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Dopamine effects on identified rat vagal motoneurons

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Abstract

Catecholaminergic neurons of the A2 area play a prominent role in brain stem vagal circuits. It is not clear, however, whether these neurons are noradrenergic or adrenergic, i.e., display tyrosine hydroxylase (TH) and dopamine- β -hydroxylase (D β H) immunoreactivity (-IR) or dopaminergic (i.e., TH- but not D β H-IR). Our aims were to investigate whether a subpopulation of neurons in the A2 area was dopaminergic and, if so, to investigate the effects of dopamine (DA) on the membrane of gastric-projecting vagal motoneurons. We observed that although the majority of A2 neurons were both TH- and D β H-IR, a small percentage of nucleus tractus solitarius neurons were TH-IR only, suggesting that DA itself may play role in these circuits. Whole cell recordings from thin brain stem slices showed that 71% of identified gastric-projecting motoneurons responded to DA (1–300 μ M) with either an excitation (28%) or an inhibition (43%) of the membrane; the remaining 29% of the neurons were unresponsive. The DA-induced depolarization was mimicked by SK 38393 and prevented by pretreatment with SCH 23390. Conversely, the DA-induced inhibition was mimicked by bromoergocryptine and prevented by pretreatment with L741626. When tested on the same neuron, the effects of DA and NE were not always similar. In fact, in neurons in which DA induced a membrane depolarization, 77% were inhibited by NE, whereas 75% of neurons unresponsive to DA were inhibited by NE. Our data suggest that DA modulates the membrane properties of gastric-projecting motoneurons via D1- and D2-like receptors, and DA may play different roles than norepinephrine in brain stem vagal circuits.

Keywords

brain stem; electrophysiology; gastric

The dorsal vagal complex (DVC), i.e., the nucleus tractus solitarius (NTS), the dorsal motor nucleus of the vagus (DMV), and the area postrema (AP), plays an important role in the control of homeostatic and autonomic functions, including the modulation of functions in the gastrointestinal (GI) tract. Sensory information from the GI tract is received and integrated by NTS neurons where it is modulated and transferred to, among other areas, the preganglionic neurons of the DMV; DMV neurons then provide a regulated efferent motor vagal outflow to the GI tract (reviewed recently in Refs. 38,39).

A vast array of neurotransmitters are present in the DVC; by modulating the neuronal membrane and/or the synaptic connections between the tractus solitarius, the NTS, and the DMV, each neurotransmitter may contribute to the fine tuning of selected circuits devoted to specific functions (reviewed recently in Ref. 33). Among these neurotransmitters catecholamines, originating either from neurons in the A6, A5, A2, and A1/C1 areas and/or

the hypothalamus (2,20,21,36,41), appear to play an important modulatory role of vagal brain stem circuits. In fact, distension of the stomach or esophagus induces *c-fos* activation in catecholaminergic neurons of the DVC (17,34,42), as does systemic administration of elevated doses of cholecystokinin (31,35). Norepinephrine (NE) has been shown to act directly on DMV neurons via actions at both α_1 - and α_2 -adrenoceptors (26) and to modulate glutamatergic transmission between the NTS and the DMV via actions at α_2 -adrenoceptors (6). Both α_1 - and α_2 -adrenoceptors have been identified as mediators of the esophageal distension-induced gastric relaxation (34), and catecholaminergic neurons of the DVC have also been shown to be involved in lithium-induced anorexia (30).

Although robust functional evidence indicates that NE plays a role in brain stem vagal circuits, scant evidence is available with regard to the presence and role of dopamine (DA) in GI-related vagal brain stem circuits. Studies on the relative distribution and colocalization of tyrosine hydroxylase (TH; whose presence implies that neurons synthesize DA but not necessarily NE) and dopamine- β -hydroxylase (D β H; whose presence implies the enzymatic transformation of DA to NE) report different degrees of colocalization in neurons of the A2 area, which encompasses both the NTS and the DMV (18,20,21,40,42). Although it is not clear whether specific GI-function related brain stem vagal circuits express D1- and D2-like dopamine receptors (15,29), a recent electrophysiological study focusing on cardiorespiratory circuits reports that DA activates D2-like receptors only and inhibits synaptic transmission between the tractus solitarius and NTS neurons (25).

Using a combination of immunohistochemical and electrophysiological recordings from identified gastric-projecting vagal motoneurons, we aimed to 1) determine whether subgroups of neurons in the A2 area were dopaminergic [i.e., TH-immunoreactive (IR) only] or noradrenergic (i.e., TH-IR and D β H-IR); 2) characterize the cellular effects of DA on gastric-projecting DMV neurons; and 3) investigate whether the neurons responding to DA and NE had distinct effects in GI brain stem vagal circuits.

METHODS

Research reported in the present manuscript fully conforms to National Institute of Health guidelines and was approved by the Pennington Biomedical Research Center-Louisiana State University System Institutional Animal Care and Use Committee.

Immunohistochemistry

Four Sprague-Dawley rats of either sex (250–350 g) were injected with fluorogold (Fluorochrome, Englewood, CO; 20 μ g/1 ml saline/rat ip) 3–4 days before experimentation, to label vagal preganglionic neurons innervating the subdiaphragmatic viscera, thus allowing delineation of the boundaries of the DMV (17,44). On the day of experimentation, the rats were anesthetized deeply (abolition of foot pinch withdrawal reflex) with isoflurane and perfused transcardially with chilled saline followed by Zamboni's fixative (see *Solution composition*).

The brain stems were extracted and postfixated in Zamboni's fixative overnight at 4°C. Brain stems were rinsed with phosphate buffer solution (PBS; see *Solution composition*) containing Triton X-100 (PBS-TX), before cutting of 40- μ m-thick coronal sections encompassing the rostrocaudal extent of the DVC using a freezing microtome. Every third slice was mounted onto gelatin-coated coverslips. The portion of the DVC located caudal to the posterior tip of the AP was defined as the "caudal DVC" and the portion of DVC located rostral to the anterior tip of the AP as the "rostral DVC." The area comprised by the extension of the AP was defined as the "intermediate DVC."

The slices were rinsed for 30 min with a fresh solution of PBS-TX containing bovine serum albumin (BSA; 1% final concentration) and incubated at 37°C for 2 h with the primary antibodies (mouse-anti-TH, 1:1,000; rabbit-anti-D β H, 1:500; both antibodies were diluted in PBS-TX containing 0.1% BSA). The slices were rinsed with PBS-TX-BSA and incubated at 37°C for 30 min with appropriate secondary antibodies (goat-anti-mouse FITC, 1:100 and goat-anti-rabbit Texas red, 1:100 in PBS-TX containing 0.1% BSA, respectively). The specimens were again rinsed with PBS-TX-BSA solution containing 1% goat antiserum, before being allowed to air dry and mounted with Fluoromount-G (Southern Biotechnology Associated, Birmingham, AL). Control experiments were carried out to ensure that the antibody labeling was selective, namely: 1) incubation of primary or secondary antibodies only and 2) reaction of primary antibody with mismatched secondary antibody. All control tests proved negative, indicating that the secondary antibodies were selective for their primary antibodies and that the antibodies themselves exhibited neither nonspecific binding nor excessive autofluorescence. Photomicrographs were taken using a Nikon E-400 microscope ($\times 400$ final magnification) equipped with tetramethylrhodamine isothiocyanate (to visualize D β H-IR), FITC (to visualize TH-IR) and UV (to visualize fluorogold-IR) epifluorescent filters and a SPOT Insight camera and software (Diagnostic Instruments, Sterling Heights, MI) connected to a PC. Overlapping panels of the whole DVC area were digitally enhanced and assembled into a montage using ImageJ (developed at the U.S. National Institutes of Health and available from the Internet at <http://rsb.info.nih.gov/ij>) and Adobe Photoshop software (Adobe Systems).

Retrograde tracing

The neuronal tracer 1,1'-diiodo-3,3',3'-tetramethylindocarbocyanine perchlorate (DiI; Molecular Probes, Eugene, OR) was used to label gastric-projecting DMV neurons, as described previously (9). In brief, 14-day-old rat pups of either sex were anesthetized deeply with isoflurane (2.5% with air, 600 ml/min; abolition of the foot-pinch withdrawal reflex). The abdominal area was cleaned with Novalsan before an abdominal laparotomy was performed. Crystals of DiI were applied to one gastric region per rat, either along the major curvature of the fundus or corpus, or to the antrum or pylorus. The crystals were embedded in the application site by a fast-hardening epoxy compound that was allowed to cure for several minutes before the entire surgical area was washed with warmed, sterile saline. The abdomen was closed with 5-0 suture and the rat allowed to recover for 10–15 days before experimentation.

Electrophysiology

The brain stems were removed as described previously (9,37,43). Briefly, the rats were anesthetized deeply with isoflurane (abolition of the foot pinch withdrawal reflex) and killed by administration of a bilateral pneumothorax. The brain stem was then removed and placed in oxygenated Krebs solution at 4°C (see *Solution composition*). Brain stems were sliced only from those animals in which the epoxy compound covering the site of DiI application was still in place at the time of the experiment.

Using a vibratome, six to eight coronal sections (200–300 μ m thick) containing the DVC were cut and stored in oxygenated Krebs solution at $30 \pm 1^\circ\text{C}$ for at least 1 h before use. A single slice was transferred to a custom-made perfusion chamber (volume 500 μ l; Michigan Precision Instruments, Parma, MI) and kept in place with a nylon mesh. The chamber was maintained at $35 \pm 1^\circ\text{C}$ by perfusion with warmed, oxygenated Krebs solution at a rate of 2.5–3.0 ml/min.

Before electrophysiological recording, DiI-labeled GI-projecting DMV neurons were identified using a Nikon E600-FN microscope equipped with epifluorescent filters suitable

for visualizing DiI. Once the identity of the labeled neuron was confirmed, whole cell recordings were made under brightfield illumination using DIC (Normarski) optics.

Whole cell recordings were made with patch pipettes (3–8 M Ω resistance) filled with a potassium gluconate solution (see *Solution composition*) using an Axoclamp 2B or an Axopatch 1D clamp amplifier (Molecular Devices, Union City, CA). Recordings were made only from neurons labeled unequivocally with DiI.

Data were sampled every 100 μ s and filtered at 2 kHz, digitized via a Digidata 1322A interface (Molecular Devices) acquired, stored and analyzed on an IBM PC utilizing pClamp 8 software (Molecular Devices Corp). The junction potential was corrected manually and recordings were accepted only if the series resistance was <15 M Ω .

Drugs were made immediately before use and were applied to the bath via a series of manually operated valves for time periods sufficient for the response to reach plateau, usually 1–3 min. Concentration-response curves were constructed from gastric-projecting neurons in which at least three different concentrations of DA were tested. When measuring the DA-induced depolarization or hyperpolarization, neurons were hyperpolarized to –65 mV via injection of direct current. Cells were defined as responders if DA (30–100 μ M) or NE (100 μ M) induced a change in the membrane of at least 5 mV.

Statistical analysis

Results are expressed as means \pm SE. Inter-group comparisons were analyzed with one-way ANOVA followed by the conservative Bonferroni test for individual post hoc comparisons, Student's paired *t*-test, or χ^2 test. Significance was defined as $P < 0.05$.

Drugs and chemicals

DiI was purchased from Invitrogen (Eugene, OR) and FluoroGold was purchased from Fluorochrome. All other drugs were purchased from Sigma Chemical (St. Louis, MO); stock solutions were prepared and diluted to the final concentration in Krebs just before use. The mouse anti-TH antibody was purchased from Immunostar (Hudson, WI) and the rabbit anti-D β H antibody was purchased from Calbiochem (San Diego, CA). Both secondary antibodies were purchased from Sigma Chemical.

Solution composition

Krebs solution consisted of (in mM) 126 NaCl, 25 NaHCO₃, 2.5 KCl, 1.2 MgCl₂, 2.4 CaCl₂, 1.2 NaH₂PO₄, and 5 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, 10 HEPES, 1 EGTA, 2 ATP-Na, 0.25 GTP-Na; adjusted to pH 7.35 with KOH. Zamboni's fixative consisted of 1.6% (wt/vol) paraformaldehyde, 19 mM KH₂PO₄, and 100 mM Na₂HPO₄·7H₂O in 240 ml saturated picric acid-1,600 ml H₂O; adjusted to pH 7.4 with HCl. PBS-TX consisted of (in mM) 115 NaCl, 75 Na₂HPO₄·7H₂O, 7.5 KH₂PO₄, and 0.15% Triton X-100.

RESULTS

Immunohistochemistry

Sections of brain stem containing the catecholaminergic A2 area were analyzed, from ~2 mm caudal to the posterior tip of the AP to the anterior tip of the AP. Only those neurons with a distinct fluorogold-stained profile were counted as DMV (17,44); the remaining neurons were considered as NTS.

The DMV comprised 103 ± 10 TH-IR cells, of which 18 ± 2 were also D β H-IR positive ($N = 4$ rats). In contrast, the NTS comprised 440 ± 75 TH-IR neurons of which 411 ± 68 contained both TH- and D β H-IR whereas 29 ± 10 displayed TH-IR only ($N = 4$ rats; Fig. 1). Within NTS, the relative distribution of TH- compared with TH- and D β H-IR-positive neurons did not differ along the rostrocaudal axis; in fact, at the level of the AP (i.e., intermediate DVC), 247 ± 49 neurons were both TH- and D β H-IR whereas 17 ± 7 were only TH-IR. Conversely, at a level caudal to the posterior tip of the AP (i.e., caudal DVC), 164 ± 46 neurons were TH- and D β H-IR and 12 ± 4 were only TH-IR. These data indicate that although the vast majority of A2 neurons in DMV are dopaminergic, a small subpopulation of NTS neurons synthesize DA but not NE.

Electrophysiology

Whole-cell patch clamp studies were conducted on 279 identified gastric-projecting neurons. The percentage of responsive cells, amplitude, or mechanism of action in response to perfusion with DA did not show significant differences among DMV neurons projecting to fundus, corpus, or antrum-pylorus; the data were thus pooled.

Effects of dopamine on DMV neurons

Of the 279 gastric projecting DMV neurons in which the response to DA was assessed, 198 neurons, i.e., 71%, responded to DA ($1\text{--}300\text{ }\mu\text{M}$) with a change in membrane potential in a concentration-dependent manner, the remaining 81 neurons (i.e., 29%) did not respond to DA. Gastric-projecting DMV neurons responded to DA with either a depolarization (75 of 198 neurons, i.e., 38% of the responsive neurons, Fig. 2) or a hyperpolarization (116 of 198 neurons, i.e., 59% of the responsive neurons, Fig. 3). In 7 neurons (i.e., 3%) perfusion with DA induced a biphasic response; in 4 of these neurons a $-6.7 \pm 0.8\text{ mV}$ hyperpolarization was followed by a $9.7 \pm 2.5\text{ mV}$ depolarization; conversely, in 3 of these neurons a $4.7 \pm 0.3\text{ mV}$ depolarization was followed by a $-11 \pm 2.1\text{ mV}$ hyperpolarization. The infrequent occurrence of such biphasic responses precluded further analysis.

Regardless of the type of response to perfusion with DA, i.e., depolarization or hyperpolarization, the response threshold was $\sim 1\text{ }\mu\text{M}$, the EC_{50} was $\sim 4\text{ }\mu\text{M}$, and the E_{max} was obtained at $100\text{ }\mu\text{M}$.

The response to DA did not show tachyphylaxis. In fact, 1-min superfusions of DA repeated at 5-min intervals gave similar results. The first superfusion with $100\text{ }\mu\text{M}$ DA depolarized the membrane by $7.0 \pm 1.1\text{ mV}$ ($P < 0.05$ vs. control; $n = 8$) whereas the second superfusion depolarized the membrane by $8.5 \pm 1.5\text{ mV}$ ($P < 0.05$ vs. control; $P > 0.05$ vs. first application, data not shown). Similarly, in a different set of neurons, the first application of DA ($100\text{ }\mu\text{M}$) hyperpolarized the membrane by $-9.9 \pm 1.8\text{ mV}$ ($P < 0.05$ vs. control; $n = 15$) whereas the second superfusion hyperpolarized the membrane by $-9.1 \pm 1.2\text{ mV}$ ($P < 0.05$ vs. control; $P > 0.05$ vs. first application, data not shown).

To ascertain whether the DA-induced effects on the DMV membrane were due to direct actions on the DMV membrane or mediated by local release of other neurotransmitters, the amplitude of the DA-induced membrane displacement was compared in the absence and presence of tetrodotoxin (TTX, $1\text{ }\mu\text{M}$), which blocks action potential-dependent synaptic transmission. In eight neurons, perfusion with DA ($30\text{--}100\text{ }\mu\text{M}$) induced a $9 \pm 0.71\text{ mV}$ depolarization that recovered to baseline upon washout. Following 10 min of perfusion with TTX, reapplication of DA in the presence of TTX induced a $7.4 \pm 0.9\text{ mV}$ depolarization (i.e., $83 \pm 8.9\%$ of control, $P > 0.05$ vs. DA alone, data not shown). Similarly, in six other neurons, DA ($30\text{--}100\text{ }\mu\text{M}$) induced a $-9.8 \pm 0.9\text{ mV}$ hyperpolarization that recovered to baseline upon washout. Following 10 min of perfusion with TTX, reapplication of DA in the

presence of TTX induced a -9.2 ± 0.7 mV hyperpolarization (i.e., $95 \pm 7.1\%$ of control, $P > 0.05$ vs. DA alone, data not shown). These data indicate that DA induces its effects principally via direct actions on the DMV membrane.

The DA-induced depolarization is mediated by D1-like receptors

In 13 cells, perfusion with $100 \mu\text{M}$ DA induced a 7.2 ± 0.9 mV depolarization. Following washout and recovery, perfusion with the D1-like receptor selective agonist SKF 38393 ($10\text{--}30 \mu\text{M}$) induced a 6.7 ± 0.8 mV depolarization ($P > 0.05$ vs. DA alone).

In four other cells perfusion with DA ($30\text{--}100 \mu\text{M}$) induced a 9.8 ± 0.9 mV depolarization. Following washout and recovery, the slice was perfused for 10 min with the D1-like receptor selective antagonist SCH 23390 ($5\text{--}10 \mu\text{M}$), which per se did not have any effect on the membrane potential. Perfusion with DA in the presence of SCH 23390 induced a 0.5 ± 0.5 mV depolarization ($P < 0.05$ vs. DA alone). Similarly, in three cells perfusion with the D1-like selective agonist SKF 38393 ($10 \mu\text{M}$) depolarized the membrane by 6.3 ± 1.2 mV. Following washout and 10-min pretreatment with SCH 23390, application of SKF 38393 in the presence of SCH 23390 induced a 0.3 ± 0.3 mV depolarization ($P < 0.05$ vs. SKF 38393 alone). These data indicate that the DA-induced depolarization is mediated by D1-like receptors and that the D1 receptors are not tonically active (Fig. 4).

The DA-induced hyperpolarization is mediated by D2-like receptors

In eight cells, perfusion with DA ($30 \mu\text{M}$) induced a -11.5 ± 1.79 mV hyperpolarization. Following wash out and recovery, perfusion with the D2-like receptor selective agonist bromoergocryptine ($100 \mu\text{M}$) induced a -6.9 ± 1.9 mV hyperpolarization ($P > 0.05$ vs. DA alone).

In a further seven cells, perfusion with DA ($30 \mu\text{M}$) induced a -9.7 ± 1.6 mV hyperpolarization. Following washout and recovery, the slice was perfused for 10 min with the D2-like receptor selective antagonist L741626 ($10 \mu\text{M}$), which per se did not have any effect on the membrane potential. Perfusion with DA in the presence of L741626 induced a -4.0 ± 2.0 mV hyperpolarization ($P < 0.05$ vs. DA alone). Similarly, in five cells, perfusion with bromoergocryptine ($100 \mu\text{M}$) hyperpolarized the membrane by -11.0 ± 2.9 mV whereas application of bromoergocryptine in the presence of L741626 induced a -1.2 ± 0.7 mV hyperpolarization ($P < 0.05$ vs. bromoergocryptine alone). These data indicate that the DA-induced hyperpolarization is mediated by D2-like receptors and that the D2 receptors are not tonically active (Fig. 5).

As further proof of their selectivity, D1- and D2-like receptor selective agonists and antagonists were then cross-analyzed. In three DMV neurons, perfusion with the D1-like receptor selective agonist SKF 38393 ($10 \mu\text{M}$) depolarized the DMV membrane by 10 ± 2.5 mV, whereas reperfusion with SKF 38393 in the presence of the D2-like receptor selective antagonist L741626 ($10 \mu\text{M}$) induced a 7.3 ± 1.8 mV depolarization ($P > 0.05$; Fig. 4). Similarly, in four DMV neurons, pretreatment with the D1-like receptor selective antagonist SCH 23390 had no effect on the D2-like receptor selective agonist (bromoergocryptine)-induced hyperpolarization (-5.2 ± 0.6 mV in bromoergocryptine and -4.5 ± 1.6 mV in bromoergocryptine plus SCH 23390; $P > 0.05$; Fig. 5). These data indicate that the D1 and D2 agonists and antagonists we used are selective for their respective receptors and that, apart from the seven neurons that had a biphasic response to DA, DMV neurons have either one of the receptors.

Differential effects of DA or NE on the same cells

DA and NE induced nonhomogeneous responses in some DMV neurons. Fifty-four gastric-projecting DMV neurons were perfused first with DA (100 μ M) followed by NE (100 μ M) after suitable washout and recovery.

In 32 of these neurons, perfusion with DA induced a -10.1 ± 0.8 mV hyperpolarization, whereas perfusion with NE induced a -14.7 ± 1.2 mV hyperpolarization in 29 neurons, a 7-mV depolarization in one neuron, and no effect in two cells. Conversely, in 13 cells, perfusion with DA induced a 6.2 ± 0.7 mV depolarization, whereas perfusion with NE induced a -10.8 ± 1.8 mV hyperpolarization in 10 neurons and a 4.3 ± 0.6 mV depolarization in three cells. Finally, in the remaining 12 cells that showed no response to perfusion with DA, NE induced a -8.4 ± 1.7 mV hyperpolarization in nine cells and no response in three cells (data not shown). These data indicate a differential distribution of DA and NE receptors on the membrane of gastric projecting DMV neurons and distinct responses to these neurotransmitters.

DISCUSSION

In the present study we have shown that 1) whereas the vast majority of NTS neurons in the A2 area are noradrenergic or adrenergic (i.e., contain both TH- and D β H-IR), ~10% of the NTS neurons contain TH-IR only, indicating that this neuronal subpopulation is dopaminergic; 2) the vast majority of catecholaminergic DMV neurons contain only TH-IR (i.e., they are dopaminergic), but ~7% of these neurons display both TH- and D β H-IR, indicating that they are noradrenergic or adrenergic; 3) 43% of gastric-projecting DMV neurons respond to DA perfusion with a concentration-dependent hyperpolarization mediated by activation of D2-like receptors; 4) 28% of gastric-projecting DMV neurons respond to DA perfusion with a concentration-dependent depolarization mediated by activation of D1-like receptors; and 5) DA and NE may induce different membrane responses in the same DMV neuron. Our data, then, support a role for dopaminergic transmission in gastrointestinal brain stem vagal circuits and suggest that DA and NE may play distinct roles in the modulation of these circuits.

The location and neurochemical phenotype of the neurons comprising the A2 area has been the subject of many manuscripts for several decades, stemming from the first definition of the A2 cell group by Dahlstroem and Fuxe in the early 1960s (12). Although it is not our intention to settle the dispute about the neurochemical phenotype of these neurons, i.e., whether all the TH-IR-positive cells in the A2 area are also D β H-IR (i.e., noradrenergic and/or adrenergic) or some of these cells are TH-IR only (i.e., dopaminergic but not noradrenergic and/or adrenergic) (18,20,21,40,42), we, however, conducted the present experiments with the intention of investigating whether a subpopulation of brain stem neurons is dopaminergic. The present manuscript confirms the previous reports that the vast majority of catecholaminergic DMV neurons are dopaminergic, since they contain TH- but not D β H-IR (3,21,32). We also show that a small, but significant, subpopulation of NTS neurons of the A2 area contains TH-IR only, indicating that these catecholaminergic neurons are dopaminergic. These local neurons of the A2 area are the most likely source of dopaminergic input to vagal motoneurons, although it is possible that minor inputs deriving from other areas of the central nervous system also impinge on A2 DMV neurons (36). Our pharmacological data also indicate that the dopaminergic input to DMV neurons is not tonically active.

Having established that some NTS neurons in the A2 area are dopaminergic, we conducted current clamp experiments to determine whether DA itself was capable of modulating the membrane of DMV neurons. We found three separate populations of gastric-projecting

DMV neurons based on their response to exogenously applied DA. The majority of these neurons (i.e., 43%) were hyperpolarized via the activation of D2-like receptors present on the DMV membrane, whereas a smaller (i.e., 28%) subpopulation of DMV neurons was depolarized via activation of membrane-bound D1-like receptors; a third subpopulation, comprising 29% of the gastric-projecting DMV neurons, was unresponsive to DA administration.

Our pharmacological evidence supports the presence of both D1- and D2-like receptors on the DMV membrane, although it appeared that the vast majority of the neurons express either receptor, but not both. In fact, the response to DA was unchanged in the presence of the action potential-dependent synaptic transmission blocker TTX, indicating that the effects of DA are mediated via interaction with receptors present on the membrane of the neuron, rather than on presynaptic terminals impinging on the cell. Additionally, the DA-induced inhibition (i.e., hyperpolarization) of a subpopulation of DMV neurons was mediated by D2-like receptors since perfusion with the D2-like receptor agonist, bromoergocryptine, mimicked the effects of DA; both DA and bromoergocryptine-induced hyperpolarization was prevented by pretreatment with the D2-like receptor selective antagonist, *L*-741676. These data are in agreement with previous reports of the inhibitory actions of DA being mediated by D2-like receptor activation (28). Finally, the DA-induced excitation (i.e., depolarization) of a subpopulation of DMV neurons was mediated by D1-like receptors since perfusion with the D1-like selective agonist SKF 38393 mimicked the excitatory effects of DA. The depolarization induced by both DA and SKF 38393 was prevented by pretreatment with the selective D1-like antagonist SCH 23390. These data are in agreement with the already reported D1-like mediated excitatory actions of DA (16,28). Interestingly, in thalamic nuclei, DA at high (200 μ M) but not at low (10 μ M) concentration has been shown to interact with α_1 -adrenoceptors (16,27), raising the possibility that this type of interaction occurs in DMV neurons too. Our data, however, show that this scenario is unlikely, at least at the concentrations of DA that we studied. In fact, when tested on the same neuron, DA and NE induced effects that were often dissimilar and, in most cases, resulted in a NE-induced membrane hyperpolarization, i.e., an effect associated with activation of α_2 -adrenoceptors (26).

Such dissimilar responses of vagal motoneurons to DA and NE suggest that these neurotransmitters are either devoted to the control of different vagal functions or differentially regulate the same function. Assuming that most of the catecholaminergic inputs to DMV originate from the A2 area, our observation further supports our hypothesis of a selective organization of brain stem vagal circuits controlling gastric or/and homeostatic functions (10). Although the role of hindbrain NE is well established in the esophagogastric reflex (34), in swallowing circuits (23,24), in lithium-induced anorexia (30), and in brain stem-hypothalamic circuits, including, perhaps, those controlling hypovolemia and hypoglycemia (13), the role of DA in these vagal circuits is far from being established.

A series of reports from Sodersten's group point to a role of DA in feeding-related circuits in the brain stem. In an anatomical study (29), Qian and colleagues report the proximity of D2, CCK-A, but not CCK-B, and ionotropic glutamate receptors in the NTS and suggest that brain stem dopaminergic circuits are involved in the CCK-A-mediated inhibition of food intake. Indeed, recent electrophysiological evidence links brain stem CCK-A receptors to fast (ionotropic) glutamate transmission (1,4). Although the relationship with TH-IR neurons was not firmly established in these electrophysiological recordings, the NTS areas of interest included both the commissuralis and centralis subnuclei of the NTS, where a robust TH-IR presence has been reported (3,20,21,34). Behavioral studies by Bednar and colleagues (5) demonstrate that activation of both D1- and D2-like receptors inhibits food intake; the inhibitory effects induced by DA receptor activation are present also in

decerebrate animals (22), further implicating dopaminergic transmission in brain stem reflexive circuits involved in feeding behavior.

Another possible physiological role of dopaminergic neurotransmission in vagal brain stem circuits relates to swallowing, which is known to involve neurons of the A2 area (7,11,19). Indeed, Kessler and Jean (24) showed that microinjection of DA or the agonist apomorphine into the NTS induced an immediate decrease in the number and amplitude of swallows initiated by stimulation of the superior laryngeal nerve. Although speculative, since the DA deficit may reflect a visceral impairment, it is interesting to note the possible relationship between brain stem dopaminergic circuits within the A2 area and the gastrointestinal dysfunctions reported by Parkinson's disease patients, including sialorrhea and dysphagia, both of which reflect disordered swallowing (8,14).

In conclusion, although the effects of DA and its pharmacological characterization have been studied extensively in many areas of the central nervous system, the present study is the first report to analyze the effects of DA in identified, gastric-projecting vagal motoneurons. Overall, our study indicates a potentially important role of D1- and D2-like receptors in influencing the excitability of vagal motoneurons and, ultimately, the role of vagally mediated functions in the control of gastric and/or homeostatic circuits.

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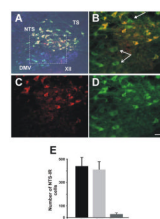
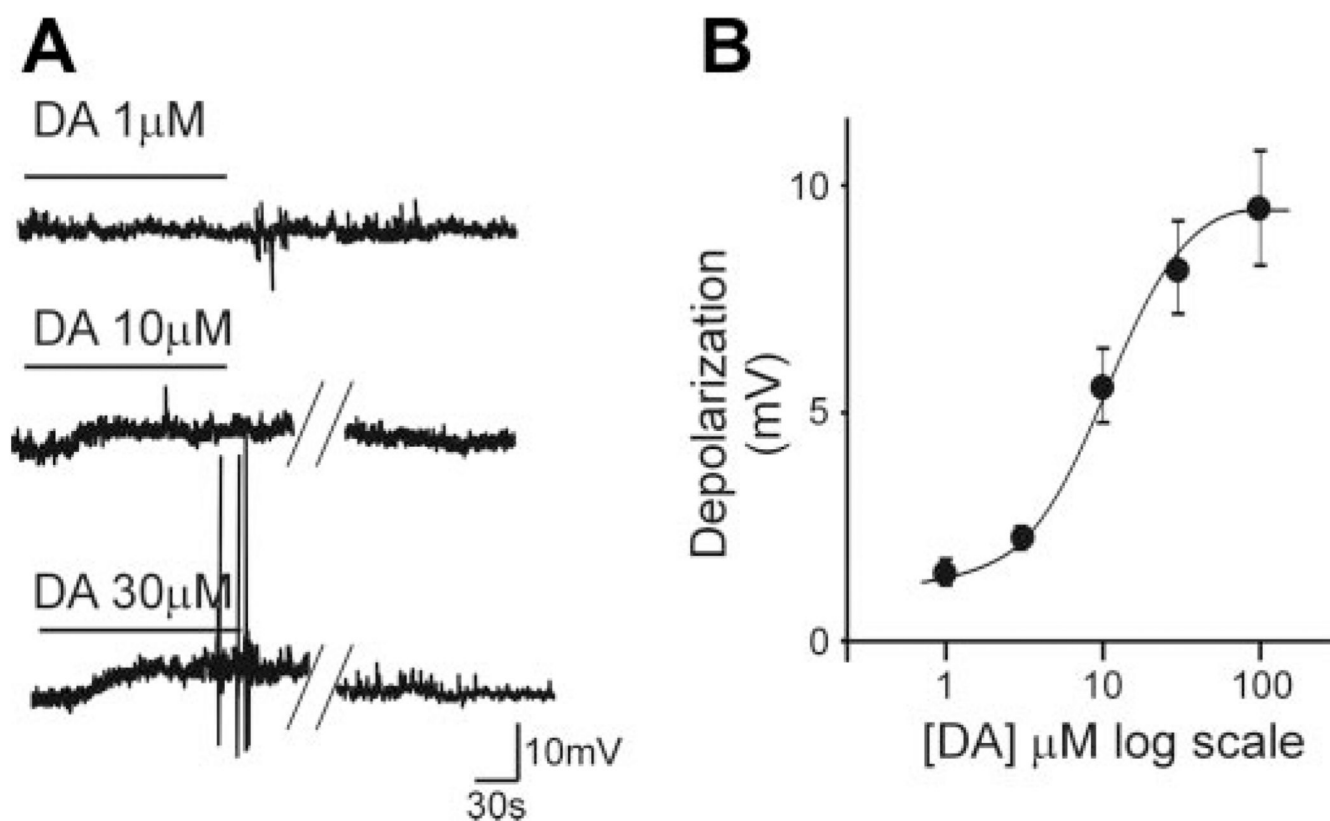


Fig. 1.

Tyrosine hydroxylase (TH)- and dopamine- β -hydroxylase (D β H)-immunoreactivity in the A2 area. *A*: representative low-magnification micrograph of the A2 area encompassing the nucleus tractus solitarius (NTS) and the dorsal motor nucleus of the vagus (DMV). *B*: high-magnification micrograph of the area enclosed by the dotted line in *A*. Arrowheads indicate neurons positive to TH-immunoreactive (IR) (green) only in the DMV (open arrowheads) as well as in the NTS (solid arrowhead). *C*: high-magnification micrograph of the area enclosed by the dotted line in *A* using tetramethylrhodamine isothiocyanate filters to show neurons positive to D β H-IR (red). Note that the neurons are confined to the NTS area mainly. *D*: high-magnification micrograph of the area enclosed by the dotted line in *A* using FITC filters to show neurons positive to TH-IR (green). *E*: summary graphic showing the number of labeled neurons in NTS. Note that the neurons are located in both the DMV and NTS areas. Blue, fluorogold-IR; green, TH-IR; red, D β H-IR; yellow, TH- and D β H-IR; XII, hypoglossus; TS, tractus solitarius. Scale bar: 25 μ m in *B*, *C*, and *D*; 50 μ m in *A*. *A* and *B* are merged images.

**Fig. 2.**

Dopamine (DA) depolarizes a subpopulation of identified gastric-projecting DMV neurons. **A**: representative traces from a gastric-projecting DMV neuron illustrating a concentration-dependent depolarization induced by DA. A recovery period of at least 5 min was allowed between successive applications. Parallel lines indicate a 2- to 3-min break in the recording. The upstroke of the action potential was truncated digitally. **B**: concentration-response curve for the DA-induced depolarization. The EC_{50} for the DA response was $\sim 4 \mu$ M. Each neuron was tested with at least 3 different concentrations of DA. Holding potential = -65 mV.

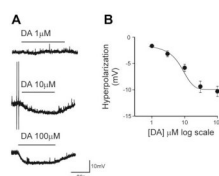


Fig. 3.

Dopamine hyperpolarizes a subpopulation of identified gastric-projecting DMV neurons. *A*: representative traces from a gastric-projecting DMV neuron illustrating the dopamine (DA) induced concentration-dependent hyperpolarization. A recovery period of at least 5 min was allowed between successive applications. The upstroke of the action potential was truncated digitally. *B*: concentration-response curve for the DA-induced hyperpolarization. The EC₅₀ for the DA response was ~ 4 μ M. Each neuron was tested with at least 3 different concentrations of DA. Holding potential = -65 mV.

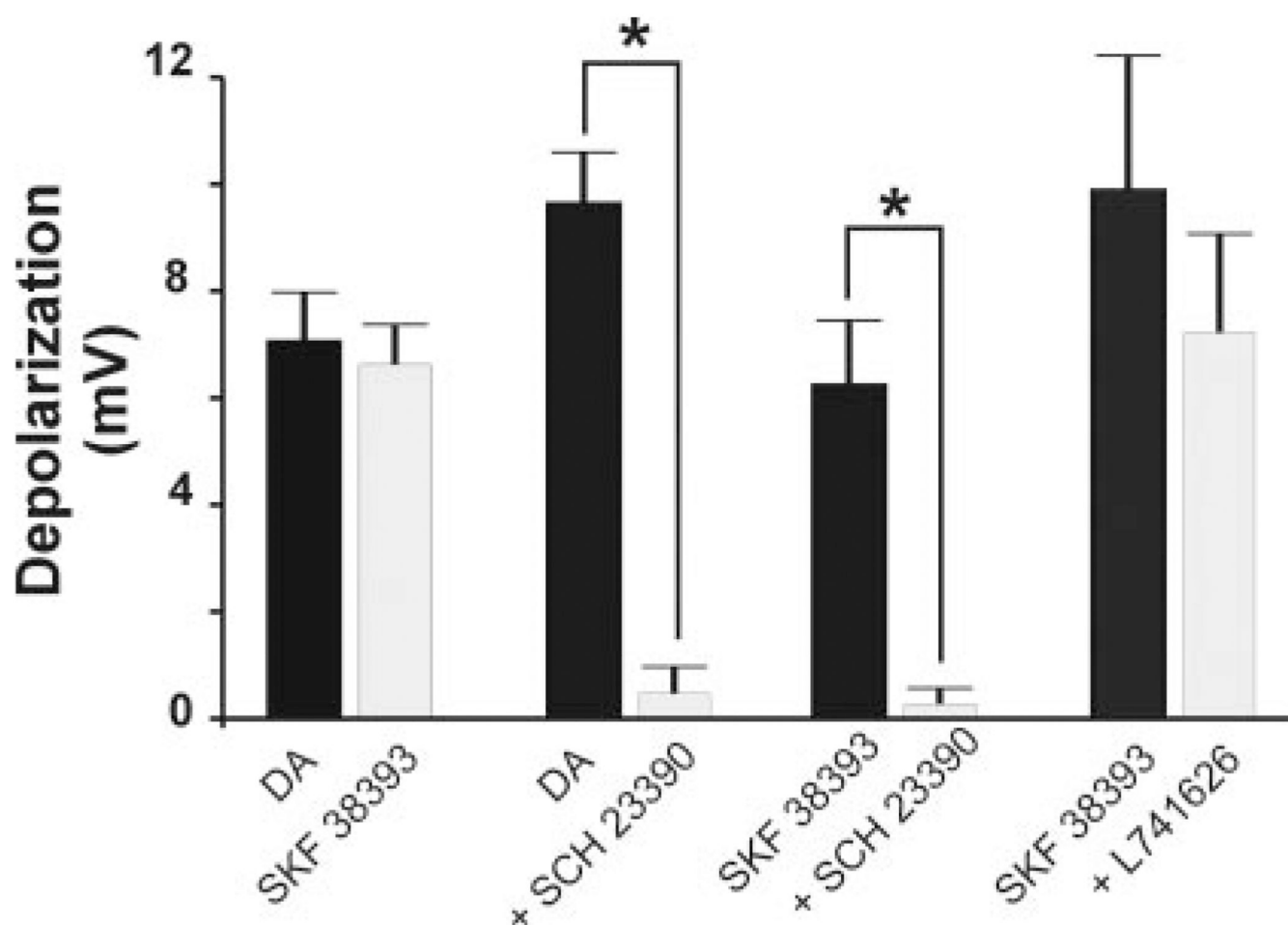


Fig. 4.

Pharmacology of the dopamine-induced depolarization. The DA-induced depolarization was mimicked by the D1-like receptor selective agonist SKF 38393 (5 μ M) and was prevented by pretreatment with the D1-like receptor selective antagonist SCH 23390 (10 μ M). Similarly, the SKF 38393-induced depolarization was antagonized by pretreatment with SCH 23390 but not by pretreatment with the D2-like receptor selective antagonist L741626 (10 μ M). * $P < 0.05$.

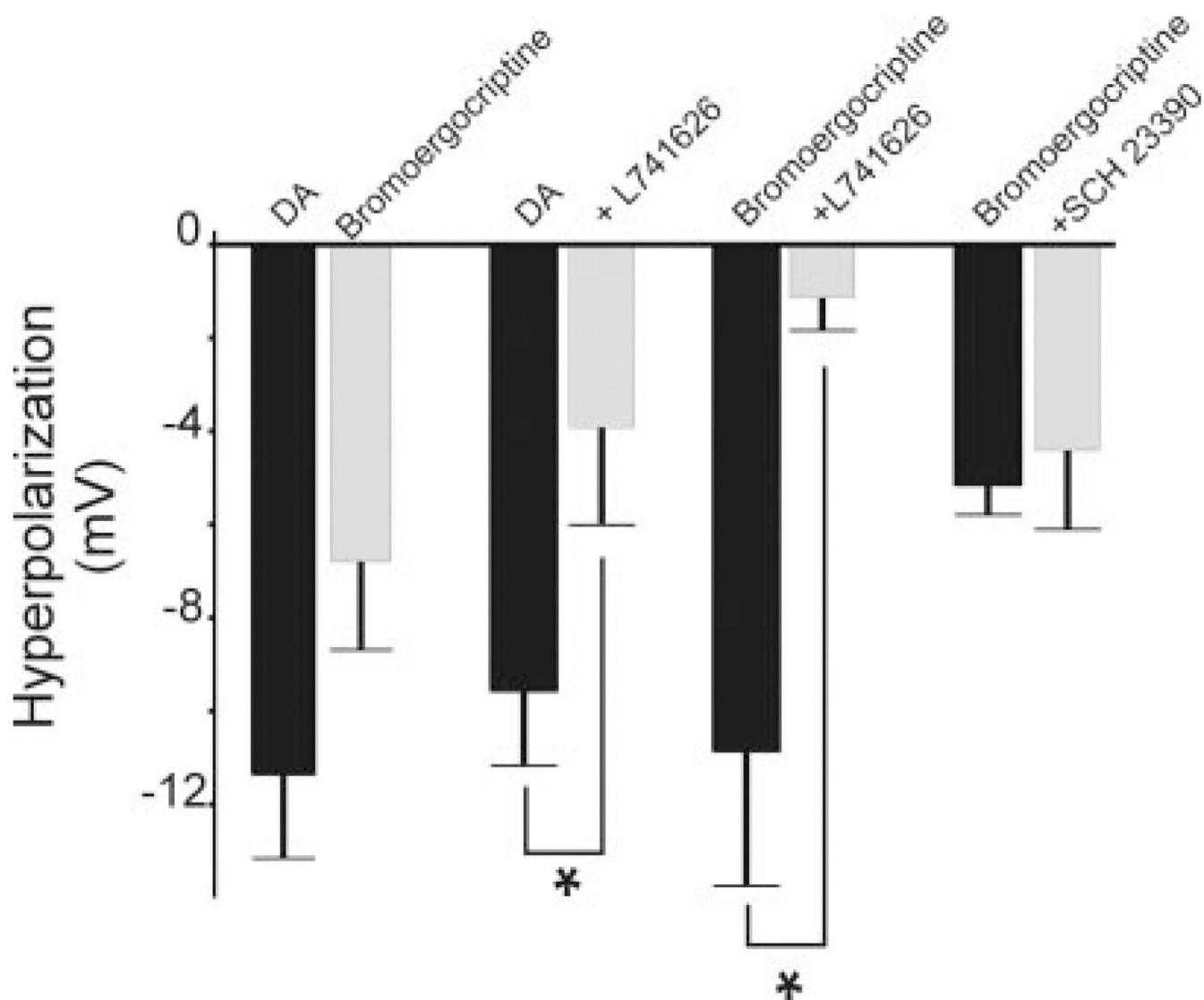


Fig. 5. Pharmacology of the DA-induced hyperpolarization. The DA-induced membrane hyperpolarization was mimicked by the D2-like receptor selective agonist bromoergocryptine (100 μ M) and was prevented by pretreatment with the D2-like receptor selective antagonist L741626 (10 μ M). Similarly, the bromoergocryptine-induced hyperpolarization was antagonized by pretreatment with L741626 but not by pretreatment with the D1-like receptor selective antagonist SCH 23390 (10 μ M). * $P < 0.05$.