Mechanism of action of baclofen in rat dorsal motor nucleus of the vagus

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Browning, K. N., and R. A. Travagli. Mechanism of action of baclofen in rat dorsal motor nucleus of the vagus. Am J Physiol Gastrointest Liver Physiol 280: G1106–G1113, 2001.—Using whole cell patch-clamp recordings, we investigated the effects of the GABA_B receptor agonist baclofen in thin slices of rat brain stem containing identified gastric- or intestinal-projecting dorsal motor nucleus of the vagus (DMV) neurons. Perfusion with baclofen (0.1–100 μM) induced a concentration-dependent outward current (EC_{50}, 3 μM) in 54% of DMV neurons with no apparent differences between gastric- and intestinal-projecting neurons. The outward current was attenuated by pretreatment with the selective GABA_B antagonists saclofen and 2-hydroxysaclofen, but not by the synaptic blocker TTX, indicating a direct effect at GABA_B receptors on DMV neurons. Using the selective ion channel blockers barium, nifedipine, and apamin, we showed that the outward current was due to effects on potassium and calcium currents as well as calcium-dependent potassium currents. The calcium-mediated components of the outward current were more prominent in intestinal-projecting neurons than in gastric-projecting neurons. These data indicate that although baclofen inhibits both intestinal- and gastric-projecting neurons in the rat DMV, its mechanism of action differs among the neuronal subpopulations.

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The aims of this study were to investigate 1) whether GABAB receptors are functionally present on the DMV membrane, and if so, 2) what the mechanism of action of the GABAB agonist baclofen is on the DMV membrane, and 3) whether there are differences in the responses of DMV neurons identified as per their peripheral projections.

METHODS

Retrograde tracing. The retrograde tracer DiI was applied to discrete gastrointestinal regions in 12-day-old Sprague-Dawley rat pups of either sex, as described previously (8). Briefly, rats were anesthetized deeply with a 6% solution of halothane in accordance with the guidelines of the Animal Care and Use Committee of the University of Michigan Medical Center (Ann Arbor, MI). The depth of anesthesia (abolition of the foot pinch withdrawal reflex) was monitored before and during surgery. The abdominal area was shaved and cleaned with alcohol before a laparotomy was performed. Crystals of DiI were applied to either the major curvature of the gastric fundus or corpus, the antrum/pylorus, the duodenum (the antimesenteric surface at the level of the hepatic and pancreaticoduodenal arteries), or the minor curvature of the cecum (at the level of the ileocecal junction). The application site was embedded in a fast-hardening epoxy resin before the entire surgical area was washed with warm, sterile saline. The wound was closed with 4-0 sutures, and the animal was allowed to recover for 10–15 days.

Electrophysiology. The method used for tissue slice preparation was as described previously (32). Briefly, rats were anesthetized deeply (halothane bubbled with air) before being killed through severing of the major blood vessels in the chest. The brain stem was removed and placed in chilled (4°C) oxygenated Krebs solution (see below for composition). Using a vibratome, we cut six to eight coronal slices containing the DMV. Slices were stored in oxygenated Krebs solution at 32°C for at least 1 h before use. A slice was then placed on a custom-made perfusion chamber (vol 500 μl) and maintained at 35°C by continual perfusion with warmed oxygenated Krebs solution at a rate of 2.5 ml/min.

Retrogradely labeled neurons were identified before electrophysiological recording using a Nikon E600FS microscope fitted with DIC (Nomarski) optics and tetramethylrhodamine isothiocyanate epifluorescent filters. The brief periods of illumination required to detect the fluorescent neurons have not been observed to cause any damage (16, 21). Once a labeled neuron was identified, electrophysiological recording using a Nikon E600FS microscope was conducted per each brain stem slice and only one experiment of each type was conducted per animal.

Chemicals and solutions. Krebs solution was composed of (in mM): 126 NaCl, 25 NaHCO3, 2.5 KCl, 1.2 MgCl2, 2.4 CaCl2, 1.2 NaH2PO4, and 11 dextrose, maintained at pH 7.4 by bubbling with 95%O2-5% CO2. Intracellular solution consisted of (in mM): 128 potassium gluconate, 10 KCl, 0.3 CaCl2, 1 MgCl2, 1 HEPES, 1 EGTA, 2 ATP, and 0.25 GTP, adjusted to pH 7.35 with KOH. 1,1-‘Dioctadecyl-3,3,3’,3’-tetramethylindocarbocyanine perchlorate [DiIC1(3); DiI] was purchased from Molecular Probes (Eugene, OR). Baclofen and 2-hydroxysaclofen were purchased from RBI (Natick, MA). Halothane and all other drugs and chemicals were purchased from Sigma Chemical (St. Louis, MO).

RESULTS

Baclofen induces outward current in subpopulation of DMV neurons. The effects of the GABAB receptor agonist baclofen were assessed in 394 gastrointestinal-projecting neurons of the DMV (211 gastric projecting and 187 intestinal projecting). Baclofen induced a concentration-dependent (0.1–100 μM) outward current in 211 (i.e., 54%) of these neurons. No differences were observed in the magnitude of the baclofen-induced current among the gastric and intestinal groups; data were thus pooled and provided an estimated EC50 of 3 μM (Fig. 1). The percentage of responsive neurons did not differ between the gastric- and intestinal-projecting neurons; in fact, 112 of 211 gastric-projecting neurons (i.e., 53%) and 99 of 187 intestinal-projecting neurons (i.e., 54%) responded to perfusion with 10 μM baclofen. Similarly, the distribution of the magnitude of the response to 10 μM baclofen did not differ between gastric- and intestinal-projecting neurons (Fig. 1).

In six neurons (3 gastric and 3 intestinal), the effects of baclofen (10 μM) were assessed before and after exposure to GABAB receptor antagonists. Baclofen induced a 51 ± 4 pA outward current in control conditions. After perfusion with the selective antagonists saclofen (300 μM, n = 2) or 2-hydroxysaclofen (100 μM, n = 4), the baclofen-induced current was reduced to 10 ± 3 pA, i.e., 17% of control (P < 0.05). However, incubation with the synaptic blocker TTX (1 μM) had no effect on the baclofen-induced outward current. In fact, baclofen induced a current of 45 ± 8 and 46 ± 8 pA in control conditions and in the presence of TTX, respectively (n = 6, 3 gastric and 3 intestinal; P > 0.05).
Baclofen effect involves several ionic conductances. The effects of the nonselective potassium channel blocker barium (2 mM) on the outward current induced by baclofen (10 μM) were assessed in 14 neurons (7 gastric and 7 intestinal). The baclofen-induced current was reduced from 66 ± 14 pA in control conditions to 25 ± 6 pA in the presence of barium, i.e., 41 ± 3.6% of control (P < 0.05; data not shown, however, see Fig. 3). No significant differences were observed in the actions of barium between gastric- and intestinal-projecting neurons. In detail, barium reduced the baclofen-induced current from 73 ± 24 to 25 ± 7 pA in gastric-projecting neurons and from 60 ± 17 to 24 ± 8 pA in intestinal-projecting neurons, i.e., 41 ± 5% and 41 ± 6% of control in gastric- and intestinal-projecting neurons, respectively.

The effects of the L-type calcium channel blocker nifedipine (3 μM) on the baclofen (10 μM)-induced outward current were assessed in 34 neurons (19 gastric and 15 intestinal). The baclofen-induced current was reduced from 76 ± 8.9 pA under control conditions to 40 ± 4.7 pA in the presence of nifedipine, i.e., 53 ± 2.4% of control (P < 0.05; data not shown, however, see Fig. 3). Significant differences were uncovered, however, in the magnitude of this reduction between gastric- and intestinal-projecting neurons. In gastric-projecting neurons, nifedipine reduced the baclofen-induced current from 85 ± 14 to 47 ± 7 pA, i.e., 58 ± 3% of control, whereas in intestinal-projecting neurons, nifedipine reduced the baclofen-induced current from 66 ± 9 to 32 ± 6 pA, i.e., 47 ± 3% of control (P < 0.05).

The involvement of both potassium and calcium conductances in mediating the actions of baclofen was confirmed by assessing the reversal potential for the baclofen-induced outward current in the absence and presence of both channel blockers. The baclofen-induced current reversed at approximately −95 mV (Fig. 2). In the presence of barium, however, the reversal of the baclofen-induced current shifted to the more posi-
between gastric- and intestinal-projecting neurons were observed in the total inhibition of the baclofen current by the combined antagonists.

Given that perfusion with the nonselective potassium channel blocker barium in combination with the selective calcium-L type channel antagonist nifedipine did not block the baclofen-induced outward current completely, in five neurons (3 gastric and 2 intestinal), we perfused the slice first with nifedipine alone and then in combination with the nonselective calcium channel blocker cadmium (200 μM). Perfusion with nifedipine reduced the baclofen-induced current from 76 ± 4 to 43 ± 2 pA, i.e., a 44 ± 3.9% reduction of the baclofen-induced current; addition of cadmium to the nifedipine perfusate caused the baclofen-induced current to be reduced to 34 ± 4 pA, i.e., an additional 11 ± 4.5% (nifedipine vs. nifedipine + cadmium, P < 0.05). These data then suggested that calcium currents other than the L-type may be involved in the baclofen-induced outward current.

Because there appeared to be some overlap between the inhibitory actions of the potassium channel blocker barium and the calcium channel blocker nifedipine (i.e., 59 ± 3.5% reduction by barium, 47 ± 2.4% reduction by nifedipine but an 81% to 88% reduction when combined), we investigated the involvement of a calcium-dependent potassium conductance using the small-conductance potassium (SK) channel blocker apamin. The outward current induced by baclofen (10 μM) was assessed before and after apamin (100 nM) in 19 neurons (12 gastric, 7 intestinal). In the presence of apamin, the baclofen-induced current was reduced from 107 ± 15 to 63 ± 9 pA, i.e., 62 ± 3.5% of control (P < 0.05; Fig. 4A). Differences were again apparent between gastric- and intestinal-projecting neurons. In detail, in gastric-projecting neurons, apamin reduced the baclofen-induced current from 95 ± 18 to 62 ± 12 pA, i.e., 68 ± 4% of control, whereas in intestinal-projecting neurons, apamin reduced the baclofen-in-

Fig. 2. Current-voltage relationship for the baclofen-induced outward current. Dorsal motor nucleus of the vagus (DMV) neurons were voltage clamped at −50 mV and stepped to −120 mV for 800 ms every 5 s in −10-mV increments. Each data point represents an average of 6–24 neurons. In control medium, the baclofen equilibrium potential (E_b) was −95 mV (A). After perfusion with the potassium channel blocker barium (2 mM), the E_b shifted to less negative potentials (approximately −55 mV) (B). Conversely, pretreatment with the L-type calcium channel antagonist nifedipine (3 μM) shifted E_b closer to potassium equilibrium potential.

Fig. 3. Baclofen-induced outward current is antagonized by a combination of nifedipine and barium. Representative trace showing that in the voltage-clamp configuration (holding potential (HP) = −50 mV), perfusion with baclofen (10 μM) induced an outward current in a DMV neuron. After pretreatment with a solution containing the selective calcium channel L-type antagonist nifedipine (3 μM) decreased the baclofen-induced current to −50% of control, addition of the nonselective potassium channel blocker barium (Ba; 2 mM) to the nifedipine-containing solution further reduced the baclofen-induced outward current.
baclofen-induced current was reduced from 88 ± 6 pA, i.e., 29 ± 5% of control (P < 0.05).

Because extracellular barium is not an effective blocker of calcium-dependent potassium currents (15), we tested its effects on the baclofen-induced current in combination with apamin. In 11 cells (6 gastric and 5 intestinal), perfusion with apamin decreased the baclofen-induced current from 88 ± 13 to 48 ± 5 pA, i.e., 60 ± 5.5% of control; the addition of barium to the apamin perfusate caused the baclofen-induced current to be reduced to 24 ± 3 pA, i.e., 30 ± 2.5% of the initial control value (apamin vs. apamin + barium, P < 0.05). Likewise, in 10 cells (5 gastric and 5 intestinal), perfusion with apamin decreased the baclofen-induced current from 136 ± 21 to 79 ± 15 pA, i.e., 58 ± 2.8% of control; addition of nifedipine to the apamin perfusate caused the baclofen-induced current to be reduced to 57 ± 12 pA, i.e., 40 ± 2.4% of the initial control value (apamin vs. apamin + nifedipine, P < 0.05; Fig. 4A).

No statistically significant differences between gastric- and intestinal-projecting neurons were observed on the total inhibition of the baclofen current by the combined antagonists. Interestingly, though, when apamin was perfused in combination with, and after exposure to, nifedipine, a further 10 ± 3.1% inhibition of the baclofen-induced current was observed in intestinal-projecting neurons. In detail, in intestinal-projecting neurons, the baclofen-induced current was reduced from 50 ± 5 pA under control conditions to 30 ± 6 pA in the presence of nifedipine and 25 ± 5 pA in the presence of nifedipine plus apamin (n = 4; P < 0.05). In gastric-projecting neurons, the baclofen-induced current was reduced from 61 ± 8 pA under control conditions to 34 ± 2 pA in the presence of nifedipine and 34 ± 3 pA in the presence of nifedipine plus apamin (n = 5; P > 0.05; Fig. 4B). These data suggested that, unlike gastric-projecting neurons, the apamin-sensitive current in intestinal-projecting neurons is activated by a combination of calcium currents rather than by the L-type calcium current only. Data are summarized in Fig. 5.

To confirm the involvement of a calcium-dependent potassium conductance, the actions of baclofen to alter the action potential afterhyperpolarization (previously demonstrated to involve the SK-channel; Refs. 8, 24, and 29) were assessed in 20 neurons (9 gastric, 11 intestinal). Baclofen caused a 16 ± 1.6% reduction in the action potential afterhyperpolarization (25 ± 1.0 vs. 20 ± 0.9 mV with baclofen; P < 0.05), a 42 ± 4% reduction in the afterhyperpolarization rate of decay (115 ± 10 vs. 62 ± 4.7 ms with baclofen; P < 0.05), and an 8 ± 1.6% increase in the action potential duration (2.61 ± 0.14 vs. 2.82 ± 0.15 ms with baclofen; P < 0.05).

![Fig. 4. Baclofen-induced outward current is antagonized differentially by a combination of apamin and nifedipine or nifedipine and apamin. Representative trace showing that in the voltage-clamp configuration (HP = −50 mV), perfusion with baclofen (10 μM) induced an outward current in a DMV neuron. A: after pretreatment with a solution containing the selective calcium-dependent potassium antagonist apamin (100 nM) decreased the baclofen-induced current to −60% of control, addition of the selective L-type calcium channel blocker nifedipine (3 μM) to the apamin-containing solution further reduced the baclofen-induced outward current. B: in contrast, in a gastric-projecting neuron, addition of apamin to the nifedipine-containing solution did not further reduce the baclofen-induced outward current.](http://ajpgi.physiology.org/)

![Fig. 5. Summary of the effects of baclofen in the presence of ion channel antagonists. A: after incubation with barium (2 mM), nifedipine (3 μM), or apamin (100 nM), the amplitude of the baclofen (10 μM)-induced outward current was reduced in gastric- and intestinal-projecting DMV neurons. Note that nifedipine and apamin both produced a larger inhibition of the baclofen-induced current in intestinal compared with gastric neurons. B: in gastric-projecting neurons, nifedipine (3 μM) reduced the current induced by 10 μM baclofen; addition of apamin (100 nM) had no additional effect. Conversely, in intestinal-projecting neurons, addition of apamin to the nifedipine superfusate resulted in an additional decrease in the baclofen-induced current. *P < 0.05, **P < 0.05 compared with relevant control.](http://ajpgi.physiology.org/)
(data not shown). No differences were observed in the effects of baclofen on action potentials from gastric vs. intestinal neurons.

**DISCUSSION**

The present study provides the first direct evidence that functional GABAB receptors are located on the membrane of DMV neurons. In fact, the GABAB agonist baclofen evoked a concentration-dependent outward (inhibitory) current in a subpopulation of gastrointestinal-projecting DMV neurons via direct activation of postsynaptic GABAB receptors. The baclofen-induced outward current was mediated by several ionic conductances, namely a potassium conductance, a calcium conductance, and a calcium-dependent potassium conductance.

Although the proportion of neurons responding to baclofen and the magnitude of response were similar in gastric- and intestinal-projecting neurons, the action of baclofen, however, appeared to differ. In fact, in intestinal-projecting neurons, the calcium-mediated components of the outward current appeared to exert a more prominent role than in gastric-projecting neurons. Such differential effects provide further evidence for the nonuniformity of gastrointestinal-projecting DMV neurons (8, 9).

In both gastric- and intestinal-projecting neurons, ~60% of the baclofen-induced response was mediated via activation of a barium-sensitive potassium conductance. Gastric- and intestinal-projecting neurons differed, however, in the proportion of the baclofen-induced response attributable to an effect on the inward L-type calcium conductance (42% vs. 53%, respectively; see nifedipine experiments), in the proportion of the response attributable to an effect on the apamin-sensitive calcium-dependent potassium (SK) conductance (32% vs. 48%, respectively; see apamin experiments), and in the contribution of the L-type calcium conductance to the effect on the SK conductance (100% vs. 84%, respectively; see nifedipine and apamin experiments).

Baclofen has been reported previously (14, 18, 35) to inhibit L-, N- and P/Q-type calcium channels, depending on the neuronal type investigated. For example, in rat hippocampus inhibitory neurons, baclofen inhibits L-, N- and P/Q-type channels (18), whereas in rat supraoptic nucleus neurons, baclofen inhibits only N- and P/Q-type channels (14) and in cerebellar granule cells, baclofen inhibits L-type channels (35). Although the effects of baclofen on calcium channels other than the L-type channel were not investigated in detail in the present study, it would appear that the major proportion of the baclofen-induced inhibition of calcium channels in vagal motoneurons is mediated via inhibition of L-type calcium channels. Indeed, the combination of nifedipine and barium almost completely abolished the baclofen-induced outward current. However, after pretreatment with nifedipine alone, a small proportion of the baclofen-induced current was further inhibited by perfusion of nifedipine in combination with the nonselective calcium channel blocker cadmium. These data indicate that calcium channels other than the L-type are also affected, although such channels play a minor role in the overall baclofen response.

The present study seems to indicate differences between gastric- and intestinal-projecting neurons with regard to the source of calcium necessary to activate the apamin-sensitive calcium-dependent potassium current. In fact, the baclofen current obtained in the presence of nifedipine compared with nifedipine in combination with a supramaximal concentration of apamin (24) did not differ in gastric-projecting neurons. When the same cocktail of antagonists was tested in intestinal-projecting neurons, however, a further 10% inhibition in the baclofen-induced current was observed after pretreatment with nifedipine and apamin. These data would suggest that the apamin-sensitive current in gastric-projecting neurons is fully activated by calcium entry via L-type channels, whereas in intestinal neurons sources other than the L-type calcium channels also play a role. Indeed, a different complement of voltage-dependent calcium currents has been shown to be present in rat vagal motoneurons (24, 28), although their selective localization has not been investigated.

Although elucidating the sources of calcium necessary to activate calcium-dependent potassium currents is beyond the scope of the present study, the observed differences between gastric- and intestinal-projecting neurons are a further indication of the distinct basic characteristics of DMV neurons projecting to separate areas of the gastrointestinal tract (8, 9).

Activation of central GABAB receptors results in several gastrointestinal effects, such as increase in gastric and intestinal motility (3, 10), increase in gastric acid secretion (13), increase in lower esophageal sphincter (LES) pressure (4), and decrease of gluteal-induced LES relaxation (1). A similar dichotomy, i.e., both excitatory and inhibitory central effects of baclofen, has also been seen in a recent clinical study (19). In their work on healthy volunteers, Lidums and colleagues (19) showed that baclofen decreased the rate of transient LES relaxations, but at the same time, increased basal LES pressure, probably via a vagally mediated pathway.

To the best of our knowledge, no in vivo studies in animal models have been performed in which baclofen has been microinjected directly in the DVC while gastrointestinal effects were monitored. However, convincing evidence (1, 3, 4, 10, 13) points toward the DVC as the central site of action of baclofen on gastrointestinal function.

In an in vivo study using a ferret model, Andrews and colleagues (3) administered baclofen subcutaneously and observed an increase in gastric pressure as well as an increase in the amplitude of rhythmic contractions. Both effects were abolished by vagotomy (3). In the same study, Andrews et al. (3) reported that, in the presence of cholinergic and sympathetic blockade, the actions of baclofen were restricted to an increase in gastric corpus pressure. Such actions can be explained
if one considers baclofen to have dual central effects: 1) an increase in vagal excitatory cholinergic drive to mediate the increase in rhythmic contractions and 2) a decrease in tonic vagal drive to NANC inhibitory neurons to mediate the increase in gastric pressure (3). At the cellular level, then, such an apparent contradiction can be resolved if one assumes that the increase in vagal cholinergic drive occurs as a result of the vagal disinhibition that follows blockade of GABAergic NTS neurons impinging on DMV. On the other hand, direct inhibition of DMV neurons by baclofen would result in the relief of inhibitory NANC drive to the stomach.

Indeed, electrophysiological studies have shown that baclofen acts directly on all NTS neurons to produce a membrane hyperpolarization (7), as well as indirectly to inhibit synaptic transmission from vagal afferents (7, 23). Such actions would relieve the tonic inhibition that the GABAergic NTS neurons exert over the DMV and lead to an increased vagal motor output (31). Similarly, the baclofen-mediated increase in gastric acid secretion (13) can be explained by an increase in vagal activity obtained by disinhibition of NTS GABAergic neurons.

In contrast, the present study has shown that activation of GABA<sub>B</sub> receptors by baclofen is also capable of inducing a direct outward current, or membrane hyperpolarization, in a subpopulation of DMV neurons. The direct inhibition by baclofen of DMV neurons, which, in our hypothesis, control inhibitory NANC intramural neurons, provides a cellular substrate to explain the atropine-insensitive increase in gastric corpus pressure (3) and the excitatory effects on the LES (1, 4).

In conclusion, we have shown that 1) GABA<sub>B</sub> receptors are functionally present on the membrane of a large percentage of DMV neurons, 2) activation of GABA<sub>B</sub> receptors by baclofen inhibits several ionic conductances, namely a potassium conductance, a calcium conductance, and a calcium-dependent potassium conductance; and 3) the action of baclofen appeared to differ between gastric- and intestinal-projecting neurons. In fact, in intestinal-projecting neurons, the calcium-mediated components of the outward current appeared to exert a more prominent role than in gastric-projecting neurons.

**Perspectives.** Our data, would then provide an explanation at the cellular level of the apparently contradictory (i.e., excitatory and inhibitory) effects of baclofen on vagal motor activity. In fact, the overall effect of brain stem GABA<sub>B</sub> receptor activation seems to be highly dependent on the neuronal circuit under study as well as the complement and activity of ionic conductances within the DMV gastrointestinal motoneurons. Future studies combining in vivo and in vitro experiments aimed at separating these circuits will be required to resolve the neurochemical phenotype and the membrane properties of the neurons involved in the effects of GABA<sub>B</sub> receptor activation in the dorsal vagal complex.

Portions of this work have been presented previously in abstract form (39).

**REFERENCES**


