Neurobiology of panic and pH chemosensation in the brain
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Introduction

Panic disorder is a common psychiatric illness with a lifetime prevalence of about 4.5%. The hallmark of the disorder is recurring panic attacks, which can appear suddenly and unexpectedly. Panic symptoms include shortness of breath, palpitations, shaking, sweating, and fear of losing control. These symptoms resemble those of other serious medical problems and lead some sufferers to think they are having a heart attack or a stroke. One of the most debilitating features of the illness is agoraphobia, a condition in which patients begin to avoid situations and places where a panic attack and the associated discomfort and embarrassment might occur. Consequently, many sufferers learn to avoid daily activities, greatly limiting their productivity and quality of life. Major depression often co-occurs. When severe, these symptoms can be debilitating, particularly for the large number of patients who are refractory to current therapies. Identifying new therapies may require understanding of why panic attacks occur and what triggers them, knowledge that is currently lacking (see Box below). Crucial advances might be made if panic attacks could be evoked in the laboratory so that the underlying...
mechanisms might be deconstructed. This review discusses progress based on this approach, which raises the possibility that brain pH and pH-sensitive receptors may contribute to the pathophysiology of panic disorder.

Panic provocation

Provocation challenges offer potential for unique insights into panic

Panic disorder is relatively unique among psychiatric illnesses, in that symptoms resembling the illness can be provoked by a number of chemicals called panicogens. Because naturally occurring panic attacks are unpredictable, the ability to induce an attack becomes a powerful tool for research. Moreover, the biological mechanisms of the panicogens themselves might tell us a lot about the neurobiology of the illness. Therefore, it has long been hoped that provocation challenges might shed light on the mechanisms underlying panic. Examples of agents with the potential ability to evoke panic attacks include carbon dioxide (CO₂),10,11 sodium lactate,12 doxapram,17 cholecystokinin (CCK-4) and related agonists,18,19 flumazenil,20 caffeine,21 adrenergic agonists (isoproterenol, yohimbine, epinephrine),22 serotonin receptor activators (d-fenfluramine, metachlorophenylpiperazine (m-CPP)),23 and perhaps opioid receptor antagonists.22,23 The symptoms provoked by these agents can closely resemble naturally occurring attacks.22,24 Unfortunately, many of these provocation challenges have been studied too little to ascertain their mechanisms of action and relative potency.23,25 Interestingly, one twin study found a high concordance rate for CO₂ sensitivity,26 suggesting a genetic etiology, although much remains to be learned about the heritability of panicogen sensitivity.

Rodent models of panic provocation challenges

The technology revolutionizing neurobiological research in rodents is rapidly expanding knowledge of mechanisms underlying behavior. The ability to explore panicogens in animal models provides a powerful research opportunity. However, progress in this area has been limited in comparison with the advancements made in other behavioral models. Yet, several examples are notable. Shekhar and colleagues have developed rodent models of lactate-evoked panic27,28 and have found that orexin-expressing neurons in the hypothalamus play a critical role.29 In another example, doxapram potentiated fear and anxiety-related behaviors in rats and induced expression of the immediate early gene c-Fos in the amygdala.30 A few investigators have also begun to explore the effects of CO₂ on fear and anxiety in rodents.31-33 For example, Mongeluzzi et al found that high

Theories of panic etiology

Although the mechanisms for panic remain obscure, several theories provide useful foundations for conceptualizing the bases of the attacks; (i) Some theories focus on cognitive distortions and misinterpretations of somatic experiences. For example, sensing one’s heartbeat may be misinterpreted as an impending heart attack, triggering uncontrolled fear (reviewed in ref 4). (ii) Other theories focus on ventilation. Klein’s false suffocation alarm theory highlights the similarities between panic attacks and the powerful fear that suffocation evokes; this theory posits that a “suffocation alarm” is falsely triggered, thus inadvertently producing panic.3 Interestingly, patients with a history of respiratory disease have a greater risk of panic disorder than the general population.64 Similarly, panic disorder patients with prominent respiratory symptoms were more likely to have a prior history of respiratory insult.7 Thus, previous experience and adaptive plasticity and/or conditioning might play a role in panic.13 (iii) Growing knowledge of the anatomy underlying fear conditioning led Gorman and others to speculate that a supercharged fear circuit could produce panic in response to a wide variety of arousing stimuli.65 This fear circuit is thought to include at least 5 components: (i) Sensory input from viscera via the nucleus of the solitary tract and sensory thalamus. (ii) Processing and conscious control via the prefrontal cortex, cingulate cortex, and insula. (iii) Processing context and fear through the hippocampus and amygdala. (iv) Coordinated output of behavioral, autonomic, and neuroendocrine manifestations from the amygdala via the hypothalamus, periaqueductal gray, locus coeruleus, and parabrachial nucleus.14,15 (v) modulation by monoamines including serotonin and the raphe nuclei.15 Supporting this final component is the well-established benefit of selective serotonin reuptake inhibitors.
CO₂ concentrations can serve as an unconditioned stimulus in Pavlovian fear conditioning. Johnson et al observed that CO₂ inhalation can induce c-Fos expression in fear circuit structures and may thus activate brain regions thought to be responsible for panic. Despite these examples, the mechanisms underlying panicogen action and panic attacks remain largely unknown.

Clinical clues about panicogen action

Perhaps the most well-studied panicogens are CO₂ and lactate. CO₂ provocation challenges vary between investigators, but generally consist of breathing single or multiple breaths of CO₂ at concentrations ranging from 5% to 35%. Protocols for lactate provocation challenges typically include intravenous infusion of 0.5 M sodium DL-lactate up to 10 mg/kg body weight over 20 minutes or until panic occurs. Several observations led investigators to suggest that CO₂ and lactate may share mechanisms of action. For example, most CO₂-sensitive panic attacks are also lactate-sensitive. In addition, CO₂ and lactate produce stereotypic responses. In particular both induce prominent ventilatory symptoms, suggesting a degree of neuroanatomical or physiological overlap. Interestingly, both CO₂ and lactate may be more likely to affect panic disorder patients who report strong respiratory symptoms during their naturally occurring attacks.

CO₂ and lactate may also induce less hypothalamic-pituitary-adrenal (HPA) axis activation than other panicogens, suggesting that their effects may be cortisol-independent. Consistent with this observation, inhibiting cortisol synthesis failed to prevent CO₂-evoked panic. Furthermore, both CO₂ and lactate play prominent roles in metabolism and share the potential to alter systemic acid-base balance.

Panic and acid-base balance

CO₂ and brain acidosis

CO₂ is constantly produced in the brain and throughout the body as a final product of carbohydrate metabolism. CO₂ readily crosses cell membranes and the blood-brain barrier. In a reaction catalyzed by carbonic anhydrase, CO₂ is hydrolyzed to carbonic acid (H₂CO₃), which readily dissociates into HCO₃⁻ and H⁺. The resulting acidosis is thought to be responsible for most of the physiological effects of CO₂, including stimulating acid-activated respiratory chemoreceptors in the brain stem. These chemoreceptors stimulate breathing to expel CO₂ and thus correctively raise systemic pH. Inhalation of CO₂ increases the partial pressure of CO₂ in the blood and lowers pH throughout the body. Thus, the CO₂ provocation challenges used in psychiatric research are likely to acutely and transiently acidify brain pH.

Lactate and brain acidosis

Endogenous lactate is generated by glucose and glycogen metabolism. In the brain, astrocytes are thought to convert glucose and stored glycogen into lactate, which is exported to the interstitial space where it can be taken up by neurons to produce energy via oxidative metabolism. Recent experiments suggest that shuttling lactate to neurons may be crucial for learning and memory. The effects of intravenous lactate on systemic pH are more complex than those of CO₂. One reason is that intravenously administered lactate can be metabolized by the liver to HCO₃⁻, which might raise blood pH. In addition, in order to cross membranes such as the blood-brain barrier, lactate requires monocarboxylate transporters (MCTs). Because these MCTs cotransport H⁺, they effectively move lactic acid, thus providing a mechanism that might lower pH in specific compartments, for example in the central nervous system (CNS). A recent review covers these complex effects of lactate on systemic pH. Importantly, intravenous lactate causes hyperventilation, suggesting that, like CO₂ inhalation, lactate likely reduces pH at ventilatory chemoreceptors and perhaps elsewhere in the brain. These observations suggest that CO₂ and lactate may share an ability to acidify interstitial pH in the brain. Interestingly, another panicogen, doxapram, may act by a related mechanism. Both doxapram and protons stimulate ventilation and both can inhibit two-pore domain K⁺ channels (TWIK)-related acid-sensitive K⁺ (TASK) channels.

pH abnormalities in panic disorder?

Increasing evidence suggests that pH may be abnormally regulated in panic disorder. Brain pH is largely controlled by the CO₂/HCO₃⁻ buffering system, which is acutely regulated by breathing. Multiple investigators have reported irregular breathing in panic disorder, including greater tidal volume variability, which may be due to more frequent sighing. Consistent with a persis-
tent breathing irregularity, panic disorder patients exhibit a chronically low end-tidal CO₂ and a compensatory decrease in serum bicarbonate. Those who exhibit breathing irregularities may also be more likely to have respiratory symptoms during an attack. Symptom cluster analyses have identified a subtype of panic disorder, in which respiratory symptoms appear to predominate. Interestingly, the respiratory subtype may be the most sensitive to CO₂ and lactate. This subtype may also respond best to the antidepressant imipramine, and may be more likely to be associated with a family history of panic disorder. Supporting a role for pH in panic pathophysiology, correcting blood gas abnormalities through breathing control or pharmacology has been suggested to produce clinical improvement.

**Endogenous lactate and pH abnormalities in panic disorder**

Lactate is a weak acid that can be an independent determinant of pH in biological systems. Several studies using H-magnetic resonance spectroscopy suggest endogenous lactate levels may be elevated in panic disorder patients. Panic disorder patients had higher lactate levels than controls in response to visual cortex activation, following hyperventilation, and during lactate-induced panic. Fiberoptic biosensor measurements of pH in primates suggest that intravenous lactate infusion reduces brain pH. Phosphorus spectroscopy further suggests that the elevated brain lactate in panic disorder patients may change pH buffering capacity. It was suggested that a vascular or metabolic abnormality might be responsible for the lactate elevation. Consistent with this view, probands who had a family history of panic and an atypical CO₂ ventilatory response were more likely to carry a polymorphism in a gene encoding lactate dehydrogenase, which catalyzes the conversion of lactate to pyruvate.

**CNS chemosensitivity**

**CO₂ and acid chemosensitivity in the CNS**

The potential associations between panic disorder, the action of panicogens, and brain pH begs the question of how the brain normally senses and responds to pH change. The majority of research on chemosensitivity in the CNS has focused on respiratory control. Thus, understanding how pH regulates breathing could provide critical insights into panic disorder. Breathing rate and volume are exquisitely sensitive to CO₂ in the blood, largely through interstitial pH and activation of pH-sensitive chemoreceptors. Although the precise sites of CO₂-mediated ventilatory control are uncertain, they are thought to lie in the brainstem (medulla and pons). Neurons in multiple brain stem sites can be activated by CO₂ and low pH, suggesting the relevant chemosensitivity might reside at multiple locations. The retrotrapezoid nucleus (RTN) of the medulla has been implicated in respiratory chemosensation, particularly the cells expressing Phox2b, which is mutated in congenital hypoventilation syndrome. Other brainstem nuclei are sensitive to pH and have been implicated in pH-mediated ventilatory control; these regions include the medullary raphe nuclei, nucleus of the tractus solitarius, and locus coeruleus. Thus, multiple chemosensitive sites are possible. The CO₂ sensitivity in panic patients, and the associations between panic and ventilation, make it tantalizing to speculate that abnormalities in these chemosensitive neurons and receptors might contribute to panic attacks. Knowledge of pH-sensitive molecules in the brain and their physiological roles is rapidly growing, but much remains to be learned.

**pH-sensitive receptors and respiratory chemosensation**

Understanding the molecules that underlie pH effects on ventilatory control could pave the way for understanding pH sensitivity in the brain in general. Thus far no single molecule has been found to be responsible for respiratory chemosensation. A number of molecules have the potential to detect falling pH and stimulate breathing. Members of the TWIK family are pH-sensitive; a subset, the TASK channels, have garnered attention as potential respiratory chemoreceptors. Because TASK channels help maintain membrane voltage near the resting potential, inhibiting these channels increases excitability and the likelihood of generating action potentials. TASK channels can be inhibited by small reductions in extracellular pH. For example, reducing pH by just 1/10th of a unit from pH 7.4 to pH 7.3 inhibits TASK-1. TASK-1 and TASK-3 are widely expressed in brain, while TASK-2 expression in brain is limited to a few brain stem nuclei, including the retrotrapezoid
nucleus (RTN), which has been implicated in pH control of ventilation. Nevertheless, disrupting the genes encoding TASK-1, TASK-2, or TASK-3 in mice failed to eliminate the centrally mediated hypocapnic ventilatory response,\textsuperscript{24,26} suggesting that the TASK channels are not required. However, some pH-sensitive responses were affected. Loss of TASK-1, TASK-3, or both reduced the pH sensitivity of cultured raphe neurones, but not that of RTN neurons.\textsuperscript{26} TASK-1 disruption also reduced peripheral chemosensitivity to hypocapnia in the carotid body.\textsuperscript{27} Additionally, TASK-2 disruption in mice increased the respiratory response to mild hypocapnia (1.5 and 2% CO\textsubscript{2}), suggesting a modulatory role.\textsuperscript{26}

**pH-sensitive ion channels, G-protein coupled receptors, and intracellular signaling molecules**

Besides the TASK channels, a wide number of additional molecules might sense pH in the brain. Examples of pH-sensitive ion channels include transient receptor potential (TRP) channels,\textsuperscript{77} P2X receptors,\textsuperscript{86,97} voltage-dependent Ca\textsuperscript{2+} channels,\textsuperscript{90} N-methyl-D-aspartate (NMDA) receptors,\textsuperscript{90} acid-sensing ion channels (ASICs),\textsuperscript{82,84} and inward rectifier K\textsuperscript{+} channels.\textsuperscript{85} Examples of pH-sensitive G-protein coupled receptors include OGR1, GPR4, TDAG8, adenosine A1 receptors, and metabotropic P2Y receptors.\textsuperscript{72,86} pH-sensitive intracellular signaling molecules include Pyk2 and soluble adenyl cyclase (sAC).\textsuperscript{72} All of these molecules are sensitive enough to detect pH changes that occur during physiology or pathophysiology. Further, all of these molecules have been suggested as candidates for pH chemosensitivity.\textsuperscript{72,86} Though more investigation is needed, some of these molecules have already been implicated in pH sensing. For example, voltage-dependent Ca\textsuperscript{2+} channels and NMDA receptors modulate synaptic plasticity in response to changes in extracellular pH.\textsuperscript{80,84} Adenosine A1 receptors, adenosine triphosphate (ATP) receptors (P2X and P2Y), and ASIC1a have been implicated in the ability of CO\textsubscript{2} and low pH to inhibit seizure activity.\textsuperscript{32,33} Recent studies also investigated the potential role in the inward rectifier K\textsuperscript{+} channel Kir5.1, which is highly sensitive to extracellular pH when heteromerically coupled to Kir4.1. Disrupting Kir5.1 produced abnormal respiration and metabolic acidosis in mice, however central hypocapnic ventilatory responses remained intact. Instead, impaired sensory afferent nerve conduction was thought to be responsible for the abnormal respiratory phenotype.\textsuperscript{88}

**Effects of chemosensation on arousal and emotion circuits**

pH-sensitive respiratory chemosensors in the brain stem medulla and pons comprise a powerful mechanism for controlling systemic CO\textsubscript{2} and pH. Slow or shallow breathing acidifies systemic pH, while fast or deeper breathing raises systemic pH, making it more alkaline. There may also be a need for a new level (more rostral) brain structure to monitor pH, for example to produce appropriate cognitive or behavioral responses to rising CO\textsubscript{2}. Rising CO\textsubscript{2} heralds the potential threat of suffocation, a terrifying situation that demands immediate detection and action to ensure survival. The clusters of pH-sensitive neurons in the medulla and pons that stimulate breathing might communicate this need for action to higher level structures. Alternatively, it might be advantageous if sites above the medulla and pons sensed pH more directly.\textsuperscript{86,89} A prominent example is midbrain serotonergic neurons. Midbrain raphe neurons are highly pH-sensitive and increase firing when CO\textsubscript{2} rises and pH falls.\textsuperscript{87} These neurons are well positioned to deliver serotonin (5-HT) to forebrain, cortical, and subcortical structures and thus alter mood and cognition in response to CO\textsubscript{2} and low pH. In sleep, a rising CO\textsubscript{2} and failing pH might signal the need to reposition the airway or to relieve an obstruction. During sleep CO\textsubscript{2} inhalation causes wild-type mice to wake up, whereas CO\textsubscript{2} fails to wake mice lacking pH-sensitive serotonin neurons.\textsuperscript{88} Thus, dysfunction of these neurons might play a critical role in sudden infant death syndrome,\textsuperscript{89} where a failure to wake may lead to suffocation. Neurons in even higher order brain areas are also activated by low pH, including orexin-expressing neurons in the hypothalamus.\textsuperscript{80} These orexin-expressing neurons have been implicated in narcolepsy and arousal, and are positioned to influence diverse physiological functions including adaptive behaviors, metabolism, respiration, and panic.\textsuperscript{29} Recently the amygdala was also implicated in CO\textsubscript{2} and acid chemosensation and CO\textsubscript{2}-evoked fear.\textsuperscript{87}

**The amygdala is a chemosensor that detects CO\textsubscript{2} and acidosis to elicit fear**

It is well established that the amygdala integrates sensory input from other brain structures to orchestrate fear behavior; however, the amygdala itself was not previously known to act as a pH sensor. Ziemann et al sus-
pected this possibility after observing that the acid sensing ion channel-1a (ASIC1a) was abundantly expressed in the basolateral amygdala and other fear circuit structures.\textsuperscript{6,8} and it was found that breathing 10% CO\textsubscript{2} lowered pH to levels sufficiently to activate ASIC1a in amygdala neurons.\textsuperscript{7} To test CO\textsubscript{2}-triggered fear in mice, four behavioral paradigms were developed: (i) CO\textsubscript{2}-evoked freezing; (ii) CO\textsubscript{2}-potentiated center avoidance in the open field; (iii) CO\textsubscript{2} aversion; and (iv) CO\textsubscript{2}-enhanced fear conditioning.\textsuperscript{8} Genetically disrupting or pharmacologically inhibiting ASIC1a reduced fear-like behavior in these paradigms.\textsuperscript{9} Particularly striking was the freezing behavior, which is often used as a correlate of fear and panic in mice. Like other fear-evoking stimuli, breathing 10% CO\textsubscript{2} induced a dramatic freezing response in wild-type mice. Disrupting or inhibiting ASIC1a significantly blunted this response.\textsuperscript{10} To test whether the amygdala itself might sense pH, acidic artificial cerebrospinal fluid was microinfused into the amygdala to lower pH to ~6.8 from normal pH 7.35. Acidifying the amygdala produced freezing behavior in wild-type mice that resembled the freezing evoked by CO\textsubscript{2} inhalation. Interestingly, in the ASIC1a knockout mice amygdala acidosis induced little or no freezing. The freezing deficit was likely specific to low pH because the ASIC1a knockouts froze normally when the amygdala was electrically stimulated. Finally, the authors tested whether ASIC1a in the amygdala might be sufficient to produce CO\textsubscript{2}-evoked freezing. Restoring ASIC1a expression to the amygdala of ASIC1a-null mice with an ASIC1a-expressing adeno-associated virus corrected the CO\textsubscript{2}-evoked freezing deficit (Figure 1). Together these findings suggest that the amygdala itself can act as a chemosensor. These experiments further identify ASIC1a as key molecular mediator of this chemosensitive response.

**Interception and false alarms**

It is intriguing that a brain structure that mediates fear has a chemosensory role. The ability to sense or monitor internal bodily states (interception) is a common human experience, ranging from vague sensations to powerful and uncontrollable emotions. Often the language needed to communicate these sensations seems inadequate. Yet, interceptional sensations may be critical for survival. pH might be one of a variety of signals that could produce interceptional sensations by activating pH-
sensitive receptors in the brain to evoke adaptive responses. The survival value of rapidly detecting CO₂ to prevent suffocation seems clear. Nearly 20 years ago Donald Klein drew from this observation to hypothesize that the suffocation detection system might be falsely triggered to produce panic attacks. Conceivably, heightened pH sensitivity could constitute such a false alarm.

Summary

We don’t yet know why panic attacks occur. Nor do we completely understand why those who suffer panic attacks are hypersensitive to panicogens. However, the potential ability of CO₂ and lactate, the two most well-studied panicogens, to alter brain pH suggests that pH chemosensation could be instrumental. Acid-sensitive molecules are widely distributed in fear circuit structures and elsewhere in the brain. Consistent with this observation, a variety of brain sites have been implicated in pH chemosensation including brain stem respiratory nuclei, midbrain raphe neurons, hypothalamus, and amygdala. However, a number of questions remain. For example, what specific role(s) do each of these pH-sensitive sites and pH-sensitive molecules play? Could there be additional sources of acidosis and pH fluctuation besides CO₂ or lactate that might activate these chemosensory pathways? Finally, might genetic or epigenetic variability in chemosensation lead to panic disorder or other psychiatric and neurological illnesses? That we are now in a position to ask these questions is in itself a significant advance. As we continue to learn more about CO₂ and pH chemosensation in the brain, the answers to these questions may be within reach. Moreover, an improved understanding of pH signaling and dysregulation might very well lead to an entirely new avenue of therapeutic intervention.

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REFERENCES


10. Drury AN. The percentage of carbon dioxide in the alveolar air, and the tolerance to accumulating carbon dioxide in case of so-called “irritable heart”. Heart. 1918;7:165-173.


Neurobiología del pánico y de la quimiosensación al pH en el cerebro

El trastorno de pánico es una enfermedad común e incapacitante para la cual los tratamientos con gran frecuencia resultan ineficaces. El mayor conocimiento de las bases biológicas podría ayudar al descubrimiento de mejores terapias. Aunque los ataques de pánico son impredecibles, la capacidad de provocarlos en el laboratorio con protocolos de estimulación permite una gran oportunidad para introducirse en la neurobiología del pánico. La inhalación de CO₂ y la infusión de lactato son dos de las pruebas de provocación de pánico más estudiadas. Aunque aún no está aclarado cómo provocan pánico estos estímulos, han comenzado a aparecer modelos animales de la acción de CO₂ y de lactato, los que permiten oportunidades insospechadas para investigar las moléculas y circuitos que están a la base de los ataques de pánico. Tanto el CO₂ como el lactato afectan el balance del pH y pueden generar acidosis, la que puede alterar el funcionamiento neuronal mediante una cantidad creciente de receptores sensibles al pH. Estas observaciones sugieren que una clave para una mejor comprensión del trastorno de pánico puede encontrarse en un mayor conocimiento de la regulación del pH cerebral y en los receptores sensibles al pH.

Neurobiología de la panique et chimiosensibilité du cerveau

Le trouble panique est une pathologie courante et invalidante aux traitements trop souvent inefficaces. Une meilleure connaissance de la biologie sous-jacente pourrait faciliter la découverte de traitements plus efficaces. Malgré l'imprévisibilité des attaques de panique, la possibilité de les reproduire au laboratoire par des protocoles de provocation offre une opportunité de compréhension de la neurobiologie de la panique. Deux des épreuves de provocation de panique les mieux étudiées sont l'inhalation de CO₂ et la perfusion de lactate. Bien que la façon dont ces épreuves provoquent la panique reste obscure, des modèles animaux de l'action du CO₂ et du lactate commencent à émerger et offrent des opportunités sans précédent pour explorer les molécules et les circuits sous-tendant les attaques de panique. Le CO₂ et le lactate changent tous les deux l'équilibre du pH et peuvent provoquer une acidose pouvant influer sur la fonction neuronale par l'intermédiaire d'une liste croissante de récepteurs sensibles au pH. Ces observations suggèrent que la clé d'une meilleure compréhension du trouble panique pourrait reposer sur une connaissance plus approfondie de la régulation cérébrale du pH et des récepteurs sensibles au pH.
69. Nattie E, Li A. Central chemoreception is a complex system function that involves multiple brain stem sites. J Appl Physiol. 2006;100:1464-1466.