K⁺ channel blockade in the NTS alters efficacy of two cardiorespiratory reflexes in vivo

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Butcher, James W., and Julian F. R. Paton. K⁺ channel blockade in the NTS alters efficacy of two cardiorespiratory reflexes in vivo. Am. J. Physiol. 274 (Regulatory Integrative Comp. Physiol. 43): R677–R685, 1998.—We investigated the role of potassium conductances in the nucleus of the solitary tract (NTS) in determining the efficacy of the baroreceptor and cardiopulmonary reflexes in anesthetized rats. The baroreceptor reflex was elicited with an intravenous injection of phenylephrine to evoke a reflex bradycardia, and the cardiopulmonary reflex was evoked with a right atrial injection of phenylbiguanide. Microinjection of two Ca-dependent potassium channel antagonists (apamin and charybdoxin) into the NTS potentiated the baroreceptor reflex bradycardia. This may reflect the increased neuronal excitability observed in vivo with these blockers. In contrast, the Ca-dependent potassium channel antagonist attenuated the cardiopulmonary reflex, whereas voltage-dependent potassium channel antagonists (4-aminopyridine and dendrotoxin) attenuated both the baro- and cardiopulmonary reflexes when microinjected into the NTS. The possibility that the reflex attenuation observed indicates a predominant distribution of certain potassium channels on γ-aminobutyric acid interneurons is discussed.

baroreceptor reflex; cardiopulmonary reflex; 4-aminopyridine; dendrotoxin; apamin; nucleus of the solitary tract

It has been well documented that primary afferent fibers from cardiorespiratory receptors project to, and terminate in, the nucleus of the solitary tract (NTS) located within the dorsomedial medulla (for review, see Refs. 14 and 28). However, the neural mechanisms by which this afferent information is integrated within the NTS to produce reflex cardiovascular and respiratory responses are not fully understood. In this regard, we have hypothesized that the sensitivity of an NTS neuron to afferent input is dependent on its own specific set of intrinsic membrane properties and that modulation of one or more of these conductances in a population of such neurons would alter the magnitude of an evoked reflex response.

On the basis of previous in vitro recordings of NTS neurons (5, 7–9, 16, 21) and recent simulation experiments (24), our current approach is to investigate the functional role of a variety of potassium conductances, including both voltage- and Ca-dependent (Iₖ and IₖCa, respectively) channels, that control neuronal firing properties in regulating reflex sensitivity in vivo. Studies by Richter and co-workers (22) have also used in vivo approaches to demonstrate a functional role for IₖCa: intracellular injection of the calcium chelator BAPTA into postinspiratory neurons, which are responsible for switching off inspiration irreversibly, prolonged action potential discharge, and reduced adaptation. It was suggested that blockade of such channels in postinspiratory neurons could lead to a lengthening of the postinspiratory phase and a decrease in respiratory frequency (22). From in vitro studies on the NTS, two IₖCa conductances were identified (5, 8, 10) that were sensitive to either charybdoxin [large-conductance IₖCa antagonist (BK)]; Ref. 16) or apamin [small-conductance IₖCa antagonist (SK); Ref. 21]. Additionally, voltage-dependent channels included both a transient potassium current (Iₖ) and a delayed, more slowly inactivating potassium current (Iₖ) that were both blocked by 4-aminopyridine (5, 8, 9, 16, 21). Another voltage-dependent potassium channel antagonist used in this study, dendrotoxin, has not previously been used on the NTS; although in cells isolated from the rat nodose ganglia this toxin had little effect on Iₖ, with a much larger effect on Iₖ (29, 30).

We have chosen to study two reflexes with contrasting roles in the intact animal: the baroreceptor reflex acts on a beat-by-beat basis to maintain arterial pressure in a regulatory manner around a given set point; in contrast, the cardiopulmonary reflex, which can be elicited experimentally with a right atrial injection of phenylbiguanide, has been postulated to act in a defensive way (6) to protect, for example, against a rise in pulmonary interstitial pressure during exercise (19). Despite a qualitatively similar pattern of reflex response [i.e., falls in arterial pressure, heart rate (HR), and respiratory frequency], the cardiopulmonary reflex is quantitatively more potent than its regulatory counterpart. In this regard, it has recently been shown, using both extra- and intracellular recording techniques, that there is very little convergence of baroreceptor and pulmonary C fiber synaptic inputs onto NTS neurons in both the mouse (20) and cat (27). One might conclude, therefore, that these reflexes are mediated by two separate populations of NTS neurons with distinct intrinsic membrane properties. Moreover, a differential distribution of potassium conductances could underlie the contrasting potency of these two reflexes.

Because in vitro studies (16, 21) revealed that blockade of potassium currents (either voltage- or Ca-dependent) increased the responsiveness of the neuron during depolarizing current injection, one might anticipate that blockade of either Iₖ or IₖCa in NTS would increase reflex efficacy. However, we show that in the in vivo rat blockade of IₖCa counterintuitively attenuates the cardiopulmonary reflex, whereas blockade of Iₖ attenuates both this and the baroreceptor reflex.

METHODS

Surgical Procedures

Experiments were carried out on mature male Sprague-Dawley rats (220–270 g; n = 54) in which anesthesia was
induced with a mixture of α-chloralose-urethan-pentobarbital sodium (69, 690, 30 mg/kg ip, respectively) and supplemented with 3–8 mg intravenous α-chloralose when required, as judged from the withdrawal of a hindlimb to a paw pinch.

The trachea was intubated below the larynx, and the left femoral artery and vein were cannulated for recording arterial pressure (Gould Stratham) and subsequent injections of anesthetic, respectively. The left jugular vein was cannulated for administration of phenylephrine. Phenylbiguanide was injected into the right atrium via a cannula inserted into the right jugular vein. Rectal temperature was monitored and injected into the right atrium via a cannula inserted into the right jugular vein. Rectal temperature was maintained at 37.5 ± 0.5°C. Animals were placed in a stereotaxic head holder (Kopf Instruments) and ventilated artificially using humidified oxygen-enriched air. End-tidal CO2 was sampled and monitored and maintained at 4.5 ± 5% by altering tidal volume. The dorsal medulla was exposed by retraction of the nuchal muscles and removal of the dura. When necessary, the posterior cerebellar vermis was retracted to expose the dorsal surface of the medulla.

Recording Procedures

The electrocardiogram (ECG) was recorded via stainless steel pins placed subcutaneously in a fore- and hindlimb, and the right phrenic nerve activity (PNA) was recorded using a bipolar hook electrode. Both signals were amplified (Neurolog module 104) and filtered (Neurolog module 125). Recordings of arterial pressure, HR, and rectified and integrated PNA were relayed to a CED 1401 interface (Cambridge Electronics Design), displayed on a computer monitor, and stored digitally (CED; Spike2).

Microinjection Techniques

Seven-barreled glass microelectrodes were used with the tips cut back to 35–40 µm to allow pressure injection. Microinjection volumes were 45 nl, calculated by observing the distance the meniscus moved down the glass capillary using a binocular microscope fitted with an eye piece graticule. Microelectrodes were placed (under visual guidance of a binocular microscope) into two anatomically distinct regions of the NTS using reference landmarks on the medullary surface, such as area postrema and calamus scriptorius. From preliminary mapping studies our histological data revealed that microinjection sites that were selective in altering the magnitude of the baroreceptor reflex were located ~200 µm caudal to obex (the point at which the central canal opens into the fourth ventricle), 600 µm lateral to midline, and 500 µm ventral to the dorsal surface. In contrast, sites that were selective in altering the efficacy of the cardiopulmonary reflex were located ~900 µm caudal to obex, 100 µm lateral to the midline, and 400 µm ventral to the dorsal surface. At the end of the experiment, Pontamine sky blue (2%) was microinjected and the brain stem was excised from the skull and fixed with a solution containing 2% paraformaldehyde and 20% sucrose. Transverse sections were cut (100 µm) on a freezing microtome, mounted on subbed slides, stained with neutral red, and placed under a coverslip. The microinjection sites were reconstructed with reference to obex and the solitary tract and documented on standard outlines (taken from Ref. 2).

Experimental Protocol

The baroreceptor reflex was evoked by an intravenous injection of the α2-adrenergic receptor agonist phenylephrine (PE; 40 µg/ml; 1–3 µg bolus), a 5-hydroxytryptamine 3 agonist (5-HT3), used to stimulate J receptors located within the lungs (19). Both drugs were prewarmed to 38°C. At the end of some experiments, the position of the tip of the cannula was checked to be within, or very close to, the right atrium. Because all microinjections were unilateral, in some experiments the pulmonary vagal C fiber afferent input was restricted to one side of the NTS by cutting the vagus nerve contralateral to the microinjection site; however, even with both vagi intact it was possible to modulate the efficacy of the cardiopulmonary reflex with unilateral microinjections. After at least three successive and consistent control reflex responses, a potassium channel antagonist was microinjected into the NTS. Two minutes later this was followed by an intravenous injection of either PBG or PE (depending on the reflex studied); these injections continued every 10 min until recovery was obtained. To test microinjection site specificity in some experiments both reflexes were elicited in quick succession after microinjection into the NTS. Control microinjections of artificial cerebrospinal fluid were made using similar volumes (45 nl).

Analysis

The peak magnitude of the reflex falls in HR and mean arterial pressure (MAP), as well as the changes in inspiratory duration and frequency of PNA, was compared before and after a microinjection of a potassium channel antagonist, and the differences were statistically tested (paired Student’s t-test). To ascertain that measurements of the baroreceptor reflex bradycardia were on the linear part of the sensitivity curve, multiple measurements of MAP and the corresponding HR fall, were made during the rising phase of PE-induced pressor responses. Moreover, the gain of the reflex (expressed as fall in HR (beats/min) per mmHg rise in MAP) together with the rate of rise of the pressor response (mmHg/s) was calculated for each PE injection. Data are presented as means ± SE.

Drugs

The microelectrodes were filled with Pontamine sky blue (2%) and artificial cerebrospinal fluid (aCSF; constituents as in Ref. 21) along with a selection of the following chemicals dissolved in aCSF: 4-aminopyridine (4-AP; 2 mM; Sigma), dextrotoxin (DTX; 10 µM; Alamone), apamin (100 µM; Alamone), and charybdotoxin (ChTX; 5 µM; Alamone). PBG (Sigma) and PE (Sigma) were dissolved in 0.9% NaCl at concentrations of 100 and 40 µg/ml, respectively.

RESULTS

In 54 animals, the resting MAP was 96 ± 2 mmHg, and the resting HR was 465 ± 6 beats/min. Analysis of intravenously integrated PNA revealed a respiratory cycle length of 1.26 ± 0.08 s and a burst duration of 0.47 ± 0.03 s (n = 30). Microinjection of both IK and IKCa antagonists resulted in transient falls in MAP from baseline: apamin 13 ± 5 mmHg (n = 11; P < 0.01), ChTX 25 ± 5 mmHg (n = 13; P < 0.001), 4-AP 12 ± 2 mmHg (n = 20; P < 0.001), DTX 9 ± 3 mmHg (n = 19; P < 0.01; Fig. 1A). Only the IKCa antagonists produced a significant (Fig. 1B) reduction in HR: apamin 13 ± 5 beats/min (P < 0.05), ChTX 18 ± 6 beats/min (P < 0.01). None of the antagonists had a consistent effect on either respiratory cycle length or phrenic burst duration. There were no significant differences in these cardiovascular re-
responses between the rostral and caudal microinjection sites (see METHODS). During reflex testing after a microinjection the basal levels of MAP, HR, and PNA were not significantly different from control values (Tables 1 and 2). The effects of the antagonist on the evoked reflex usually occurred within 2 min after the microinjection and usually recovered within 80 min (Tables 1 and 2). Vehicle injection of aCSF (45 nl) had no effect, either on baseline cardiorespiratory variables or on reflex responsiveness.

Baroreceptor Reflex

The baroreceptor reflex was elicited via an intravenous injection of PE, which increased arterial pressure and induced a reflex bradycardia. As seen in Fig. 2B, measurements were taken on the linear portion of the baroreceptor reflex response curve. A 1- to 3-µg bolus of PE raised MAP by 73 ± 3 mmHg, which elicited a reflex bradycardia of 92 ± 6 beats/min (n = 25). Microinjection of potassium channel antagonists had no effect on the slight respiratory slowing observed during the induced pressor responses under control conditions or on the amplitude or burst duration of the PNA. In all experiments microinjections into the NTS had no significant effect on either the magnitude or the rate of rise of the PE-induced pressor response (Table 1). All effective microinjection sites were restricted to regions 100–200 µm caudal to obex as described in METHODS (see above and Fig. 7).

Effect of blockade of I\textsubscript{KCa}. Microinjection of either apamin or ChTX into the NTS potentiated the baroreceptor reflex (Table 1; Fig. 2); apamin increased the reflex bradycardia from 12 ± 3 to 25 ± 5% (n = 6; P < 0.05) and ChTX from 12 ± 2 to 24 ± 5% (Table 1; n = 6; P < 0.05).

Effect of blockade of I\textsubscript{K}. In contrast to apamin and ChTX, 4-AP attenuated the baroreceptor reflex bradycardia, reducing it from 23 ± 3 to 13 ± 2% (n = 8; P < 0.01; Table 1; Fig. 3). Similar effects were observed with DTX: the evoked bradycardia was reduced from 32 ± 5 to 17 ± 6% (Table 1; n = 6; P < 0.01).

Site specificity of antagonists into NTS. In 14 of these experiments, as soon as the PE-induced increase in arterial pressure had returned to control (i.e., ~5 min after injection), a second bolus of PE was given into the NTS. In all experiments, the second PE bolus again raised MAP by 70 ± 7 mmHg and elicited a reflex bradycardia of 90 ± 3 beats/min (n = 14). The antagonists had no effect on the baseline cardiorespiratory variables or on reflex responsiveness.
after a microinjection) the cardiopulmonary reflex was evoked with a right atrial injection of PBG. In 12 of 14 animals, the reflex was of similar magnitude to controls (i.e., that seen before microinjections into NTS). For example, microinjection of apamin into a site that was 0.1 mm caudal to obex (see Fig. 7) potentiated the baroreceptor reflex bradycardia from 5 to 30% but left the PBG-evoked bradycardia of 82% unaffected.

Cardiopulmonary Reflex

Right atrial injection of PBG elicited a 227 ± 14 beats/min decrease in HR and a 32 ± 2 mmHg decrease in MAP (n = 43) together with an increase in phrenic cycle length to 2.38 ± 0.38 s (n = 17). The doses used resulted in reproducible, submaximal effects with a latency of 1 to 1.5 s. This is within the pulmonary circulation time of the rat, indicating a relatively specific action of PBG on pulmonary vagal C fibers. All effective microinjections were restricted to more caudal regions of the NTS (i.e., 900–1,000 µm caudal to the obex; see METHODS and Fig. 7).

Effect of blockade of I_{KCa}. Blockade of apamin- and ChTX-sensitive I_{KCa} in NTS neurons attenuated the cardiopulmonary reflex (Table 2; Figs. 4 and 6). After a microinjection of apamin into the NTS the reflex falls in HR and MAP were reduced from 63 ± 11 to 21 ± 3% (P < 0.01) and from 35 ± 3 to 23 ± 3% (n = 5; P < 0.05), respectively. Moreover, the duration of the reflex prolongation in phrenic nerve cycle length was reduced by 46 ± 7% (n = 4; P < 0.05). In seven other animals, a microinjection of ChTX reduced the bradycardia from 54 ± 9 to 15 ± 2%, the depressor response from 44 ± 4 to 29 ± 2%, and the reflex increase in phrenic nerve cycle length by 34 ± 17% (n = 7; P < 0.05).

Effect of blockade of voltage-dependent K channels. Both 4-AP and DTX microinjected into the NTS produced qualitatively similar attenuating effects to the I_{KCa} antagonists (see above; Table 2; Figs. 5 and 6). In 12 animals, a microinjection of 4-AP attenuated the bradycardia from 51 ± 6 to 20 ± 4% (P < 0.01) and the depressor response from 33 ± 3 to 18 ± 5% (P < 0.01) and reduced the duration of the reflex prolongation in phrenic nerve cycle length by 27 ± 9% (n = 6; P < 0.05; Fig. 5). Similarly DTX attenuated the bradycardia from 42 ± 5 to 19 ± 3% and the depressor response from 30 ± 3 to 17 ± 3% (n = 13; P < 0.01; Fig. 6A; PNA not recorded).

Site specificity of antagonists in NTS. In 10 of these experiments, as soon as the PBG-evoked falls in HR and arterial pressure had returned to control (i.e., ~4 min after the microinjection), the baroreceptor reflex was elicited. In eight animals, the baroreceptor reflex gain was not affected by microinjections into these caudal regions (i.e., 900–1,000 µm caudal to obex; see Fig. 7); for example, 2 min after ChTX was microinjected into a caudal NTS site, the PBG-evoked bradycardia of 82% was unaffected.
response from 40 to 28%, whereas the baroreceptor reflex bradycardia was not substantially altered (7% before and 4% after ChTX microinjection). The effective microinjection sites for both the baroreceptor and cardiopulmonary reflexes are shown in Fig. 7.

DISCUSSION

As far as we know this is the first study to investigate the physiological significance of \( I_K \) and \( I_{KCa} \) in the NTS for cardiorespiratory reflex function. Blockade of \( I_{KCa} \) potentiated the baroreceptor reflex but attenuated the cardiopulmonary reflex, whereas antagonizing \( I_K \) produced a qualitatively similar depression of both reflexes.

Specificity of the Microinjection Technique

The microinjection technique is a useful first approach for understanding the physiological significance of channels or receptors and is dependent on sufficient diffusion of injectate to produce a systems level response. As suggested previously (13, 18), this technique does, however, have numerous disadvantages, particularly concerning the degree of specificity. With the use of equations derived by Nicholson (18) it can be estimated that a microinjection of 50 nl will spread \( \approx 550 \mu \text{m} \); however, the “effective” spread may well be less because the drug concentration will decrease from its source, and, furthermore, in the intact animal, it will be removed by the circulation.

Specificity of Microinjection Sites in NTS

Our results indicate that the effective spread of drugs was within previously determined estimates (18), because we could demonstrate site specificity of drugs on either the baroreceptor reflex or the cardiopulmonary reflex after microinjections into anatomically distinct regions of the NTS (i.e., 100–200 \( \mu \text{m} \) and 900–1,000 \( \mu \text{m} \) caudal to the obex; see Fig. 7 and RESULTS). Although baroreceptor and pulmonary C fiber afferent innervation of NTS partially overlap, anatomic evidence suggests that there is a difference in the density of labeling of these afferents within this nucleus. Studies in the rat (12) and rabbit (33) suggest that aortic nerve afferents project, in part, to the commissural NTS, a region that has been described as playing an integral role in the cardiopulmonary reflex (3); however, the density of this baroreceptor innervation, particularly in the very medial regions that we microinjected into, which contain high densities of pulmonary vagal afferents (see below; Ref. 3), is not as great as that seen at the level of the obex. Because the microinjection technique relies on recruiting sufficient neurons to effect a change at the...
systems level, this could account for the large degree of specificity for the cardiopulmonary reflex at the commissural level. Similarly, it has been demonstrated with the use of microinjections of cobalt chloride into NTS that the primary site of pulmonary C fiber afferent termination lies between 0.7 and 1.1 mm caudal to obex (3), a region that was effective in modulating the cardiopulmonary reflex but not the baroreceptor reflex, and which lies at least 0.5 mm caudal to the sites where we could modulate the baroreceptor reflex consistently. Moreover, recent electrophysiological studies in the mouse (20) and cat (27) suggest that NTS neurons, which are synaptically driven by pulmonary C fiber stimulation, receive little convergent input from baroreceptors.

Transient Effects of Potassium Channel Blockers on MAP and HR

All four potassium channel antagonists evoked transient falls in baseline MAP and HR (see RESULTS and Fig. 1), indicating that both $I_K$ and $I_{KCa}$ are “tonically” active in NTS in the anesthetized rat. These data in combination with our results showing changes in reflex performance suggest roles in both static as well as dynamic circulatory control.

Effects of $I_{KCa}$ Antagonists on the Baroreceptor Reflex

A microinjection of either apamin or ChTX into regions of the NTS slightly caudal to obex resulted in an increase in the magnitude of the baroreceptor-mediated reflex bradycardia. This might be explained by an increase in the excitability of primary afferent terminals or of NTS neurons after potassium channel blockade and is consistent with the effect of blocking $I_{KCa}$ in NTS neurons recorded in vitro: both a reduction in the after-hyperpolarization and an increase in firing response to injected current was observed (16, 21). The absence of an effect of apamin on dissociated NTS neurons reported previously (16) might reflect 1) that there is heterogeneity of NTS neurons mediating the baroreceptor reflex or 2) that the neurons studied did not belong to the population of cells mediating the baroreceptor reflex or 3) that these channels were lost during the enzymatic isolation procedure.

It is perhaps surprising that antagonism of SK channels with apamin and BK channels with charybdotoxin produced a similar potentiation of the baroreceptor reflex and an almost identical attenuation of the cardiopulmonary reflex. There is no a priori reason to expect that the distribution of these two channel types would be similar on a given neuron or indeed that their blockade would produce a quantitatively similar systems effect. The limitations of the present experiments do not permit further interpretation; a detailed cellular analysis investigating the relative distribution and density of the two $I_{KCa}$ conductance types on characterized NTS neurons is required.

Anomalous Effects of Blocking $K^+$ Channels on the Baroreceptor and Cardiopulmonary Reflexes

We found that ChTX and apamin, in contrast to their effects on the baroreceptor reflex, attenuated the cardiopulmonary reflex. Furthermore, we demonstrated that blockade of $I_K$ also reduced both baro- and cardiopulmonary reflexes. One possible interpretation of these data is that $I_{KCa}$ and $I_K$ are predominantly located on inhibitory [e.g., $\gamma$-aminobutyric acid (GABA)] interneurons impinging on NTS cells mediating cardiorespiratory reflexes. In this regard, only 40% of dorsal NTS neurons at the level of, and caudal to, the area postrema recorded in vitro contained 4-AP-sensitive currents and 64% apamin-sensitive currents (21). Thus, after blockade of potassium channels in GABA interneurons, excitability would be expected to increase (e.g., Refs. 7, 24), which would augment levels of tonic inhibitory drive thereby reducing reflex efficacy. This is
supported by a previously published observation of ours (4): antagonism of GABA\textsubscript{A} receptors within the NTS using bicuculline led to an increase in the magnitude of the cardiopulmonary reflex bradycardia; moreover, a concomitant microinjection of DTX and bicuculline prevented the attenuating effect of DTX on the PBG-evoked bradycardia. It should be emphasized, however, that these data do not reveal whether this is the result of the summation of two dependent or mutually exclusive events. Interestingly, preliminary in vitro data from our laboratory indicate that 4-AP can both decrease the ongoing firing rate and the firing response to injected current of NTS neurons (Butcher and Paton, unpublished observations); the neuronal mechanisms responsible for these observations are unknown at present but may underlie the reduction in reflex performance seen in vivo.

The attenuating effects of potassium channel blockers on cardiorespiratory reflex control reported here are analogous to recent studies where activation of 5-HT\textsubscript{3} receptors within the NTS reduced the gain of baroreceptor, chemoreceptor, and cardiopulmonary reflexes (15, 25, 26). Because 5-HT\textsubscript{3} receptors are known to mediate excitatory actions (23), it was suggested that the effects of 5-HT\textsubscript{3} receptors were mediated via a local GABAergic system within the NTS (26). This was further supported by the finding that bicuculline prevented the inhibitory effect of 5-HT on the baroreceptor reflex (15). In this regard, >90% of 5-HT\textsubscript{3} receptors expressing cells in the neocortex and hippocampus are GABAergic (17). By analogy, our data would predict that GABA-containing interneurons within the NTS contain high densities of I\textsubscript{K} channels and those that control the cardiopulmonary reflex also have high numbers of I\textsubscript{K,ca} conductances.

The involvement of potassium channels on presynaptic membrane terminals must also be considered. It is possible that our microinjections of potassium channel antagonists caused a depolarization block of the peripheral afferent endings. Alternatively, potassium channels may exist on the terminals of local GABA-containing neurons (see above). There is substantial evidence that the \textit{Aplysia} gabaergic system contains both inward rectifier and A-type potassium channels (16). Furthermore, I\textsubscript{K} channels allow for the generation of two different types of excitatory postsynaptic potentials (EPSPs) at the gabaergic terminals in \textit{Aplysia}: a small and a large EPSP (16). It is possible that the smaller EPSP activates the small potassium channels, whereas the larger EPSP activates the large one. If this is the case, then the larger potassium conductance could be responsible for the inhibition of the gabaergic response. This hypothesis is supported by the finding that bicuculline prevented the attenuating effect of DTX on the PBG-evoked bradycardia.

Values are means ± SE. For control, test, and recovery for all potassium channel blockers microinjected into NTS, the magnitude of the phenylbiguanide (PBG)-evoked falls in MAP and HR both in absolute terms and as a percentage, together with the average time to recovery, is shown.

### Table 1. Grouped data for baroreceptor reflex experiments

<table>
<thead>
<tr>
<th></th>
<th>MAP at Rest, mmHg</th>
<th>PE-induced Rise in MAP, mmHg</th>
<th>dP/dt, mmHg/s</th>
<th>HR at Rest, beats/min</th>
<th>Baroreflex Fall in HR, beats/min</th>
<th>Gain, 1·mmHg</th>
<th>Avg. Time to Recovery, min</th>
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<tbody>
<tr>
<td>Control</td>
<td>87 ± 5</td>
<td>74 ± 4</td>
<td>21 ± 2</td>
<td>462 ± 7</td>
<td>107 ± 14</td>
<td>-1.44 ± 0.14</td>
<td>66 ± 10</td>
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<tr>
<td>Post 4-AP</td>
<td>85 ± 5</td>
<td>74 ± 5</td>
<td>21 ± 1</td>
<td>464 ± 8</td>
<td>64 ± 10</td>
<td>-0.79 ± 0.1</td>
<td>25 ± 7</td>
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<tr>
<td>Recovery</td>
<td>88 ± 3</td>
<td>73 ± 5</td>
<td>20 ± 2</td>
<td>459 ± 10</td>
<td>87 ± 10</td>
<td>-0.41 ± 0.1</td>
<td>71 ± 13</td>
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<tr>
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<td>82 ± 5</td>
<td>19 ± 2</td>
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<td>142 ± 22</td>
<td>-1.76 ± 0.3</td>
<td>61 ± 13</td>
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<td>Post DTX</td>
<td>92 ± 9</td>
<td>86 ± 7</td>
<td>20 ± 1</td>
<td>464 ± 20</td>
<td>74 ± 25</td>
<td>-0.87 ± 0.33</td>
<td>61 ± 13</td>
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<tr>
<td>Recovery</td>
<td>91 ± 8</td>
<td>84 ± 5</td>
<td>18 ± 1</td>
<td>456 ± 13</td>
<td>132 ± 25</td>
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<td>91 ± 2</td>
<td>70 ± 6</td>
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<td>Post Apamin</td>
<td>91 ± 3</td>
<td>73 ± 4</td>
<td>19 ± 1</td>
<td>445 ± 10</td>
<td>108 ± 20</td>
<td>-1.58 ± 0.41</td>
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<tr>
<td>Recovery</td>
<td>87 ± 3</td>
<td>76 ± 5</td>
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<td>455 ± 6</td>
<td>65 ± 21</td>
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<td>64 ± 5</td>
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<td>Post ChTX</td>
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<td>92 ± 3</td>
<td>71 ± 4</td>
<td>19 ± 1</td>
<td>454 ± 7</td>
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<td>-0.84 ± 0.11</td>
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### Table 2. Grouped data for the cardiopulmonary reflex experiments

<table>
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<tr>
<th></th>
<th>MAP at Rest, mmHg</th>
<th>PBG-Evoked Fall in MAP, mmHg</th>
<th>%</th>
<th>HR at Rest, beats/min</th>
<th>PBG-Evoked Fall in HR, beats/min</th>
<th>Time to Recovery, min</th>
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<tbody>
<tr>
<td>Control</td>
<td>94 ± 3</td>
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<td>33 ± 3</td>
<td>489 ± 10</td>
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<tr>
<td>Post 4-AP</td>
<td>87 ± 4</td>
<td>16 ± 4</td>
<td>18 ± 5</td>
<td>483 ± 10</td>
<td>96 ± 18</td>
<td>20 ± 4</td>
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<tr>
<td>Recovery</td>
<td>87 ± 3</td>
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<td>29 ± 3</td>
<td>495 ± 10</td>
<td>206 ± 37</td>
<td>42 ± 8</td>
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<tr>
<td>Control</td>
<td>99 ± 4</td>
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<td>30 ± 3</td>
<td>455 ± 11</td>
<td>187 ± 19</td>
<td>42 ± 5</td>
</tr>
<tr>
<td>Post DTX</td>
<td>95 ± 4</td>
<td>17 ± 3</td>
<td>17 ± 3</td>
<td>459 ± 13</td>
<td>86 ± 11</td>
<td>19 ± 3</td>
</tr>
<tr>
<td>Recovery</td>
<td>98 ± 4</td>
<td>23 ± 3</td>
<td>23 ± 2</td>
<td>460 ± 12</td>
<td>188 ± 20</td>
<td>41 ± 5</td>
</tr>
<tr>
<td>Control</td>
<td>96 ± 7</td>
<td>33 ± 3</td>
<td>35 ± 3</td>
<td>451 ± 14</td>
<td>283 ± 47</td>
<td>63 ± 11</td>
</tr>
<tr>
<td>Post Apamin</td>
<td>90 ± 9</td>
<td>21 ± 3</td>
<td>23 ± 3</td>
<td>458 ± 12</td>
<td>98 ± 15</td>
<td>21 ± 3</td>
</tr>
<tr>
<td>Recovery</td>
<td>91 ± 6</td>
<td>23 ± 8</td>
<td>24 ± 9</td>
<td>469 ± 9</td>
<td>291 ± 34</td>
<td>62 ± 7</td>
</tr>
<tr>
<td>Control</td>
<td>97 ± 4</td>
<td>42 ± 3</td>
<td>44 ± 4</td>
<td>444 ± 12</td>
<td>240 ± 41</td>
<td>54 ± 9</td>
</tr>
<tr>
<td>Post ChTX</td>
<td>94 ± 5</td>
<td>27 ± 2</td>
<td>29 ± 2</td>
<td>448 ± 15</td>
<td>68 ± 7</td>
<td>15 ± 2</td>
</tr>
<tr>
<td>Recovery</td>
<td>94 ± 2</td>
<td>34 ± 2</td>
<td>37 ± 3</td>
<td>445 ± 15</td>
<td>205 ± 34</td>
<td>47 ± 8</td>
</tr>
</tbody>
</table>
evidence supporting the presence of $I_K$ in GABAergic terminals: in synaptosomal preparations both 4-AP (32) and DTX (34) have been shown to release GABA, whereas inhibitory neurotransmission was enhanced by DTX in pyramidal cells (11). To our knowledge there are no such reports detailing the existence of $I_{KCA}$ in presynaptic GABAergic terminals.

In conclusion, our data indicate that $I_K$ and $I_{KCA}$ play an important role in determining the efficacy of both the baroreceptor reflex and the cardiopulmonary reflex in vivo. The exact mechanism, whether pre- and/or postsynaptic, by which blockade of potassium channels in the NTS leads to a reduction in cardiorespiratory reflex efficacy requires further investigation at the cellular level. Nevertheless, on the basis of our data obtained with bicuculline (4) together with previous reports of enhanced GABA-mediated neurotransmission after potassium channel blockade, the notion that there is a predominance of potassium channels on GABAergic interneurons within the NTS is supported.

Perspectives

With the exception of studies on invertebrates such as aplysia (e.g., 31), the functional implications of potassium channel activity remain largely uninvestigated. Clearly though, these currents play a pivotal role in determining the excitability of neurons and, as such, are prime targets for regulation by neurotransmitters and second messengers. Indeed, such neuromodulation has been implicated in mechanisms of plasticity, such as learning and memory, and perhaps, by analogy, may prove important in the NTS for determining, for example, baroreceptor reflex set point. Potassium conductances may also account for more hard-wired mechanisms such as the differing potencies of the baroreceptor versus cardiopulmonary reflexes. Neurons receiving afferent input on a beat-by-beat basis from baroreceptors may have a higher density of $I_{KCA}$ conductances, which are needed to dampen their activity. When these channels are blocked the neurons would fire action potentials at a higher frequency, accounting for the potentiation of reflex efficacy that we observed. In contrast, pulmonary C fibers discharge at very low frequencies at rest (0.3 Hz; Ref. 1), which may negate the need for a central adapting mechanism. Whether or not the firing behavior of NTS neurons dictates the type and density of potassium channel that is expressed is open for experimental investigation.

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