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Local Antagonism of Intertrigeminal Region Metabotropic Glutamate Receptors Exacerbates Apneic Responses to Intravenous Serotonin

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Abstract

Injections of a broad spectrum glutamate receptor antagonist into the pontine intertrigeminal region (ITR) exacerbate vagal reflex apnea produced by intravenous serotonin infusion. This effect is not reproduced by ITR injections with either NMDA or AMPA receptor antagonists. Here, we tested the hypothesis that ITR injection with a metabotropic glutamate antagonist would alter respiratory responses to serotonin (5-HT) intravenous infusions. In anesthetized adult male rats (N = 20; Sprague-Dawley) AIDA (1-aminoindan-1,5-dicarboxylic acid), a specific antagonist of the type 1 metabotropic glutamate receptor (mGlu1R), was microinjected unilaterally into the ITR to block 5-HT evoked apnea. Respiratory pattern changes evoked by ITR glutamate injection and by intravenous serotonin (5-HT) infusion (0.5 μ l, 0.05 M; or 2.5×10^{-8} moles) were characterized according to apnea expression and duration, as well as coefficients of variation for breath duration (CVT_T) and amplitude (CVV_T) before and after ITR AIDA injection.

Unilateral AIDA blockade of the ITR significantly increased the duration of apnea evoked by 5-HT infusion ($p < 0.03$ for each dose tested) during the 30 s following infusion in a dose-dependent fashion, with the two highest doses resulting in intermittent apneas for at least 10 minutes following a bolus 5-HT infusion. Similar prolonged increases in CVT_T and CVV_T with respect to control were associated with ITR AIDA injections. These findings suggest that brief perturbations of vagal afferent pathways can produce ongoing respiratory dysrhythmia, including spontaneous apnea, and that glutamatergic neurotransmission within ITR may be important for damping such disturbances. The present observations also suggest that such respiratory damping may be mediated by mGlu1 receptors. These findings extend our understanding of the role of the intertrigeminal region in modulating respiratory reflexes.

Keywords

intertrigeminal region (ITR); glutamate; pressure injection; rats; metabotropic receptors; 5-HT induced reflex apnea

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2. Introduction

The pontine intertrigeminal region (ITR) first described by Brodal in 1981 has been the subject of investigations into its potential role in the control of respiration. A series of studies has established that respiratory disturbance, including apnea, can be produced by microinjections of glutamate (Glu) into the ITR and the neighboring parabrachial (PB) and Kölliker-Fuse nuclei of the pons in rats (Chamberlin and Saper, 1994, 1998, 2003; Radulovacki et al., 2003, 2007). Chamberlin and Saper (1998) extended this observation with neuroanatomical evidence postulating that ITR neurons may participate in apneic airway protective reflexes and may integrate afferent input from different portions of the airway, relaying this information to the central respiratory pattern generator. Further evidence for a function of this region in respiratory control derives from our demonstration that small ibotenic acid lesions of the ITR increased sleep related apneas in conscious rats (Radulovacki et al., 2004). Collectively, these observations suggested the importance of defining the mechanisms underlying glutamatergic neurotransmission or neuromodulation within the ITR, in terms of their impact on respiration. In accordance, we showed (Radulovacki et al., 2003, 2007) that ITR-glutamate-induced apnea can be completely abolished by pre-injecting the same site with kynurenic acid, a broad spectrum antagonist which blocks NMDA, AMPA and kainate as well as metabotropic mGlu1 receptors (Perkins & Stone, 1982). ITR-glutamate-induced apnea also was abolished by the selective NMDA receptor antagonist AP5 but was only partially reduced by the AMPA receptor antagonist NBQX, indicating the functional expression and importance of ITR ionotropic glutamate receptors in the respiratory responses evoked by glutamate injection (Radulovacki et al., 2007; Isenovic et al., 2007). Blocking endogenous ITR glutamatergic neurotransmission with kynurenic acid also increased the duration of vagally mediated reflex apnea induced by intravenous injection of serotonin (5-HT) (Radulovacki et al., 2003). In contrast, neither AP5 nor NBQX, when injected into the ITR, affected 5-HT induced reflex apnea duration (Radulovacki et al., 2007; Isenovic et al., 2007). These differential effects between kynurenic acid, AP5 and NBQX on 5-HT induced apnea suggested that ionotropic receptors in the ITR, both NMDA and AMPA, were not involved in modulation of vagally mediated reflex apnea.

The aim of the present study was to test the hypothesis that blockade of endogenous activity at mGlu1 receptors within the ITR modulates vagally mediated reflex apnea in rats. Specifically, we administered AIDA (1-aminoindan-1, 5-dicarboxylic acid), a selective antagonist at mGlu1 receptors (Pellicciari et al., 1995), into the ITR and observed its effects on vagally mediated reflex apnea induced by intravenous infusion of serotonin.

3. Methods

3.1 General Procedures

Experiments were done in spontaneously breathing, anesthetized, adult male Sprague Dawley rats (270 – 300 g, Harlan, Indianapolis, IN). All surgical and experimental procedures were conducted in accordance with the “Guide for the Care and Use of Laboratory Animals” (National Academy of Science Press, Washington, DC, 1996) and were reviewed and approved by the University of Illinois at Chicago Animal Care Committee.

Twenty rats were anesthetized with a combination of 80 mg/kg ketamine (Hospira Inc., Lake Forest, IL) and 5 mg/kg Xylazine (Phoenix Scientific Inc., St. Joseph, MO) given intraperitoneally. Depth of anesthesia throughout the experiment was monitored by the absence of corneal and tail-pinch reflexes. Rats were placed in a stereotaxic apparatus (Stoelting Co., Wood Dale, IL) with the incisor bar set at interaural zero and a unilateral craniotomy was performed to allow access to rostral lateral pons. Multibarrel micropipettes with an outer tip diameter of 10 – 20 μ m were made from capillary glass (1 mm $\times$ 0.25 mm, A-M Systems, Carlsberg, WA) using a vertical puller (model No 50–39, Harvard Apparatus Ltd, Kent,

England) and mounted on a stereotaxic micromanipulator. Each barrel was connected via polyethylene tubing to a pulse pressure injector (Picospritzer II, General Valve Co., Fairfield, NJ). Separate barrels were filled with glutamate (L-glutamic acid monosodium salt, ICN Biomedicals, Aurora, OH), AIDA (1-aminoindan-1,5-dicarboxylic acid, Tocris Bioscience, Ellisville, MO), phosphate buffered saline (PBS) and oil red O-dye (Sigma, St. Louis, MO; solution of 7 mg in 1 ml ethanol). The first two drugs, dissolved in 0.2 M PBS (pH = 7.4), were used for glutamatergic manipulation of the ITR, PBS served as a sham control, whereas dye was used to mark the injection site at the end of the experiment.

The micropipette was introduced into the brain on a dorso-ventral axis to allow pressure microinjections into the ITR (A/P: -9.30 from bregma; L: 2.4; D/V: -8.0, Paxinos and Watson, 1986). The injection volume was measured by observing the movement of the pipette fluid meniscus along a calibrated eyepiece reticule of a binocular stereozoom microscope (model 48920-10, Cole-Parmer, Vernon Hills, IL).

Intravenous bolus infusions of 5-HT (5-Hydroxytryptamine hydrochloride, MP Biomedicals LLC, Aurora, OH) were accomplished through a catheter inserted into the left femoral vein and using a Hamilton syringe mounted on an infusion pump (model KDS210, KD Scientific Inc., Hollister, MA).

3.2 Recording Procedures

All experiments were performed with two-channel recording: injection marker (logic level pulse provided by the pressure injector) and respiratory movements registered by a piezo-electric strain gauge (Sleepmate Technologies, Midlothian, VA). The latter allowed measurements of the respiratory timing and uncalibrated respiratory volume. Recordings were performed using BrainWave for Windows software (Datawave Systems, Longmont, CO). Each channel was continuously digitized at sampling frequency of 100/s and saved to disk for later analysis.

3.3 Experimental Protocol

Four-barrel micropipettes were used to pressure inject glutamate (10 mM; 3×10^{-7} moles), AIDA (5, 25 or 50 mM; 1.5×10^{-10} , 7.5×10^{-10} or 1.5×10^{-9} moles, respectively), PBS and red oil O-dye. Three groups of animals were studied. The first group (8 animals) was given 50 mM AIDA, the second group (6 animals) was given 25 mM AIDA and the third group (6 animals) was given 5 mM AIDA. Each experiment began with a 10-minute recording prior to any injections. Following that, three successive infusions (0.5 μ l over 5 s) of 5-HT (0.05 M; or 2.5×10^{-8} moles) were made into the left femoral vein at 10-minute intervals. To locate an ITR apneic site the micropipette was positioned according to the stereotaxically defined coordinates most dorsal margin of the ITR, advanced ventrally in 100 μ m increments, with glutamate (30 nl) being injected at each site. This procedure continued until pressure injection of glutamate evoked an immediate apnea of at least 2.5 s duration. If the stereotaxically defined ventral margin of the ITR was reached without identifying an apneic site, the pipette would be removed and reintroduced on a parallel track. Histologically verified ITR apneic sites were identified in every animal described here. After the recovery of the breathing pattern, which usually occurred within next 3 minutes, an intravenous bolus of 5-HT again was infused. As an additional control, 10 minutes later PBS (30 nl) was injected into the same ITR site at which response to glutamate was observed, followed by another series of three successive intravenous infusions of 5-HT given at 10-minute intervals. Ten minutes following the third 5-HT infusion, AIDA (30 nl) was injected at the same ITR apneic site. To test whether AIDA would block the ITR-glutamate induced apnea, an additional glutamate microinjection was administered at the same site. Subsequently, three intravenous 5-HT (0.5 μ l) infusions were administered at 10-

minute intervals. At the end of the experiment red oil O-dye was injected to mark the identified ITR site for histological verification.

3.4 Histological Procedures

Upon the completion of the experiment, rats were deeply anesthetized and prepared for transcardial perfusion. The perfusion started using saline (pH = 7.4) at a perfusion speed of 40 ml/min until the liver had cleared, continued with 4% paraformaldehyde solution in 0.1 M PBS (200 ml at 40 ml/min, then 30 ml/min) and ended with 10% sucrose solution in 0.1 M PBS (200 ml at 30 ml/min). Following the perfusion, the brain was removed from the skull, cleared of meninges and blood vessels and stored in 4% paraformaldehyde overnight, and then in 30% sucrose solution (in 0.1 M PBS) for several days. After that, the brain was cut into 40 μ m serial sections using a cryostat microtome (Leica CM1900, Nussloch, Germany). After rinsing in PBS, the tissue sections were mounted on microscopic slides, dried, treated with cresyl violet Nissl stain, dehydrated in graded ethanols, cleared in xylene and coverslipped using Permount.

3.5 Statistical Analysis

Offline analysis was made using Experimenter's Workbench software (Datawave Systems Longmont, CO) to identify the duration (T_T) and amplitude (V_T) of each breath throughout each recording. Apnea was operationally defined as a reduction of tidal amplitude $\geq 85\%$ from the mean of the preceding 10 breaths and with a duration of at least 2.5 s. The respiratory response to 5-HT infusion was quantified as the total duration of apnea and as CVT_T and CVV_T over 30 s intervals during baseline conditions and immediately preceding and following each 5-HT administration. To define precisely the immediate, early, and long-lasting effects of 5-HT, we then grouped the values of respiratory parameters obtained during the initial 30 s interval after each 5-HT intravenous infusion, and 30–150 s ($N = 4$), 150 – 300 s ($N = 5$), 300 – 450 s ($N = 5$) and 450 – 600 s ($N = 5$) post-infusion intervals for comparison to the pre-injection values. Further, the triplicate responses to 5-HT infusion made before ITR injection were averaged, as were the triplicate responses to 5-HT obtained after AIDA injection into the ITR for each animal. Thus, for final analysis, we extracted the time-dependent responses of CVV_T , CVT_T , and apnea duration under control (pre-ITR injection), post-glutamate ITR injection, and post AIDA ITR injection conditions. These observations were then used for statistical interpretation, with the individual animal being the unit of observation.

CVV_T , CVT_T , and apnea duration were initially determined to be suitable for parametric analysis according to normality and homoscedasticity. The effects of AIDA ITR injections on apnea duration and respiratory pattern were then tested using a two-way analysis of variance with a drug-dose main effect and within subjects time effect. Subsequent one-way analysis of variance was performed on the individual effects to examine individual post-hoc contrasts which were controlled by Fisher's protected least significant difference (PLSD). In all cases, statistical significance was inferred for $p < 0.05$.

4. Results

Apnea induced by glutamate microinjection as shown in Fig. 1 (insert) was used for functional identification of ITR sites analyzed in the present study. This immediate, glutamate-induced transient inhibition of respiration was not significantly altered by any dose of AIDA (5 mM, 25 mM and 50 mM) microinjected in the same ITR site. Figure 1A illustrates a typical reflex apnea induced by intravenous serotonin administration. Although it had no effects on ITR-glutamate induced apnea, as shown in Fig. 1B AIDA markedly increased total apnea duration and respiratory instability following intravenous 5-HT.

Fig. 2 presents the group data (mean $\pm$ S.E.M.) comparing the effects of different doses of AIDA (5 mM, 25 mM and 50 mM) injected into the ITR on 5-HT induced total apnea duration over the period of 600 s. Initial two-way analysis of variance demonstrated significant dose (DF = 4; F = 13.95; $p < 0.0001$), time (DF = 4; F = 109.9; $p < 0.0001$) and dose*time interaction (DF = 16; F = 2.27; $p = 0.004$) effects. Using one-way ANOVA and post-hoc contrasts to further examine these effects confirmed that under control conditions 5-HT injection consistently produced an initial apnea and sometimes additional brief apneas with a total duration during the first 30 s following injection of 8.8 ± 0.9 s. However, after the first 30 s following injection, secondary apneas were extremely rare under control conditions, with the subsequent mean total apnea duration being less than 0.1 s (mean difference > 0.08 ; PLSD critical difference = 0.054; $p < 0.005$ for contrast of each subsequent time interval with the initial 0 – 30 s time interval).

The duration of 5-HT induced apnea was not altered by the process of identifying an ITR site and subsequent glutamate injection into that site (Fig. 2; post Glu vs. control; mean difference = -0.231 ; PLSD critical difference = 0.85; $p = 0.59$ from two-way ANOVA). During the first 30 s following 5-HT infusion all three doses of AIDA (5 mM, 25 mM and 50 mM) significantly increased apnea duration (mean difference < -1.44 ; PLSD critical difference > 1.049 ; $p < 0.03$ for each). The increase ranged from 40 – 67% depending on the antagonist dose applied. In addition, as depicted in Fig. 2, the 25 mM and 50 mM doses of AIDA produced a more sustained disturbance of respiratory pattern that was marked by additional apneas and a significance increase in total apnea duration extending across every interval for a full 10 minutes after infusion (mean difference < -2.21 ; PLSD critical difference > 1.05 ; $p < 0.001$ for each dose versus control, from two-way ANOVA).

CVT_T and CVV_T following intravenous 5-HT infusion before and after AIDA (5 mM, 25 mM and 50 mM) injections into the ITR are shown in Figs. 3 and 4, respectively. CVT_T and CVV_T increased significantly during the first 30s following 5-HT infusion (mean difference = 0.76; PLSD critical difference = 0.089; $p < 0.0001$ for CVT_T and mean difference = 0.093; PLSD critical difference = 0.054; $p = 0.00098$ for CVV_T vs. preinjection), which correlates with the presence of significant apnea during this interval following every 5-HT infusion. The increases of CVT_T and CVV_T during the first 30 s were not altered by any AIDA dose tested (DF = 4; F = 0.53; $p = 0.71$ for dose effect from one-way ANOVA; no significant pair-wise contrasts among doses by PLSD). Under control conditions both CVT_T and CVV_T returned to baseline within 30–150s. After 5 mM AIDA injection, CVT_T significantly differed from control only during the 30–150s response interval (mean difference = -0.15 ; PLSD critical difference = 0.136; $p = 0.05$), whereas 25 mM and 50 mM AIDA increased CVT_T for at least 600s following 5-HT infusion in comparison to control ($p < 0.03$ for each dose in each time interval controlled by PLSD). CVV_T values following 5-HT infusion were not altered by administration of 5 mM AIDA to the ITR, but remained elevated during all post-30 s time intervals after injection of 25 mM or 50 mM AIDA ($p < 0.05$ vs control for each time interval controlled by PLSD). In addition, our analysis demonstrated no significant difference in responses to 5-HT intravenous infusion before and after saline injection into the ITR. Also, at the doses tested, AIDA injections exerted no significant impact on the respiratory responses to subsequent ITR glutamate injection at the same site. There were no significant changes in mean TT and VT during any interval following 5-HT infusion, either before or after AIDA administration.

Fig. 5 provides a histological example of a typical injection site and Fig. 6 schematically summarizes the anatomical distribution of ITR sites injected across the group of animals. As noted above, each injection site included in this analysis was verified to be within the ITR, but Fig. 6 illustrates that these sites were widely distributed within this region.

5. Discussion

The main finding of this study is that respiratory disturbances caused by serotonergic activation of the peripheral vagus nerves are increased by ITR injection of the metabotropic glutamate receptor antagonist AIDA in a dose-dependent manner (Fig 2). This finding is in sharp contrast to the previously reported effects of ionotropic glutamate receptor antagonists, which blocked or attenuated ITR-glutamate induced apnea, but had little or no impact on vagal reflex apnea (Radulovacki et al., 2007; Isenovic et al., 2007).

In anesthetized rats, Yoshioka et al. (1992a,b, 1995) demonstrated that reflex apnea following intravenously infused 5-HT is vagally mediated, as this effect was completely prevented by bilateral vagotomy above the nodose ganglia (Yoshioka et al., 1995). In fact, intravascular infusion of 5-HT produces a reflex apnea by activating vagal afferent fibers via distal type 2 receptor and somatodendritic (within the nodose ganglia) type 3 receptor activation (Yoshioka et al. 1992a,b, 1995). This evoked apnea is in many ways similar to activation of the Hering-Brueur reflex by mechanical distension of the pulmonary stretch receptors. Here, and in a recent series of studies, we used 5-HT venous infusions to test the role of the ITR in central modulation of vagally mediated reflex apnea. For this purpose, we administered various glutamate receptor antagonists to ITR sites shown to evoke immediate respiratory disturbance in the form of apnea following glutamate microinjection. The impact of these antagonists on subsequent 5-HT induced respiratory reflexes was then examined.

Initially, we demonstrated that kynurenic acid, a non-selective antagonist both ionotropic and metabotropic glutamate receptors (Perkins and Stone, 1982), altered 5-HT induced respiratory disturbances both by increasing apnea duration and by prolonging the overall respiratory disturbance (Radulovacki et al., 2003). Blockade of ITR by the specific NMDA receptor antagonist AP5, at doses sufficient to abolish the respiratory response to subsequent glutamate injections, had no impact on the duration of 5-HT-induced apnea. However, like kynurenic acid, AP5 prolonged the overall respiratory disturbance following 5-HT infusion for at least 300 s (Radulovacki et al., 2007). In contrast to kynurenic acid and AP5, the AMPA receptor antagonist NBQX had no impact on any aspect of the 5-HT-induced respiratory response (Isenovic et al., 2007).

In the present study, ITR administration of AIDA produced an increase in 5-HT-induced apnea duration (Fig 2) and also prolonged increases in CVT_T and CVV_T following intravenous infusion of serotonin (Fig. 3 and Fig. 4). These effects were dose-dependent: all three doses tested exerted similar effects on apnea duration CVT_T and CVV_T during the first 30 s post-infusion interval; but these effects were present throughout the full 600 s post-infusion time only for the 25 mM and 50 mM doses (Figs 2–4).

These findings are of interest because they suggest a strong impact of metabotropic mGlu1 receptors within the physiological role of the ITR in dampening respiratory disturbances, at least following vagal afferent activation. Taken together, the present findings and previous observations highlight important differences in mechanisms underlying apnea following ITR stimulation by glutamate – a pharmacological phenomenon – and vagal reflex apnea, a physiological phenomenon. Thus, although the ionotropic receptor antagonists AP5 and NBQX were able to at least partially block glutamate-induced apnea, neither of these agents reproduced the exacerbation of vagally mediated reflex apnea demonstrated by kynurenic acid, suggesting that metabotropic receptors might be involved in this effect. In accordance, administration of AIDA into the ITR completely replicated the effect of kynurenic acid on vagally mediated reflex apnea.

Neither the site of action nor the specific mechanism by which ITR mGlu1 receptor blockade produces augmented respiratory disturbances following 5-HT infusion can be determined from

the present study. Still, it may be noted that there is independent biochemical evidence that NMDA receptors and mGluRs are expressed within the ITR (Ohishi et al., 1998; Guthmann and Herbert, 1999; Turman et al., 2001). On this basis it is perhaps not surprising that these two types of glutamate receptors showed the largest pharmacological effects in our studies. However, these different receptors clearly exerted functionally different effects on respiratory regulation. In particular, metabotropic mGlu1 receptors within the ITR appear to exert a potent effect on respiratory reflexes, which is a generally accepted view of metabotropic glutamate receptor function in neuromodulation.

We may speculate that endogenous glutamate release within the ITR modulates ongoing respiratory pattern control based on its activity at mGlu1 receptors localized in one or more of three anatomical domains: 1) somato-dendritic sites, resulting in direct modulation of ITR neurons; 2) presynaptic nerve terminals, resulting in modulation of inputs to ITR neurons, or 3) axonal sites, resulting in modulation of ITR outputs or outputs from other areas with projection axons passing through the ITR. It is known that mGlu1 receptors can be expressed in each of these anatomical neuronal domains (Ohishi et al., 1998). In a previous study, we showed that unilateral lesions of the ITR increased sleep related apnea in unanesthetized rats, a finding correlated with significant cell loss in the ITR but not with destruction of axons of passage (Radulovacki et al., 2004). In view of this, we consider AIDA-induced modulation of axons of passage within the ITR to be the least likely explanation for the present effects; with either direct modulation of ITR neurons or modulation of inputs to ITR neurons as more likely mediators of the effects observed in this study. Vagally mediated reflex apneas and spontaneous sleep-related apneas are certainly different phenomena with at least partially distinct underlying mechanisms. Still, the observation that ITR manipulations can alter both forms of respiratory dysrhythmia suggests that neurons of this region participate in modulation of a wide range of respiratory phenomena. Also, AIDA administration to the ITR resulted in numerous apparently spontaneous intermittent apneas occurring over at least a 10-minute interval following the initial serotonin-evoked apnea (Fig. 2).

Presynaptic mGlu1 inhibitory autoreceptors are well-known and widely distributed in the brain (for a recent review see Raiteri, 2008). Furthermore, the primary vagal afferent relay neurons of the nucleus of the solitary tract are largely glutamatergic and send projections to the ITR (Chamberlin and Saper, 1998). Thus it is possible that AIDA may act on presynaptic autoreceptors to inhibit glutamate release in the ITR, ultimately rendering the vagal modulation of respiration less damped. In any case, the present data support the conclusion that functional integrity of mGlu1 receptor signaling within the ITR is necessary to maintain normal physiological control of respiratory pattern following vagal stimulation. More detailed immunohistochemical and functional studies will be necessary to define the specific mechanisms and pathways underlying this effect.

It should be noted that there are important limitations to interpretation of the present findings. First, ketamine was employed as part of the anesthetic regimen, and this agent acts as an NMDA channel blocker. Thus, it is possible that the observed effects of ITR AIDA injection either require or are modified by the co-inhibition of NMDA ionotropic receptors. This possibility cannot be ruled out. Still, we believe that if ketamine (80 mg/kg) diminished the activity at ITR NMDA receptors at all in the present study, this effect was limited. In a previous study (Radulovacki et al. 2007), we found that administration of AP5, an NMDA receptor antagonist, eliminated the robust apneic response to ITR glutamate injection. This argues that significant functional NMDA receptors remained available within the ITR despite the use of ketamine at the same dose employed in the present study.

Another limitation is that we cannot be certain of the exact relevant population of neurons that produced the observed effects. Specifically, although we were able to verify that the pipette

tip location was within the ITR (as summarized in Fig. 6) in each of these experimental animals, we cannot be certain to what extent the AIDA spread to nearby regions. Thus, it is possible that neurons within the sensory and motor trigeminal areas, for example, may have played a role in the observed effects. It is clear, however, that when exogenous glutamate is injected via the pipette, the apneic response is specifically associated with a tip location in the ITR. If glutamate is injected into the sensory or motor regions, whisker movement and jaw movement, respectively, are typically observed. Thus it seems most likely that the dominant neuronal population accounting for the observed respiratory effects reside within the ITR, rather than neighboring regions.

In summary, the present data provide functional evidence that AIDA, a metabotropic glutamate receptor antagonist, when injected into the ITR significantly alters vagal reflex apnea, an important physiological phenomenon which may be a part of the protective mechanism of reflex apneas. These findings are consistent with the possible existence of functional mGlu1 receptors in respiratory modulating areas of the ITR and suggest that the observed effects may have been mediated by mGlu1 receptors.

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Figure 1.

Typical respiratory responses evoked by Apnea evoked by glutamate (Glu) pressure injection into the ITR (upper right inset). Intravenous administration of 5-HT before (panel A) and after (panel B) AIDA (50 mM) pressure injection into the ITR. Arrows mark the time of 5-HT i.v. infusion. Horizontal bars illustrate the timing of the induced apneas. Note that the small oscillations apparent during the apneas are at a distinctly higher frequency ($\sim 3/s$) and arise from cardiogenic pulsation of the chestwall detected by the piezo-electric strain gauge. Inset shows the functional identification of an ITR site according to the immediate apnea induced by glutamate injection into the ITR. Although the strain gauge was not calibrated to quantitative lung volume (arbitrary units) the amplification of the signal remained constant between tracing A and tracing B.

Figure 2.

Group data for reflex apnea duration (mean $\pm$ S.E.M) after 5-HT i.v. infusion before (control, postGlu and saline) and after injection of different concentrations of AIDA (5, 25 and 50 mM). Injection of 5 mM AIDA (N = 6) significantly prolonged reflex apnea duration only in the first 30 s (5 mM AIDA vs. control, **p = 0.0097). 25 mM AIDA (N = 6) and 50 mM AIDA (N = 8) significantly (*p < 0.03 for each dose at all time intervals) and equivalently (p > 0.1 for difference between doses at all time intervals) prolonged reflex apnea following 5-HT infusion.

Figure 3.

Effects of AIDA on 5-HT induced respiratory timing variability measured as coefficient of variation of breath duration (CVT_T). Preinj: CVT_T measured for 10 minutes prior to any 5-HT infusion in the experiment. CVT_T values obtained after each 5-HT i.v. infusion were grouped for the following consecutive intervals: 0–30 s, 30–150 s, 150–300 s, 300–450 s and 450–600 s. Each point represents the mean $\pm$ SEM. Prior to AIDA injections into the ITR CVT_T returned to baseline within 30 - 150 s. After 5mM AIDA injection CVT_T significantly differed from control only during 30 - 150 s response interval (* $p = 0.0052$). After ITR injections of 25 mM and 50 mM AIDA CVT_T remained elevated in comparison to control for at least 600 s following 5-HT infusion. (* $p < 0.01$; ** $p \leq 0.0005$).

Figure 4.

Effects of AIDA on 5-HT induced tidal volume variability (CVV_T). Preinj: CVV_T measured for 10 minutes prior to any 5-HT infusion in an experiment. CVV_T values obtained after each 5-HT i.v. infusion were grouped as in Fig. 3. Each point represents the mean $\pm$ SEM. The 25 mM and 50 mM doses of AIDA increased CVV_T in all measured time intervals in comparison to control (* $p < 0.05$; ** $p < 0.01$).

Figure 5. Photomicrograph illustrating the histological identification of a typical injection site, showing pipette damage with oil red-O dye track in the ITR (bold arrow); Mo5: motor trigeminal nucleus; Pr5: principal sensory trigeminal nucleus; ITR: intertrigeminal region.

Figure 6.

Schematic summary diagram of the histologically verified injections into ITR sites. Each dot represents the AIDA injection for one experiment. Collectively, injections were widely distributed through the anatomical extent of the ITR (−9.16 to −9.68 mm from bregma). Mo5: motor trigeminal nucleus; Pr5: principal sensory trigeminal nucleus; ITR: intertrigeminal region.