Cellular mechanisms involved in carotid body inhibition produced by atrial natriuretic peptide

L. HE, B. DINGER, AND S. FIDONE
Department of Physiology, University of Utah School of Medicine, Salt Lake City, Utah 84108

He, L., B. Dinger, and S. Fidone. Cellular mechanisms involved in carotid body inhibition produced by atrial natriuretic peptide. Am J Physiol Cell Physiol 278: C845–C852, 2000.—Atrial natriuretic peptide (ANP) and its analog, atriopeptin III (APIII), inhibit carotid body chemoreceptor nerve activity evoked by hypoxia. In the present study, we have examined the hypothesis that the inhibitory effects of ANP and APIII are mediated by cyclic GMP and protein kinase G (PKG) via the phosphorylation and/or dephosphorylation of K⁺ and Ca²⁺ channel proteins that are involved in regulating the response of carotid body chemosensory type I cells to low-O₂ stimuli. In freshly dissociated rabbit type I cells, we examined the effects of a PKG inhibitor, KT-5823, and an inhibitor of protein phosphatase 2A (PP2A), okadaic acid (OA), on K⁺ and Ca²⁺ currents. We also investigated the effects of these specific inhibitors on intracellular Ca²⁺ concentration and carotid sinus nerve (CSN) activity under normoxic and hypoxic conditions. Voltage-dependent K⁺ currents were depressed by hypoxia, and this effect was significantly reduced by 100 nM APIII. The effect of APIII on this current was reversed in the presence of either 1 µM KT-5823 or 100 nM OA. Likewise, these drugs retarded the depression of voltage-gated Ca²⁺ currents induced by APIII. Furthermore, APIII depressed hypoxia-evoked elevations of intracellular Ca²⁺, an effect that was also reversed by OA and KT-5823. Finally, CSN activity evoked by hypoxia was decreased in the presence of 100 nM APIII, and was partially restored when APIII was presented along with 100 nM OA. These results suggest that ANP initiates a cascade of events involving PKG and PP2A, which culminates in the dephosphorylation of K⁺ and Ca²⁺ channel proteins in the chemosensory type I cells.

THE CAROTID BODY, an arterial chemoreceptor organ located at the bifurcation of the common carotid artery, is excited by hypoxia, hypercapnia, and acidosis. The parenchyma of the organ consists of groups or lobules of specialized type I cells that lie interspersed among a network of capillaries and sinusoids. Contemporary views suggest that chemosensory activity is initiated in type I cells that activate synaptically apposed carotid sinus nerve (CSN) afferent terminals (11, 12). Although early investigations of the carotid body envisioned a classical chemical synapse between type I cells andafferent terminals involving the action of a single excitatory transmitter agent, more recent studies have revealed a neurochemically complex synaptic apparatus (11, 12, 27). The available evidence suggests that type I cells synthesize and release multiple excitatory and inhibitory neurotransmitter agents, and moreover, that the CSN afferent fibers themselves contain substances capable of potently influencing the activity of type I cells (1, 27, 32). It is likely, therefore, that in at least some physiological conditions, carotid body output measured in terms of chemoreceptor nerve activity is the product of multiple excitatory and inhibitory influences acting simultaneously.

Two potent inhibitory agents recently found in the carotid body, nitric oxide (NO) and atrial natriuretic peptide (ANP), have been postulated to participate as modulators of chemosensory activity (see Ref. 1). NO is synthesized in a specialized subpopulation of inhibitory afferent fibers that appear to mediate the efferent inhibition of chemoreceptor activity, a phenomenon that was first documented some 30 years ago by Neil and O’Regan (24) and Fidone and Sato (14). ANP, initially discovered in cardiac myocytes, is also present in type I cells, and available data indicate that these cells express ANP receptors as well (see Ref. 1). Aside from the fact that both NO and ANP elevate cyclic GMP (cGMP) in type I cells (36, 37), and that exogenous cGMP inhibits hypoxia-evoked CSN activity (31), virtually nothing is known about the cellular mechanisms that mediate the chemosensory inhibition produced by these agents. In the present study, we have examined the hypothesis that the inhibitory effects of ANP are mediated by the primary target for cGMP, namely, protein kinase G (PKG), in a mechanism that alters the phosphorylation status of voltage-activated Ca²⁺ and O₂-sensitive K⁺ channel proteins. In freshly dissociated cultured type I cells, we examined the effects of a specific PKG inhibitor, KT-5823 (see Refs. 20 and 21), and an inhibitor of protein phosphatase 2A (PP2A), okadaic acid (OA) (see Ref. 8), on K⁺ and Ca²⁺ currents. We also investigated the effects of these specific inhibitors on intracellular Ca²⁺ concentration ([Ca²⁺]) and CSN activity under normoxic and hypoxic conditions. Our results suggest that ANP initiates a cascade of events that culminates in the dephosphorylation of K⁺ and Ca²⁺ channel proteins.

METHODS

Dissociation of carotid body cells. Young New Zealand White rabbits (1.4–2.0 kg) were anesthetized with pentobarbital sodium (35 mg/kg iv), tracheostomized, and artificially ventilated. The carotid arteries, including the bifurcations,
were surgically exposed, removed, and placed into ice-cold modified Tyrode's solution containing (in mM): 112 NaCl, 4.7 KCl, 2.2 CaCl\(_2\), 1.1 MgCl\(_2\), 42 sodium glutamate, 5.6 glucose, and 5 HEPES buffer (pH 7.43 at 37°C) and equilibrated with 100% O\(_2\). The carotid bodies were dissected free of surrounding connective tissue and transferred to Ham's F-12 medium (Ca\(^{2+}\)-free and Mg\(^{2+}\)-free) containing 0.2% collagenase and 0.2% trypsin. Each carotid body was cut into 6–12 pieces and incubated for 40 min in a CO\(_2\) incubator (5% CO\(_2\)-95% air) at 36.5°C. Tissue fragments were rinsed (2 × 10 min, room temperature) in Hanks' balanced salt solution (Ca\(^{2+}\)- and Mg\(^{2+}\)-free) and then transferred to poly-L-lysine-coated glass coverslips, where they were tritucrated in a small volume of Ham's F-12 medium, plus 10% FCS and 5 µg/ml insulin. The coverslips containing dissociated type I cells were placed in the CO\(_2\) incubator for at least 2 h before use.

Perforated whole cell recordings. Coverslips containing type I cells were placed in a 0.3-mL flow chamber mounted on the stage of a Zeiss phase-contrast inverted microscope. Cells were bathed in modified Tyrode's solution delivered at 0.5 ml/min via a peristaltic pump. Bath temperature was maintained at 35–36.5°C. The bath was grounded via an Ag-AgCl electrode. Patch pipettes were fabricated from borosilicate glass tubing (outer diameter = 1.5 mm; internal diameter = 0.75 mm; Sutter Instrument) in a Flaming/Brown micropipette puller, model P-87 (Sutter Instrument). Pipette resistance varied between 2 and 10 MΩ. Stock solutions of nystatin (Sigma Chemical) were prepared by ultrasonication in DMSO at a concentration of 5 mg/100 µl for 30 s. Final pipette concentrations of nystatin were 125–200 µg/ml. Maximal whole cell currents were recorded 5–25 min after exposure to the nystatin solution in the cell-attached mode. For K\(^+\) current (I\(_K\)) measurements, bath solutions contained 135 mM choline-Cl, 5 mM KCl, 50 µM CaCl\(_2\), 1.0 mM MgCl\(_2\), 5.6 mM glucose, and 10 mM HEPES buffer, pH 7.43 at 37°C. The pipette solution contained (in addition to nystatin) 145 mM K-glutamate, 15 mM KCl, 2 mM MgCl\(_2\), and 20 mM HEPES, pH 7.2 at 37°C. I\(_K\) was evoked by step voltage changes from a holding potential of −70 mV. Ca\(^{2+}\) currents were measured in a bath solution containing 140 mM choline-Cl, 10 mM CaCl\(_2\), and 10 mM HEPES, pH 7.43 at 37°C. In addition to nystatin, the pipette solution contained 120 mM CsCl, 25 mM tetraethylammonium chloride, 0.5 mM CaCl\(_2\), 5.5 mM EGTA, and 30 mM HEPES, pH 7.2 at 37°C. From a holding potential of −70 mV, I\(_{Ca}\) was evoked by applying ramp voltages of 200 ms duration between −40 and −80 mV.

Under control conditions, cells were superfused in a solution equilibrated with air (P\(_O_2\) ≈ 128 Torr). Hypoxic solutions were equilibrated with air and contained 0.5 or 1.0 mM sodium dithionite, resulting in Po2 values of −58 and −32 Torr, respectively. These Po2 values obtained using an oxygen scavenger are in the range measured internally in the intact carotid body during moderate hypoxia (5, 6). Bubbling of superfuses occurred in a reservoir separate from the superfusion chamber by the shortest possible lengths of peristaltic pump tubing; nonetheless, final Po2 values were undoubtedly influenced by exchange with ambient air. “Normoxic,” therefore, refers to superfusion with air-equilibrated media, and “hypoxia” or “hypoxic” indicates treatment of the superfuse with air plus sodium dithionite.

Patch-clamp data analysis. Whole cell currents were recorded with an Axopatch 200A patch-clamp amplifier and a CV 201A headstage (Axon Instruments). Records were simultaneously displayed on an oscilloscope and digitized with a DigiData 1200 computer interface for analysis using pCLAMP version 5.0 software (Axon Instruments). The series resistance was typically 40 MΩ for perforated whole cell recordings and was not compensated in these experiments. Junction potentials, which varied from 2 to 4 mV, were canceled at the beginning of the experiment. Current-voltage (I-V) relations were plotted after subtraction of any capacitance and leakage currents.

Intracellular Ca\(^{2+}\) measurements. Freshly dissociated type I cells attached to coverslips were incubated in F-12 medium containing 0.5 µM fura 2-AM for 10–15 min in a CO\(_2\) incubator at 36.5°C. Coverslips were placed into a flow chamber where they were superfused with modified Tyrode solution at 0.75 to 1.0 ml/min. The temperature was maintained at 35–36.5°C. Drugs were delivered via a 500-µl sampling loop; the bath exchange rate was 30–40 s. The chamber was mounted on the stage of a Zeiss inverted microscope incorporated into a Zeiss/Attofluor workstation equipped with an excitation wavelength selector (filter changer) and an intensified charge-coupled device camera system. Fura 2 fluorescent emission was measured at 520 nm in response to alternating excitation wavelengths of 334 and 380 nm. Data were collected and analyzed using Attofluor RatioVision software (version 6.0). The 334/380 ratio was used to calculate intracellular Ca\(^{2+}\) in accord with the expression

\[
[Ca^{2+}]_i = K_d \frac{(R_0 - R_{min})}{(R_{max} - R_0)} \beta
\]

where \(R_0\) is the observed fluorescence ratio, \(R_{min}\) is the ratio at 0 Ca\(^{2+}\), \(R_{max}\) is the ratio at a saturating concentration of Ca\(^{2+}\), \(K_d\) is the dissociation constant for Ca\(^{2+}\), with fura 2, and \(\beta\) is the 380 nm fluorescence at 0 Ca\(^{2+}\), divided by the 380 nm fluorescence at the saturating Ca\(^{2+}\) concentration. The \(K_d\) was assigned 145 nM in accord with the value published by Molecular Probes (Eugene, OR) (16).

Typically, fluorescence observations were obtained from isolated type I cells. However, some data were also obtained from multiple cells aggregated into clusters. In these instances, data from each cell was analyzed separately. We did not observe any consistent difference in the basal or stimulus-evoked responses from isolated vs. clustered cells, in contrast to a report showing an absence of Ca\(^{2+}\) responses in duster rat glomus cells (4). Also, cells selected for analysis in the present study displayed morphology typical of type I cells, and they responded to the low-O2 stimulus with at least a doubling of the basal [Ca\(^{2+}\)].

Electrophysiological recording of CSN activity. Under pentobarbital anesthesia (35 mg/kg), carotid bodies along with their attached nerves were removed from New Zealand White rabbits and placed in a lucite chamber containing 100% O\(_2\)-equilibrated modified Tyrode solution at 4°C. With the aid of a dissecting microscope, each carotid body was carefully cleaned of surrounding connective tissue. The preparation was then placed in a conventional superfusion/flow chamber where the carotid body was continuously superfused (up to 4 h) with modified Tyrode solution maintained at 37°C and equilibrated with a selected gas mixture. The CSN was drawn through a tiny hole in a glass coverslip into an adjoining chamber containing mineral oil, where it was positioned on two platinum wire electrodes for differential recording of chemoreceptor activity. Neural activity was led to an alternating current-coupled preamplifier, filtered, and transferred to a window discriminator and a frequency-to-voltage converter. Signals were processed by an AD/DA converter for display of frequency histograms on a PC monitor. In these intact superfused organs, basal neural activity was established in solutions equilibrated with 100% O\(_2\). Chemoreceptor responses were evoked in solutions equilibrated with 20% O\(_2\), a
moderate hypoxic stimulus that evokes submaximal activity in the superfused carotid body/CSN preparation (13).

Statistical comparisons. Multiple observations obtained from identical experimental conditions were combined to calculate means and SE of the mean. Between-group comparisons were made using Student's t-test (two-tailed) or ANOVA where appropriate.

RESULTS

Figure 1 shows the effects of hypoxia and the ANP analog, atriopeptin III (APIII), on voltage-activated outward K⁺ current. A typical result is presented (left) that shows currents evoked by a voltage step from a holding potential of −70 mV to +60 mV. The four numbered traces illustrate 1) the current evoked in normoxic media, 2) depression of the sustained current by hypoxic superfusion media, 3) the effect of 100 nM APIII on the hypoxia-evoked depression, and 4) recovery of the current after a return to normoxic media. The voltage sensitivity of this current is plotted under the same four experimental conditions (right). In agreement with previous reports by others (22, 23), the outward current is activated at voltages of −20 mV and above, and furthermore, it is noticeably depressed by a hypoxic challenge (22). However, we also show for the first time that the effects of hypoxia are substantially mitigated in the presence of 100 nM APIII. The data presented in the inset summarize the effects of APIII in seven cells, indicating that this agent significantly (P < 0.001) retards the depression of the outward current by hypoxia. We did not observe any increase in the outward current when APIII was present in normoxic media equilibrated with air (data not shown). This latter result is in accord with our earlier reports that show APIII does not alter basal CSN activity (31).

In similar experiments, we examined the effects of agents capable of interfering with intracellular signaling cascades on the ability of APIII to retard the hypoxia-evoked depression of the K⁺ current. Figure 2 presents data from a different cell, where the depression of the outward current by hypoxia was reduced by 100 nM APIII (compare traces and plots 1, 2, and 3 in Fig. 2). When APIII was introduced into the superfusate along with the specific PKG inhibitor, KT-5823 (1 µM), the hypoxia-induced depression of the current was partially restored (Fig. 2, trace and plot 4). These effects were reproducible in 11 cells (see inset) where treatment with hypoxia in the presence of APIII plus KT-5823 resulted in a significantly greater depression (P < 0.001) of the current than in hypoxic cells treated with APIII alone.

Figure 3 demonstrates the effects of the PP2A inhibitor, OA, on the ability of APIII to retard the hypoxia-induced depression of the K⁺ current. Again, the data demonstrate that the ANP analog potently inhibits the current depression evoked by hypoxia, yet, in the presence of 100 nM OA, the depression is almost fully restored. Observations from five cells (inset) indicate that in the presence of APIII, the current is significantly larger than with hypoxia alone (P < 0.001) or with hypoxia plus APIII and OA (P < 0.001). Voltage-gated Ca²⁺ channels in rabbit type I cells are not sensitive to hypoxia in the physiological range of PO₂ (see Refs. 22 and 23). In the present experiments, Ca²⁺ currents were evoked in normoxic media. Voltage ramps (200 ms duration) applied between −40 and +80 mV evoked inward currents that activated near 0 mV and peaked near +20 mV. These I-V characteristics are similar to Ca²⁺ currents in rabbit type I cells previously
documented by others (29). The superimposed current traces in Fig. 4 show that 100 nM APIII depressed the voltage-activated inward current, and further, when 1 µM KT-5823 was introduced into the bath along with 100 nM APIII, the evoked current was nearly normal. The inset summarizes data from five cells, showing that APIII depresses the inward current by 32 ± 8% (means ± SE; P < 0.01). In the presence of KT-5823 plus APIII, the current was restored to >90 ± 1% of the current observed in the absence of drugs (P = 0.01 vs. APIII alone). In some experiments the activation voltage appeared to be elevated in the presence of APIII; this phenomenon was not analyzed further. In a similar manner, the PP2A inhibitor, OA (100 nM), reversed the APIII-induced inhibition, which amounted to a 27 ± 3% reduction of the Ca2+ current (Fig. 5; P < 0.001). In 11 cells, OA restored the current to 91 ± 3% of the control value (P < 0.001 vs. APIII alone; Fig. 5, inset).

The effects of OA on the Ca2+ influx in type I cells were confirmed in experiments that measured changes in intracellular Ca2+ levels in response to hypoxic stimulation. The record of [Ca2+]i, presented in Fig. 6

---

**Fig. 2.** Protein kinase G inhibitor, KT-5823, partially reverses retardation of K+ current induced by APIII. Superfusion times and other details same as in Fig. 1. Inset summarizes peak currents recorded at +40 mV from 11 cells, and indicates that treatment with hypoxia in presence of 100 nM APIII plus 1 µM KT-5823 (condition 4) resulted in a significantly greater depression (***P < 0.001) of current than when cells were treated with APIII alone (condition 3). ***P < 0.001 vs. control current recorded in normoxia; ***P < 0.001 vs. current recorded in hypoxia without APIII.

**Fig. 3.** Effect of protein phosphatase 2A inhibitor, okadaic acid (OA), on retardation of K+ current induced by APIII. Details same as in Fig. 1. Inset summarizes data from 5 cells: hypoxia significantly depressed current (***P < 0.001), and 100 nM APIII retarded hypoxia-induced depression of current (**P < 0.01). Presence of 100 nM OA significantly reversed effects of APIII (***P < 0.001). Peak current values obtained at +40 mV for statistical comparisons.

**Fig. 4.** Voltage-current relations (I-V plots) from control and hypoxic (15 min exposure) conditions in carotid body chemoreceptor cells in the absence (1) and presence of 100 nM APIII (2) or 100 nM APIII plus 1 µM KT-5823 (3). Hypoxia caused a significant reduction in inward current amplitude (hypoxia vs. control; P < 0.001; condition 3). Inset summarizes data from five cells: hypoxia significantly depressed current (***P < 0.001), and 100 nM APIII retarded hypoxia-induced depression of current (**P < 0.01). Presence of 100 nM OA significantly reversed effects of APIII (***P < 0.001). Peak current values obtained at +40 mV for statistical comparisons.
demonstrates, in sequence, the response of an isolated type I cell to 1) hypoxia, 2) hypoxia plus 100 nM APIII, 3) hypoxia plus APIII and 100 nM OA, 4) a second exposure to hypoxia plus APIII, and finally, 5) hypoxia. The record illustrates the inhibition of the Ca$^{2+}$ response in the presence of APIII, and the reversal of this effect by OA. In 12 similarly treated cells, we observed that 100 nM APIII reduced the peak response by 39.7% ($P < 0.001$; see Fig. 6, inset) and that with the addition of 100 nM OA, the response was restored to 97.6% ($P < 0.001$ vs. APIII alone) of the control Ca$^{2+}$ levels evoked by hypoxia. In similar experiments, the effects of 100 nM APIII on the hypoxia-evoked Ca$^{2+}$ response were reversed in the presence of 1.0 µM KT-5823 (Fig. 7). APIII depressed the hypoxic response by 43.6%, but in the presence of KT-5823 plus APIII, the peak Ca$^{2+}$ levels were restored to 95.4% of normal ($P < 0.001$ vs. APIII alone).

The effects of KT-5823 and OA, examined at the cellular level on isolated type I cells, predict that these agents should reverse chemoreceptor inhibition produced by APIII. To test this hypothesis, we recorded CSN activity in vitro and evaluated the ability of OA to reverse chemoreceptor inhibition in the presence of APIII. The integrated nerve discharges (3 superimposed traces) presented in Fig. 8 show a control response to hypoxia, and the substantial inhibition produced by 100 nM APIII, confirming our previous demonstrations of the potent nature of this agent (31). As we have also shown previously, the peptide did not alter basal discharge activity established in normoxic conditions.
The introduction of 100 nM OA into the superfusate likewise did not alter the basal discharge activity. However, OA partially restored the response to hypoxia nearer to the value observed in the absence of drugs. In six similar experiments (Fig. 8, inset), 100 nM APIII inhibited the response to hypoxia by 44 ± 6% (P < 0.001), and this inhibition was reduced to 26 ± 7% in the presence of 100 nM OA (P < 0.01 vs. APIII alone).

**DISCUSSION**

ANP and its analog, APIII, are potent inhibitors of hypoxia-evoked activity in the rabbit carotid body (30, 31). ANP has been immunocytochemically localized to type I chemosensory cells (30), and these cells also express ANP receptors that are known to incorporate guanylate cyclase (7, 30). Exposure to submicromolar concentrations of ANP/APIII generates high levels of cGMP in type I cells (31, 37), and cell-permeant cGMP analogs likewise inhibit stimulus-evoked CSN activity (31). The present findings that low concentrations of APIII retard the hypoxia-induced depression of K⁺ currents, and reduce the magnitude of voltage-activated Ca²⁺ currents and intracellular Ca²⁺ levels, suggest that APIII and ANP potently modulate the excitation of type I cells by hypoxia. The involvement of cGMP in the inhibitory mechanism is further suggested by the finding that the specific PKG antagonist, KT-5823, reverses the effects of APIII on the voltage-dependent currents and intracellular free Ca²⁺ concentration. Thus it appears that the inhibitory actions of APIII in the carotid body are mediated by the cGMP-dependent activation of PKG.

Although a role for PKG is strongly implicated by these data, the mechanism of kinase modulation of K⁺ and Ca²⁺ channels appears to be indirect. If PKG had phosphorylated the channels directly, we would have expected to find that phosphatase inhibition enhanced the effects of APIII. That the process instead involves dephosphorylation of these proteins is indicated by our results with the PP2A inhibitor, OA, which, like KT-5823, retards the effects of the ANP analog. Moreover, the parallel reduction in chemoreceptor discharge induced by APIII is likewise reversed by exposure to OA. On average, however, the reversal of the APIII inhibition of nerve activity by OA was incomplete, whereas at the cellular level the PP2A inhibitor restored [Ca²⁺]i levels to 97% of control. This apparent discrepancy may indicate that APIII acts via additional mechanisms or sites to inhibit chemoreceptor activity (e.g., direct action on CSN endings). Alternatively, in the intact superfused organ, APIII and OA may not gain equal access to type I cells.

Previous studies have shown that the activity of specific types of K⁺ channels is downregulated by phosphorylation (19, 28) and that dephosphorylation by PP2A reverses this effect (19). Likewise, numerous data indicate that dihydropyridine-sensitive Ca²⁺ channels, similar to those found in type I cells, can be modulated by the action of cAMP-dependent protein kinase (PKA) and PP2A. In the case of these channels, however, phosphorylation appears to enhance their activity (2, 3, 18, 25). Findings similar to ours have been reported by White et al. (38), who used pituitary-derived GH₄C₁ cells to show that ANP enhances voltage-sensitive outward K⁺ currents and inhibits inward Ca²⁺ currents. Based on these and other data, White and colleagues concluded that ANP, via cGMP, activates PKG, which in turn phosphorylates PP2A or its regulatory subunit, thus initiating the dephosphorylation...
tion step (38). Although other possible mechanisms cannot be ruled out, the current results are consonant with this hypothesis.

In any case, our data strongly favor a role for second messenger-mediated modulation of specific cell currents in type I cells. Hypoxic excitation of the carotid body is known to increase cAMP in these cells (9, 26, 30), and previous studies have shown that the presence of ATP and the catalytic subunit of PKA in patch pipettes retards the “rundown” of Ca^{2+} currents in type I cells (10, 17), suggesting that channel protein function during excitation is modulated by specific kinases. In contrast, inhibition of the carotid body by “efferent” fibers in the CSN has been shown to involve NO, which like ANP, elevates cGMP in type I cells (35, 36). Consequently, modulation of type I cell activity may involve antagonistic signaling pathways controlled by either cAMP or cGMP. In contrast to our findings, Hatton and Peers recently reported that cell-permeant analogs of cyclic nucleotides, including specific activators of PKA and PKG, fail to alter the properties of voltage- and hypoxia-sensitive currents in rat type I cells (15). However, these data were collected using the whole cell configuration of the patch-clamp technique (not the nystatin perforated-patch technique employed here), where intracellular constituents are diazylated against the patch-pipette solution.

In summary, our findings suggest that chemoreceptor inhibition produced by APIII is initiated by the formation of cGMP, which activates PKG, and in turn, PP2A. During hypoxic stimulation in the presence of APIII, dephosphorylation of specific proteins appears to enhance K^{+} currents and depress Ca^{2+} influx via Ca^{2+} channels. Consequently, the state of ion channel phosphorylation may be an important determinant of type I cell excitability and chemoreceptor output. Type I cell excitability could be influenced via feedback loops involving autoreceptors coupled to adenylate and guanylate cyclases and multiple secretory agents released from these cells and CSN terminals.

This work was supported by National Institute of Neurological Disorders and Stroke Grants NS-12636 and NS-07938.

Address for reprint requests and other correspondence: S. J. Fidone, Dept. of Physiology, Univ. of Utah School of Medicine, 410 Chipeta Way, Research Park, Salt Lake City, UT 84108 (E-mail: toni.gillett@m.cc.utah.edu).

Received 13 August 1999; accepted in final form 15 November 1999.

REFERENCES


