

(between 150 and 200 μm below the surface) was found, from which it was possible to drive ventilation by electrical microstimulation before histological mapping (45,46). These neurons confirmed later by electron microscopy (47, 48) were characterized by a random firing pattern, reminiscent of peripheral chemoreceptors, and were sensitive to both inhaled CO_2 and $\text{pH}/[\text{HCO}_3^-]$ changes of the superfused CSF (49,50).

A functional localization of chemosensitive substrates was achieved by a modified version of the early perfusion experiments (25), carried out by Berndt et al. (51–54), now directly superfusing the ventral medullary surface in combination with CO_2 inhalation. The central chemosensors would thus be accessible to CO_2 of the blood and to H^+ of the CSF. Regarding the diffusion laws for CO_2 and HCO_3^- in brain tissue, the extracellular pH value was calculated at different depths below the ventral medullary surface. The highest correlation between pulmonary ventilation and extracellular pH was found in a depth of 250 to 300 μm . This apparently supported a “unique function (reaction) theory” and the idea of rather superficially located chemosensitive structures, distant from the respiratory centers as such.

Neurophysiological studies on ventral medullary surface neurons have been carried out in all three areas in vivo, as reviewed by Schläpke (42): among a total number of 185 units, 45% responded to changes in surface pH . Detailed studies of quantitative relationships between neuronal discharge rates and lowered surface pH in the “intermediate area” revealed comparable results (55): Among 49 neurons, 31 significantly increased their impulse rate to any kind of acidification, irrespective of whether it was induced by CO_2 inhalation, intravenous acid injection, or local superfusion with low HCO_3^- buffer solutions. There were, however, two strikingly different sensitivity ranges, whereby about one-fifth of the cells showed a sensitivity as low as reported for brain slices (about +35% per 0.1 units decrease in pH), whereas the majority showed an approximately tenfold higher sensitivity (+300%/–0.1 pH), reaching the order of magnitude of pulmonary ventilation in response to medullary surface pH -changes induced by CO_2 -inhalation (56–58). The neurons were likewise activated in different ways, but coordination between sensitivity and mode of acidification is still unknown.

After the pioneer work of Fukuda and Honda (59), in vitro brainstem slice recordings were continued under different respiratory and metabolic acid-base conditions. More than 50% of the cells investigated in superficial slices of the ventral medulla were found to be H^+ -sensitive, but only 13% of those in the dorsal medulla as in whole brain. The H^+ -sensitive neurons in the ventral medullary surface slices showed an average rise of impulse activity by not more than about 25% in response to a decrease in pH of 0.1 units (60), whereby CO_2 and H^+ responses were not differentiated.

In order to furnish proof of a cholinergic mechanism underlying central H^+ chemosensitivity, pharmacological experiments with cholinergic agonists

and antagonists were conducted on intact animals in vivo and on brainstem slices in vitro.

In vivo, it could be shown that the spatial distribution of the respiratory responses to locally applied acetylcholine (ACh) or nicotine coincided rather accurately with that of the respiratory responses to locally varied pH or electrical microstimulation (42,45,61). These responses could be diminished or abolished by prior application of cholinergic blocking agents, whereas inhibition of ACh-esterase (AChE) enhanced resting ventilation without a change in CO_2 -sensitivity (62). Recently, these findings were extended by Trouth et al. (50). The H^+ and CO_2 responses of non-respiratory modulated neurons in the “caudal area” were depressed by topically applied atropine and enhanced by ACh, suggesting cholinergic involvement at muscarinic receptor sites. Naloxone potentiating the excitatory responses to low pH , ACh, and physostigmine pointed to cholinergic and opioid interaction.

In vitro, it could be demonstrated that H^+ -sensitive excitation of neurons depended on intact cholinergic synaptic transmission (63). Thus, three possible roles of H^+ were discussed, either increasing the sensitivity of cholinceptive neurons to ACh, or augmenting the release of ACh from presynaptic terminals due to greater ionization of calcium, or inhibiting AChE activity.

Since then, an enormous number of studies have been carried out dealing with putative transmitters involved in central and peripheral chemoreception by others. Some recent attempts by Prabhakar should be mentioned (64), since, when he was a young graduate student, his views were decisively shaped by Loeschcke.

Signal Transmission from Blood to Brain ECF

In addition to the theoretical calculations of brainstem extracellular fluid (ECF) pH by Berndt et al. (51), direct measurements were attempted by Ahmad and Loeschcke (65–67). Based on the morphological evidence of free access of substances from the subarachnoid to the intercellular spaces (47,48), ECF pH and PCO_2 could be measured with sufficient accuracy immediately at the ventral surface of the medulla by balanced noninvasive macroelectrodes. Following on-and-off step changes of inspired CO_2 , the time courses of pulmonary ventilation and medullary (but not cortex) surface pH were closely correlated with each other without hysteresis, pointing to acid-base changes in the ECF environment of the central chemosensor as adequate stimulus (65).

Since the ECF does not contain considerable amounts of protein, an increment in PCO_2 was expected to reduce pH (according to the Henderson-Hasselbalch relationship) with almost no change in $[\text{HCO}_3^-]$. However, marked increases in ECF (HCO_3^-) were calculated from simultaneous measurements of pH and PCO_2 . Adjacent glial cells containing carbonic anhydrase (68) were thus assumed to serve as cellular buffer for the ECF, in much the same way as red cells in

contact with blood plasma (Hamburger shift). To support the idea of cellular buffering, a (third) chloride electrode was applied, whereby in the ECF of the ventral medulla a one-to-one monoexponential increase in $[\text{HCO}_3^-]$ and decrease in $[\text{Cl}^-]$ could be demonstrated in response to step rises in end-tidal PCO_2 (66).

By analogy, a nonelectrogenic carrier-mediated $\text{HCO}_3^-/\text{Cl}^-$ exchange across the blood-brain barrier was postulated for metabolic acid-base changes in the blood. Indeed, correspondingly fast inverse changes of both anions in response to a sudden bicarbonate load at constant PCO_2 could be demonstrated. It was suggested that rapid signal transmission from blood to brain should provide rapid adjustment of pulmonary ventilation by central chemosensitivity during both respiratory and metabolic acid-base disturbances (67).

The topic of the last doctoral thesis initiated by Loeschcke was to compare signal transmission from blood to brainstem ECF with ventilatory responses during acute respiratory and metabolic acidosis. In his thesis, Shams studied the ventilatory reactions to inhaled CO_2 and to intravenously infused sulfuric acid. Unexpectedly, pulmonary ventilation during respiratory acidosis reacted about 15 times more sensitively to the same decrease in ECF pH than during metabolic acidosis (58). This coincided at least qualitatively with our own findings (56,57), when metabolic acidosis was not caused by infusion of strong mineral acids but instead by endogenously accumulated lactic acid. In spite of a considerable reduction in ECF pH, no ventilatory drive was thereby elicited at all, although the CO_2 sensitivity was not disturbed.

From their early perfusion experiments, Loeschcke et al. (25) had not recognized the rather low H^+ -sensitivity of ventilation to a decrease in CSF $[\text{HCO}_3^-]$ at constant PCO_2 . Tidal volume (V_T) increased only by 12% per 0.1 unit fall in CSF pH upon low $[\text{HCO}_3^-]$ perfusion, being quite in the order of magnitude observed later on for ventilatory responses to ECF pH changes induced by intravenous acid infusions (58,69,70). Furthermore, it had been concluded from the superfusion studies that only H^+ ions, and not CO_2 , were able to drive lung ventilation, since tidal volume was depressed by a rise in superfused CSF PCO_2 at constant $[\text{HCO}_3^-]$. However, a CO_2 drive in addition to that of ECF H^+ could not generally be ruled out, since inhaling CO_2 to keep CSF (or ECF) pH constant at enhanced $[\text{HCO}_3^-]$ rather increased than decreased pulmonary ventilation markedly (37,38,58).

Thus, Loeschcke (43), trying to explain these discrepancies, suggested that H^+ ions, unlike CO_2 , might not under all circumstances have free access to the receptor, e.g., possibly the synaptic cleft of cholinergic neurons.

B. Persisting Objections Against the Reaction Theory

Hypoxia and Endogenous Lactacidosis

Before the discovery of the peripheral chemoreceptors, the difficulty of reconciling hypoxic dyspnea and blood alkalinity was solved by assuming "asphyxiating

substances" in the centers according to the second version of the reaction theory (17). After the discovery of the peripheral chemoreceptors, Winterstein (22) claimed that the hypoxic ventilatory depression and CO_2 rise observed in chemoreceptor-deprived animals was an "experimentum crucis" against the traditional CO_2 theory, for once not considering the role of hypoxia-induced acid metabolites for central chemosensitivity any more.

To explore the role of hypoxia-induced lactic acid, we recorded the ventral medullary surface pH and pulmonary ventilation in chemodenervated cats after repeated periods of hypoxia (56,57). The distinct acidification of ECF (by about 0.15 pH units), which persisted during posthypoxic recovery, unexpectedly did not lead to any ventilatory response at all (Fig. 7). On the other hand, a strong and reproducible rise in ventilation (by about +360%/–0.1 pH units) could be observed when ECF pH was reduced by CO_2 inhalation. Under the same experimental condition, we found extraordinarily high concentrations of lactic acid in the arterial blood of chemodenervated rabbits but likewise no ventilatory responses (71).

To explain the discrepancy between the lack of ventilatory reaction to endogenous brainstem lactacidosis and the high sensitivity of pulmonary ventilation against CO_2 -induced changes in ECF pH, the unique significance of brainstem ECF $[\text{H}^+]$ for central chemoreception had to be questioned. Kiwull-Schöne and Kiwull (56) speculated about taking the intracellular pH as reference value, so that, assuming chemosensory structures were affected by the intra/extracellular H^+ gradient, the simultaneous decrease of pH in both compartments would remain ineffective as respiratory drive.

More recently, this concept was substantiated by similar observations of Xu et al. (72) in awake goats, showing "anomalous" hypoxic acidification of medullary ventral surface, no ventilatory reaction to CO -hypoxia, but maintained responses of ECF pH and ventilation to inhaled CO_2 . These observations were discussed to be compatible with the hypothesis that the chemosensor cell might be stimulated by a fall of the transmembrane H^+ gradient, whereby internally generated (hypoxic) acidosis would increase the gradient and therefore depress rather than stimulate the chemoreceptors.

For hypercapnia, there is indeed experimental evidence of a decreased transmembrane H^+ gradient due to more effective buffering in the intracellular than in the extracellular compartment (73,74). For anaerobic intracellular lactic acid production, however, direct evidence of an increased transmembrane H^+ gradient is still lacking.

To explain the small or even absent ventilatory response to the significant fall in brainstem ECF pH following fixed acid infusion (57,58,69–71), a necessary implication of this rather attractive hypothesis would be that extracellular metabolic acidosis should lead to a less pronounced diminution of the gradient than respiratory acidosis. The same argument is valid for the rather strong metabolic brainstem acidification (by 0.250 to 0.730 pH units) reached by super-

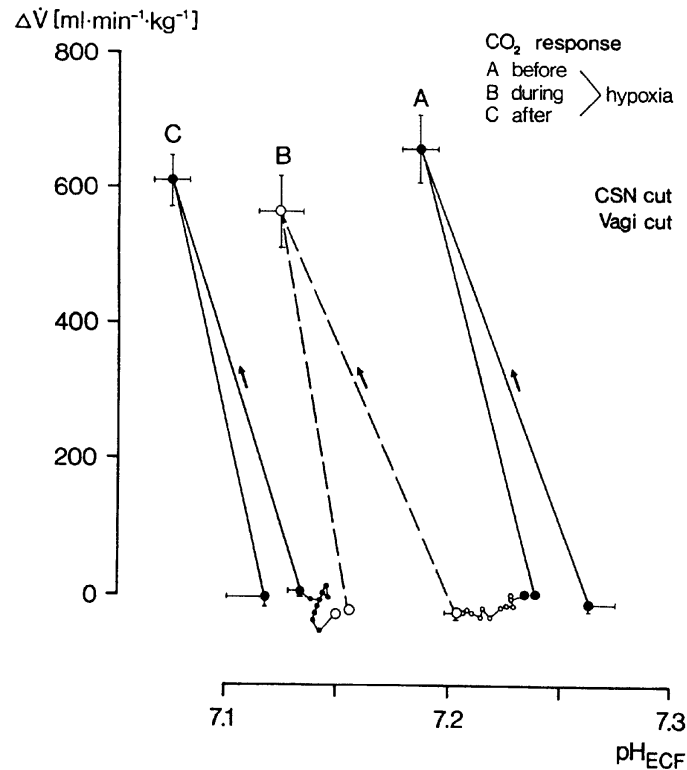


Figure 7 Lack of ventilatory reaction to hypoxia-induced metabolic brainstem acidosis despite maintained CO_2 sensitivity. Changes in pulmonary ventilation ($\Delta\dot{V} \pm \text{SEM}$) from baseline level ($304 \pm 15 \text{ mL}/\text{min}^{-1}/\text{kg}^{-1}$) as a function of the extracellular fluid (ECF) pH at the ventral medullary surface in 11 carotid chemodenervated cats during hyperoxia (●—●) and hypoxia (○—○) before and after step rises in PaCO_2 of about 1.3 kPa (A,B,C). (Slightly modified from Ref. 57, Fig. 1, courtesy of Plenum Press, New York.)

fusion with isocapnic low- $[\text{HCO}_3^-]$ mock CSF, leading to a rise in tidal volume of only 10% to 15% / -0.1 pH units in cats and rats (43,75).

Thus, the unifying transmembrane H^+ -gradient hypothesis would only explain the rather low central chemosensitivity to metabolic acid-base changes in brainstem ECF, if these were less effectively buffered inside the cell than hypercapnia.

Breathing Pattern Differences

Another argument against a unique central chemoreception for respiratory and metabolic acidosis concerns the different type of breathing pattern. The now classic spirometric recordings during perfusion of the fourth ventricle with

low $[\text{HCO}_3^-]$ CSF by Loeschcke et al. (25) demonstrate a nearly exclusive rise in tidal volume.

Similarly, the ventilatory response to acute and chronic metabolic acidosis in chemodenervated and vagotomized rabbits exclusively consisted in a tidal volume rise, respiratory rate being even reduced (71,76). On the other hand, the central chemosensitive breathing pattern response to inhaled CO_2 in these rabbits included both tidal volume and respiratory frequency in a rather definite proportion.

According to Hey et al. (77), the type of breathing pattern response can be quantified by the linear relationship between ventilation (\dot{V}) and tidal volume (V_T), $\dot{V} = m (V_T - k)$. The \dot{V}/V_T relationships we found for acute respiratory acidosis and chronic metabolic acidosis are compared in Figure 8: The CO_2 -responses are characterized by rather high values of $m \pm \text{SD} = 42.0 \pm 2.3 \text{ min}^{-1}$

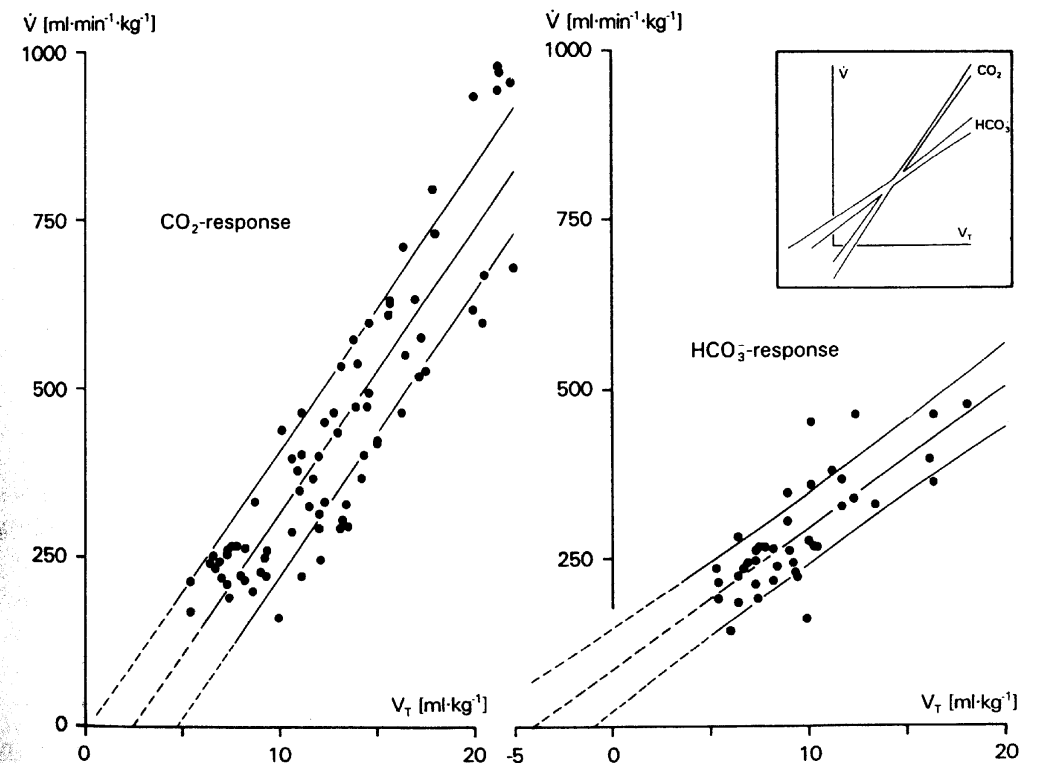


Figure 8 Quantitative comparison of breathing pattern during respiratory and metabolic acidosis. V/\dot{V}_T relationship in anesthetized chemodenervated and vagotomized rabbits during CO_2 inhalation ($n = 80$, left-hand diagram) and during chronic metabolic acidosis in the range between 13 and 28 mM $[\text{HCO}_3^-]$ ($n = 41$, right-hand diagram). Linear regression analysis $\pm \text{SD}$ (or $\pm \text{SEM}$ in the small insert) showed slopes (m) and intercepts (k) with the V_T axis to be significantly different in the two groups ($P < 0.0002$, unpaired t -test). (Data from Ref. 76.)

and positive values of $k \pm SE = 2.5 \pm 0.5$ mL/kg, pointing to an increasing involvement of respiratory rate, whereas the responses to lowered arterial $[\text{HCO}_3^-]$ (in the range between 28 and 13 mM) consist in a pure tidal volume response and even decreasing frequency, thus showing smaller values of m (20.8 ± 2.6 min⁻¹) and negative values of k (-4.2 ± 1.1 mL/kg). Interestingly, the same principle can be demonstrated when re-evaluating the experiments of Pappenheimer et al. (38) in awake goats, using ventricular-cisternal perfusion combined with CO₂ inhalation. Breathing pattern responses to CO₂ inhalation are likewise characterized by high values of m (~ 41 min⁻¹) and positive values of k (~ 6 mL/kg), whereas those to changed CSF $[\text{HCO}_3^-]$ at constant PCO₂ yield smaller values of m (~ 12 min⁻¹) and negative values of k (~ -18 mL/kg).

This consistently different quality in the breathing pattern response to either respiratory or metabolic acid-base changes suggests different underlying central chemosensory control mechanisms.

VI. Today's Research on the Ventrolateral Medulla

A. NMR Studies of Whole-Brain Intracellular pH

One promising type of modern approach to explore the still undefined nature of central chemosensitivity is noninvasive nuclear magnetic resonance (NMR) spectroscopy. Under the topic of chemical respiratory control, whole-brain intracellular pH (pH_i) was estimated by NMR spectroscopy in anaesthetized animals (74,78) and conscious humans (73,79). Based on the NMR pH measurements in brain of conscious humans, Lassen (80) raised the crucial question of whether the central chemoreceptors were sensitive to intracellular pH (pH_i) rather than to extracellular pH (pH_e). Conclusions were drawn from comparing effects of hypercapnia and oral acetazolamide: since during hypercapnia the brain pH_i was diminished and pulmonary ventilation concomitantly enhanced but following acetazolamide application neither variable was changed, pH_i but not pH_e was assumed to be the central chemoreceptor stimulus. This rather "economic" supposition may be taken as some resuscitated intracellular reaction theory.

On the other hand, the simultaneous determination of intracellular and extracellular brain pH at different levels of arterial PCO₂ by Portman et al. (74) lent support to the transmembrane H⁺ gradient hypothesis (72) at least for hypercapnia, since better buffering of CO₂ inside the cell should markedly decrease in the pH gradient between the extracellular and the intracellular space and may influence central respiratory control. As pointed out above, the experimental proof of the H⁺ gradient hypothesis for endogenous and exogenous metabolic acidosis is still lacking.

Thus, in spite of this very elegant noninvasive technique to determine intracellular brain pH, the adequate stimulus for central chemosensitive control of breathing is not yet finally determined, but unifying theories are supported.

B. Reduced Central Chemosensitive Systems

Another main line of modern approaches to the problem of central chemosensitivity is the development of reduced chemosensitive subsystems in vitro to eliminate complex whole-system interactions.

The isolated "brainstem-spinal cord preparation", including cranial and phrenic nerve roots to record respiratory motor output, is usually taken from neonatal rats and superfused with oxygen-enriched mock CSF at reduced temperature. Besides rhythmogenesis, this preparation serves to explore central chemosensitivity and differential effects of respiratory and nonrespiratory acidosis.

Harada et al. (81) found maintained respiratory responses directly related to extracellular $[\text{H}^+]$, when $[\text{HCO}_3^-]$ in the superfusate was changed at constant PCO₂. The average pH sensitivity of phrenic minute activity was 15%/0.1 pH units and both phrenic amplitude and frequency were involved. Unexpected was the distinct transient rise in phrenic discharge amplitude alone, elicited by superfusion with high CO₂ at constant pH, pointing to some specific CO₂ sensing mechanism. Furthermore, there was no response to extracellular acidification by HCl in HEPES buffer free of CO₂/HCO₃⁻, comparable to in vivo findings by Leusen (30,39,40). Central chemoreceptors eliciting strikingly different breathing pattern responses appeared to be independently sensitive to either H⁺/HCO₃⁻ or CO₂.

Contrarily, a pure frequency response to hypercapnic superfusion was found by Okada et al. (82) in a similar preparation. However, depth profiles of pH and PO₂ have shown that the superfused brainstem-spinal cord preparations are more or less anoxic and acidic except for a small layer below the surface (83), possibly causing this functional variability.

Thus, it is reasonable to perfuse the vertebral arteries additionally, as done in preparations of adult guinea pigs by Morin-Surun et al. (84). This better supplied preparation did react to high CO₂ at constant pH in the superfusate by increasing again the burst amplitude of hypoglossal nerve discharge. However, if the same solution was delivered via the vertebral arteries, respiratory frequency was accelerated. Nonrespiratory acidosis at constant PCO₂ affected the frequency throughout, disregarding the mode of application. In agreement with Harada et al. (81), this preparation also revealed results in favor of different central chemosensitive subsystems, some sensitivity to CO₂ at ventral surface affecting burst amplitude, but no specific H⁺-sensitivity.

The next step was to reduce the system to progressively thin brainstem slices. An extensive study of respiratory and nonrespiratory acid-base effects on the neuronal activity in this slices of the ventral medullary surface layer was performed by Fukuda (85). Only a small number of the neurons studied (12%) responded as expected from the reaction theory, namely to the both kinds of pH reduction either by high CO₂ at normal $[\text{HCO}_3^-]$ or by low $[\text{HCO}_3^-]$ at normal PCO₂. The majority (72%) was activated by high CO₂ even at normal pH.

A neuronal subpopulation of about 30% did respond to low pH only when induced by high CO_2 , another of about 40% only when low pH was induced by low $[\text{HCO}_3^-]$. Thus, this slice approach, in much the same way as the isolated brainstem preparation, did not support the unifying reaction theory, but CO_2 and HCO_3^- appeared to act rather differently on chemosensitive elements.

Another study by Jarolimek et al. (86) on ventral medullary slices in the rat revealed that 43% of the investigated neurons were activated by low bicarbonate acidification, but in all cases only transiently. Increased PCO_2 at constant pH, however, generally depressed the activity, except for a few chemosensitive neurons, in which the response to the same extracellular pH reduction was potentiated by hypercapnia. This observation, pointing at least to some prominent role of CO_2 over fixed acids, led these authors to suggest a role for intracellular pH in the central chemosensitive process.

The next promising approach to explore the nature of central chemosensitivity is based on organotypic tissue cultures of small but defined brainstem regions, adequately supplied by oxygen and substrates, and disconnected from extended neuronal networks. Results available up to now are, however, contradictory (87,88).

Bingmann et al. (87) performed intracellular recordings in neurons of cultured (horizontal) medullary slice explants under different respiratory and nonrespiratory acid-base conditions in the superfusate. He found 62 neurons with periodic (respirationlike) activity not confined to any dorsal or ventral region. This periodic activity consisted in depolarizing (D-) waves of 100 to 300 ms duration, generating short trains of action potentials. In part of the neurons, occurrence of these periodic events was tested for decreased $[\text{HCO}_3^-]$ at constant PCO_2 , for changes in PCO_2 at constant $[\text{HCO}_3^-]$ and for HCl administration. Periodic activity changed with all kinds of acid-base deviation from the control pH (7.4) with an average sensitivity of about 7%/0.1 pH units. This appeared to be consistent with the hypothesis of a unique dependence on H^+ activity of medullary neurons involved in central respiratory chemosensitivity.

Neubauer et al. (88) came to the opposite conclusion. They likewise used explant tissue cultures of ventral and dorsal medulla. All ventral brainstem neurons showed steady pattern firing. About half of the dorsal neurons showed either "steady" or "bursting" firing pattern. Part of both kinds of neurons were investigated for chemosensitive behavior. About one-third of both ventral and dorsal neurons increased firing frequency in response to hypercapnic acidosis (in one representative response +10%/0.1 pH unit). In some neurons tested for lowered $[\text{HCO}_3^-]$, no reaction was found. Thus, chemosensitive neurons appeared to be present in explant tissue cultures of both the ventral and dorsal medulla, but these cells responded to changes in pH only in association with changes in CO_2 tension.

The role of carbonic anhydrase has also been questioned in this respect. Martin and Neubauer (89) studied the effect of acetazolamide on CO_2 sensitivity

of cultured medullary neurons using the perforated-patch technique for whole-cell recording. Respiratory acidosis produced an overshooting depolarization and spike discharge, more rapidly adapting after the addition of acetazolamide. It appeared that in these neurons, carbonic anhydrase was required to sustain the membrane responses to CO_2 -induced acidosis. Unfortunately, nothing is known about concomitant intracellular pH changes in these neurons.

E. The Peripheral Chemoreceptors Model for H^+ Sensitivity

There are recent publications dealing with intra/extracellular pH relationships in cultured carotid body type I cells under acid-base conditions comparable to those studied in the central chemosensitive system. The idea to take the peripheral chemoreceptors as a model for H^+ -chemosensitivity arose as early as the third and fourth version of the reaction theory (10,21). The acid theory to explain the peripheral chemoreception was followed up by Torrance (90), initially postulating the extracellular pH as being the unique and adequate stimulus during hypoxia, hypercapnia, and acidosis. Thereby, the question arose analogous to that raised earlier for central chemosensitivity, namely, whether arterial CO_2 was sensed independently of pH (91) or not (92).

More recent experiments, comparing membrane permeating and nonpermeating carbonic anhydrase inhibitors, led to the replacement of the extracellular theory of peripheral chemoreception by an intracellular one (93), suggesting that intracellular carbonic anhydrase and a rise in $[\text{H}^+]$ were involved at least in the quick response to CO_2 .

Indeed, isolated carotid body type I cells, stained with a pH-sensitive fluorescent dye, exhibited intracellular acidification upon changing extracellular non-bicarbonate buffer to isohydric $\text{CO}_2/\text{HCO}_3^-$ solution. The rate of intracellular acidification, probably due to CO_2/H^+ conversion, was considerably slowed by the membrane permeant carbonic anhydrase inhibitor acetazolamide (94). Likewise, switching from bicarbonate-free to prolonged $\text{CO}_2/\text{HCO}_3^-$ buffer perfusion of the isolated carotid body in vitro caused biphasic responses of sinus nerve discharge. Thereby, intracellular carbonic anhydrase inhibition, affecting only the peak-response but not the persisting response, substantiated that the CO_2 response of the peripheral chemoreceptors most probably is mediated by a rise of intracellular $[\text{H}^+]$, independent of extracellular $[\text{H}^+]$ (95).

The possible role of intracellular $[\text{H}^+]$ for chemoreception in metabolic acidosis has been investigated in isolated glomus cells by varying either external $[\text{HCO}_3^-]$ or PCO_2 independently. Wilding et al. (96), using a pH sensitive fluorescent dye, made the fundamental observations that the steady state relationship between intra- and extracellular pH was the same under different respiratory and nonrespiratory acid-base conditions as well as in the presence and absence of extracellular $\text{CO}_2/[\text{HCO}_3^-]$. The exceptional sensitivity of the intracellular to the extracellular pH suggested pH_i of the glomus cell to be a link in the chemoreceptor's response to external acidity.

With the same technique, Buckler et al. (97) investigated dynamic responses of intracellular pH responses to extracellular hypercapnic acidosis, isocapnic acidosis, and isohydric hypercapnia. Hypercapnic acidosis led to a rapid and isocapnic acidosis to a slower reduction in pH_i , reaching the same steady-state level. Isohydric hypercapnia, however, although leading to rapid transient reductions in pH_i , did not significantly affect steady-state pH_i over a wide range of PCO_2 . By comparing these dynamics of intracellular pH to those of carotid sinus nerve discharge recorded elsewhere in situ under comparable acid-base conditions (92), the authors indirectly concluded that peripheral chemoreceptors should uniquely respond to intracellular pH.

However, there are no direct experiments to support intracellular $[H^+]$ as being the adequate stimulus for peripheral chemoreceptor activation. The only report on the resting potential and intracellular pH in glomus cells measured simultaneously by microelectrodes is that of He et al. (98): independent of pH_e , the membrane potential was hyperpolarized by low pH_i , whereas depolarization was correlated with a lower pH_i - pH_e difference, much in agreement with the membrane H^+ gradient hypothesis for central chemosensitivity proposed by Xu et al. (72).

Thus, it would be useful to extend the techniques developed for pH_i measurement in isolated glomus cells to the reduced preparations of central chemosensitive structures, to verify or to discard unifying intracellular H^+ or transmembrane H^+ gradient theories.

VII. Open Questions and Future Perspectives

The history of research on chemical control of breathing is characterized by partial acceptance of the unique role of $[H^+]$ sensitivity but also by repeated objections against the rather reductive reaction theory. By means of whole-system approaches, several research groups have substantiated unifying theories, earlier concerning central nervous extracellular fluid $[H^+]$ (25,26), including within tissue $[H^+]$ gradients (38,51-54). Since, however, techniques for direct measurement of brainstem ECF pH became available, its unique role in ventilatory responses to inhaled CO_2 and to intravenous acid infusion has been challenged (57,58,68,70,71).

With the possibility to determine whole-brain pH_i by NMR techniques, the unique role of extracellular $[H^+]$ as adequate stimulus for central chemosensors has been replaced by intracellular $[H^+]$ (80). For instance, the behavior of pH_i in glomus cells could serve as a model for central chemosensitive neurons and explain the repeatedly observed respiratory responses to proportionate rise in CSF PCO_2 and $[HCO_3^-]$ in vivo (30,39,40) and in vitro (81,85,88) by CO_2 more easily penetrating cell membranes and acidifying the cytosol.

If, for the central chemosensitive process, CO_2 had to be converted to intracellular H^+ , then for quick CO_2 reactions of the respiratory system carbonic

anhydrase would be required. Carbonic anhydrase appeared indeed to act at different sites in the region of medullary chemoreceptors, on either side of the blood-brain barrier (99). More recently, focal application of small amounts of acetazolamide (AZ) to different brainstem sites was performed by Coates et al. (100). The induced slope reduction and slowing of the respiratory response to CO_2 step rises speak in favor of H^+ as being the adequate stimulus for detection of transient changes. Since respiratory responses to AZ were not uniquely correlated with medullary surface pH, this altogether led to the assumption that central chemoreceptors responded to changes in intracellular rather than extracellular fluid pH.

However, this assumption of an attenuated intracellular acidification cannot explain why considerable respiratory effects can be elicited by microinjections of AZ at "widespread sites" of the medulla (100,101) and by systemic carbonic anhydrase inhibition in servoventilated animals (57,102). Likewise, another important observation is not compatible with the intracellular H^+ theory, namely the lack of ventilatory reaction to posthypoxic brainstem lactacidosis in spite of a maintained CO_2 response (56,57). This led to transmembrane H^+ gradients being considered earlier (56) and substantiated more recently (72,103).

Transmembrane H^+ gradients have also been proposed by Severinghaus (103) to explain the respiratory drive by carbonic anhydrase inhibition: The seeming paradox that systemic administration of AZ does acidify brain ECF at constant brain tissue PCO_2 with no fall in ICF pH (78) could be due to a rise in brain tissue $[HCO_3^-]$, opposed by metabolically generated carbonic acid, which, better buffered inside the cell and diffusing out, would lead to a greater decrease in extracellular than intracellular pH. This, in agreement with the transmembrane H^+ gradient hypothesis, could account for respiratory stimulation by AZ due to extracellular carbonic acidosis in much the same way as for the lack of stimulation due to intracellular hypoxic acidosis (103).

Interestingly, there is one report on simultaneous recording of intracellular pH and membrane potential pointing directly to a possible role for the transmembrane H^+ gradient in glomus cell depolarization (98).

There are, however, several findings on central chemosensitive systems in vivo and in vitro that are not compatible with either intracellular or transmembrane $[H^+]$ theories. The intracellular $[H^+]$ or transmembrane H^+ gradient in peripheral chemoreceptor type I cells, when taken as a model for central H^+ sensitivity, could not generally explain the differing respiratory responses observed. The intra/extracellular pH relationship in the glomus cell model remained the same under all respiratory and metabolic acid-base conditions and was independent of whether bicarbonate or nonbicarbonate buffer was used. Thus, neither the differing effects of metabolic and respiratory acidosis in isolated brainstem-spinal cord preparations, slices, and tissue cultures (81,85,88) would be explained, nor the lacking effects of acid nonbicarbonate buffers on respiratory responses (81), unless central chemosensitive neurons underwent transmembrane H^+ distributions different from those in glomus cells. Further-

more, it is also inexplicable in the light of this theory why respiration in vivo does not react to acid nonbicarbonate buffers at the ventral medullary surface (30,39,40) and why, in spite of predicted intracellular $\text{CO}_2/[\text{H}^+]$ conversion, local high CO_2 /saline injections in central chemosensitive areas remain ineffective (101).

There is persisting doubt about unifying hypotheses throughout, based on strikingly different sensitivities to respiratory and metabolic blood or CSF acidosis observed in vivo, either during ventriculo-cisternal perfusion and medullary superfusion (26,38,51-54) or during intravenous acid infusion (57,58, 69,70,71). Likewise, respiratory pattern analysis of CO_2 responses and acid responses in vivo (76) led to the postulate of different central chemosensitive subsystems for CO_2 and $[\text{H}^+]$ or $[\text{HCO}_3^-]$. Furthermore, the observation that AZ did not affect the sensitivity of the steady state CO_2 response (57,100,102) but did affect the apnoeic threshold and breathing pattern (102) does not rule out a possible role of CO_2 independent of $[\text{H}^+]$.

Experimental evidence also from reduced central chemosensitive systems pointed to the possibility of at least two different subsystems of central chemosensitivity. This has been proposed for medullary slices by Fukuda (85) and for explant tissue cultures by Neubauer et al. (88) as well as for isolated brainstem-spinal cord preparations by Harada et al. (81) and Morin-Surun et al. (84), the latter approaches also showing characteristic differences in breathing pattern. Intracellular pH measurement in addition to neuronal activity in reduced brainstem preparations would be necessary to verify or falsify the significance of intracellular H^+ or the transmembrane H^+ gradient as unique stimulus for central CO_2 and acid sensitivity and, if falsified, to substantiate the involvement of at least two different subsystems, tentatively a more sensitive blood-related CO_2 system and a less sensitive but rather effective CSF-related acid-base system.

In spite of the considerable development of experimental techniques up to now, the exact nature of central chemosensitivity is still undefined. This may be due to inherent dangers in highly sophisticated techniques that sometimes the problem gets lost. Winterstein, although remaining up to date with new techniques throughout his scientific life, never took a method as an end in itself but always kept close to the primary question. Originating from Winterstein's theory there is still the problem whether a unique stimulus of central chemosensitivity or CO_2 different from H^+ elicit the drives to pulmonary ventilation during respiratory and metabolic acidosis.

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References

1. Honda Y. Acid-Base Balance: Review in Basic and Clinical Medicine. 2nd ed. Tokyo: Shinko-Koeki Medical Book Publisher, 1984.
2. Kellogg RH. Central chemical regulation of respiration. In: Handbook of Physiology: Vol. 1, Sec. 3. Respiration. Washington DC: American Physiological Society, 1964:507-534.
3. Perkins JF Jr. Historical development of respiratory physiology. In: Fenn WO, Rahn H, eds. Handbook of Physiology: Vol. 1, Sec. 3. Respiration. Washington DC: American Physiological Society, 1964:1-62.
4. Winterstein H. Skizzen aus meinem Leben. In: Ärzte unserer Zeit in Selbstdarstellungen. Hippokrates 1962; 33:79-83.
5. Weber HH, Loeschke HH. In Memoriam Hans Winterstein. *Ergebn Physiol* 1964; 55:1-27.
6. Burmeister J. Geschichte der Physiologie an der Universität Rostock. *Wiss Z Universität Rostock* 1989; N-Reihe 38/8:35-41.
7. Winterstein H. Über die Wirkung der Kohlensäure auf das Centralnervensystem. *Arch Anat Physiol* 1990 (suppl):177-192.
8. Gad J. Über das Atemzentrum in der Medulla oblongata. *Arch Anat Physiol* 1893; 175-184.
9. Winterstein H. Die Regulierung der Atmung durch das Blut. *Pflügers Arch* 1911; 138:167-184.
10. Winterstein H. Die chemische Steuerung der Atmung. *Ergebn Physiol* 1955; 48: 328-528.
11. Winterstein H. Kausalität und Vitalismus vom Standpunkt der Denkökonomie: 2. Aufl. Berlin: Springer, 1928.
12. Hasselbalch KA. Neutralitätsregulation und Reizbarkeit des Atemzentrums in ihren Wirkungen auf die Kohlensäurespannung des Blutes. *Biochem Z* 1912; 46:403.
13. Winterstein H. Neue Untersuchungen über die physikalisch-chemische Regulierung der Atmung. *Biochem Z* 1915; 70:45-73.
14. Gray JS. Pulmonary Ventilation and Its Physiological Regulation. Springfield, IL: Charles C. Thomas, 1950.

15. Jacobs M. The influence of ammonium salts on cell reaction. *J Gen Physiol* 1922; 2:181-187.
16. Gesell R, Hertzman AB. The regulation of respiration: IV. Tissue acidity, blood acidity and pulmonary ventilation: A study of the effects of semipermeability of membranes and the buffering action of tissues with the continuous method of recording changes in acidity. *Am J Physiol* 1926; 78:610-629.
17. Winterstein H. Die Reaktionstheorie der Atmungsregulation. *Pflügers Arch* 1921; 187:293.
18. Winterstein H. Atmungsregulation und Reaktionsregulation. *Naturwissenschaften* 1923; 625:644.
19. Heymans JF, Heymans C. Sur les modifications directes et sur la régulation réflexe de l'activité du centre respiratoire de la tête isolée du chien. *Arch Int Pharmacodyn* 1927; 33:273.
20. von Euler US, Liljestrand G, Zotterman Y. The excitation mechanism of the chemoreceptors of the carotid body. *Skand Arch Physiol* 1939/40; 83:132.
21. Winterstein H. The "reaction theory" of respiratory regulation. *Experientia (Basel)* 1949; 5:221.
22. Winterstein H. Die Atmung ohne Chemoreceptoren. *Arch Int Pharmacodyn* 1950; 83:80.
23. Winterstein H, Gökhan N. Ammoniumchlorid-Acidose und Reaktionstheorie der Atmungsregulation. *Arch Int Pharmacodyn* 1953; 93:212.
24. Winterstein H. The actions of substances introduced into the cerebrospinal fluid and the problem of intracranial chemoreceptors. *Pharmacol Rev* 1961; 13:71-107.
25. Loeschcke HH, Koepchen HP, Gertz KH. Über den Einfluss von Wasserstoffionenkonzentration und CO₂-Druck im Liquor cerebrospinalis auf die Atmung. *Pflügers Arch* 1958; 266:569-585.
26. Mitchell RA, Loeschcke HH, Massion WH, Severinghaus JW. Respiratory responses mediated through superficial chemo-sensitive areas on the medulla. *J Appl Physiol* 1963; 18:523-533.
27. Winterstein H. Die Entdeckung neuer Sinnesflächen für die chemische Steuerung der Atmung. *Naturwissenschaften* 1960; 47:99-133.
28. Wiemer W, Winterstein H, Kiwull P, Ott N. Interaction of intracranial and extracranial respiratory mechanisms. In: Brooks Ch McC, Kao FF, Lloyd BB, eds. *Cerebrospinal Fluid and the Regulation of Ventilation*. Oxford: Blackwell Scientific Publications, 1965:303-330.
29. Perkins JF Jr. The relation of the cerebrospinal fluid to respiration. A brief historical introduction. In: Brooks Ch McC, Kao FF, Lloyd BB, eds. *Cerebrospinal Fluid and the Regulation of Ventilation*. Oxford: Blackwell Scientific Publications, 1965:7-42.
30. Leusen I. Aspects of the acid-base balance between blood and cerebrospinal fluid. In: Brooks Ch McC, Kao FF, Lloyd BB, eds. *Cerebrospinal Fluid and the Regulation of Ventilation*. Oxford: Blackwell Scientific Publications 1965:55-105.
31. Severinghaus JW, Mitchell RA, Richardson BW, Singer MM. Respiratory control at high altitude suggesting active transport regulation of CSF pH. *J Appl Physiol* 1963; 18:1155-1166.

32. Severinghaus HW. Electrochemical gradients for hydrogen and bicarbonate ions across the blood-CSF barrier in response to acid base balance changes. In: Brooks Ch McC, Kao FF, Lloyd BB, eds. *Cerebrospinal Fluid and the Regulation of Ventilation*. Oxford: Blackwell Scientific Publications, 1965:247-267.
33. Mitchell RA. The regulation of respiration in metabolic acidosis and alkalosis. In: Brooks Ch McC, Kao FF, Lloyd BB, eds. *Cerebrospinal Fluid and the Regulation of Ventilation*. Oxford: Blackwell Scientific Publications, 1965:109-140.
34. Wiemer W, Ott N, Winterstein H. Reflektorische und zentrale Anteile der Hyperpnoe bei HCl-Acidose. *Z Biol* 1964; 114:299-308.
35. Loeschcke HH. A concept of the role of intracranial chemosensitivity in respiratory control. In: Brooks Ch McC, Kao FF, Lloyd BB, eds. *Cerebrospinal Fluid and the Regulation of Ventilation*. Oxford: Blackwell Scientific Publications, 1965: 183-210.
36. Loeschcke HH, Sugioka K. pH of cerebrospinal fluid in the cisterna magna and on the surface of the choroid plexus of the 4th ventricle and its effect on ventilation in experimental disturbances of acid-base-balance. *Pflügers Arch* 1969; 312:161-188.
37. Fencel V, Heisey SR, Held D, Pappenheimer JR. Role of cerebrospinal fluid in the respiratory response to CO₂ as studied in unanaesthetized goats. In: Brooks Ch McC, Kao FF, Lloyd BB, eds. *Cerebrospinal Fluid and the Regulation of Ventilation*. Oxford: Blackwell Scientific Publications, 1965:141-149.
38. Pappenheimer JR, Fencel V, Heisey SR, Held D. Role of cerebral fluids in control of respiration as studied in unanaesthetized goats. *Am J Physiol* 1965; 208: 436-450.
39. Leusen IR. Chemosensitivity of the respiratory center: Influence of CO₂ in the cerebral ventricles on respiration. *Am J Physiol* 1954; 176:39-44.
40. Leusen IR. Chemosensitivity of the respiratory center: Influence of changes in the H⁺ and total buffer concentrations in the cerebral ventricles on respiration. *Am J Physiol* 1954; 176:45-51.
41. Van Vaerenbergh P, van der Mijnsbrugge K, Leusen I. The influence on respiration of the substitution of bicarbonate by tris buffer in the cerebral ventricles. *Arch Int Pharmacodyn Ther* 1962; 138:334-337.
42. Schläfke ME. Central chemosensitivity: A respiratory drive. *Rev Physiol Biochem Pharmacol* 1981; 90:171-244.
43. Loeschcke HH. Central chemosensitivity and the reaction theory. *J Physiol (Lond)* 1982; 332:1-24.
44. Kiwull-Schöne H. Chemical signals and the control of breathing. In: Lüttgau HCh, Necker R, eds. *Biological Signal Processing*. VCH Weinheim 1989:112-121.
45. Trouth CO, Loeschcke HH, Berndt J. Histological structures in the chemosensitive regions on the ventral surface of the cat's medulla oblongata. *Pflügers Arch* 1973; 339:171-183.
46. Trouth CO, Loeschcke HH, Berndt J. A superficial substrate on the ventral surface of the medulla oblongata influencing respiration. *Pflügers Arch* 1973; 339:135-152.
47. Dermietzel R. Central chemosensitivity, morphological studies. In: Loeschcke HH, ed. *Acid-Base Homeostasis of the Brain Extracellular Fluid and the Respiratory Control System*. Stuttgart: Thieme Verlag 1976:52-65.

