Acute exposure to systemic hypoxia activates various responses which act to alleviate tissue hypoxia. A prime example is the ventilatory response to hypoxia (hypoxic ventilatory response: HVR) mediated by peripheral arterial chemoreceptors (chemoreflexes). This chemoreflex consists of initial augmentation of ventilation (respiration) followed by inhibition. Using the method of progressive isocapnic hypoxia (rate of decrease in $P_{O_2}$, 10–20 mmHg/min), $P_{aO_2}$ levels down to approximately 60 mmHg augmented ventilation (hypoxic ventilatory stimulation; HVS), but further reduction in $P_{aO_2}$ (e.g. ≤40 mmHg) suppressed ventilation (hypoxic ventilatory depression: HVD) [1–3]. In addition, sustained isocapnic hypoxia ($P_{aO_2}$, 50 mmHg) elicited a maximum excitatory response within 2–3 min; thereafter ventilation gradually decreased and stabilized at a level slightly greater than that during air breathing (biphasic response [3–5]). Hence, the direction and magnitude of HVR depend upon activity from peripheral chemoreceptor. Two underlying mechanisms contributing to the depression have been advocated. (1) Change in synaptic transmission: Within the neuronal network controlling the hypoxic respiratory response, hypoxia might induce the enhancement of inhibitory neurotransmission (modulation), disfacilitation of excitatory neurotransmission or both. (2) Change in the membrane property of respiratory neurons: Hypoxia might suppress the membrane excitability of respiratory neurons composing the hypoxic respiratory response via modulating ion channels, leading to hyperpolarization or depolarization blocking of the neurons. However, the quantitative aspects of $P_{aO_2}$ (degree and duration of hypoxic exposure) to induce these changes and the susceptibility of both mechanisms to the $P_{aO_2}$ level have not yet been clearly elucidated. [Japanese Journal of Physiology, 50, 15–24, 2000]
logical and neuroanatomical studies, including ablation studies of the brainstem neural axis and the brainstem, and the involvement of neuromessengers (neurotransmitters and neuromodulators) in hypoxic respiratory response (HRR) is now being elucidated. Recent topics have been directed to the cellular and molecular mechanisms of hypoxic respiratory stimulation (HRS) and hypoxic respiratory depression (HRD) as well as their integrative functions.

The central mechanisms of HRR have been studied at the neuronal membrane and neural pathway levels. The first concerns the effects of local hypoxia and associated metabolic changes on the properties of respiratory neurons. Since the membrane properties of these neurons are heterogeneous, they have varied responses to changes in \( P_{O_2} \) [6]. The analysis of respiratory neuronal pathways, on the other hand, is concerned with the activation or inhibition of multi-neuronal streams leading to motor output and neural correlations, phrenic nerve activity through individual inhibitory and excitatory mechanisms. According to this hypothesis, HRD results from the activation of a specific neuronal pathway which enhances inhibitory and/or attenuates excitatory synaptic transmission controlling ventilatory motor output. Thus, the research is focused on (1) identifying the neuronal circuitry of HRS and its messenger substances, and (2) elucidating the effects of hypoxia on this system.

Another recent interest concerns the time-dependent component of hypoxia-induced responses. Neuronal plasticity is important for understanding the HRS. Stimulation of the carotid sinus nerve (CSN) by hypoxia or electrical impulses is known to elicit changes which outlast the duration of the stimulus [7–9]. Thus, the time course of HRR after cessation of stimulation depends on the history of stimulation. The contribution of this “time-dependent response” may be difficult to separate from that of ongoing response transients. Time-dependent responses are, however, established as important in the behavior of the respiratory control system response to hypoxia, and have been shown to differ among various respiratory motor outputs including the phrenic and external intercostal nerves and for cranial nerves with inspiratory or expiratory activity [10–12].

In this review, we discuss the following issues from a functional perspective: (1) neuronal pathways constituting the HRR, (2) time-dependent stimulatory responses and their contributory neuronal pathways, (3) HRD (or biphasic response to hypoxia) and its related neuronal circuits, and (4) time-dependent depressive responses.

**Neuronal Circuitry of HRS**

1. **Integration of afferent signals in the lower brainstem**

A general schema of the neuronal circuits for HRS is depicted in Fig. 1. Information from type I glomus cells in the carotid body is transmitted to sensory terminals of the CSN, which projects to the commissural subnucleus of NTS [10, 11]. Glutamate, nitric oxide (NO) [12, 13] and substance P [14] are considered to be neuromessengers at the synapses in the NTS. N-methyl-D-aspartate (NMDA) receptors on NTS neurons are involved in the production of short-term potentiation (STP) [15]. Glutamate [16] and inhibitory amino acids such as GABA and glycine are also implicated as neuromessengers for HRD, although their source has not been identified. Richter et al. [17, 18] have proposed that neurons located in the reticular formation might be third-order interneurons for HRR, and there is some evidences for this hypothesis [19].

2. **Suprapontine influences**

The involvement of suprapontine structures to HRS has been studied extensively. These structures are extremely sensitive to anesthesia, limiting experimental approaches. The activation of diencephalic mecha-
Fig. 2. Schematic representation of various types of time-dependent responses. Stimulus, hypoxia or electrical stimulation of carotid sinus nerve; VT, tidal volume; f, respiratory frequency. 1) Immediate facilitatory response elicited by peripheral chemoreceptor activation; 2) short-term potentiation or afterdischarge; operates as a damper for respiratory control and prevents the undershoot of respiratory motor output seen after hyperventilation; 3) long-term potentiation; 4) rapid adaptation of f during stimulation (accommodation); 5) short-term depression (or post hypoxic frequency decline (phfd))

The cellular mechanisms of glomus (type I) cells in the carotid body have been reviewed in recent literature [39, 40]. The type I cells, which are thought to be depolarized in hypoxia via PO2-sensitive K+ channels, lead to an opening of the voltage-dependent Ca2+ channels [39]. An increase in cytosolic Ca2+ then elicits exocytosis of vesicular dopamine causing depolar-
2. Short-term potentiation

Short-term potentiation (STP), also known as after-discharge, refers to increase in respiratory motor output outlasting carotid chemoreceptor stimulation by several seconds to 10 min [7, 9, 42] (cf. long-term potentiation or facilitation (LTP or LTF), which lasts for at least 30 min (see below)). Since STP or LTP can be induced in central neuronal circuits by the activation of carotid body afferents, they can be regarded as short- and long-term memory in the respiratory control network [7–9]. The induction of STP serves as a damper on the respiratory control system, preventing undershoot of PaO₂ during sudden increases in PaO₂ following hypoxia (the “off-response”). STP mainly affects peak phrenic nerve activity (which corresponds to tidal volume) and, to a lesser extent, respiratory frequency [9, 43, 44]. In experiments using paralyzed, vagotomized, artificially ventilated animals under isocapnic conditions, the time constant of STP was 30–60 s [9, 42–44]. In experiments with awake human subjects, STP was also observed [45]. Regarding the site(s) and mechanisms responsible for STP, NMDA receptors have been implicated in both the NTS [18, 46] and phrenic nucleus [47].

STP might also be contributory to hyperpnea after the cessation of exercise, which occurs even when no chemical stimulus is present (no systemic metabolic acidosis) [44, 48]. Thus, the neuronal pathway for STP may not be specific for hypoxia-induced activation of respiration.

3. Long-term potentiation

LTP lasts for 30 min to 24 h after the cessation of stimulation [7–10]. In the decerebrate cat, the main component of LTP was integrated phrenic discharge amplitude [38], but in anesthetized rats, both amplitude and frequency of respiration were affected [9]. Serotonergic neurons located in the Raphe nuclei have been shown to project to the phrenic nucleus and implicated in the induction of LTP in phrenic nerve activity [7, 39, 40]. Pretreatment with an antagonist for 5-hydroxytriptamine (5-HT; serotonin) receptors abolished the development of LTP indicated the involvement of serotonergic mechanism in the induction of LTP [7, 49, 50]. CSN stimulation activates Raphe neurons [51]. Serotonergic neurons located in the Raphe nuclei receive input from carotid chemoreceptors [52] and project directly or indirectly to the phrenic nucleus [53, 54]. The serotonin-induced bistable neuronal behaviour [55] in phrenic motoneurons might contribute to LTP. LTP may also be important for high altitude (hypoxic) acclimatization [7, 49, 56].

Hypoxic Respiratory Depression

Oxygen supply to the brain is of prime significance since brain functions are necessary for the control of other organs. If brain oxygenation is not improved with HVR, a second phase of response to hypoxia, HVD occurs (biphasic response), in which the excitatory HVR is greatly attenuated. Although HVD may reduce tissue oxygenation, the reduction of cellular activity during prolonged hypoxia may provide an opportunity for the survival of neurons and other cells by reducing their metabolic functions and energy requirements. In response to maternal hypoxia, respiration and heart rate in the mammalian fetus undergo a marked reduction [5, 57]. The hypoxic response of the neonate is notably biphasic, the initial excitatory response being followed by a pronounced inhibitory component, and VE usually stabilizes during hypoxia below baseline levels [58, 59], whereas in adult animals, the initial excitatory response is marked compared with the subsequent decline. These observations strongly suggest that the inhibitory component of HVR might be a relic of the fetal period.

HRD, as an active adaptive mechanism, has been a recent focus of attention in the field of respiratory physiology. If oxygen delivery (blood flow times arterial oxygen content) is reduced below a critical level, oxygen consumption (VO₂) decreases [58, 59]. This “oxygen delivery-dependent oxygen consumption” is described in several species, particularly small mammals [60, 61]. Body temperature control mechanisms are also important for protective hypometabolic reflexes.

Several theories have been proposed to account for HRD: (1) adaptation of peripheral chemoreceptor activity [9, 62], (2) rise in brain extracellular fluid pH due to increased cerebral blood flow [63], and (3) hypoxic depression of neurons in the central nervous system. The third hypothesis may be further elaborated as attributing HRD either to (3a) anoxic failure of function of respiratory neurons, or (3b) alterations in neurotransmitter mechanisms, in particular, within
neuronal circuits subserving HRR. The efficacy of synaptic transmission might be modulated by events occurring presynaptically (e.g., rate of synthesis, secretion, or metabolism of neurotransmitters) or postsynaptically (e.g., rate of transmitter degradation or change in sensitivity of receptors). These three theories and two variants will be discussed below.

Neubauer et al. have classified HRD into three types [3, 64]. Type I is characterized by an increase in cerebral blood flow. In type II, enhanced inhibitory neuromessengers (neurotransmitters or neuromodulators) are the main causes of HRD. Type III is due to direct disturbances of neuronal function and is only seen in severe hypoxia ($\text{SaO}_2 < 20\%$).

1. Peripheral chemoreceptors

The proposed involvement of chemoreceptor adaptation in HVD was based on experiments with on- and off-responses to hypoxia in awake human subjects [62]. However, in anesthetized animals, CSN activity did not accommodate, at least during the acute phase of sustained exposure to hypoxia [65, 66]. In addition, ventilation during normoxia decreased after chemodenervation. Finally, selective intra-arterial perfusion of the lower brainstem with hypoxic blood during maintained systemic hypoxia resulted in ventilatory depression [67]. HVD is therefore unlikely due to the accommodation of CSN activity at the periphery. Components of HVD, such as the marked decline in $f$ within 10–20 s we noted during continuous CSN stimulation [9], are presumably due to accommodation at higher-order interneurons such as those of the NTS core.

2. Increased cerebral blood flow and alkaline shift in brain extracellular fluid

Hypoxia-induced dilatation of cerebral vessels increases cerebral blood flow causing a washout of $\text{CO}_2$ from central chemosensitive areas, resulting in an increase in local pH [63, 68, 69]. HVD might be a response of central chemosensitive mechanisms to this alkalosis. This hypothesis is not compatible with the following observation. In response to hypoxic exposure, the pH of the cerebrospinal fluid at the medullary surface initially shifted to alkaline but became acidic during prolonged hypoxia which was accompanied by HVD [68, 69].

3. Central nervous system effects

In considering hypoxia-induced alterations in brainstem functions, two components of HVD should be considered separately, the effects on $f$ (rhythm generator) and effects on amplitude of inspiratory activity and $V_T$ (pattern generator). In goats and humans [4], HVD reduced $V_T$ but not $f$, although the extent of hypoxia was limited in human experiments. However, in newborn mammals with profound HRD, reduction in $f$ was the main component [70, 71]. In anesthetized or arterially perfused rats, respiratory arrest reversibly occurred before marked changes in burst pattern [72–74]. Respiratory rhythm in adult animals is considered to be maintained by mutual inhibition among three classes of respiratory neurons; namely, decrementing inspiratory, decrementing expiratory and augmenting expiratory neurons [20, 21]. Disruption of this circuit by hypoxia would cause an arrest of respiratory output. As discussed previously, higher brain structures also contribute to respiratory output during hypoxia.

Is respiratory neuronal activity affected by hypoxia itself or by hypoxia-induced changes in neuromessengers? Although hypoxia undoubtedly influences both the metabolism and synaptic activity of neurons constituting the respiratory network [75, 76], it is difficult to assess their relative importance. The reasons for this are: (1) intracellular recordings in vivo are technically difficult to maintain during hypoxic exposure [75, 76], and (2) in slice preparations or dissociated cells of the medulla oblongata, synaptic events could not be related definitely to respiratory neuronal function because these reduced preparations did not exhibit rhythmic activity [6]. Thus, care must be taken in attributing neuronal circuit responses to intra- or inter-neuronal disturbances due to hypoxia.

1) Hypoxia and changes in neuronal membrane properties. In general terms, severe hypoxia or anoxia ($\text{PaO}_2 < 20–25 \text{ mmHg}$) disrupts aerobic metabolism, resulting in the depletion of ATP, which is required for the operation of ionic pumps such as those for $\text{Na}^+ / \text{K}^+$ and $\text{Ca}^{2+}$. Hence, $\text{K}^+$ escapes from the neuron, causing depolarization of the membrane potential [6]. This has been confirmed in experiments using medullary slices, which showed increased extracellular [K$^+$] within the hypoglossal nucleus and dorsal motor nucleus of vagus after hypoxic exposure [6, 77]. In addition, the excitatory amino acid glutamate is released by hypoxia from pre-synaptic terminals. The activation of glutaminergic receptors on the post-synaptic membrane induces massive entry of $\text{Na}^+$, $\text{Ca}^{2+}$ and $\text{Cl}^-$ into cells. This ionic influx causes an increase in osmotic pressure, with the movement of water resulting in cellular edema [6, 78–80]. The increase in cytosolic [Ca$^{2+}$]1 hydrolyzes phospholipids of the plasma membrane, releasing harmful free fatty acids and causing the destruction of cell membranes [81] with a low pH in hypoxia due to
metabolic acidosis. Neuronal death may be immediate or delayed. In the latter case, neurons which survive anoxic exposure start to die by apoptosis within 3–7 d [82, 83], probably due to a glutamate-mediated increase in cytosolic $[\text{Ca}^{2+}]$ as this type of the death could be alleviated if extracellular $[\text{Ca}^{2+}]$ was lowered [84].

There is evidence that HRD is not due to irreversible cellular damage from hypoxia. Animals with experimental HRD were fully responsive to other respiratory stimuli such as hypercapnia [72]. Hypoxia-induced arrest of the respiratory rhythm was immediately reversible with reoxygenation [73, 85].

### 2) Neurotransmitters and neuromodulators.  

HVD shows reversibility and commences at a $\text{PaO}_2$ level higher than that which causes neuronal damage, suggesting that hypoxia may initially disrupt communications among neurons. In sensitive hippocampal neurons, brief hypoxia elicited membrane hyperpolarization with the attenuation of evoked potentials followed by depolarization [86]. In respiratory modulated neurons in the hypoglossal nucleus and dorsal motor nucleus of the vagus, hypoxia depolarized membrane potentials, although prior hyperpolarization (as seen in hippocampal neurons) was sometimes detected [78, 87]. The initial hyperpolarization was due to inhibitory synaptic input and was considered to be related to reduction in the level of consciousness [86]. Using an *in vitro* spinal cord preparation, we found an increase in evoked potentials in $C_7$–$T_1$ ventral roots after brief hypoxia, suggesting greater susceptibility of inhibition as compared with excitatory neurotransmission [88].

In medullary respiratory neurons, HRD might be due to the disruption of synaptic transmission by hypoxia [75]. Post-synaptic excitatory and inhibitory potentials (EPSPs and IPSPs) were depressed by hypoxia [75, 76]. Several neuromessengers are known to be involved in rhythmogenesis, including adenosine, gamma amino butyric acid (GABA), and endogenous opioids [3].

Medullary respiratory neurons can be hyperpolarized or depolarized by the application of inhibitory or excitatory amino acids, respectively, and these effects reversed by their antagonists [89–91]. A decrease in integrated phrenic amplitude was found after microinjection of the GABA antagonist bicuculline into most parts of the ventral respiratory group (VRG) [92]; a possible explanation is that inhibitory input to neurons themselves inhibitory to respiratory neurons was blocked. Such inhibitory neurons would normally be inhibited by GABA, leading to the disinhibition of respiratory neurons; although the location of such a pathway has not yet been identified. Thus, interactions in inhibitory neurotransmitter circuits may be central to the mechanism of HRD. Several investigators have indicated [93–95] that GABA content in the medulla oblongata increases in response to systemic hypoxia, and the failure of GABA reuptake has been quoted to explain this increase [96]. GABA antagonists have been found to reverse respiratory depression [97]. However, there is a paucity of direct evidence that hypoxia causes the accumulation of inhibitory neuromessengers within the neuronal circuit for HRR, and in turn, suppresses the respiratory motor output. We have recently shown using an *in vitro* preparation obtained from newborn rats that superfusion containing strychnine and naloxone (glycine and opioid receptor antagonists, respectively) greatly alleviated HRD [98].

Adenosine has been shown to accumulate in the brain during hypoxia [99] and depress ventilation [100, 101]. Superfusion of an *in vitro* preparation with the adenosine receptor antagonist theophylline alleviated HRD [102]. Pretreatment with aminophylline suppressed the reduction in $f$ caused by hypoxia, but was ineffective in reducing $V_T$ [103, 104]. Adenosine inhibits neuronal activity and synaptic transmission, possibly through glycolysis [105]. A marked decrease of IPSPs in respiratory neurons was observed after the application of adenosine, possibly due to a pre-synaptic action. The disruption of inhibitory synaptic inputs by hypoxia in a circuit of mutually inhibitory respiratory neurons could result in depression of the respiratory output.

Hypoxia causes the accumulation of GABA in the brain, probably by transient activation of glutamic acid decarboxylase, the enzyme responsible for its synthesis [106]. Since GABA superfusion of the ventral medullary surface or by intracisternal injection greatly decreased $V_T$ but not $f$ [93, 94], GABA is likely to be involved in the control of respiratory “pattern” during HVD. Melton et al. [97] have shown in the anesthetized cat that a significant component of HVD could be reversed by the intravenous injection of bicuculline. Hence, they believe that GABA has a major role in HVD induced by moderate hypoxia.

Endogenous opioids operate mainly via $\mu$-receptors to depress respiratory motor output [107, 108]. Various kinds of physiological stresses release endogenous opioids, which alleviate stress responses [109, 110]. The pronounced HRD of newborn animals can be blocked by pretreatment with the opioid receptor antagonist naloxone. However, opioid receptor antagonists are ineffective in the HVD of adult humans and animals [108, 110]. It has been reported for mature
animals that endogenous opioids may be involved in HVD only during extreme hypoxic conditions. It remains possible that opioids have a significant role in HRD in the neonate.

In summary, adenosine release in hypoxia might cause disruption of the inhibitory synaptic transmission among respiratory neurons, thereby interfering with respiratory rhythm generation. In addition, GABA and glycine may depress the amplitude of the inspiratory burst.

### Time-Dependent Depressive Response

#### 1. Short-term depression

The reinstitution of hyperoxia after hypoxic gas induces short-term depression (STD) or post-hypoxic frequency decline (phfd), a decrease in $f$ below the control level [9, 52] which accompanies the increase (STP, see above), in the amplitude of phrenic nerve activity. STD is seen more prominently in the anesthetized rat than in the cat [7, 9]. Noradrenergic neurons located in the ventrolateral pons might be involved in the inhibition of VRG neurons via $\alpha_2$-noradrenergic receptor mechanisms [52, 111].

The lesioning of high lateral pontine regions abolished the induction of HVD in fetal lambs [112]. Lesioning of neurons located in the ventrolateral pons in the $A_5$ noradrenergic area attenuated the STD of respiratory frequency following hypoxia [52]. Neurons in the $A_3$ area were found to be activated after CSN stimulation using Fos immunohistochemistry [49].

In summary HVD is probably caused by the activation of inhibitory mechanisms coupled with disfacilitation of the excitatory inputs.

#### 2. Long-term depression

In decerebrate chemodenervated cats, severe hypoxemia ($P_{aO2}<26\text{ mmHg}$) gave rise to a long-lasting depression (LTD) of both amplitude and $f$ via mesencephalic structures [33, 34]. Since LTD was abolished by pretreatment with the adenosine receptor antagonist aminophylline, adenosine might be involved in the induction of LTD [33]. It is unclear whether input from the carotid body is significant for LTD.

### Acquisition of Hypoxic Tolerance by the Respiratory Center

Rats die in 2–3 min with the inhalation of 4% oxygen and within 1 h at 5%, which corresponds to an altitude of 10,000 m. In 7–8% $O_2$, rats do not survive long-term, indicating that acclimatization does not take place. The altitude to which human beings as well as rats can acclimatize fully is 5,000–5,500 m, corresponding to approximately 11% inspired $O_2$. The mechanism for this is not yet fully understood.

The administration of sodium cyanide (NaOCN) in the rat induces tissue hypoxia by carbamation of the hemoglobin and a left-shift in the oxygen dissociation curve. In such rats, HVD was not evident at a $P_{aO2}$ of 40 mmHg [113, 114]. This hypoxic tolerance of the respiratory center might be due to decreased diffusion distance since increased capillary density in the medulla oblongata was observed after NaOCN administration [114].

### REFERENCES

15. Chitravanshi VC and Sapru HN: Chemoreceptor-sensi...
R851–R858, 1995


42. Eldridge FL: Central neural respiratory stimulatory effect of active respiration. J Appl Physiol 37: 723–735, 1974


46. Mifflin SW: Short-term potentiation of carotid sinus nerve inputs to neurons in the nucleus of the solitary tract. Respir Physiol 110: 229–236, 1997


49. Erickson JT and Millhorn DE: Hypoxia and electrical stimulation of the carotid sinus nerve induce fos-like immunoreactivity within catecholaminergic and sero-
52. Coles SK and Dick TE: Neurones in the ventrolateral pons are required for post-hypoxic frequency decline. J Physiol (Lond) 497: 79–94, 1996
60. Mortola JP: Hypoxic hypometabolism in mammals. NIPS 8: 79–82, 1993
87. Jiang C and Haddad GG: Effect of anoxia on intracel-


112. Gluckman PD and Johnston BM: Lesions in the upper lateral pons abolish the hypoxic depression of breathing in unanaesthetized fetal lambs in utero. J Physiol (Lond) 382: 373–383, 1987


F. HAYASHI and Y. FUKUDA