Differential Modulation of Respiratory Neuronal Discharge Patterns by GABA\textsubscript{A} Receptor and Apamin-Sensitive K\textsuperscript{+} Channel Antagonism


\textsuperscript{1}Zablocki Veterans Affairs Medical Center, Research Service, Milwaukee 53295; \textsuperscript{2}Department of Anesthesiology, Medical College of Wisconsin, Milwaukee, Wisconsin 53226; and \textsuperscript{3}Department of Physiology and Institute for Neuroscience, Northwestern University Medical School, Chicago, Illinois 60611

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Tonkovic-Capin, V., A. G. Stucke, E. A. Stuth, M. Tonkovic-Capin, M. Krolo, F. A. Hopp, D. R. McCrimmon, and E. J. Zuperku. Differential modulation of respiratory neuronal discharge patterns by GABA\textsubscript{A} receptor and apamin-sensitive K\textsuperscript{+} channel antagonism. \textit{J Neurophysiol} 86: 2363–2373, 2001. The discharge patterns of respiratory neurons of the caudal ventral respiratory group (cVRG) appear to be subject to potent GABA\textsubscript{A}ergic gain modulation. Local application of the GABA\textsubscript{A} receptor antagonist bicuculline methochloride amplifies the underlying discharge frequency (F\textsubscript{m}) patterns mediated by endogenous excitatory and inhibitory synaptic inputs. Gain modulation can also be produced by alterations in the amplitude of spike afterhyperpolarizations (AHPs) mediated by apamin-sensitive small-conductance Ca\textsuperscript{2+}-activated K\textsuperscript{+} (SK) channels. Since methyl derivatives of bicuculline (BICm) have also been shown to reduce the amplitude of AHPs, in vitro, it is possible that the BICm-induced gain modulation is due to a block of SK channels. The purpose of these studies was to determine the mechanisms by which BICm produces gain modulation and to characterize the influence of SK channels in the control of respiratory neuron discharge. Six protocols were used in this in vivo study of cVRG inspiratory (I) and expiratory (E) neurons in decerebrate, paralyzed, ventilated dogs. The protocols included block of SK channels and GABAAergic shunting inhibition rather than a reduction in AHPs by block of SK channels in canine cVRG neurons.

\textbf{INTRODUCTION}

The discharge patterns of respiratory neurons of the caudal ventral respiratory group (cVRG) appear to be subject to potent GABA\textsubscript{A}ergic gain modulation. Local application of the methyl derivatives of the competitive GABA\textsubscript{A} receptor antagonist bicuculline (BICm) (MacDonald and Olsen 1994) amplifies the underlying discharge frequency (F\textsubscript{m}) patterns that are mediated by endogenous excitatory and inhibitory synaptic inputs (Dugas et al. 1998; McCrimmon et al. 1997). These results imply a tonic GABA\textsubscript{A}-ergic mechanism that constrains the control and reflexly induced activities of these bulboespinal premotor respiratory neurons to \textasciitilde35–50\% of the discharge rate without this inhibitory input. Such gain control could provide a powerful mechanism for the central respiratory control system to optimize the breathing pattern in response to changes in conditions or state (McCrimmon et al. 1997).

Some recent in vitro studies have shown that BICm can also block Ca\textsuperscript{2+}-dependent, apamin-sensitive, small-conductance K\textsuperscript{+} (SK) channels in concentrations that are used for blocking GABA\textsubscript{A} receptors (Debarbieux et al. 1998; Johnson and Seutin 1997; Seutin et al. 1997). The SK channels are activated rapidly by increases in intracellular Ca\textsuperscript{2+} concentration following a single action potential and produce a hyperpolarization that decays with a time constant of \textasciitilde150 ms (Vergara et al. 1998), termed the medium afterhyperpolarization (AHP) (Kobayashi et al. 1997; Viana et al. 1993). Blockade of the SK channels reduces the amplitude of the AHPs and causes a marked increase in the minimal repetitive firing frequency and slope of the frequency versus injected depolarizing current plot for hypoglossal motoneurons, suggesting an alternative mechanism for gain control or modulation (Viana et al. 1993). Thus it is possible that the BICm-induced gain modulation responses of the cVRG neurons may be due to a block of SK channels rather than GABA\textsubscript{A} receptors. Alternatively, the BICm-induced gain modulation may be due to effects on both SK channels and GABA\textsubscript{A} receptors or to GABA\textsubscript{A} receptors alone.

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The purpose of the present studies was to gain further insight into the mechanisms underlying BICm-induced gain modulation using a multifaceted approach. Evidence from six protocols was used in this in vivo study of cVRG inspiratory (I) and expiratory (E) neurons in decerebrated, paralyzed, ventilated dogs. The protocols included characterizations of the neuronal responses to the separate application of BICm and apamin on the same neuron and to the sequential co-application of one during the maximum-induced effect of the other. In addition, the responses to the specific GABA_A receptor antagonist, (+)-β-hydrastine, the specific GABA_A receptor agonist, muscimol, and the GABA uptake inhibitor, nipeptic acid, were characterized. Evidence from these protocols suggest that gain modulation is altered by both SK channel and GABA_A receptor activation, but the effect of BICm on gain modulation is most likely due to a reduction of GABAergic shunting inhibition rather than a reduction in AHPs due to a block of SK channels.

Methods

This research was approved by the Subcommittee on Animals Studies of the Zablocki VA Medical Center, Milwaukee, WI, in accordance with provisions of the Animal Welfare Act, the National Institutes of Health Guide for the Care and Use of Laboratory Animals, and VA policy. Data were obtained from 46 mongrel dogs of either sex weighing from 8 to 17 kg. Mask induction with a volatile anesthetic (isoflurane or halothane) was used, and anesthesia was maintained during the surgical procedure with isoflurane (1.4–2.0% end-tidal concentration). Airway, isoflurane, CO_2, and O_2 concentrations were continuously monitored with an infrared analyzer (POET II, Criticare Systems, Waukesha, WI). The animals were monitored for signs of inadequate anesthesia (e.g., salivation, lacrimation, and/or increases in blood pressure and heart rate), and if required, depth of anesthesia was increased immediately.

Surgical procedure

Dogs were intubated with a cuffed endotracheal tube and mechanically ventilated with an air-O_2-isoflurane mixture. The surgical procedure, monitoring, and maintenance of body homeostasis have been previously described in detail (Dogas et al. 1998). Briefly, after cannulating the femoral axis (for blood pressure recording and blood-gas sampling) and vein (for infusion of maintenance blood-gas sampling) and vein (for infusion of maintenance and VA policy. Data were obtained from 46 mongrel dogs of either sex weighing from 8 to 17 kg. Mask induction with a volatile anesthetic (isoflurane or halothane) was used, and anesthesia was maintained during the surgical procedure with isoflurane (1.4–2.0% end-tidal concentration). Airway, isoflurane, CO_2, and O_2 concentrations were continuously monitored with an infrared analyzer (POET II, Criticare Systems, Waukesha, WI). The animals were monitored for signs of inadequate anesthesia (e.g., salivation, lacrimation, and/or increases in blood pressure and heart rate), and if required, depth of anesthesia was increased immediately.

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Protocols

Following decerebration and discontinuation of the anesthetic, a period of at least an hour was allowed for washout of isoflurane (airway concentration, <0.05%) and for stabilization of the breathing pattern (i.e., PNG). Upon establishing a stable recording of single unit activity, a 2- to 3-min period was used to obtain the control discharge pattern prior to picoejection. Picoejections were made using step increases in dose rate via increases in the volume ejection-rate, which was measured as a change in meniscus height/time with a ×100 magnification microscope equipped with a reticule. Each dose rate was maintained until a steady-state neuronal response was achieved. Step increases in dose rate were continued until no further effect was observed. Picoejections of the ACSF vehicle were routinely used to verify that the ACSF constituents and/or ejected volumes were without effect.

In protocol 1, the effects of BICm and apamin were compared on the same neuron. Sufficient time (20–40 min) was allowed between picoejections for the discharge frequency to return to prejection baseline levels.

In protocol 2, we tested the ability of BICm to produce additional increases in F_0 during maximum block of the SK channels with apamin. For this purpose, apamin was picoejected at increasing rates until a maximum steady-state response was obtained. BICm was then picoejected concurrently with apamin at a dose rate that is known (protocol 1) to produce a near maximum response when given alone.

In protocol 3, we tested the ability of apamin to produce additional increases in F_0 during the maximum BICm-induced response. For this purpose, BICm was picoejected at increasing rates until a maximum steady-state response was obtained. Then during continued picoejection of BICm, the dose rate of apamin was increased until the maximum effect was seen.

In protocol 4, the neuronal responses to the competitive GABA_A receptor agonist (+)-β-hydrastine were characterized and compared statistically with the responses to BICm and apamin.

In protocol 5, the neuronal response characteristics to picoejection of the specific GABA_A agonist muscimol were compared for effects consistent with those produced by BICm, but opposite in direction.

In protocol 6, the neuronal responses to the GABA uptake inhibitor nipeptic acid, which is expected to increase the level of endogenously released GABA, were characterized and compared with those of muscimol.

Data analysis

Cycle-triggered histograms (CTHs; binwidth: 50 ms), triggered from either the onset of the E or I phase and based on 5–25 respiratory cycles were used to quantify the discharge frequency patterns at each dose rate. The values of F_0 for each bin were calculated as the number of spikes per bin/bin duration in seconds. For each time (bin) increment within the triggered cycle, these values were averaged over the number of cycles used to generate the CTH. Drug-induced changes in the gain and offset of the discharge frequency pattern were analyzed via plots of the F_0(drug) versus F_0(control) values obtained from corresponding-time bin values from each CTH. Thus each point is an average based on the number of cycles used for the CTHs. This

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Comparison of responses to apamin and BICm on the same neuron

INSPIRATORY NEURONS. Figure 1 shows a typical example of the responses to picoejections of apamin (top) and BICm (bottom) for an I augmenting neuron. The dose rates were gradually increased until a maximal response was obtained. The effect of apamin was usually visible in <1 min from the onset of picoejection, and full recovery usually required ∼20–40 min. CTHs show that the apamin-induced increases in discharge frequency (F_n) occurred mainly in the higher F_n range near the end of the I phase (Fig. 2, top left). Using corresponding data from the CTHs, the plot of F_n during apamin versus F_n control was highly linear and indicated that the changes in discharge pattern resulted from an increase in the gain (slope) but with a negative offset (y intercept) (Fig. 2, top right). In this example, apamin increased the average F_n by 31% and peak F_n by 40%. Gain was increased by 64% with a negative offset of −18.8 Hz.

The neuronal response to BICm was usually visible in <1 min after the start of picoejection, and full recovery usually required 30–40 min (Fig. 1, bottom). The BICm-induced increases in F_n were evident throughout the I phase (Fig. 2, bottom left) and were due to an increase in the gain with a positive offset (y intercept) (Fig. 2, bottom right). In this example, BICm increased the average F_n by 104% and peak F_n by 102%; gain was increased by 97% with a positive offset of 4.2 Hz.

The pooled data from nine I neurons comparing the responses to apamin and BICm are shown in Fig. 3. BICm produced a greater increase than apamin in both average F_n (97.5 ± 26.9 vs. 36.5 ± 13.4%, P < 0.05) and peak F_n.
(103.7 ± 24.9 vs. 50.4 ± 13.6%), but only the difference in average $F_n$ was statistically significant ($P < 0.05$, Fig. 3, left). The gain increases produced by apamin and BICm were not significantly different (72.0 ± 11.7 vs. 97.6 ± 18.5%, Fig. 3, top right). However, the offset produced by apamin was significantly more negative than that due to BICm ($-22.1 ± 3.5$ vs. $-2.7 ± 8.6$ Hz, $P < 0.05$, Fig. 3, bottom right).

**Expansory neurons**

The responses of the cVRG E neurons to apamin and BICm were qualitatively similar to those of the I neurons. Only the gain and peak $F_n$ increase produced by BICm was significantly greater for I neurons than for E neurons ($P = 0.002$ and $P = 0.032$, respectively). The CTHs of the responses of an E decrementing neuron showed that the apamin-induced increases in $F_n$ were also greater for the higher $F_n$ range, whereas BICm produced marked increases in both the high and low $F_n$ ranges (Fig. 4, left). The average and peak $F_n$ responses to BICm were greater than those due to apamin. Both, apamin and BICm, produced marked increases in gain (50 vs. 70%, respectively), but striking differences in offset ($-12.2$ vs. $+37.2$ Hz, respectively).

The pooled responses of 10 cVRG E neurons to apamin and BICm are compared in Fig. 5. BICm produced greater in-

**FIG. 2. Effects of apamin and BICm on the discharge patterns of the I neuron of Fig. 1 are compared via cycle-triggered histograms (CTHs) of unit activity (left). Right: corresponding plots of control discharge frequency pattern [$F_n$(control)] vs. drug-induced discharge frequency pattern [$F_n$(drug)] were used to analyze changes in the gain (slope) and offset ($y$ intercept) of the discharge patterns. Top: apamin increased the gain by 64% and produced a negative offset of $-18.8$ Hz. Bottom: BICm increased the gain by 97% and produced a positive offset of 4.2 Hz. BICm also produced a much greater increase in peak $F_n$ than apamin. $F_n$: neuronal discharge frequency. $-$ - $-,$ line of identity. $r$, correlation coefficient.

**FIG. 3. Pooled data obtained from cVRG inspiratory neurons. BICm and apamin were applied on the same 9 I neurons. Hydrastine was applied on an additional 7 I neurons. Left: BICm produced the greatest average increases in averaged and peak $F_n$. The difference between responses to BICm and apamin was only significant for the average $F_n$ data. Top right: all 3 drugs produced significant increases in gain; however, the observed differences among these gain increases were not significantly different from each other. Bottom right: BICm and hydrastine produced significantly different offsets than apamin ($*P < 0.05$). Offsets produced by BICm and hydrastine were not significantly different from each other.
creases than apamin in average $F_n$ (76.3 ± 15.5 vs. 21.8 ± 8.4%, $P < 0.05$), while the increases in peak $F_n$ were not significantly different (63.4 ± 11.0 vs. 29.3 ± 7.0%). The gain increases produced by apamin and BICm were not significantly different from one another (54.0 ± 6.1 vs. +18.9 ± 9.8 Hz, $P < 0.01$, Fig. 5, bottom right).

**BICm responses during maximum apamin-induced block of SK channels**

Protocol 2 tested the hypothesis that if the BICm-induced neuronal response was due to antagonism of GABA_A receptors, then a BICm response should be observable during full block of the apamin-sensitive SK channels. Figure 6A shows an example in which apamin alone, at maximal effective dose rates, increased peak $F_n$ by 56% (i.e., from 81 to 126 Hz, left part of trace), and the concurrent application of BICm produced an additional 164% increase relative to preejection control $F_n$ (i.e., from 126 to 259 Hz, right part of trace). To demonstrate that the block of the apamin-sensitive SK channels was complete, additional studies were performed on nine neurons comparing the effects of different concentrations of apamin (e.g., 150 and 300 nM, and 125, 250, and 500 nM, respectively). These data indicated that no further increase in peak $F_n$ occurred beyond that produced by the lowest apamin concentration. Figure 6B shows the response of another E neuron to picoejection of 150 nM apamin (3.9 fmol/min), which produced a maximum increase of 93% in peak $F_n$ (i.e., from 75 to 145 Hz, middle). Subsequent picoejection of 300

**FIG. 4.** Responses of a cVRG expiratory neuron to picoejection of apamin and BICm at maximally effective dose rates. Left: CTTHs of unit activity during the control period and during ejection of drugs (bold patterns). Right: corresponding plots of $[F_n(drug)]$ vs. $[F_n(control)]$ used to estimate gain (slope) and offset (y intercept) changes. Top: apamin (125 nM) increased the gain by 50% and caused a negative offset of ~12.2 Hz. Bottom: BICm (200 μM) increased the gain by 70% and caused, in contrast, a positive offset of 37.2 Hz. $F_n$: neuronal discharge frequency. - - -, line of identity.

**FIG. 5.** Pooled data obtained from cVRG expiratory neurons. BICm and apamin were applied on the same 10 E neurons whereas hydrastine was applied on an additional 14 E neurons and muscimol was applied on another 26 E neurons. **Left:** BICm produced greater increases than apamin in average $F_n$ (*$P = 0.034$). The average $F_n$ for hydrastine was less than that for BICm (#$P = 0.042$). The peak $F_n$ data were similar to the average $F_n$ data, but significant differences were not found. Muscimol produced a decrease in average and peak $F_n$. **Top right:** apamin, BICm, and hydrastine produced significant increases in gain; however these gain increases were not significantly different from each other. Muscimol produced a decrease in the gain. **Bottom right:** the offsets produced by BICm and hydrastine were significantly different from that of apamin (***$P < 0.01$) but not significantly different from each other. Muscimol produced a negative offset.
nM apamin (10.9 fmol/min) produced no further increase (Fig. 6B, bottom). Concurrent application of BICm increased peak $F_n$ by an additional 154% (i.e., from 145 to 260 Hz). The pooled data of eight neurons indicate that apamin increased peak $F_n$ by 67.3 ± 7.9% and BICm increased peak $F_n$ by an additional 112.4 ± 17.9% relative to the preapamin control, both at maximum effective dose rates.

**Apamin responses during maximum BICm-induced effect**

Protocol 3 tested whether apamin could produce additional neuronal response, due to a block of SK channels, during the maximum BICm-induced effect. This response was expected to be absent if BICm already blocked the SK channels. BICm was picoejected at increasing dose rates until the maximum response was obtained. While continuing BICm picoejection, co-application of apamin was initiated and continued at increasing dose rates until the maximum neuronal response was obtained. A typical example of the responses (CTHs) of an E decrementing neuron is shown in Fig. 7, left. The responses are shown for the maximum effective dose rates of BICm and the combined apamin plus BICm picoejections. Note that the slope as well as peak $F_n$ increased. Plots of $F_n$(BICm) versus $F_n$(control) indicate that BICm increased the gain by 37% (Fig. 7A). The plot of $F_n$(BICm + AP) versus $F_n$(BICm) indicates that apamin produced an additional gain increase of 77% (Fig. 7B). The slope for the effect of both drugs [$F_n$(BICm + AP) vs. $F_n$(control)] suggests that the two gain mechanisms act in a cascade fashion (i.e., the overall gain is the product of the gains from each mechanism, slope = $2.42 = 1.37 \times 1.77$, Fig. 7C). For five E neurons, the average slope (gain) increase produced by BICm was 61 ± 16%, and apamin produced an additional 71 ± 25% increase in slope. The two cascaded gains result in an overall gain increase of 175%. BICm increased peak $F_n$ by 114.8 ± 26.6% and apamin increased peak $F_n$ by an additional 175.6 ± 74.5%, both at maximum effective dose rates.

**Neuronal responses to (+)-β-hydrastine**

Typical examples of the neuronal responses to the specific GABA<sub>A</sub> receptor antagonist (+)-β-hydrastine are shown for cVRG I and E neurons in Fig. 8, A and B, respectively. The effects of hydrastine could be observed within 1 min after the start of picoejection and lasted 10–20 min after discontinuation. The hydrastine-induced increases in $F_n$ were due to a combined increase in the gain and a positive offset as indicated by the $F_n$(HYD) versus $F_n$(control) plots of Fig. 8, bottom. The pooled responses from seven I neurons are shown in Fig. 3. Hydrastine produced increases of 71.1 ± 14.5 and 68.9 ± 8.8% in average and peak $F_n$, respectively. These values were not significantly different from those produced by either apamin or BICm. Hydrastine increased the gain of the neuronal discharge pattern by 65.9 ± 10.9%, and the corresponding average offset was +6.3 ± 11.2 Hz. Similar to BICm, the hydrastine-induced offset was significantly different from that produced by apamin ($P < 0.05$).

The pooled data from 14 cVRG E neurons for hydrastine were similar to those for the cVRG I neurons (Fig. 5). Hydrastine produced increases in average and peak $F_n$ of 47.7 ± 5.8 and 46.3 ± 4.4%, respectively. The increase in gain was 35.9 ± 8.6% with an offset of +8.5 ± 7.3 Hz, where the latter was significantly greater than that produced.
by apamin. Only average $F_n$ for hydrastine was significantly less than that for BICm ($P < 0.05$), while the peak $F_n$ and slope values were not significantly different from those for BICm.

Neuronal responses to muscimol

Picoejection of the specific GABA$_A$ receptor agonist muscimol resulted in a dose-dependent reduction of $F_n$. A typical example of the responses of an E neuron with a decrementing discharge pattern is shown in Fig. 9. In this protocol, submaximal dose rates were used to preserve the contour of the $F_n$ pattern, which can be distorted when the lower levels of activity are suppressed below discharge threshold. Higher dose rates of muscimol will completely inhibit neuronal activity. Analysis of the CTHs of this neuron’s activity indicates that the reduction in $F_n$ was due to a decrease in gain to 59% of control and a negative offset of $-2.14.4$ Hz (Fig. 10).

The pooled data from 26 E neurons is shown in Fig. 5. Muscimol reduced the average and peak $F_n$ by 68.2 ± 2.7 and

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**FIG. 7.** Apamin-induced response of cVRG E neuron during the maximum BICm-induced response. Left: CTHs of unit activity during the control period and during ejection of drugs (bold patterns). BICm increased the gain of the $F_n$ pattern by an estimated 37% [plot of $F_n$(BICm) vs. $F_n$(control), A]. Subsequent, concurrent picoejection of apamin produced a further 77% increase in gain [plot of $F_n$(BICm + APA) vs. $F_n$ (BICm), APA: apamin, B]. Both drugs produced an estimated increase of 142% [plot of $F_n$(BICm + APA) vs. $F_n$(control), C]. The corresponding offsets from A–C were 1.4, −24.7, and −3.0, respectively.

**FIG. 8.** Responses of a cVRG I and E neuron to picoejection of the specific GABA$_A$ antagonist (+)-hydrastine (HYD). A: CTHs show effect of hydrastine on the discharge pattern of an I neuron. The plot of $F_n$(HYD) vs. $F_n$(control) indicates a 31% increase in the gain (slope) and a positive offset (y intercept) of $11.5$ Hz. **B:** CTHs show effect of hydrastine on the discharge pattern of an E neuron. The plot of $F_n$(HYD) vs. $F_n$(control) indicates a 48% increase in gain and a positive offset of $13.4$ Hz. **C:** CTHs show effect of hydrastine on the discharge pattern of an E neuron. The plot of $F_n$(HYD) vs. $F_n$(control) indicates a 48% increase in gain and a positive offset of $13.4$ Hz. **C:** CTHs show effect of hydrastine on the discharge pattern of an E neuron. The plot of $F_n$(HYD) vs. $F_n$(control) indicates a 48% increase in gain and a positive offset of $13.4$ Hz.
57.8 ± 2.1%, respectively. The gain was reduced by 38.4 ± 3.2% with an offset of −26.2 ± 4.6 Hz.

**Neuronal responses to nipecotic acid**

A typical response of an E augmenting neuron to picoejection of the GABA uptake inhibitor nipecotic acid is shown in Fig. 11. The amount of reduction of $F_n$ was dose dependent (Fig. 11A), and at the highest dose rate, it was possible to inhibit neuronal activity almost completely. Recovery following discontinuation of the picoejection was relatively rapid (Fig. 11A, right). The analysis of the CTH data with plots of $F_n$(NIPA) versus $F_n$(control) (Fig. 11B) indicates that the reduction of $F_n$ at the intermediate dose rate of 154 pmol/min was the result of a reduced gain (to 84%) and a negative offset (−39.6 Hz). The pooled mean data for the reduction of average and peak $F_n$ were 54.8 ± 23.6 and 50.2 ± 19.4%, respectively. The gain was reduced by 36.7 ± 19.3% with an offset of −11.3 ± 6.0 Hz (E neurons; n = 3). Except for the neuron of Fig. 11 in which a submaximal dose rate was used, the pooled data were analyzed at maximum effective dose rates, which were able to attenuate but not shift the $F_n$ pattern below the discharge threshold and confound the gain analysis.

**Neuronal responses to the ACSF vehicle**

A routine procedure that was incorporated in all protocols was the test of the effects of the constituents and volume of the ACSF vehicle. Very little or no noticeable effect was observed with pressure ejection of the ACSF vehicle alone at ejection rates equal to or greater than those used during the drug protocols.

**DISCUSSION**

The present studies provide several lines of evidence suggesting that GABA_A receptors mediate a gain modulatory response in cVRG I and E neurons. An increase in the gain of the neuronal discharge frequency pattern was produced by the specific GABA_A receptor antagonists, BICm and hydrastine, while a reduction in gain was produced by both the specific GABA_A receptor agonist muscimol as well as an increase in endogenous GABA by the GABA uptake inhibitor, nipecotic acid.

In those studies that compared the responses of the same neuron to apamin and BICm, the changes in gain were of
similar magnitude. However, the offset or y intercept associated with the gain change produced by apamin was significantly more negative than that produced by BICm. This difference in offset was on average 19.4 and 43.9 Hz for I and E neurons, respectively, and in part accounts for the smaller increase in average and peak $F_n$ produced by apamin versus BICm.

From an analysis viewpoint, phasic discharge patterns, which are a characteristic of respiratory neurons, make it possible to distinguish between changes in the gain versus changes in baseline offset of neuronal activity. This distinction would not be possible with tonic or plateau-like, “flat,” phasic patterns without the use of additional perturbations. Mechanistically, a change in gain is analogous to a change in neuronal excitability, whereas a change in offset is analogous to a change in tonic polarization of the resting membrane potential. For example, if the Cl$^{−}$ equilibrium potential was more negative than the resting membrane potential, then a reduction in Cl$^{−}$ conductance would produce a relative depolarization. If, in addition, the Cl$^{−}$ conductance was of sufficient magnitude and/or strategically located, for example on a main dendritic trunk, then the sensitivity of the neuron to other synaptic inputs would also be affected. Neuronal responses to the latter would manifest as a gain change.

During each action potential, Ca$^{2+}$ enters the neuron via voltage-activated Ca$^{2+}$ channels and rapidly activates small-conductance Ca$^{2+}$-activated K$^{+}$ channels. Because the increase in intracellular Ca$^{2+}$ is rapidly buffered, this effect is transient and produces the AHP, a form of relative refractoriness. A reduction in the amplitude of the AHP by low bath Ca$^{2+}$ concentration, block of Ca$^{2+}$ channels, sequestration of Ca$^{2+}$ by intracellular chelators, or block of SK channels results in an increase in neuronal excitability or gain (Viana et al. 1993). The mechanism by which apamin produces a negative offset is not clear, since a reduction in K$^{+}$ conductance would also be expected to produce a relative depolarization of the membrane potential.

The results with hydrastine are similar to those of BICm but significantly different from those of apamin with regard to the offset of the discharge pattern. The molecular structure of hydrastine is very similar to that of bicuculline (Huang and Johnston 1990). It has been shown that bicuculline-free base can also block AHPs but only at much higher concentrations than those used for GABA$\text{\textsubscript{A}}$ receptor antagonism (Debarbieux et al. 1998; Khawaled et al. 1999). However, bicuculline-free base is unstable in aqueous solutions. We thus used hydrastine, which is relatively stable and stays in solution at a pH of 7.0. Apparently, the presence of the quaternary N-methyl group, which makes BICm water soluble, is also responsible for acting as a plug of the SK channels because their block by BICm is voltage dependent (Debarbieux et al. 1998).

Different concentrations of apamin (125–500 nM) were picoejected on the same neuron to assure that a complete block of the apamin-sensitive SK channels (i.e., SK2) was achieved. The reported IC$_{50}$ values for apamin at the SK2 channels are similar: 83 pM (HEK293 cells) (Strøbæk et al. 2000) and 63 pM (oocytes) (Köhler et al. 1996), and complete block was achieved at bath concentrations of 300 nM (Wolfart et al. 2001). Our pipette concentrations should have been sufficient to block both SK channel subtypes. Thus the additional marked increases in gain and in peak and average $F_n$ produced by
BICm during apamin block suggest that such responses are due to effects that are not mediated by a possible BICm block of SK channels but rather by GABA$_A$ receptors.

This finding alone does not rule out the possibility that BICm, when given alone, could affect both mechanisms. In this regard, the reported IC$_{50}$ values for BICm for the apamin-sensitive SK channels are 26 mM for rat dopaminergic neurons (Seutin et al. 1997) and 25 mM for cloned rat SK2 channels expressed in HEK 293 cells (Straback et al. 2000), but only 1.1 mM for SK2 channels expressed in Xenopus oocytes (Kha- waled et al. 1999). Complete block in these preparations was achievable with bath concentrations of 100–300 mM. Thus it is possible that our BICm pipette concentration of 200 mM could have considerably blocked SK channels. However, the additional finding that apamin was capable of producing a marked increase in gain and in peak and average $F_n$ during maximum effective dose rates of BICm strongly suggests that SK channels were not blocked by BICm on these E neurons. The data from this protocol show that the two different mechanisms for gain change act in a cascade fashion (e.g., Fig. 7), that is, within the same neuron the overall gain is determined by the product of the gains from the two mechanisms.

Recent studies show that the SK channels can be subdivided into apamin-sensitive (SK2) and apamin-insensitive (SK1) channels, which may or may not coexist in the same neuron (Kha- waled et al. 1999; Vergara et al. 1998). For example, the AHPs of hippocampal interneurons are sensitive to apamin, whereas those of hippocampal pyramidal neurons are not (Kha- waled et al. 1999). Both, apamin-sensitive and -insensitive AHP components are present in vagal motoneurons, neocortical neurons, and pyramidal neurons of the sensorimotor cortex. Furthermore, BICm appears to block both SK1 and SK2 currents (Kha- waled et al. 1999).

Apamin-sensitive AHPs are activated rapidly following a single action potential and decay with a time constant of 150 ms. In contrast, apamin-insensitive AHPs exhibit slow rise times, taking 0.5–1 s to peak, and decay with a time constant of 1.5 s. Apamin-sensitive AHPs serve to set the discharge frequency, whereas activation of the apamin-insensitive AHPs leads to spike-frequency adaptation (Sah 1996; Stocker et al. 1999). While it is not known if apamin-insensitive channels exist in the membranes of respiratory neurons, Richter and Heyde (1975) have shown that after the second spike of a single action potential and decay with a time constant of 150 ms. In contrast, apamin-insensitive AHPs exhibit slow rise times, taking 0.5–1 s to peak, and decay with a time constant of 1.5 s. Apamin-sensitive AHPs serve to set the discharge frequency, whereas activation of the apamin-insensitive AHPs leads to spike-frequency adaptation (Sah 1996; Stocker et al. 1999). While it is not known if apamin-insensitive channels exist in the membranes of respiratory neurons, Richter and Heyde (1975) have shown that after the second spike of a single action potential and decay with a time constant of 150 ms. In contrast, apamin-insensitive AHPs exhibit slow rise times, taking 0.5–1 s to peak, and decay with a time constant of 1.5 s. Apamin-sensitive AHPs serve to set the discharge frequency, whereas activation of the apamin-insensitive AHPs leads to spike-frequency adaptation (Sah 1996; Stocker et al. 1999). While it is not known if apamin-insensitive channels exist in the membranes of respiratory neurons, Richter and Heyde (1975) have shown that after the second spike of a single action potential and decay with a time constant of 150 ms. In contrast, apamin-insensitive AHPs exhibit slow rise times, taking 0.5–1 s to peak, and decay with a time constant of 1.5 s. Apamin-sensitive AHPs serve to set the discharge frequency, whereas activation of the apamin-insensitive AHPs leads to spike-frequency adaptation (Sah 1996; Stocker et al. 1999). While it is not known if apamin-insensitive channels exist in the membranes of respiratory neurons, Richter and Heyde (1975) have shown that after the second spike of a single action potential and decay with a time constant of 150 ms. In contrast, apamin-insensitive AHPs exhibit slow rise times, taking 0.5–1 s to peak, and decay with a time constant of 1.5 s. Apamin-sensitive AHPs serve to set the discharge frequency, whereas activation of the apamin-insensitive AHPs leads to spike-frequency adaptation (Sah 1996; Stocker et al. 1999). While it is not known if apamin-insensitive channels exist in the membranes of respiratory neurons, Richter and Heyde (1975) have shown that after the second spike of a single action potential and decay with a time constant of 150 ms. In contrast, apamin-insensitive AHPs exhibit slow rise times, taking 0.5–1 s to peak, and decay with a time constant of 1.5 s. Apamin-sensitive AHPs serve to set the discharge frequency, whereas activation of the apamin-insensitive AHPs leads to spike-frequency adaptation (Sah 1996; Stocker et al. 1999). While it is not known if apami...


