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Vagally-regulated gastric motor activity: evidence for kainate and NMDA receptor mediation

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Abstract

Blockade of $GABA_A$ receptors in the dorsal vagal complex produces marked gastric motor excitation. This effect is abolished by a prior microinjection of a non-selective excitatory amino acid receptor antagonist. Here we present functional evidence for kainate and NMDA receptor-mediated gastric excitation in the dorsal vagal complex. Microinjections into the dorsal vagal complex were performed in α-chloralose-anesthetized rats using multi-barrelled glass micropipettes while recording intragastric pressure and motility. Kainic acid (30 and 100 pmol in 30 nl) and NMDA (100 and 300 pmol) produced dose-related increases in intragastric pressure and motility. The gastric responses to kainate (30 pmol) and NMDA were selectively abolished by prior microinjection 6,7-dinitroquinoxaline-2,3-dione (600 pmol, 60 nl) and DL-2-amino-5-phosphanopentanoic acid (2 nmol), respectively. Atropine (1 mg/kg, i.v.) pretreatment blocked kainate-, NMDA- and L-glutamate-induced gastric excitation. Thus, both kainate- and NMDA-receptors in the dorsal vagal complex can independently cause vagally-mediated gastric motor excitation.

Keywords: Hindbrain; Dorsal vagal complex; Vagus; Gastric motility

1. Introduction

In the rat hindbrain, the nucleus of the solitary tract and the dorsal motor nucleus of the vagus are anatomically adjacent structures that are often considered as one regulatory unit, the dorsal vagal complex. While preganglionic vagal neurons in the dorsal vagal complex are known to regulate gastric motility and secretion, the role of various neurotransmitters involved in this process is only partially characterized (Krowicki and Hornby, 1995a).

In the dorsal motor nucleus of the vagus, microinjection of a $GABA_A$ receptor antagonist, bicuculline methiodide, increases gastric motility and secretion in cats (Feng et al., 1990; Washabau et al., 1995) and rats (Sivarao et al., 1998). Thus, there is a tonic GABAergic inhibition of the vagal neurons, which when blocked unleashes an underlying excitation. The identity of the receptors which mediate vagal excitation in the presence of GABAergic blockade is likely to be glutamatergic. This is because, kynurenic acid, a non-selective antagonist of excitatory amino acid receptors, microinjected into the dorsal vagal complex prior to bicuculline, abolished the bucuculline-mediated gastric motor excitation (Sivarao et al., 1998). Furthermore, using in vitro slice recordings, it has been demonstrated that both NMDA and non-NMDA type of glutamate receptors are involved in neurally mediated depolarization of preganglionic vagal motoneurons (Travagli et al., 1991). Finally, both excitatory amino acid and GABAA receptors are co-localized within the dorsal motor nucleus of the vagus (Broussard et al., 1997), and provide anatomical support for these observations.

There is also a lot of evidence for glutamate as a neuro transmitter in the nucleus tractus solitarius. Preliminary data indicate that glutamate in the nucleus tractus solitarius transmits primary afferent information from the gut (Rogers et al., 1990) and both NMDA (Monaghan and Cotman, 1985; Broussard et al., 1996) and kainate (Petralia et al., 1994) receptors are present in this region. However, to the best of our knowledge, there are no functional studies that demonstrate a role for NMDA and non NMDA receptors in the dorsal vagal complex in the control of gastric motor function. Therefore, we microinjected selected excitatory amino acid receptor agonists and antagonists into the

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dorsal vagal complex of anesthetized rats while recording gastric motor activity.

2. Materials and methods

2.1. General

Male Sprague–Dawley rats (250–350 g; Charles River Laboratories, Wilmington, DE) were used in this study. Animals were kept in 12:12 h light–dark cycle with ad libitum food and water available except before laparotomy, when they were denied access to food but not water overnight. The procedures were approved by LSUMC Animal Care and Use Committee.

2.2. Drug solutions employed

Kainic acid, L-glutamic acid monosodium salt, (Sigma, St. Louis, MO), kainate receptor antagonist, DNQX,

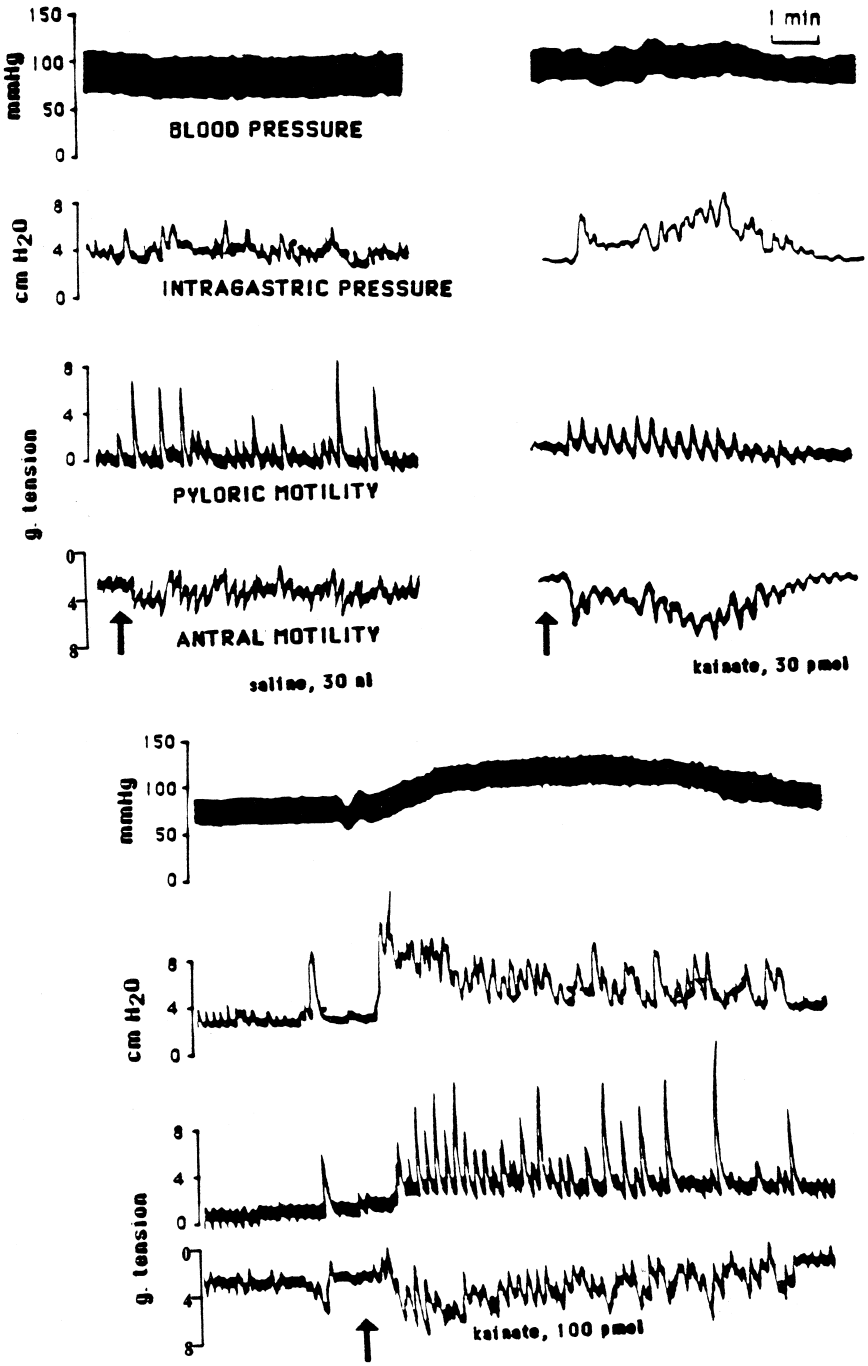

Fig. 1. Sample trace recording from an animal microinjected with vehicle and kainate into the dorsal vagal complex. Notice that in this, as well as all other trace recordings, the antral motility was recorded with a reverse polarity. Arrows mark the microinjection event.

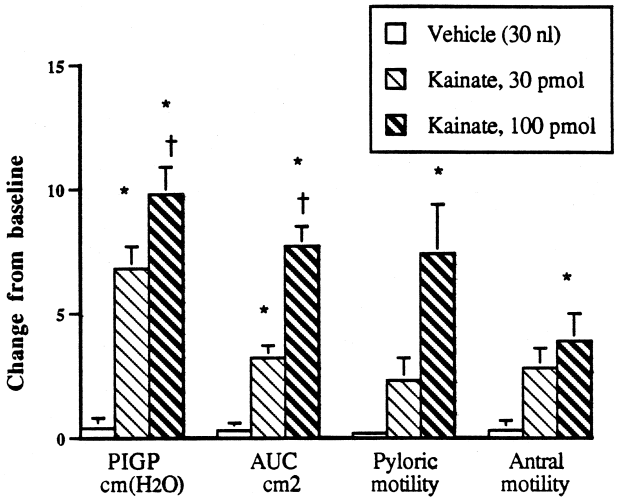

Fig. 2. Compiled data showing the effects on peak-intragastric pressure (PIGP), total-intragastric pressure (AUC), pyloric MMI and antral MMI of vehicle and kainate. ∗Significantly different from the vehicle mean of the same index; †Significantly different from lower dose mean of the same gastric index. Analysis was by repeated measures ANOVA with Newman–Keul post-test comparison for significance ($P < 0.05$).

NMDA receptor antagonist AP5 (Tocris Cookson, St. Louis, MO) and pontamine sky blue (ICN Biochemicals, Cleveland, OH) were employed in this study. DNQX was sonicated in 0.01 N NaOH and the final pH was adjusted to 7.4. Atropine methylbromide (Sigma, St. Louis, MO) was dissolved in saline and stored protected from light. All other drug solutions for microinjection were made in phosphate-buffered saline. DNQX was sonicated in 0.01 N NaOH and the final pH was adjusted to 7.4. Injectate volume of drugs was 30 nl unless otherwise stated. Doses of microinjected drugs were modified from the following references: L-glutamate (Krowicki et al., 1997); kainate (Depaulis et al., 1994), NMDA (Jensen and Yaksh, 1992), DNQX (Narita et al., 1993), AP5 (El-Mas and Abdel-Rahman, 1993), atropine methyl bromide (Sivarao et al., 1998). The doses of kainate and NMDA used in this study were based on preliminary experiments in which a range of doses was microinjected and the smallest doses that caused a reproducible increase in gastric motor activity were chosen (data not shown). Similarly, the antagonist doses employed were the smallest that completely and reversible abolished the respective agonist's effects on gastric motor activity.

A total of eight rats were employed in this study. Each rat was microinjected with various agonists and antagonists as discussed below. Following each agonist microinjection the pressure and motility traces were always allowed to return to baseline values and a further 15 min rest period was allowed before another microinjection was made. When an antagonist microinjection was followed by an agonist microinjection, a further period of 40 min was allowed for wash out. L-Glutamate (15 nmol) was first microinjected into the dorsal vagal complex to locate the site in which an increase in peak-intragastric pressure of at least 4 cm H_2O was obtained.

2.3. Animal instrumentation

Rats were anesthetized (35 mg/kg ketamine and 3.5 mg/kg xylazine, i.m.), and indwelling cannulae inserted into femoral vein (PE 50, Becton Dickinson, Parsippany, NJ) and artery (PE 10, Becton Dickinson, Parsippany, NJ). The arterial cannula was connected via a Statham pressure transducer to a Grass polygraph (Grass Instrument, Quincy, MA). α-Chloralose (80 mg/kg) was given i.v. to maintain surgical anesthesia, and rectal temperature monitored with a thermistor thermometer (Cole-Palmer, Niles, IL) and kept between 36.5 and 37.5°C with radiant heat. Supplemental analgesia was provided by a single dose of buprenorphine (0.1 mg/kg, s.c.) given about 40 min after i.v. α-chloralose.

A tracheal cannula was inserted and connected to a small animal respirator (Kent Scientific, Litchfield, IL) for positive pressure ventilation, if necessary. A laparotomy was performed and an intraluminal latex balloon was inserted into the stomach through an incision in the fundus to monitor intragastric pressure. Two small strain gauges (Figure 7 arch, 120 mΩ, Warren Research Products, SC) were sutured to the surface of the stomach for continuous recording of circular smooth muscle contractility of the pyloric region and the antral region. The rat was placed in a stereotaxic apparatus with the nose bar set at −6.5 mm below the interaural line and the atlanto-occipital membrane and adjacent bone removed to expose the dorsomedial medulla. Upon completion of the surgery, the intragastric balloon was inflated with warm saline to an imparting pressure of approximately 5 cm H_2O. Animals were allowed to stabilize for about an hour prior to further experimentation.

2.4. Manufacture and use of multi-barrelled micropipettes

Seven to eight glass barrels with 0.4 mm internal diameter (A-M Systems, Everett, WA) were pulled together using a micropipette puller (Narshige, Japan) and the tip cut back to a total external diameter of 40 μm. Each barrel was attached via PE50 tubing to a picospritzer (General Valve, Fairfield, NJ). Barrels were filled by negative pressure with vehicle, 2% pontamine sky blue, L-glutamate and any of the agonists and antagonists given below. Micropipettes were placed in the holder at an angle of 28°. Obex is defined as the point between the area postrema and calamus scriptorius, where the central canal starts to open into the fourth ventricle (Paxinos and Watson, 1986). Using this as the reference point, the tip was lowered into the dorsal vagal complex (coordinates: 0.5 mm rostral, 0.5 mm lateral from the obex and 0.4 mm ventral to the surface). Generally, a total of 30 nl of

vehicle or drug solution were microinjected by visual observation of the movement of the miniscus on calibrated tape. This was done by application of brief pulses of nitrogen through a picospritzer at a pressure of 30 psi and duration range of 20–50 ms.

Following the termination of drug microinjections, the site was marked by injecting 60 nl of 2% pontamine sky blue, for subsequent histological verification. The animal was deeply anesthetized (pentobarbital sodium 60 mg/kg, i.v.) and flushed intracardially by a perfusion pump (Cole-Palmer, Niles, IL) with 300 ml of saline, followed by 200 ml of 4% paraformaldehyde solution (in phosphate-buffered saline). Brains were removed, post-fixed in the same solution and sectioned at 50 μm by Vibratome (Int. Tech. Prod., St. Louis, MO) then counter-stained with neutral red. Thus, the bright blue dye spot, indicating the placement of the pipette tip, could be histologically located against the red nuclear counter stain.

2.5. Analyses, calculations and statistics

The change in peak-intragastric pressure (cm H_2O) was defined as the difference between the pre- and post-injection maximal levels of the intragastric pressure trace. The area under the curve (AUC) of intragastric pressure response was calculated in cm^2 using a microcomputer-based imaging system (Imaging Research, St. Catherines, Ontario, Canada). For this purpose, the baseline intragastric pressure tracing was extended across the period of the response to the point at which it returned to baseline and the area enclosed between the baseline and the curve of the response was calculated. Gastric motility was quantified by minute motility index (MMI) based on the method of Ormsbee and Bass (1976). This was calculated within 5 min before and 5 min after administration of the agents and expressed as a difference between post- and preinjection values of MMI.

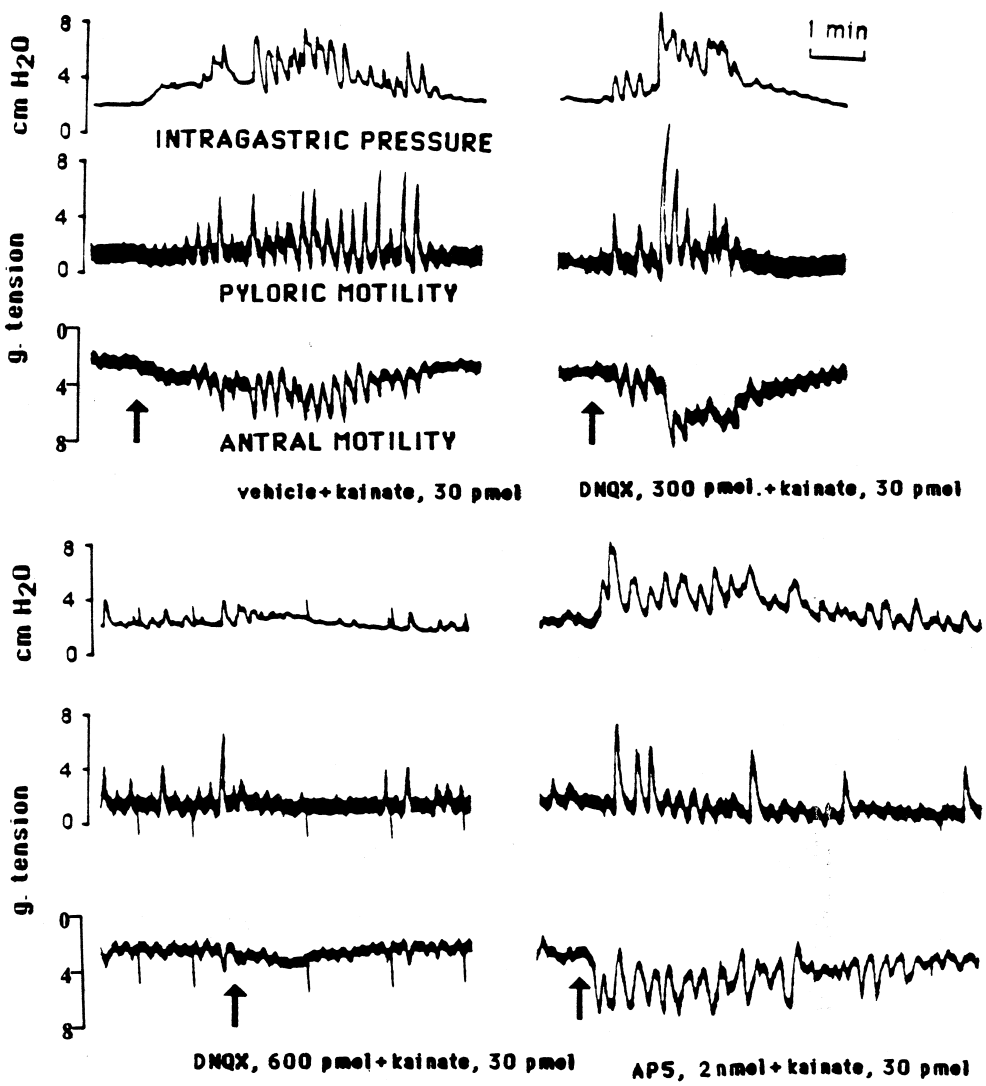

Fig. 3. Sample trace recording from an animal microinjected with kainate immediately following vehicle, DNQX, and AP5 microinjection into the dorsal vagal complex. Arrows mark the microinjection event.

The data were analyzed by one-way analysis of variance (ANOVA) with or without repeated measures as appropriate. In the variance analysis, the differences were assessed with the Newman–Keul test of significance. When comparing the effect of L-glutamate and vehicle microinjections, a two-tailed Student's *t*-test was used. A level of $P < 0.05$ was considered statistically significant. All data are submitted as means ± S.E.M.

3. Results

In all the experiments, L-glutamate was first microinjected into the dorsal vagal complex to determine the effective site for stimulation of gastric motor function. In eight animals, L-glutamate (15 nmol) significantly increased peak intragastric pressure (6.5 ± 0.8 cm H_2O), total intragastric pressure (3.1 ± 0.8 cm^2) as well as pyloric (2.3 ± 0.9 MMI) and antral motility (2.7 ± 0.7 MMI) compared to saline microinjection. Typically, these increases lasted for about 3 min. Atropine (1 mg/kg, i.v.) abolished the gastric excitatory response to L-glutamate in four animals tested; peak intragastric pressure (-0.3 ± 0.3 cm H_2O), total intragastric pressure (-0.3 ± 0.3 cm^2) and pyloric (0.0 ± 0.0 MMI) and antral motility (0.0 ± 0.0 MMI).

3.1. Kainate microinjection studies

Fig. 1 is a trace recording that illustrates typical gastric motor and cardiovascular responses obtained following microinjections of vehicle and kainate (30 and 100 pmol) into the dorsal vagal complex. Lower doses (3 and 10 pmol) of the agonist did not give reproducible responses in preliminary experiments (data not shown). Kainate (30 pmol) markedly increased intragastric pressure and motility, which returned to baseline within 6 min (Fig. 1). At the 100 pmol dose, kainate evoked more pronounced changes in peak intragastric pressure and motility, which lasted for about 10 min before returning to baseline (Fig. 1). Vehicle microinjection had no discernible effect. The compiled data is represented graphically in Fig. 2, and, based on this, kainate (30 pmol) was chosen as a submaximal dose for all subsequent microinjection studies with this agent. Only the higher dose of kaintate (100 pmol) produced significant increases in mean arterial pressure (22.5 ± 5.9 mm Hg significantly different compared to 0.0 ± 0.0 mm Hg after vehicle microinjection). Heart rate changes (32.5 ± 9.3 bpm) following microinjection of 100 pmol kainate were not significantly different from those following saline microinjection (0.0 ± 0.0; $P > 0.95$, ANOVA).

To further characterize the kainate-mediated response, we employed a selective kainate receptor antagonist, DNQX at two doses, 300 and 600 pmol. Either vehicle or DNQX was microinjected 2–3 min before 30 pmol of kainate microinjection into the dorsal vagal complex (Fig. 3). DNQX and vehicle on their own did not produce any significant changes in baseline pressure and motility traces (data not shown). The higher dose of DNQX was microinjected in a volume of 60 nl, due to DNQX's limited aqueous solubility. Vehicle microinjections into the dorsal vagal complex of 30 and 60 nl volumes were performed and no differences were observed, therefore only the ef-

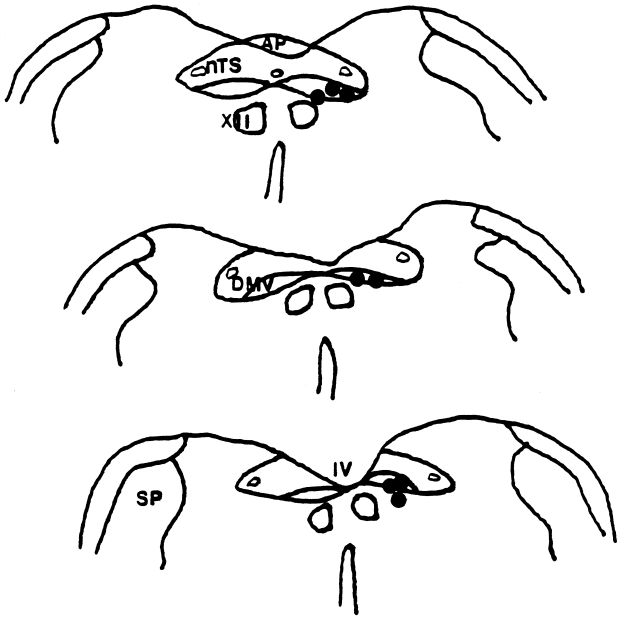

Fig. 4. Compilation of all microinjection sites in the dorsal vagal complex. Coronal sections of the hindbrain approximately −13.7 mm (top), −13.3 mm (middle), and −13.2 mm (bottom) from bregma (Paxinos and Watson, 1986). AP, area postrema; DMV, dorsal motor nucleus of the vagus; nTS, nucleus tractus solitarius; SP, spinal trigeminal tract; XII, hypoglossal nucleus.

Table 1
Effect on peak-intragastric pressure (Peak-IGP; cm of H_2O) and total-intragastric pressure (Total-IGP; cm^2) of different agents microinjected into the dorsal vagal complex

Treatment	*N*	Peak-IGP	Total-IGP
(A) Kainate microinjection			
Vehicle + kainate, 30 pmol	5	6.7 ± 1.7	4.1 ± 1.2
DNQX, 300 pmol + kainate	3	2.0 ± 0.7[a]	1.7 ± 0.6
DNQX, 600 pmol + kainate	5	0.0 ± 0.0[a,b]	-0.1 ± 0.4[a,b]
AP5, 2 nmol + kainate	3	6.5 ± 1.7	3.9 ± 1.2
(B) NMDA microinjection			
Vehicle + NMDA, 100 pmol	6	7.4 ± 2.0	2.9 ± 0.5
AP5, 2 nmol + NMDA	4	0.6 ± 0.7[a,c]	0.3 ± 0.3[a,c]
DNQX, 600 pmol + NMDA	5	6.4 ± 1.5	2.8 ± 0.6

[a] Significantly different from corresponding vehicle + agonist microinjection.
[b] Significantly different from AP5 + kainate.
[c] Significantly different from DNQX + NMDA.
$P < 0.05$, one-way ANOVA.

fects of 60 nl vehicle are shown in Table 1. DNQX dose-relatedly antagonized that gastric motor excitation evoked by kainate (Table 1; Fig. 3). The antagonism of kainate receptors by DNQX was temporally reversible in that at least 40 min following DNQX microinjection, a repeat microinjection of kainate elicited gastric responses that were similar to those obtained by kainate microinjection alone in all the rats tested ($N = 5$; data not shown). However, AP5, a selective NMDA receptor antagonist, microinjected 2–3 min before kainate, did not significantly affect the gastric motor excitation evoked by kainate microinjection (Table 1; Fig. 3). This dose of AP5 effectively abolished the NMDA mediated increases in intragastric pressure (see below).

Peripheral injection of the muscarinic antagonist, atropine methylbromide (1 mg/kg, i.v.) abolished the increase in gastric motor activity evoked by kainate microinjection into the dorsal vagal complex (peak intragastric pressure, 0.20 ± 0.20; total intragastric pressure, 0.03 ± 0.03; $N = 4$). All microinjection sites were located within

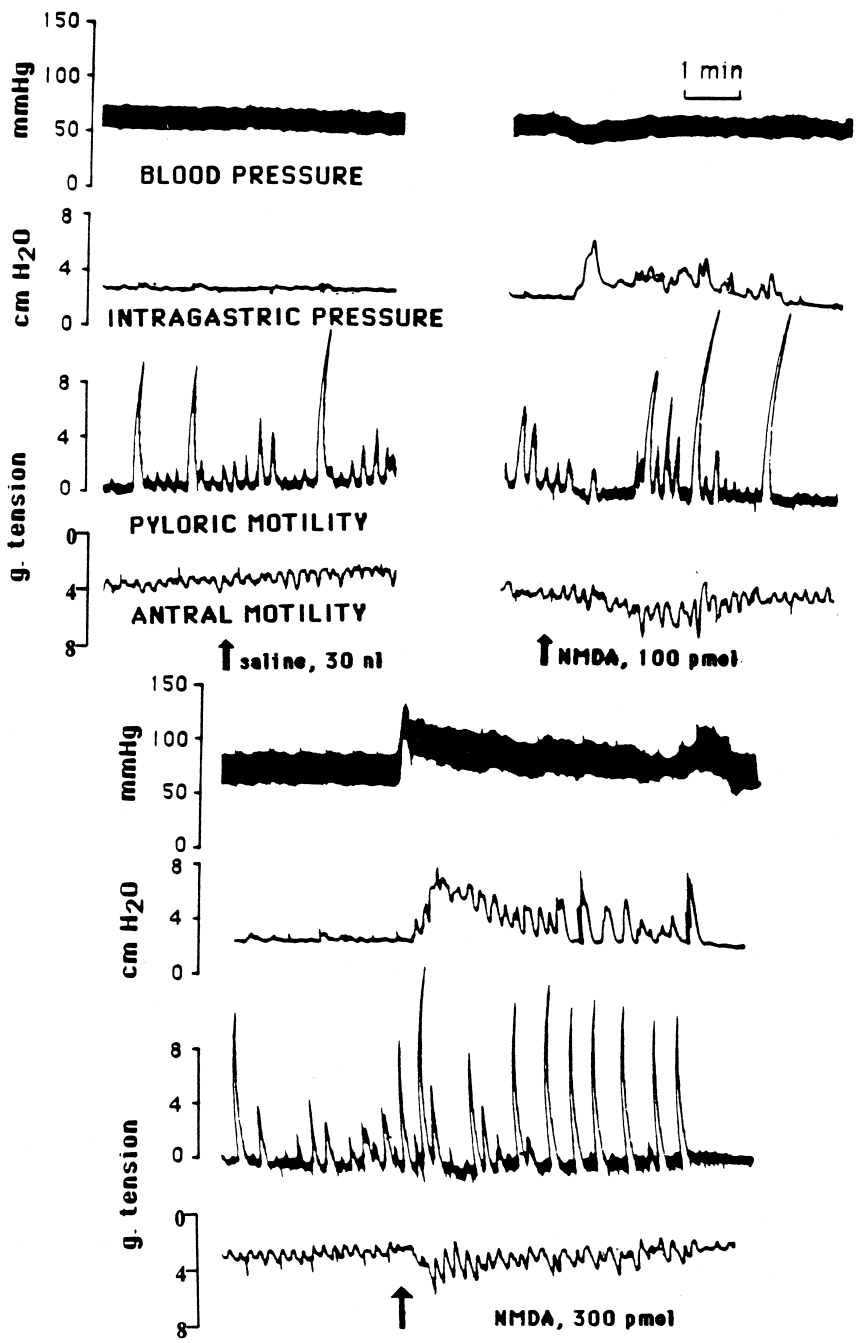

Fig. 5. Sample trace recording from an animal microinjected with vehicle and NMDA into the dorsal vagal complex. Arrows mark the microinjection event.

or close to the ventral border of the dorsal motor nucleus of the vagus (Fig. 4).

3.2. Microinjection studies with NMDA

Typical gastric motor responses obtained following NMDA microinjection into the dorsal vagal complex, are shown in Fig. 5. Microinjection of NMDA (100 pmol) significantly increased intragastric pressure only, whereas the 300 pmol dose produced consistent and significant increases in both intragastric pressure and antral motility (Fig. 6). In preliminary studies, 10 and 30 pmol of NMDA failed to elicit consistent changes in intragastric pressure indices (data not shown). Thus, a 100 pmol dose was the lowest dose tested that produced consistent increases in intragastric pressure, and this dose was employed for all subsequent studies. The cardiovascular effects of NMDA microinjections into the dorsal vagal complex were very variable, and overall, did not attain statistical significance in four animals (mean arterial pressure, 1.2 ± 8.7 mm Hg (100 pmol), 8.7 ± 18.4 mm Hg (300 pmol); heart rate, -14.2 ± 16.6 bpm (100 pmol), -0.7 ± 16.6 bpm (300 pmol) compared to vehicle microinjections (0.0 ± 0.0 mm Hg; 0.0 ± 0.0 bpm).

Microinjection of NMDA (100 pmol) after 2–3 min following microinjection of the selective NMDA receptor antagonist, AP5 (2 nmol), resulted in gastric motor changes that were similar to vehicle microinjection (Fig. 7; Table 1). On the other hand, microinjection of the selective kainate receptor antagonist DNQX prior to NMDA yielded gastric motor excitation that was indistinguishable from NMDA preceded by vehicle (Fig. 7; Table 1). The effects of AP5 on NMDA were temporally reversible in that after 40 min following AP5 microinjection, a repeat NMDA microinjection resulted in a marked gastric excitation (data not shown).

Atropine pretreatment completely abolished the gastric effects of a subsequent microinjection of NMDA (peak-intragastric pressure, -0.3 ± 0.3 cm; AUC, -0.12 ± 0.0 cm^2; $N = 4$). All microinjections were located within the dorsal vagal complex, generally within, or on the ventral border of the dorsal motor nucleus of the vagus (Fig. 4).

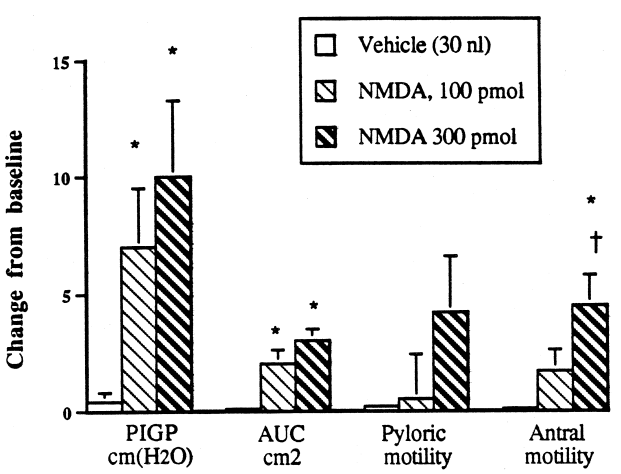

Fig. 6. Compiled data showing the effects on peak-intragastric pressure (PIGP), total-intragastric pressure (AUC), pyloric MMI, and antral MMI of vehicle and NMDA microinjection into the dorsal vagal complex. ∗Significantly different from the vehicle mean of the same index; †Significantly different from lower dose of the same gastric index. Analysis was by repeated measures ANOVA with Newman–Keul post-test comparison for significance ($P < 0.05$).

4. Discussion

We have recently reported that the gastric motor effects of GABAergic disinhibition in the dorsal vagal complex were abolished by a prior microinjection of the broad spectrum excitatory amino acid receptor antagonist, kynurenic acid (Sivarao et al., 1998). Kynurenic acid by itself did not affect the baseline gastric motor activity (Sivarao et al., 1998). In the present study, we show that both kainate and NMDA receptors in the dorsal vagal complex can be selectively activated to evoke vagally-mediated increases in gastric motor function.

Although L-glutamate has been implicated to be the neurotransmitter released by gastrointestinal visceral afferents in the nucleus tractus solitarius (Rogers et al., 1990), most research has focussed on the baroreceptor reflex in this region. However, L-glutamate microinjection in the dorsal vagal complex has been known to produce both inhibitory (Spencer and Talman, 1986a,b) and excitatory (Krowicki et al., 1997; present study) gastric responses. It is likely that L-glutamate microinjection in the nucleus tractus solitarius evokes gastric inhibition (Spencer and Talman, 1986a,b), whereas direct activation of vagal motor neurons in the dorsal motor nucleus results in gastric excitation (Ormsbee et al., 1984; Krowicki et al., 1997). In the present study, we were primarily investigating the effects of excitatory amino acid receptor activation of vagal motor neurons in the dorsal motor nucleus of the vagus. This is because the functional response to L-glutamate microinjection was gastric excitation. The localization of the microinjection tip within the dorsal motor nucleus of the vagus in the majority of our experiments supports this contention. However, we cannot rule out the possibility that the agonists were acting both at the level of the nucleus tractus solitarius and the dorsal motor nucleus of the vagus.

In the dorsal motor nucleus of the vagus, perivagal stimulation causes NMDA- and non NMDA-mediated excitation of vagal motor neurons (Travagli et al., 1991). Subsequent reports confirmed this observation and provided evidence that the source of this input was from neurons in the nucleus tractus solitarius (Willis et al., 1996; Bertolino et al., 1997). Willis and his co-investigators further characterized this input to activate NMDA and

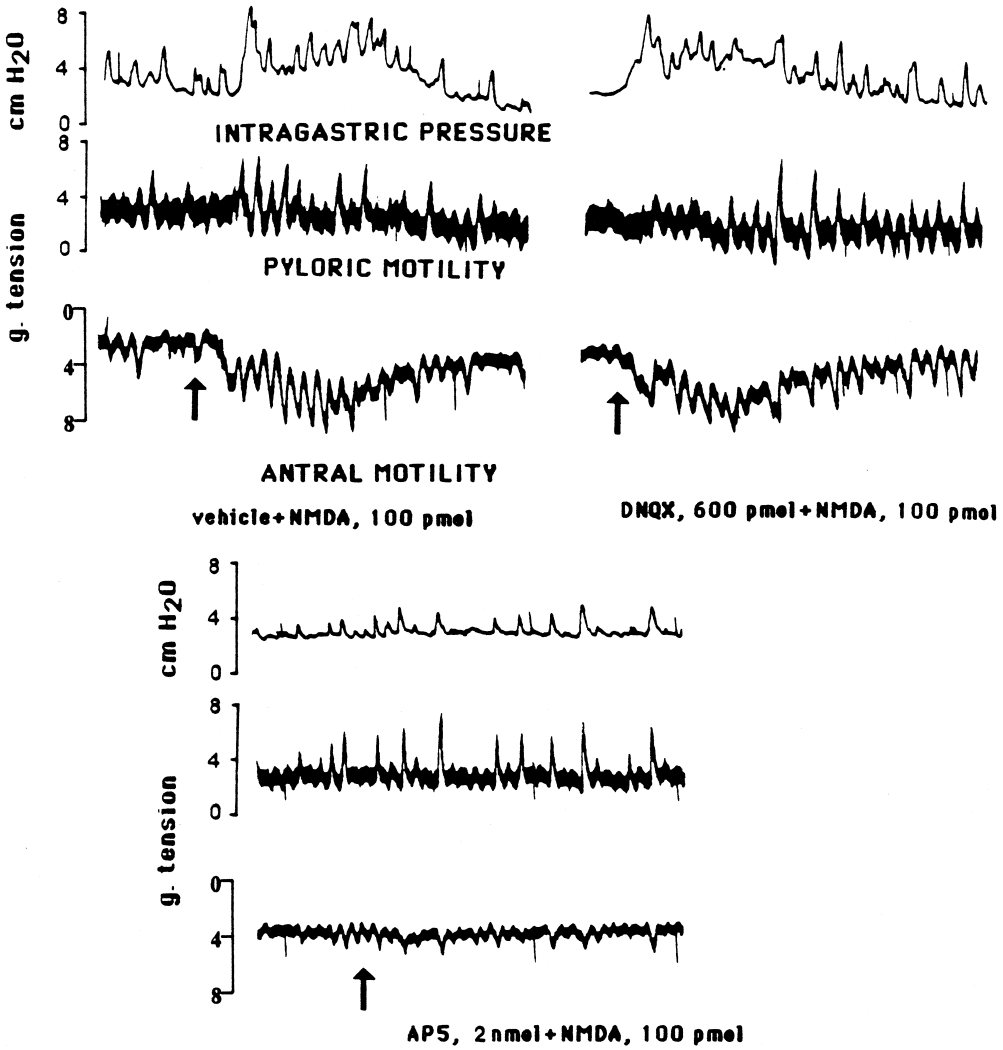

Fig. 7. Sample trace recording from an animal microinjected into the dorsal vagal complex with NMDA immediately following vehicle, DNQX or AP5 microinjection. Arrows mark the microinjection event.

kainate/AMPA receptors (Willis et al., 1996). Since these reports utilized a 'reduced' slice preparation, it was not possible to demonstrate a functional consequence to this excitation. The present study supports the idea that excitatory amino acid inputs from the nucleus tractus solitarius mediate vagal gastric motor excitation via both NMDA and kainate receptors in vagal motor neurons.

Since kainate and NMDA have been both previously employed to cause localized lesioning and gliosis in the CNS, a question may be raised as to whether the responses observed were due to excitotoxicity of these agents. For lesioning, both kainate (Schwarcz et al., 1978) and NMDA (Vasquez et al., 1992; Varner et al., 1994) are used at ≥ 300 times the currently employed doses. Previous reports that employed chemical stimulation of hindbrain raphe nuclei to obtain increases in gastric acid (Yang et al., 1993; Yang and Taché, 1995), and pancreatic hormone secretions (Krowicki and Hornby, 1995b), did so at typically higher doses of kainic acid, without observing any adverse effects. It is unlikely that excitotoxicity occurred at the doses employed in our study since L-glutamate was again microinjected at the end of each experiment to demonstrate that gastric motor excitation could still be evoked.

In our experiments, blood pressure increased after kainate (100 pmol) microinjection into the dorsal vagal complex. It is therefore possible that the observed increase in intragastric pressure and motility was secondary to this hypertension. This type of relationship between blood pressure and visceral motility has been reported previously (Chen et al., 1993). However, in this case, hypertension is unlikely to be causally related to the increase in intragastric pressure, since a lower dose of kainic acid increased gastric motor activity without increasing the blood pressure.

The observation that kainate microinjection into the dorsal vagal complex produced an increase in mean arterial pressure was by itself intriguing to us since microinjec-

tion of kainate (0.1–10 pmol) in the nucleus tractus solitarius evokes hypotension, mimicking the baroreceptor reflex (Talman et al., 1980; Reis et al., 1981; Galloudec et al., 1989). Yet, there is evidence to show that selective activation of commissural subnuclei of the caudal nucleus tractus solitarius, at the level of calamus scriptorius, causes hypertension, perhaps akin to the carotid chemoreceptor activation (Vardhan et al., 1993). In awake rats, this hypertensive effect seems to be the dominant response, overriding the baroreceptor reflex-like hypotension (Colombari et al., 1994; Colombari et al., 1996). Since our injections were centered in the dorsal motor nucleus of the vagus, it is possible that, when the higher dose of kainate was administered, it spread into this region in the nucleus tractus solitarius. Alternately, since the hypotensive responses in the nucleus tractus solitarius are obtained from typically 10–20 fold lower doses of kainate (Leone and Gordon, 1989), there may be a low affinity pressor site in the dorsal vagal complex that activates sympathetic hypertensive neurons when a high dose of kainate is encountered. These are only speculations that need to be tested in future studies.

The evidence for a role of NMDA receptors in baroreceptor reflexes mediated by the nucleus tractus solitarius is more controversial. In some studies, there is evidence for their participation (e.g., El-Mas and Abdel-Rahman, 1993; Ohta and Talman, 1994), whereas in others, they seem less relevant than non-NMDA receptors (e.g., Gordon and Leone, 1991). Our own experiments reflect this confusion since, overall, the cardiovascular responses to NMDA were not significant, but we noted very variable responses in individual cases. This is typified by the example in Fig. 5 where the low dose of NMDA evoked hypotension, whereas the higher dose in the same animal evoked a marked hypertension. We have no explanation for these disparate responses. Since our site of microinjection was primarily in the dorsal motor nucleus of the vagus we can speculate that differential diffusion from the pipette tip into the adjacent nucleus tractus solitarius might have evoked differing responses in individual cases.

While characterizing the involvement of different subtypes of glutamate receptors, it is especially important that selectivity of a particular agonist be demonstrated unequivocally as glutamate receptor subtypes have been known to co-localize in the same synapse (Bekkers and Stevens, 1989; Greenamyre and Young, 1989). Both DNQX and AP-5 antagonized their respective subtype agonists only, thus suggesting that the doses of both agonists and antagonists employed were subtype-selective.

The possibility of multiple glutamate receptors mediating a complex motor function such as gastrointestinal motility is not new. For example, in the initiation of the swallowing reflex, much evidence exists to suggest that the pattern generator in the nucleus tractus solitarius is activated both by NMDA and non-NMDA type receptors. Each subtype has been shown to confer a different firing pattern on the nucleus tractus solitarius neurons in brain slices (Tell and Jean, 1990), while triggering the swallowing reflex in anesthetized rats (Kessler et al., 1990). In the present study, it is not possible to analyze such differences based on intragastric pressure and extraluminal force traces. However, it is still an intriguing question as to why a multitude of receptor subtypes are required for mediating an apparently similar outcome—that is, increased gastric motility. Simplistically, it may be speculated that each subtype may trigger a different pattern of gastric motility; for example mixing vs. propulsive movements. Alternately, kainate and other subtype receptors like AMPA may mediate fast transmission between premotor nerve terminals and vagal motor neurons while NMDA may be involved in long term facilitation or depression of this transmission. Other investigators have reported that GABAergic disinhibition in the dorsal vagal complex causes an increase in gastric motor as well as secretory activity (Feng et al., 1990; Washabau et al., 1995). It is conceivable that different glutamate receptor subtypes in the dorsal vagal complex may differentially activate gastric secretion and motility and thus provide a framework for glutamate receptor diversity in regulating gastric function.

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