitial fluid being a capillary ultrafiltrate and bulk CSF occupying 6% of supratentorial mass. The results are shown in Fig. 8. The straight line represents the data of Kjallquist and co-workers; their individual measurements (not shown) deviated from the line by not more than about 1 mmole/kg. The agreement is quite good for the entire arterial PCO₂ range (15–90 mm Hg) over which comparison is possible.

Siesjö (17) previously found the relation between pH, physically dissolved CO₂, and total CO₂ of physiologic salt solutions to be not significantly affected by the presence of 5–42% of homogenized brain tissue. This indicates a partition of cerebral CO₂ solely between dissolved CO₂ and bicarbonate ions, with no significant bound CO₂, at least over the pH range studied (5.9–7.1). Therefore, meaningful values for intracellular bicarbonate concentration and pH can be obtained from total CO₂ content and CO₂ tension. The agreement between the two methods illustrated in Fig. 8 inspires confidence that the DMO distribution can similarly yield reliable information about these
intracellular parameters over a wide PCO₂ range. Both derivations are, of course, dependent to some extent upon assumed size and composition of the extracellular compartment.

REFERENCES


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