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NMDA receptors are involved at the ventrolateral nucleus tractus solitarii for termination of inspiration ☆

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Abstract

The purpose of the present study was to determine whether blockade of excitatory amino acid receptors at the ventrolateral nucleus of the tractus solitarius would influence respiratory activity. This was done by microinjecting excitatory amino acid receptor antagonists into the ventrolateral nucleus of the tractus solitarius of α-chloralose-anesthetized animals while monitoring respiratory activity using a Fleisch pneumotachograph and arterial blood pressure and heart rate. Bilateral microinjection of the NMDA receptor antagonist, 3-[(*R*)-carboxypiperazin-4-yl]-propyl-1-phosphomic acid (CPP), 5.62 nmol per side, produced an increase in inspiratory duration ($+4 \pm 1.6$ s, $n = 8$) which progressed to an apneustic pattern of breathing. Similar results were obtained with CPP microinjected into the ventrolateral nucleus of the tractus solitarius of three vagotomized animals. Bilateral microinjection of a second NMDA receptor antagonist, 2-amino-7-phosphono-heptanoic acid (AP7), 562 nmol per side, produced qualitatively similar effects on respiration as seen with CPP. In contrast, blockade of non-NMDA receptors with 6-cyano-7-nitroquinoxaline-2,3-dione (CNXQ), 0.125 nmol per side, had very little effect on respiration. Activation of NMDA receptors at the ventrolateral nucleus of the tractus solitarius with bilateral microinjection of NMDA, 39 pmol, produced a large increase in expiratory duration ($+11 \pm 3$ s, $n = 8$), and apnea during the expiratory phase of the respiratory cycle in half of the animals studied. Similar results were obtained with D,L-α-amino-3-hydroxy-5-methyl-4-isoxazol-proprionate (AMPA). These results indicate that an endogenous excitatory amino acid released at the ventrolateral nucleus of the tractus solitarius and acting at the NMDA receptor, plays a significant role in respiratory timing.

Keywords: Excitatory amino acid; Respiratory activity; Ventrolateral nucleus of the tractus solitarius; NMDA receptor; Apneusis

1. Introduction

We recently reported that i.v. administration of MK-801, a non-competitive antagonist of the NMDA receptor complex, produces an increase in inspiratory duration and apneustic breathing in anesthetized cats (Abrahams et al., 1988, 1993). Similar results have been reported by other investigators in cats (Foutz et al., 1988a,b), and with systemic administration of other antagonists of NMDA receptors (Foutz et al., 1988a, 1989). According to Foutz and colleagues (1988a), this effect of NMDA receptor antagonists appears to occur in the central nervous system (CNS). In an attempt to elucidate where blockade of NMDA receptors in the CNS acts to provoke such striking changes in breathing

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we initially examined the ventrolateral medulla for sites where excitatory amino acids are involved in respiratory control (Abrahams et al., 1991). Although we did not uncover any sites where apneusis can be brought about, we did find a site in the ventrolateral medulla, specifically in the caudal subretrofacial area, where an excitatory amino acid is responsible for maintaining normal breathing.

In continuing to pursue the site (or sites) in the CNS where blockade of NMDA receptors results in apneustic breathing, we have focused on the dorsal side of the brain, and on the dorsal respiratory group. The dorsal respiratory group traditionally refers to neurons of the ventrolateral nucleus of the tractus solitarius (Berger, 1977). The reasons for examining this site are based on the findings of other investigators indicating that apneustic breathing can result from perturbations in the activity of dorsal respiratory group neurons. These perturbations were produced by anatomic lesions, local cooling and local administration of lidocaine and neurotensin (Koepchen et al., 1985; Budzinska et al., 1985; Morin-Surun et al., 1986). Hence, the hypothesis tested in our study was that antagonists of the NMDA receptor act in the dorsal respiratory group to prolong inspiratory duration and to cause apneustic breathing.

2. Materials and methods

Experiments were performed on adult cats (2.5–4.0 kg) unselected as to sex. Anesthesia was induced with α-chloralose (80 mg/kg i.v.). Chloralose was chosen because it is the anesthetic we have employed in our previous studies (Abrahams et al., 1991, 1993, 1994; McManigle et al., 1994) on the effects of excitatory amino acid antagonists on respiratory function, and the purpose of the present study was to extend our findings of the earlier studies. A femoral artery and vein were cannulated for measurement of arterial blood pressure and systemic administration of drugs, respectively. In three animals, the cervical vagus nerves were isolated, and bilateral section of these nerves was performed. Heart rate was measured from electrocardiographic tracings. Rectal temperature was monitored and maintained between 37°C and 38°C by an infrared heating lamp.

The trachea of each cat was cannulated and fitted with a number 0 Fleisch pneumotachograph connected to a respiratory flow transducer (HP47630A, Hewlett-Packard, Waltham, MA, USA). The airflow signal obtained was integrated (HP8815A, respiratory integrator) to obtain tidal volume and respiratory minute volume. Respiratory rate was obtained from slow tracings (i.e., 1 or 2.5 mm/s paper speed) of the tidal volume recording, and over a period of 20 s to 1 min. Inspiratory and expiratory durations were obtained from fast tracings (i.e., 25 mm/s paper speed) of the tidal volume recording, and values for at least three respiratory cycles were averaged. End-tidal CO_2 was measured with a CO_2 infrared analyzer (Datex Airway Gas monitor PB model 252, Datex Medical Instrumentation, Tewksbury, MA, USA). All indices of respiratory and cardiovascular function were recorded continuously on an eight-channel recorder (HP model 7758B).

The animal was placed in a stereotaxic holding device with the dorsal portion of the brain uppermost, and the dorsal surface of the medulla was exposed as previously described (Pagani et al., 1984). Briefly, the dorsal surface of the brainstem was exposed by limited occipital craniotomy. The dura was cut and reflected to expose the surface of the medulla and allow the cerebrospinal fluid to drain. The medial-lateral and anterior-posterior coordinates of the obex were identified and the obex was used as a reference point for the placement of micropipettes.

Double-barrelled (ID 0.3 mm; FHC, New Brunswick, ME, USA) glass micropipettes were pulled, and the tips cut to approximately 15–20 μm outside diameter. Drugs were dissolved in 0.9% saline with 1% Fast Green added to the saline solution to mark the injection site. The pH of the drug solutions was adjusted to 7.30–7.40 before use. The solutions were drawn into the micropipette with a negative air pressure inside the micropipette produced by a syringe attached to PE-90 tubing. Next, a volume of injectate ranging from 50 to 125 nl (usually 50 nl) was microinjected into the ventrolateral nucleus of the tractus solitarius by a syringe through watching the meniscus move a calibrated distance within the micropipette, as indicated from Formaline Tape (type 900GB, Graphic Products Corp., Rolling Meadows, IL, USA) placed on the side of the micropipette and used as a guide. The coordinates for microinjection into the ventrolateral nucleus of the tractus solitarius used in our experiments were 1.2 mm rostral from obex, 2.6 mm lateral to the midline, and 1.7 mm below the dorsal medullary surface. Microinjection studies were also performed in sites adjacent to the ventrolateral nucleus of the tractus solitarius. One site was the medial nucleus of the tractus solitarius, and the coordinates used in targeting this site were 1.2 mm rostral from obex, 1.3 mm lateral to the midline, and 0.3 mm below the dorsal medullary surface. The other site was the dorsal motor nucleus of the vagus, and the coordinates used in targeting this site were 1.2 mm rostral from obex, 1.5 mm lateral to the midline, and 0.7 mm below the dorsal medullary surface.

Experiments were performed as follows: (1) the micropipettes were inserted bilaterally into the area of the ventrolateral nucleus tractus solitarius; (2) after obtaining a stable baseline of readings of indices of cardiorespiratory function, either vehicle (saline con-

Table 1
Respiratory effects produced by microinjection of excitatory amino acid antagonists into the ventrolateral nucleus tractus solitarius [a]

Dose of drugs and No. of animals studied (n)	Experimental condition	Respiratory indices measured							Cardiovascular indices measured	
		Respiratory minute volume (ml/min)	Tidal volume (ml)	Respiratory rate (breathes/min)	Inspiratory duration (s)	Expiratory duration (s)	Total cycle duration (s)	End-tidal CO_2 (%)	Mean blood pressure (mm Hg)	Hear rate (beats/min)
CPP	Control	265±14	33 ±3	8 ±0.7	2.1±0	5.3±0.5	7.4±0.5	6.1 ±0.2	84 ± 8	160±12
5.62 nmol/side	Maximal change after bilateral injection	−75±14 [b]	+11 ±5	−4 ±0.7 [b]	+4 ±1.6 [b]	+1.8±0.7 [b]	+6 ±2 [b]	+1.3 ±0.2 [b]	+6 ± 7	+4± 6
$n = 8$										
0.9% NaCl	Control	250±30	31 ±8	8 ±1	2.1±0	6 ±1	8.1±1	6.5 ±0	89 ± 3	185±13
$n = 3$	Maximal change after bilateral injection	+19± 3	+1 ±1	+0.7±0.3	0 ±0	0 ±0	0 ±0	+0.3 ±0.1	−1 ± 4	−5±10
CPP	Control	278±30	40 ±5	7.0±0.6	3.2±0.3 [b]	6.3±1.4	9.5±1.2	5.6 ±0.4	118 ±12	220±40
5.62–14.0 nmol/side vagotonized cats	Maximal change after bilateral injection	−122±23 [b]	+7.7±2.0	−3.7±0.3 [b]	+9.1±1.2 [b]	+0.0±0.5	+9.1±1.5 [b]	+2.2 ±0.6	+2.7± 5.6	0± 0
$n = 3$										
CNQX	Control	413±92	37 ±0.7	11 ±2	1.7±0.3	4 ±1	5.8±1	5.8 ±0.6	108 ±13	205±33
0.124 nmol/side	Maximal change after bilateral injection	+13±39	+3 ±2	−1 ±0	+0.3±0.3	+0.3±0.3	+0.6±0.5	+0.03±0.3	+1 ±0.6	+2± 4
$n = 3$										

[a] Values are means±S.E.M.; [b] $P < 0.05$ using Student's t-test for paired data.

taining Fast Green dye) or drug was microinjected bilaterally; and (3) in agonist-antagonists studies, the agonist (either NMDA or AMPA) was microinjected unilaterally into the ventrolateral nucleus tractus solitarius to obtain two reproducible control responses (i.e., responses were obtained within 10–20 min of each other and did not vary from each other by more than approximately 10%). Following control responses, time was allowed for complete recovery from respiratory depression (typically 15–20 min). The antagonist (CPP, AP7 or CNQX) was then microinjected unilaterally followed in 2 min by microinjection of the agonist at the same site in the ventrolateral nucleus tractus solitarius. Doses of drugs employed were in the same range as the doses that were used successfully in our earlier studies (Abrahams et al., 1991, 1994).

The drugs used in these microinjection studies were as follows: 3-[(*R*)-carboxypiperazin-4-yl]-propyl-1-phosphonic acid (CPP) (obtained from Tocris Neuramin, Essex, UK); 2-amino-7-phosphono-heptanoic acid (AP7) (obtained from Tocris Neuroamin); *N*-methyl-D-aspartate (NMDA) (obtained from Sigma Chemical Co., St. Louis, MO, USA); D,L-α-amino-3-hydroxy-5-methyl-4-isoxazol-proprionate (AMPA) (obtained from Tocris Neuroamin); 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) (obtained from Tocris Neuroamin); and Fast Green FCF (obtained from Sigma).

On completion of the experiment, brains were removed and fixed for at least 24 h in 6% buffered paraformaldehyde. The tissue was then transferred to 20% sucrose in phosphate-buffered saline for 24 h before sectioning at 50 μm. Sections were counterstained with neutral red to facilitate identification of nuclear groups, and the site of microinjection was determined as a bright green spot, with tissue damage marking the pipette tract in some cases.

Data are presented as the means ± the standard error of the mean and were taken during the control periods and at the time of peak changes and/or just before apneusis or apnea occurred. Statistical analysis was performed using the Student's paired *t*-test with $P < 0.05$ being the criterion for statistical significance.

3. Results

3.1. Effects of microinjections of excitatory amino acid receptor antagonists into the ventrolateral nucleus tractus solitarius

Our first step in examining whether an excitatory amino acid is an important neurotransmitter controlling respiratory neurons located at the ventrolateral nucleus tractus solitarius was to microinject antagonists of excitatory amino acids into the ventrolateral nucleus tractus solitarius while monitoring respiratory activity. Three antagonists were tested and consisted of the NMDA receptor antagonists CPP and AP7, and the non-NMDA receptor antagonist CNQX. Data obtained using CPP given bilaterally in a dose of 5.62 nmol per side into the ventrolateral nucleus tractus solitarius are tabulated in Table 1. Bilateral microinjection of CPP into the ventrolateral nucleus tractus solitarius produced striking changes in respiratory activity. Inspiratory duration was increased, and an apneustic pattern of breathing was noted in each animal tested.

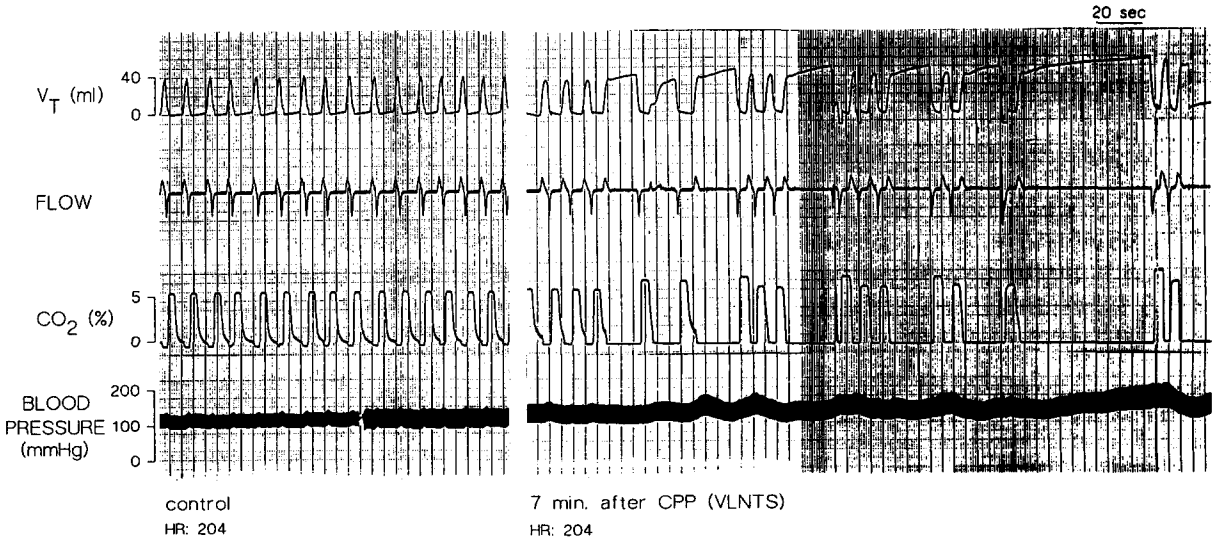

Fig. 1. The effect of CPP (5.62 nmol/site) microinjected into the ventrolateral nucleus tractus solitarius bilaterally on V_t, tracheal air flow (flow), $FECO_2$ (CO_2), blood pressure and heart rate (HR). Data shown are data obtained just prior to microinjecting CPP, and data obtained at the time of the peak effect of CPP, i.e., 7 min post-injection.

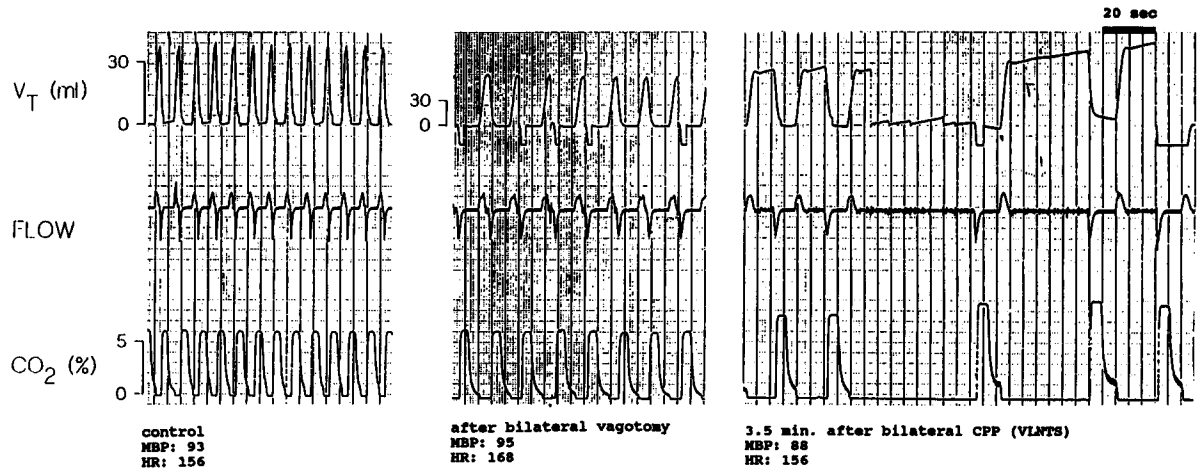

Fig. 2. The effect of CPP (5.62 nmol/site) microinjected into the ventrolateral nucleus tractus solitarius (VLNTS) bilaterally on V_t, tracheal air flow (flow), $FECO_2$ (CO_2), mean blood pressure (MBP) and heart rate (HR) of an animal subjected to bilateral cervical vagotomy. Data shown are data obtained just prior to bilateral cervical vagotomy, immediately after bilateral cervical vagotomy, and at the time of the peak effect of CPP, i.e., 3.5 min post-microinjection.

Inspiratory 'holds' of 15–80 s in duration were observed. Expiratory duration was also increased but the increase was only about one-half that observed for inspiratory duration. As expected, with increases in both inspiratory and expiratory durations, there was a decrease in respiratory rate. Microinjection of CPP resulted in a decrease in respiratory minute volume and an increase in end-tidal CO_2. No statistically sig-

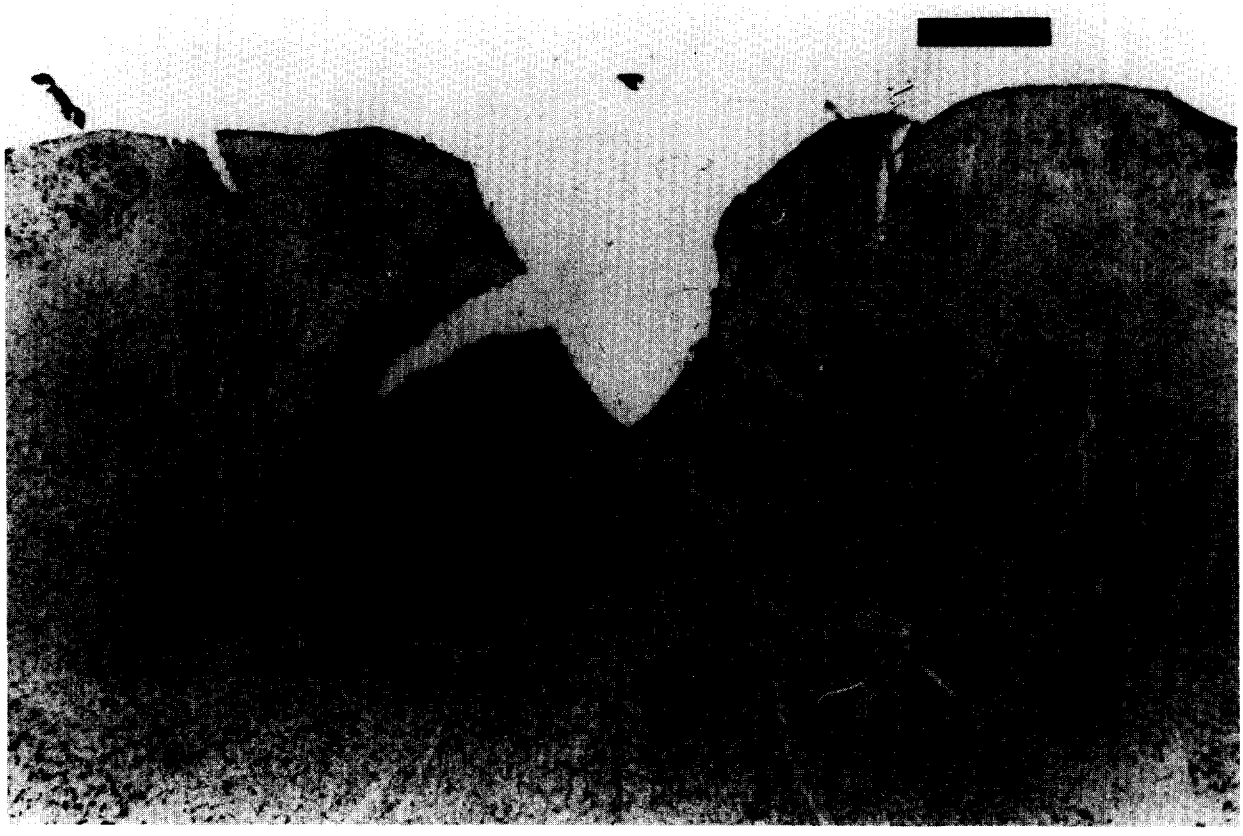

Fig. 3. Photomicrograph of the cat medulla where bilateral microinjection of 5.62 nmol of CPP resulted in the responses illustrated in Fig. 2. The tip of the micropipette is indicated on each side of the medulla by the presence of the dye in the injectate. T = tractus solitarius; X = dorsal motor nucleus of the vagus; XII = hypoglossal nucleus. Bar = 1 mm.

nificant effects were noted either on tidal volume, blood pressure or heart rate. In terms of time course of action, the peak effects of CPP were seen at 6.3 ± 1.8 min after the first microinjection of drug, and the effect persisted for the duration of the experiment (i.e., for another 1–2 h).

A representative experiment demonstrating the respiratory effects and time course of action of bilateral microinjection appears as Fig. 1. As can be noted, peak effects of CPP occurred at 7 min post-injection of CPP and were manifested as inspiratory 'holds' ranging from approximately 12 s to 60 s in duration.

Three control experiments were also carried out where bilateral microinjections of the vehicle for CPP, i.e., 0.9% NaCl solution, were tested at the ventrolateral nucleus tractus solitarius. Data obtained are tabulated in Table 1 and indicate no significant respiratory effects of the vehicle in the same volume as employed for the CPP studies.

Additional experiments were performed with bilateral microinjections of CPP administered into the ventrolateral nucleus tractus solitarius of animals with cervical vagus nerves sectioned (Table 1). The specific hypothesis that was tested was that CPP-induced apneusis did not require afferent vagal input arising from peripherally located receptors such as pulmonary stretch receptors. Data obtained in the three animals tested indicate that microinjection of CPP into the ventrolateral nucleus tractus solitarius of vagotomized animals also prolongs inspiratory duration, and produces an apneustic pattern of breathing (Fig. 2). A representative experiment demonstrating CPP-induced apneustic breathing in a vagotomized animal appears as Fig. 2, and the site of microinjection into the ventrolateral nucleus tractus solitarius is illustrated in Fig. 3.

In two animals we purposely set out to microinject CPP into sites near the ventrolateral nucleus tractus solitarius but not in the ventrolateral nucleus tractus solitarius. In the first experiment our target site was the medial nucleus tractus solitarius. Microinjection of CPP bilaterally into this target area resulted in placing the drug in the medial nucleus tractus solitarius on the left side of the medulla, an area about 1.3 mm medial from the ventrolateral nucleus tractus solitarius (Fig. 4), and in the dorsal motor nucleus vagus on the right side of the medulla, an area about 1.1 mm medial from the ventrolateral nucleus tractus solitarius (Fig. 4). A dose of 14.0 nmol CPP microinjected into these sites

Fig. 4. Photomicrograph of the cat medulla where CPP was bilaterally microinjected in the experiment illustrated as Fig. 5. On the left side of the medulla, the pipette tip is located in the medial nucleus of the tractus solitarius, while on the right side of the medulla, the pipette tip is located in the dorsal motor nucleus of the vagus. T = tractus solitarius; XII = hypoglossal nucleus. Bar = 1 mm.

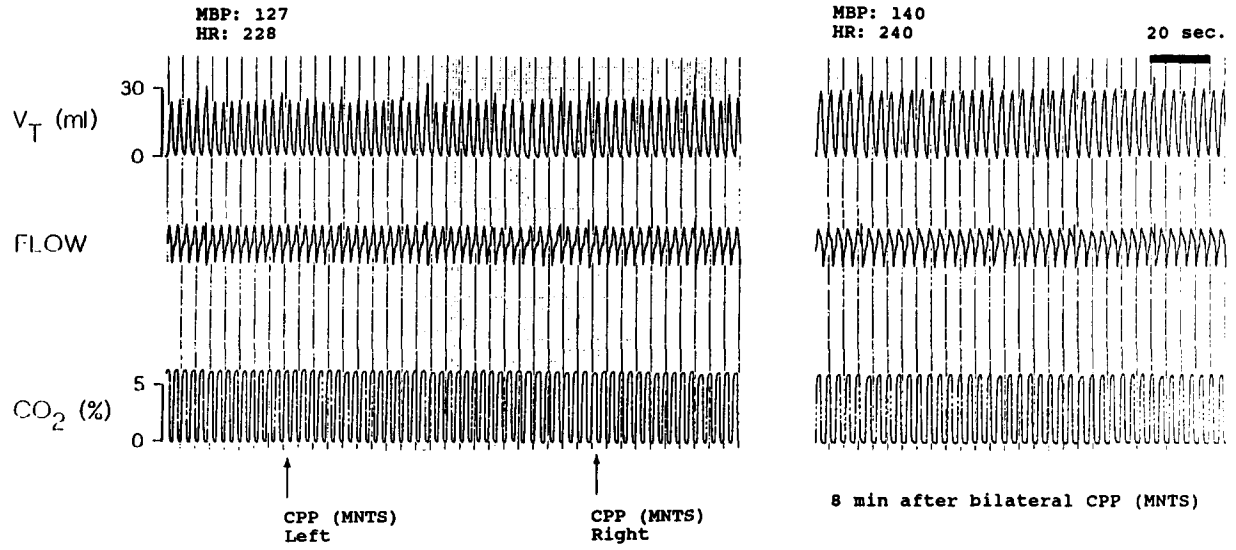

Fig. 5. Lack of effect of CPP (14 nmol/site) microinjected bilaterally into medullary areas outside of the ventrolateral nucleus tractus solitarius (VLNTS) on V_t, tracheal air flow (flow), $FECO_2$ (CO_2), mean blood pressure (MBP) and heart rate (HR). Data shown are data obtained just prior to microinjecting CPP, and data obtained 8 min post-microinjection.

had no significant effect on respiratory activity over the time period where similar microinjections into the ventrolateral nucleus tractus solitarius were found to elicit apneustic breathing (Fig. 5). In the second experiment, our target site was the dorsal motor nucleus of the vagus, and microinjection of CPP (5.62 nmol) bilaterally into this target area resulted in placing the drug in the dorsal motor nucleus of the vagus on the right side of the medulla and just below the hypoglossal nucleus on the left side of the medulla. CPP microinjections had no effect on respiratory activity over the time period where similar microinjections into the ventrolateral nucleus tractus solitarius were found to elicit apneustic breathing.

In another series of experiments, AP7 was microinjected bilaterally into the ventrolateral nucleus tractus solitarius of three animals. AP7, in a dose of 562 nmol/side, produced respiratory effects that were

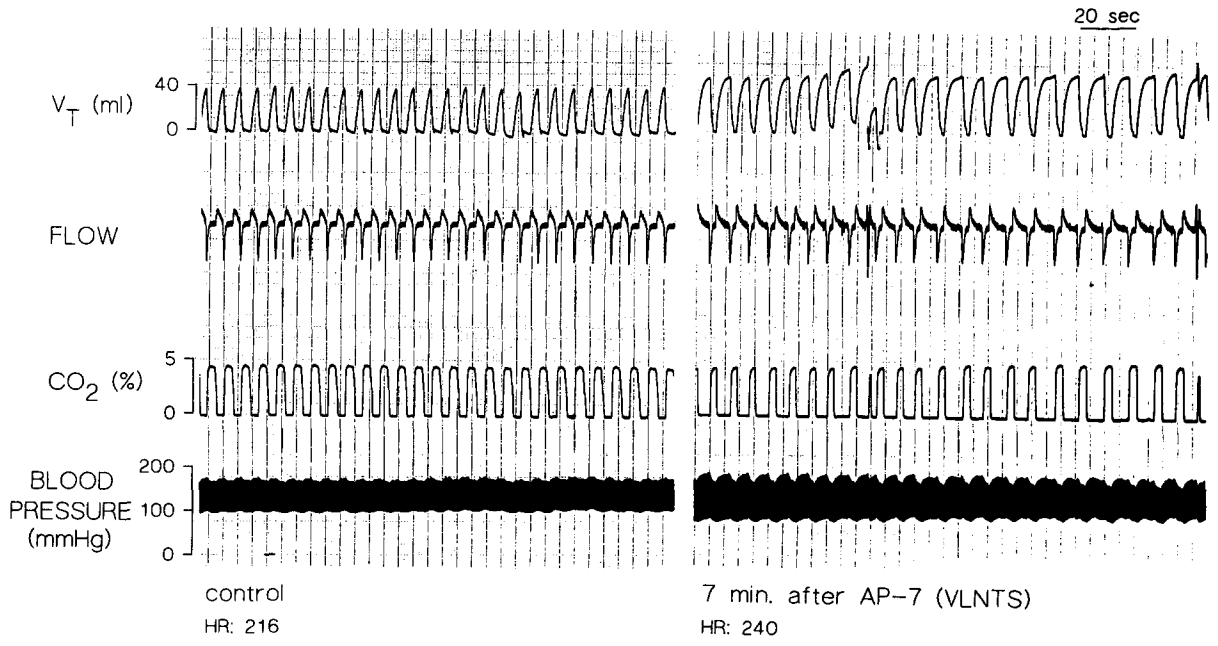

Fig. 6. The effect of AP7 (562 nmol/site) microinjected into the ventrolateral nucleus tractus solitarius (VLNTS) bilaterally on V_t, tracheal air flow (flow), $FECO_2$ (CO_2), blood pressure (BP) and heart rate (HR). Data shown are data obtained just prior to microinjecting AP7, and data obtained at the time a distinct effect of AP7 was present, i.e., 7 min post-microinjection.

qualitatively similar to those observed with CPP. Prolongation of inspiratory duration, inspiratory 'hold' and apneusis were observed. For example, in the first experiment, apneusis with gasps was noted 11 min after AP7 was microinjected. This was preceded by a prolongation in inspiratory duration. In the second experiment, inspiratory 'holds' of 15–30 s duration were noted approximately 2 min after the drug was microinjected. In the third experiment, inspiratory duration was prolonged 3.5 min after AP7 was microinjected, and this was followed by inspiratory 'holds' of 20–30 s duration. An experiment illustrating AP7-induced prolongation of inspiratory duration appears as Fig. 6.

Next, we tested the effects of microinjection of CNQX into the ventrolateral nucleus tractus solitarius of three animals. The dose used was 0.125 nmol per side and the data obtained are tabulated in Table 1. In contrast to CPP and AP7, CNQX given bilaterally produced no significant effects on respiratory activity. That is, over the time period where similar microinjections of CPP into the ventrolateral nucleus tractus solitarius were found to elicit apneustic breathing, no changes in inspiratory, expiratory, and total respiratory cycle durations, end-tidal CO_2, respiratory minute volume, respiratory rate, tidal volume, blood pressure and heart rate were observed in animals receiving CNQX.

It could be argued that the dose of CNQX used, i.e., 0.125 nmol, was too small to produce blockade of non-NMDA receptors in the ventrolateral nucleus tractus solitarius. To test this possibility, experiments were carried out wherein 0.125 nmol of CNQX was tested against AMPA-induced respiratory depression elicited from the ventrolateral nucleus tractus solitarius. AMPA-induced depression of respiration evoked by unilateral microinjection of 25 pmol of drug given unilaterally into the ventrolateral nucleus tractus solitarius appears as Fig. 7, and is described more fully in the next part of the Results section. Fig. 8 illustrates an experiment in which CNQX, 0.125 nmol, was microinjected unilaterally into the ventrolateral nucleus tractus solitarius, and completely prevented the respiratory depressant effect of subsequent administration of AMPA microinjected into the same site. Three experiments of this type were performed, and the data are tabulated in Table 2. As can be noted, in each case, CNQX, 0.125 nmol, microinjected into the ventrolateral nucleus tractus solitarius prevented the respiratory depressant effect of a subsequent administered dose of

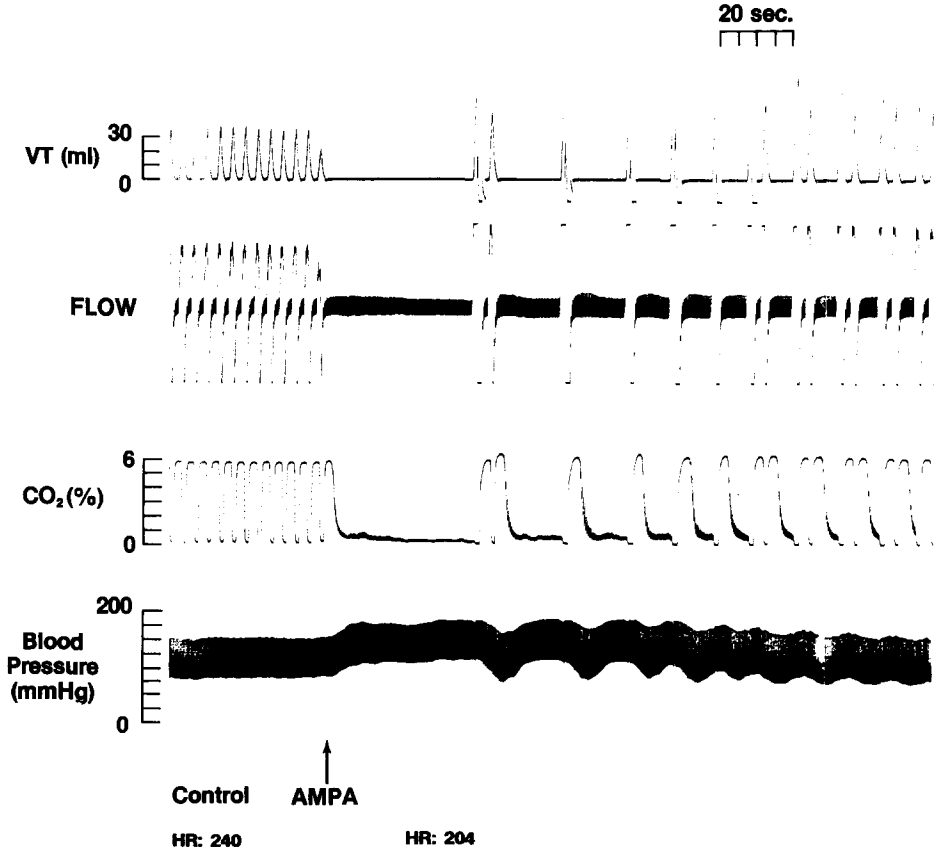

Fig. 7. The effect of AMPA (25 pmol/side) microinjected into the ventrolateral nucleus tractus solitarius unilaterally on V_t, tracheal air flow (flow), $FECO_2$ (CO_2), blood pressure and heart rate (HR).

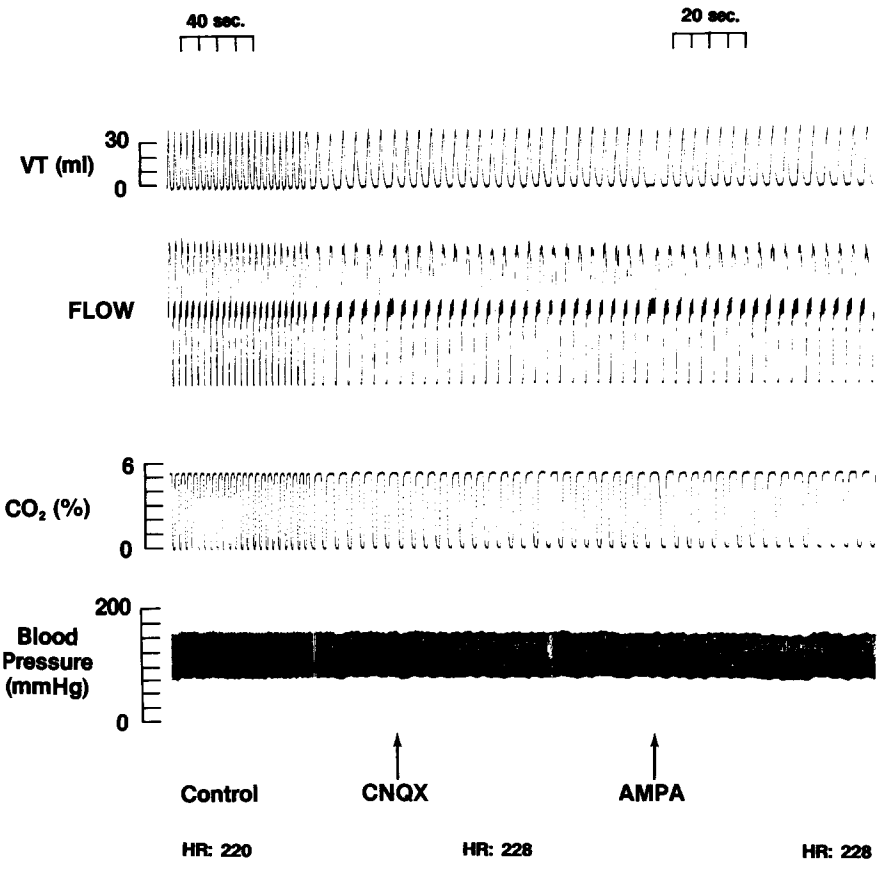

Fig. 8. The effect of CNQX (0.125 nmol/side) microinjected into the ventrolateral nucleus tractus solitarius unilaterally on subsequent administration of AMPA microinjected into the same site. VT = tidal volume (V_t); flow = tracheal air flow; CO_2 = end-tidal CO_2; HR = heart rate.

AMPA microinjected into the same site of the ventrolateral nucleus tractus solitarius.

3.2. Effects of microinjections of NMDA and AMPA into the ventrolateral nucleus tractus solitarius

Based on the positive data obtained using specific antagonists of the NMDA receptor, we then studied the spectrum of respiratory effects produced by microinjection of NMDA into the ventrolateral nucleus tractus solitarius. Bilateral microinjection of NMDA (39 pmol/side) into the ventrolateral nucleus tractus solitarius of eight animals produced striking changes in respiratory activity. The most striking change was an increase in expiratory duration (Table 3). Apnea ranging in duration from 35 to 80 s was observed in four of the eight animals studied. However, in contrast with the results obtained with CPP (and AP7), apnea occurred in expiration. No significant change in inspiratory duration was noted. The increase in expiratory duration was reflected by a decrease in respiratory rate. Respiratory minute volume was noted to decrease. On the other hand, there was an increase in tidal volume. End-tidal CO_2 was observed to increase. No changes in blood pressure occurred but heart rate was noted to increase slightly ($P < 0.05$). All of these events began to occur immediately after microinjection of NMDA into the ventrolateral nucleus tractus solitarius. Peak effects occurred at 2.8 ± 0.9 min.

A representative experiment demonstrating the respiratory effects and time course of action of bilateral microinjection of NMDA appears as Fig. 9. As can be noted, effects of NMDA occurred right after microinjection into the ventrolateral nucleus tractus solitarius, and were manifested as prolongation of expiratory duration and an increase in tidal volume.

In assessing whether the dose of CNQX tested in our study for ventrolateral nucleus tractus solitarius effects was effectively blocking non-NMDA receptors, several experiments were carried out using the non-NMDA receptor agonist drug AMPA (see Figs. 7 and 8). In these studies AMPA was used as a unilateral microinjection into the ventrolateral nucleus tractus solitarius. As can be noted in Fig. 7, AMPA produces a pronounced decrease in respiratory rate due primarily to an increase in expiratory duration, and a significant decrease in respiratory minute volume. All of these changes began to occur immediately after microinjection of AMPA into the ventrolateral nucleus tractus solitarius, and the effects noted were similar to those

Table 2
Respiratory effects produced by microinjection of AMPA into the ventrolateral nucleus tractus solitarius before and after CNQX was microinjected into the same site [a]

Dose of drugs and No. of animals studied (n)	Experimental condition	Respiratory indices measured							Cardiovascular indices measured	
		Respiratory minute volume (ml/min)	Tidal volume (ml)	Respiratory rate (breaths/min)	Inspiratory duration (s)	Expiratory duration (s)	Total cycle duration (s)	End-tital CO_2 (%)	Mean blood pressure (mm Hg)	Heart rate (beats/min)
AMPA	Control	395 ±34	25 ±4	17 ±0.6	1.3±0.1	2.1±0.0	3.4±0.1	5.3±0.2	119 ±12	233 ±7
25 pmol/side	Maximal change after bilateral injection	−191 ±33 [b]	+12 ±7	−10 ±2.2 [b]	0 ±0	+5.8±2.7	+5.8±2.7	+0.4±0.2	+8.3± 7.4	+4.0±4.0
$n = 3$										
CNQX	Control (after recovery from AMPA)	382 ±53	24 ±4	16 ±0.3	1.4±0.2	2.2±0.1	3.6±0.1	5.3±0.1	114 ±11	229 ±7
0.125 nmol/side	Maximal change after unilateral injection	+2.7±20	+1.0±1.0	−1.0±0.3	−0.1±0.0	−0.1±0.0	−0.2±0.1	0 +0	−2 ± 2.3	0 +0
$n = 3$										
Repeat of AMPA	Control (2–3 min after CNQX)	366 ±50	25 ±3	13 ±1.2	1.5±0.2	2.2±0.2	3.8±0.0	5.3±0.1	114 ±11	229 ±1
25 pmol/side after CNQX	Maximal change after unilateral injection	−4 ±14	−1.7±1.0	−1.3±1.0	+0.1±0.1	+0.8±0.6	0.9±0.7	+0.1±0.1	0 ±0	0 ±0
$n = 3$										

[a] Values are means ± S.E.M.; [b] $P < 0.05$ using Student's t-test for paired data.

Table 3
Respiratory effects proeduced by microinjection of either NMDA or AMPA into the ventrolateral nucleus tractus solitarius [a]

Dose of drugs and No. of animals studied (n)	Experimental condition	Respiratory indices measured							Cardiovascular indices measured	
		Respiratory minute volume (ml/min)	Tidal volume (ml)	Respiratory rate (breathes/min)	Inspiratory duration (s)	Expiratory duration (s)	Total cycle duration (s)	End-tidal CO_2 (%)	Mean blood pressure (mm Hg)	Heart rate (beats/min)
NMDA 39 pmol/side $n = 8$	Control Maximal change after bilateral injection	396±64 −205±51 [b]	34±4 +12±2 [b]	11±1 −7±1 [b]	1.6±02 −0.1±0.2	3.8±0.8 +11 ±3 [b]	5.2±1 +10.7±3 [b]	5.8±0.5 +0.9±0.2 [b]	106±12 +11± 7	181±12 +10± 3 [b]
NMDA 38 pmol/side $n = 5$	Control Maximal change after unilateral injection	442±58 −121±46	49±6 +3±7	11±2 −4±1 [b]	1.6±0.2 +1.5±1	4.4±0.9 +2.9±1.5	6 ±1 +4.4±1.7	5.9±0.1 +0.2±0.1	115± 6 −11± 8	202± 8 −2± 5
AMPA 25 pmol/side $n = 6$	Control Maximal change after unilateral injection	420±24 −186±20 [b]	27±4 +6±4	16±0.1 −8±2 [b]	1.4±0.1 0.0±0	2.5±0.6 +4.1±1.6	3.8±0.4 +4.1±1.6	5.3±0.2 +0.6±0.4	120± 9 +6±0.4	214±21 −2± 2

[a] Values are means±S.E.M.; [b] $P < 0.05$ using Student's t-test for paired data.

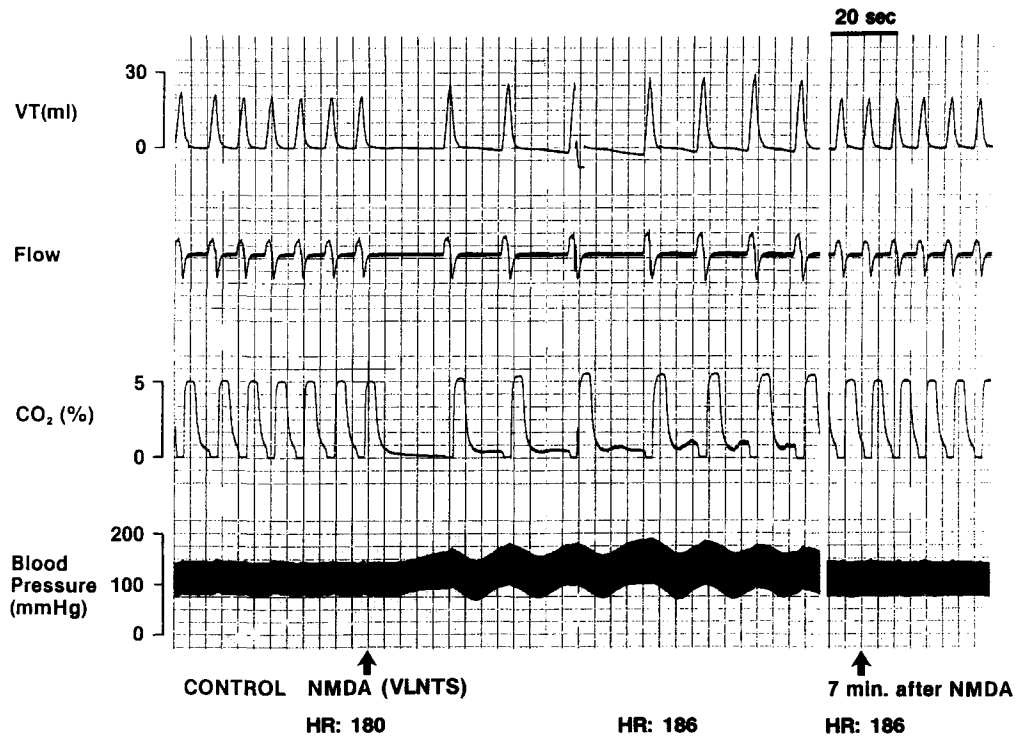

Fig. 9. The effect of NMDA (39.0 pmol/site) microinjected into the ventrolateral nucleus tractus solitarius (VLNTS) bilaterally on V_t, tracheal air flow (flow), $FECO_2$ (CO_2), blood pressure and heart rate (HR). Data shown are data obtained just prior to and immediately after bilateral microinjection of NMDA and data obtained at recovery at 7 min post-microinjection.

obtained with NMDA. Data obtained with unilateral microinjections of AMPA into the ventrolateral nucleus tractus solitarius are tabulated in Table 3.

For comparison, five experiments were performed with unilateral microinjections of NMDA, and the data are also tabulated in Table 3. As can be noted, unilateral microinjection of 39 pmol of NMDA produced qualitatively similar but less striking effects on respiratory activity than 25 pmol of AMPA.

Finally, in two animals, AMPA was tested by unilateral microinjection into the ventrolateral nucleus tractus solitarius before and after microinjecting 5.62 nmol of CPP into the same site. AMPA microinjected before CPP decreased respiratory rate and respiratory minute volume by 4.5 breaths/min and 97 ml/min, respectively, and increased end-tidal CO_2 by 0.55%. Corresponding changes in respiratory rate, and respiratory minute volume and end-tidal CO_2 with AMPA given 2 min after CPP were −4.5 breaths/min, −88 ml/min and +0.30% respectively.

4. Discussion

The purpose of our study was to determine whether the ventrolateral nucleus tractus solitarius could be a site of action whereby blockade of NMDA receptors results in prolongation of inspiratory duration and apneustic breathing in chloralose-anesthetized animals. This was tested by bilaterally microinjecting two different antagonists of NMDA receptors into the ventrolateral nucleus tractus solitarius, namely, CPP and AP7. Both of these agents produced an increase in inspiratory duration and apneustic breathing. However, bilateral microinjection of one of these agents, CPP, into brain tissue near the ventrolateral nucleus tractus solitarius but outside of the ventrolateral nucleus tractus solitarius, namely the dorsal motor nucleus of the vagus and the medial subnucleus of the tractus solitarius, did not prolong inspiratory duration or provoke apneustic breathing during the time period over which bilateral microinjection of CPP into the ventrolateral nucleus tractus solitarius produced these effects. The possibility that one of these agents, namely, CPP, was acting non-specifically was ruled out by showing that the drug does not modify AMPA-induced changes in respiratory activity elicited from the ventrolateral nucleus tractus solitarius. Of concern is the high dose of AP7 used in our study. According to Davies and colleagues (1986), AP7 is approximately 5–10 times less potent (based on data in Table 1 of their paper) than CPP. However, to mimic the effect of CPP at the ventrolateral nucleus tractus solitarius with AP7, we were required to use a dose of AP7 ranging from 40 to 100 times larger than for CPP. One possible explanation for the need for such a high concentration of AP7

is that it diffuses away from the site of microinjection much faster than does CPP. These results indicate that the ventrolateral nucleus tractus solitarius could be an important site of action whereby antagonists of NMDA receptors (e.g., MK-801, phencyclidine, AP7 and ketamine – see Foutz et al., 1988a, 1989; Abrahams et al., 1988, 1993) might act to produce apneustic breathing.

To our knowledge these are the first data indicating that blockade of NMDA receptors at the ventrolateral nucleus tractus solitarius can result in prolongation of inspiratory duration and apneustic breathing. The CNS has been clearly implicated in this effect of NMDA receptor antagonists (Foutz et al., 1988a), but the brain site that has been implicated is the circuitry comprising pontine inspiratory off-switch mechanisms (Ling et al., 1992; Feldman et al., 1992). It should be noted that data have been recently reported indicating that blockade of NMDA receptors in the nucleus tractus solitarius produces a significant prolongation of inspiratory duration in the decerebrate, paralyzed and artificially ventilated cat (Karius et al., 1994). This occurred upon microinjection of AP5 into the medial regions of the nucleus tractus solitarius. These investigators raise the possibility that prolongation of inspiratory duration might result from diffusion of AP5 into the region of the ventrolateral nucleus tractus solitarius. However, because of the rapid time course of action of AP5 they favor the notion that the AP5 effect is due to a direct action within the medial regions of the nucleus tractus solitarius.

The present results obtained with CPP and AP7 suggest that an endogenous excitatory amino acid is tonically released at the ventrolateral nucleus tractus solitarius and plays a significant role in respiratory timing. Release of the excitatory amino acid with subsequent activation of NMDA receptors on respiratory neurons results in termination of inspiration. Our data further indicate that while non-NMDA receptors are present in the ventrolateral nucleus tractus solitarius they are not activated by an endogenously released excitatory amino acid neurotransmitter. That is, the non-NMDA receptor agonist, AMPA, exerts a striking effect on breathing when microinjected into the ventrolateral nucleus tractus solitarius. The effect observed resembled the effect obtained by activating NMDA receptors at the same site. However, blockade of non-NMDA receptors with bilateral microinjection of CNQX in a dose that blocks non-NMDA receptors has little or no effect on inspiratory duration and respiratory pattern.

These findings, namely the presence of both NMDA and non-NMDA receptors at the ventrolateral nucleus tractus solitarius but only NMDA receptors responding to an endogenously released excitatory amino acid neurotransmitter, suggest that L-glutamate may not be the excitatory amino acid neurotransmitter at this site. L-Glutamate activates both NMDA and non-NMDA receptors and, hence, does not fit the criterion of neurotransmitter based on our antagonist studies with CNQX. (This assumes that the quantity of glutamate released is sufficient to activate NMDA as well as non-NMDA receptors.)

The source of endogenous excitatory amino acid present at the ventrolateral nucleus tractus solitarius, based on indirect evidence using CPP and AP7, is unclear. Afferent projections to the ventrolateral nucleus tractus solitarius consist of fibers of the superior laryngeal nerve (McCrimmon et al., 1987; Long and Duffin, 1986), carotid sinus nerves (Housley and Sinclair, 1988) and some fibers of the vagus nerve (Averill et al., 1984; Kalia and Richter, 1985). Unlikely sources are terminals of vagal afferent nerves synapsing in the ventrolateral nucleus tractus solitarius. Reasons for drawing this conclusion are as follows: (1) blockade of NMDA receptors at the ventrolateral nucleus tractus solitarius was effective in producing apneusis regardless of whether the vagus nerves were intact; and (2) although the superior laryngeal nerve was always intact in our study and its afferent terminals are known to be in close apposition with ventrolateral nucleus tractus solitarius neurons (McCrimmon et al., 1987; Long and Duffin, 1986), neurotransmitter released from superior laryngeal neurons does not appear to act on NMDA receptors (Karius et al., 1991).

Another important finding of the present study was that activation of NMDA receptors in the ventrolateral nucleus tractus solitarius increased the duration of expiration, and in four out of eight animals, expiratory duration was excessively prolonged such that apnea of 35–80 s duration was produced. Presumably, activation of NMDA receptors on ventrolateral nucleus tractus solitarius neurons would excite these neurons and this has been the finding when excitatory amino acids have been iontophoresed or microinjected onto medullary respiratory neurons (Henry and Sessle, 1985; Morin-Surun et al., 1986). A fascinating observation made by Lipski et al. (1977) is that iontophoretic application of the excitatory amino acid, homocysteic acid, onto a dorsal respiratory group neuron (Rα neuron) evokes continuous discharge of the unit (Fig. 4 of Lipski et al., 1977). On the other hand, at the same time, the phrenic nerve recording, instead of exhibiting a discharge pattern similar to that observed for the Rα neuron (which would be expected if ventrolateral nucleus tractus solitarius neurons function primarily to transmit electrical signals form the medulla to the phrenic motor nucleus by a monosynaptic pathway), exhibits a change in respiratory timing, reflected by an increase in expiratory duration (Fig. 4 of Lipski et al., 1977). It should also be noted that electrically induced excitation of the dorsal respiratory group of neurons has also been reported to

reduce phrenic nerve activity (Speck and Feldman, 1980). Thus, it appears that excitatory amino acid-induced increase in unit activity of the Rα neuron mimics electrically induced excitation of these neurons in the Speck and Feldman study (1980) in that in both cases phrenic nerve activity is reduced.

In summary, our data with NMDA receptor antagonists microinjected into the ventrolateral nucleus tractus solitarius suggest that an endogenously released excitatory amino acid is released in the vicinity of ventrolateral nucleus tractus solitarius neurons and acts on NMDA receptors to terminate inspiration. These data raise the possibility that the ventrolateral nucleus tractus solitarius may be one of the sites where systemically administered drugs that interfere with NMDA receptor function may act to produce an apneustic pattern of breathing.

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