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P2X AND NMDA RECEPTOR INVOLVEMENT IN TEMPOROMANDIBULAR JOINT-EVOKED REFLEX ACTIVITY IN RAT JAW MUSCLES

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Abstract

We have previously shown that injection of the excitatory amino glutamate into the rat temporomandibular joint (TMJ) evokes reflex activity in both anterior digastric (DIG) and masseter (MASS) muscles that can be attenuated by prior TMJ injection of a N-methyl-D-aspartate (NMDA) receptor antagonist. The aim of the present study was to test if jaw muscle activity could also be evoked by P2X receptor agonist injection into the rat TMJ region and if the reflex activity could be modulated by TMJ injection of P2X receptor antagonist or NMDA receptor antagonist. The selective P2X subtype agonist α,β -methylene adenosine 5'-triphosphate (α,β -me ATP) and vehicle (phosphate-buffered saline) or the selective P2X antagonist, 2'-(or-3')-O-(2,4,6-trinitrophenyl) adenosine 5'-triphosphate (TNP-ATP) or selective NMDA antagonist ($\pm$)-D-2-amino-5-phosphonovalerate (APV) were injected into the rat TMJ region. Electromyographic (EMG) reflex activity was recorded in both DIG and MASS muscles. Compared with the baseline EMG activity, α,β -me-ATP injection into the TMJ (but not its systemic administration) following pre-injection of the vehicle significantly increased the magnitude and the duration of ipsilateral DIG and MASS EMG activity in a dose-dependent manner. The α,β -me-ATP-evoked responses could be antagonized by pre-injection of TNP-ATP into the same TMJ site but contralateral TMJ injection of TNP-ATP proved ineffective. Furthermore, the α,β -me-ATP-evoked responses could also be antagonized by APV injected into the same TMJ site but not by its systemic injection. These results indicate the interaction of peripheral purinergic as well as glutamatergic receptor mechanisms in the processing of TMJ nociceptive afferent inputs that evoke reflex activity in jaw muscles.

Keywords

Temporomandibular joint; Purinergic; Reflex; NMDA

INTRODUCTION

There is accumulating evidence that adenosine 5'-triphosphate (ATP) may be an important ligand in the activation of nociceptive primary afferents and central nociceptive neurons

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(Burnstock, 2007; 2008; Ding et al., 2000; Donnelly-Roberts et al., 2008; Liu and Salter, 2005; MacDermott et al., 1999; North, 2002; 2004). ATP is released or co-released from various cellular elements in peripheral tissues (e.g. endothelial cells, sympathetic and cholinergic efferents, tumor cells), and may activate two families of P2 receptors, the P2Y metabotropic receptors and the P2X ionotropic receptors (P2XR). Seven P2XR subtypes have been cloned, and some occur as heteromultimers (e.g. P2X_{2/3, 1/5, 4/6} receptors). P2X₁₋₆ receptors are expressed in spinal dorsal root and trigeminal ganglion neurons, and they are reported to be selectively and uniquely expressed in a subset of predominantly small, presumed nociceptive ganglion neurons, including their central terminals in the dorsal horn (e.g. for review, Burnstock 2007; 2008; Burnstock and Knight, 2004; Ding et al., 2000; Khakh et al., 2001; MacDermott et al., 1999; North, 2002; 2004; Liu & Salter, 2005; Petruska et al., 2000a,b; Snider and McMahon, 1998). In the craniofacial area, P2X_{2, 3} receptors have been shown in various tissues, including rat tooth pulp, dura and temporomandibular joint (TMJ) (Alavi et al., 2001; Ambalavanar et al., 2005; Ichikawa et al., 2004; Kim et al., 2008; Staikopoulos et al., 2007; Vulchanova et al., 1997; 1998). The activation of P2X receptors has been reported in rat carrageenan-induced and complete Freund's adjuvant (CFA)-induced TMJ inflammatory pain models, suggesting that P2X receptors may be involved in TMJ inflammatory hyperalgesia (Oliveira et al., 2005; Shinoda et al., 2005). ATP injected into either intact or inflamed TMJ tissues can also activate nociceptive neurons in trigeminal brainstem subnucleus caudalis (Tashiro et al., 2007; 2008). Thus, it is possible that ATP receptors play a key role both in normal functioning of the TMJ as well as in pathophysiological conditions affecting the TMJ, such as some types of temporomandibular disorders.

To test further the possible involvement of P2X receptor mechanisms within the TMJ region, we have employed a TMJ reflex model that we previously developed in rats and in which we have shown that injection of mustard oil (TRPA1 receptor agonist), capsaicin (TRPV1 receptor agonist) or glutamate (excitatory amino acid receptor agonist) into the rat TMJ induces a reflex increase in jaw muscle electromyographic (EMG) activity. These reflex effects can be significantly attenuated by co-injection of N-methyl-D-aspartate (NMDA) receptor antagonists (MK801 or APV; Cairns et al., 1998; Lam et al., 2005b; Yu et al., 1996). This nociceptive reflex model manifests a characteristic increase in the EMG activity of both the anterior digastric (DIG) and the masseter (MASS) muscles that involves a brainstem reflex circuit involving trigeminal brainstem subnucleus caudalis (Cairns et al., 1998; 2001; Lam et al., 2005a,b; Tang et al., 2004; Tsai et al., 1999; Yu et al., 1995; 1996; also see Hu et al., 1997) and has proven to be an effective method for studying receptor mechanisms in peripheral craniofacial tissues. Thus, the present study utilized this model to test if jaw muscle activity could be evoked by P2X receptor agonist injection into the rat TMJ region and if the reflex activity could be modulated by TMJ injection of a P2X receptor antagonist. In addition, since there is evidence that jaw reflex responses evoked by TMJ stimulation can be modulated by NMDA receptor antagonists (see above) and that functional interactions may take place between NMDA receptors and P2X receptors in both trigeminal and spinal somatosensory systems (Gu and MacDermott, 1997; Jennings et al., 2006; Lam et al., 2005a; Nakatsuka et al., 2003; Tsuda et al., 1999), this study also tested if the evoked reflex activity could be modulated by the TMJ injection of a NMDA receptor antagonist. A portion of this data has been previously presented in abstract form (Watanabe et al., 2005).

RESULTS

1. DIG and MASS EMG activities following α,β -me ATP injection

None of the animals revealed any variation in baseline EMG activities greater than 2 S.D. during the initial 10 min period prior to α,β -me ATP injection into the TMJ region and

during all pre-load conditions (e.g. Fig. 1A,B; Fig. 2A,B; Fig. 3A,B; PBS or antagonists). The AUC of the ipsilateral DIG and MASS showed significantly higher responses to 100 mM α,β -me ATP than the PBS injection alone. Some animals also showed contralateral DIG and MASS responses but not significant AUC changes (Table 1). The TMJ application of 100 mM α,β -me ATP activated both the ipsilateral DIG and MASS muscles with a mean ($\pm$ S.D.) duration of 378.3 ± 138.8 sec. The latency to onset for 100 mM α,β -me-ATP-evoked EMG activity ranged from 5 to 19 sec (mean 9.8 ± 11.8 sec). The EMG activities were evoked in a dose-dependent manner (for AUC: ANOVA on ranks, $p < 0.01$) for the ipsilateral DIG and MASS muscles (Fig. 1C and 1D). Two repeated doses of 10 mM α,β -me ATP into the TMJ produced a desensitizing effect, i.e., markedly reduced EMG activities produced by the second injection which was significantly different from the EMG activities evoked by PBS (preload) followed with 10 mM α,β -me ATP (right portion of Fig. 1C and D). Systemic administration of 100 mM α,β -me ATP produced a very small and non-significant increase in DIG (AUC median, [25%-75%]: $0.052 [0.023-0.076]$ $\mu\text{V}\cdot\text{min}$) and MASS ($0.01 [0.002-0.014]$) activity compared to baseline and to the significant EMG increases induced by 100 mM α,β -me ATP injected into the TMJ region (Mann-Whitney U-tests, $p < 0.01$, DIG; $p < 0.05$, MASS).

2. Effect of P2X receptor antagonist TNP-ATP

In the case of the P2X receptor antagonist TNP-ATP, none of the animals revealed any variation in baseline EMG activities after the TNP-ATP (or PBS) injection alone. In both ipsilateral DIG and MASS muscles, the pre-loaded higher dose of TNP-ATP (1 μM) produced a significant reduction of the 10 mM α,β -me-ATP-evoked DIG and MASS activities, although this was not apparent at the higher dose (100 mM) of α,β -me ATP with the pre-loaded higher dose of TNP-ATP (Fig. 2). We also employed a similar approach to test whether the EMG responses to 10 mM α,β -me ATP application reflected local or systemic actions of 1 μM TNP-ATP. No evoked effect on baseline EMG activity was demonstrated after 1 μM TNP-ATP injection into the contralateral (right) TMJ region, and there was also no antagonistic effect of this contralateral injection on the increases in DIG (median[25%-75%]: $0.014 [0-0.023]$) and MASS ($0.002 [0-0.010]$) activity induced by α,β -me ATP application to the left TMJ region, compared to the significant EMG increases evoked by 10 mM α,β -me ATP application to the left TMJ region after PBS injection to the left TMJ region ($p < 0.05$, Mann-Whitney U-test). EMG activity evoked by left TMJ injection of 10 mM α,β -me ATP after 1 μM TNP-ATP to the left TMJ region was significantly lower compared with the EMG activity of left TMJ injection of α,β -me ATP following right TMJ pre-load with TNP-ATP ($p < 0.01$, Mann-Whitney U-test).

3. Effects of NMDA receptor antagonist APV

There was no change from the baseline EMG activities when APV alone was injected into the left TMJ region (Fig. 3A, B). When APV was injected into the TMJ at 5 min before the α,β -me ATP injection, the TMJ pre-injection of APV at the higher concentration tested (0.5 μM) significantly reduced the AUC of the α,β -me-ATP-induced ipsilateral DIG EMG response (but not at the lower concentration 0.05 μM) compared with the significant α,β -me-ATP-evoked EMG responses in the animals receiving pre-injection of PBS vehicle (Fig. 3C; ANOVA on ranks, Dunn's test, $p < 0.05$); a similar but a non-significant modulatory trend was noted for the ipsilateral MASS activity (Fig. 3D). Systemic administration of PBS or the higher dose (0.5 μM) of APV had no significant effect on baseline (AUC median [25% - 75%]: $0.002 [0-0.11]$ $\mu\text{V}\cdot\text{min}$, $0.033 [0-0.096]$ $\mu\text{V}\cdot\text{min}$, respectively) EMG activity (Mann-Whitney U-test, $p > 0.05$). Systemic administration of PBS also had no significant effect on α,β -me-ATP-evoked DIG ($0.31 [0.27-0.54]$ $\mu\text{V}\cdot\text{min}$) and MASS ($0.11 [0.034-0.25]$ $\text{V}\cdot\text{min}$) activity compared to the EMG increases evoked by α,β -me-ATP (10 mM) injected into the left TMJ region after PBS injection to the left TMJ region (DIG $0.42 [0.21-0.55]$ $\mu\text{V}\cdot\text{min}$,

MASS 0.04 [0.017- 0.22] $\mu\text{V}\cdot\text{min}$; $p>0.05$, Mann-Whitney U-tests). Injection of the higher dose (0.5 μM) of APV into the left TMJ had a significant effect on the evoked DIG activity (0.066 [0- 0.130] $\mu\text{V}\cdot\text{min}$) compared to the DIG activity increases evoked by α,β -me-ATP (10 mM) injected to the left TMJ region after systemic administration of 0.5 μM APV (0.35 [0.26-0.66] $\mu\text{V}\cdot\text{min}$, $p<0.05$, Mann-Whitney U-test). Systemic administration of 0.5 μM of APV also had no significant effect on the α,β -me-ATP-evoked DIG (0.35 [0.26 – 0.66] $\mu\text{V}\cdot\text{min}$) and MASS (0.047 [0.012 – 0.33] $\mu\text{V}\cdot\text{min}$) activity compared with the EMG activity increases evoked by the α,β -me-ATP TMJ injection after systemic administration of PBS (DIG 0.31 [0.27 – 0.54] $\mu\text{V}\cdot\text{min}$., MASS (0.11 [0.034 – 0.25] $\mu\text{V}\cdot\text{min}$; Mann-Whitney U-tests, $p>0.05$).

DISCUSSION

This study has provided the first documentation that the local application of an ATP agonist (α,β -me ATP) to the TMJ region produces an increase in jaw muscle activity that can be attenuated by TMJ pre-injection of the P2X receptor antagonist TNP-ATP. The α,β -me-ATP-evoked increases in EMG activity unlikely involved any “systemic” pathway since injection of TNP-ATP at the same concentration into the contralateral (right) TMJ did not produce any antagonistic action and systemic administration of the same dose of α,β -me ATP produced no significant EMG activity. We have interpreted the lack of effects of systemic α,β -me ATP and of TNP-ATP when injected into the contralateral TMJ as indicating that the α,β -me-ATP-evoked EMG activity may be attributed to P2X receptor mechanisms localized to the injected TMJ region. In addition, the study has demonstrated for the first time that P2X receptor mechanisms within the TMJ can be modulated by NMDA receptor processes within the TMJ since the TMJ injection of APV (but not its systemic administration) attenuated the α,β -me-ATP-evoked EMG activity. These results suggest that peripheral P2X and NMDA receptor mechanisms within the TMJ region may interact to influence TMJ nociceptive processes.

The increased EMG activity evoked in both jaw-opening (DIG) and jaw-closing (MASS) muscles is consistent with that documented in our previous studies of effects of mustard oil, capsaicin or glutamate injection into the TMJ region (Cairns et al., 1998; 2001; Tang et al., 2004; Lam et al., 2005b; Tsai et al., 1999; Yu et al., 1995, 1996; also see Hu et al., 1997). However, unlike the bilateral EMG responses evoked by these other algescic chemicals, the α,β -me-ATP-evoked EMG activities were restricted to the side ipsilateral to the injection and were also smaller in magnitude in terms of the AUC and time course parameters than those of the aforementioned irritants, e.g. the mean duration of DIG and MASS responses evoked by 100 mM α,β -me ATP was 378.3 ($\pm$ 138.8) sec compared with mustard oil: 19.4 min (Bakke et al., 1998a,b), glutamate: >10 min (Cairns et al., 1998), and capsaicin: >10 min (Lam et al., 2005a), and the latency to onset ranged from 5 to 19 sec (mean 9.8 $\pm$ 11.8 sec) which was somewhat longer than that reported previously for mustard oil, glutamate and capsaicin applied to the TMJ region (Bakke et al., 1998a,b; Cairns et al., 1998; Lam et al., 2005b; 2009a; Tang et al., 2004). The EMG response demonstrated in the effects of α,β -me ATP injection into the TMJ region can be attributed to the activation of P2X receptor mechanisms and not to local non-specific actions or potential confounding variables such as mechanical distension or the pH value of the injected solution, for the following reasons: 1) injection of vehicle (PBS) or antagonists (TNP-ATP or APV) at the same volume as α,β -me-ATP evoked no change of baseline EMG activities in any of the muscles; 2) injection of α,β -me-ATP, which is a mainly a P2X₁, P2X₃, P2X_{2/3} and P2X_{4/5} receptor agonist, evoked significant dose-dependent EMG activity; 3) at 10 mM (not at 100 mM) α,β -me-ATP concentration, the pre-load injection of the P2X receptor antagonist TNP-ATP (1 μM), (Javis, 2010; Lewis et al., 1998et al., 1998; Virginio et al., 1998; see Burnstock et al, 2007; North and Surprenant, 2000), produced a dose-dependent antagonism of the α,β -me-ATP

evoked EMG activity such that the matching concentrations ratio (10,000:1) between agonist (α,β -me-ATP) and antagonist (TNPATP) was similar to those previously demonstrated in *in vivo* and *in vitro* P2X receptor-related experiments in the peripheral and central nervous system (Chizh and Illes, 2001). However, the inability of TNP-ATP to antagonize the effects of the higher dose of α,β -me-ATP raises the possibility that the higher dose of α,β -me-ATP could have evoked non-specific pharmacological effects. 4) two consecutive TMJ injections of α,β -me-ATP produced a rapid desensitizing effect, which is a well-documented feature of ATP and its agonists in the case of P2X₁ and P2X₃ receptors but not heteromeric P2X_{2/3} receptor subtypes (Bland-Ward and Humphrey, 1997; Lewis et al. 1995; Ueno et al. 1998, 1999; see Burnstock, 2006; North and Surprenant, 2000).. Although the present findings from this TMJ nociceptive reflex model do not directly rule out the involvement of P2X₁ receptor subtypes, other studies suggest that the P2X₁ receptor is not involved in such nociceptive responses (see Surprenant and North, 2009) and so P2X₃ receptor mechanisms are the most likely purinergic processes accounting for the the α,β -me-ATP-evoked effects in the present study.

Our finding that APV could reverse the α,β -me-ATP-evoked EMG activity when APV was injected into the TMJ but not when the same dose was administered systemically suggests modulatory interactions occur between P2X and glutamatergic receptors in the TMJ region. Previously, we have shown the involvement of glutamatergic receptors in modulating mustard oil (TRPA1 receptor) and capsaicin (TRPV1) receptor mechanisms in the TMJ (Cairns et al., 1998; Lam et al., 2005a; 2005b; 2009a,b). Possible sources of ATP and glutamate following deep tissue injury could be from damaged cells (Cook and McCleskey, 2002) or other tissue cells (e.g. macrophages) or through their co-release from nociceptive afferent endings with subsequent autocrine or paracrine interactions involving P2X and glutamatergic receptor mechanisms that influence nociceptive afferent excitability (Cairns et al., 1998; Lam et al., 2005a; Lam et al., 2009a,b). It has been shown *in vitro* that the P2X receptors and NMDA receptors do functionally interact with each other in trigeminal spinal subnucleus caudalis neurons and that α,β -me-ATP administration in caudalis slices causes a significant increase in miniature postsynaptic currents in caudalis neurons (Jennings et al., 2006), suggesting that α,β -me-ATP acts on presynaptic afferent terminals in caudalis to increase glutamatergic neurotransmission (Gu and MacDermott, 1997). There have been several spinal studies demonstrating similar interactions, e.g., activation of P2X receptors of primary afferent terminals by α,β -me-ATP may increase glutamate release onto >80% of neurons in spinal dorsal horn slices (Nakatsuka et al, 2003), as well as interactions in a model of behavioral thermal hyperalgesia produced by intrathecal application of α,β -me-ATP (Tsuda et al, 1999). It has also been suggested that ATP might play a role in modulating the sensitivity of deep craniofacial tissues through autocrine and/or paracrine regulation of ionotropic glutamate receptor mechanisms (Lam et al., 2005a,b). However, the present findings and our previous demonstrations of NMDA receptor mechanisms for the several algic chemicals tested in our model (see above) raise the possibility of a common peripheral amplification process involving the release of glutamate as a major modulatory process in the TMJ.

METHODS AND MATERIALS

Animal preparation

All methods and experimental approaches were approved by the University of Toronto Animal Care Committee in accordance with the regulations of the Ontario Animal Research Act (Canada). Male Sprague-Dawley rats (250–400 gm) were prepared for acute *in vivo* recording of jaw muscle activity, as previously described (Cairns et al., 1998; Lam et al., 2005b; Yu et al., 1995; 1996). Briefly, under surgical anesthesia (O₂: 1 L/min; halothane: 1.5–2.5%), a tracheal cannula was inserted and artificial ventilation initiated. The rat's head

was placed in a stereotaxic frame, the skin over the dorsal surface of the skull was reflected, and two screws were inserted into the parietal bone. These screws were attached to a vertical support bar with dental acrylic to facilitate access to the TMJ region and thereby allowed the ear bars to be removed. On removal of the ear bars, the needle tip of a catheter, consisting of a 27-gauge needle connected by polyethylene tubing to a Hamilton syringe (50 μ l), was carefully inserted into the TMJ region and used for drug applications. Bipolar electrodes were fashioned out of 40-gauge Teflon-coated single-strand stainless-steel wire and inserted into the left and right DIG and MASS muscles.

After completion of all surgical procedures, the halothane level was titrated (0.9–1.2%) until noxious pressure applied to the hindpaw could not induce a flexion reflex of the hindlimb to ensure that an adequate level of anesthesia was maintained for the duration of the experiment. Heart rate and body core temperature were continuously monitored throughout the experiment and kept within the physiological range of 330–430/min and 37–37.5°C, respectively.

Because of the unstable nature of ATP that is rapidly broken down by ectoenzymes to ADP (that acts on P2Y receptors) and adenosine (that acts on P1 receptors) (Zimmermann, 2000), we employed the more stable ATP analog, α,β -me ATP which is mainly a P2X₁, P2X₃, P2X_{2/3} and P2X_{4/5} receptor agonist with affinity comparable to ATP (Lewis et al., 1998et al., 1998; Virginio et al., 1998; see Burnstock et al, 2007; North and Surprenant, 2000). In order to economize on the number of rats used, TMJ injections took place on both sides in 30% of the experiments, with a separation of at least 2 hours between injections. In the first series of experiments, only phosphate-buffered saline (PBS) vehicle (n=9) or α,β -me ATP (1, 10 and 100 mM, n=12, 12, 14; Sigma, St. Louis) was injected into the left TMJ. In the second series of experiments, PBS was injected first as the pre-load and 5 min later α,β -me ATP (1, 10 and 100 mM, n=9 per group) was injected. In order to test for possible α,β -me-ATP-induced desensitization (Bland-Ward and Humphrey, 1997), two consecutive injections of 10 mM α,β -me ATP were applied to the same TMJ site (n=8) with 5 min between the two injections. In addition, to test for possible systemic effects, 100 mM α,β -me ATP (10 μ L) was administered intravenously in an additional group of rats (n=9). In the third series of experiments, in order to test for receptor subtype specificity and antagonism, we employed 2'-(or-3')-O-(2,4,6-trinitrophenyl) adenosine 5'-triphosphate (TNP-ATP) which is an P2X receptor antagonist that competitively blocks, at nanomolar concentration, the recombinant rat P2X₁, P2X₂, P2X₃, P2X₄, P2X₇ and P2X_{2/3} receptors (Lewis et al., 1998et al., 1998; Virginio et al., 1998; see Burnstock et al, 2007; North and Surprenant, 2000). The injection needle was filled first with either PBS or TNP-ATP (1 μ M or 100 nM; 10 μ L; n=9 per group; Research Biochemicals International, Natick, MA), followed by a 2 μ L mineral oil plug, and then α,β -me ATP (10 or 100 mM; 10 μ L). The pre-load (PBS or TNP-ATP) was followed by the α,β -me ATP injection 5 min later. In the fourth series of experiments, in order to test the effects of NMDA receptor antagonists, we employed APV which is a NMDA receptor antagonist. An analogous method to the third series of experiments was used: PBS or two concentrations of APV (0.5 or 0.05 μ M; 10 μ L; n=9 per group; Research Biochemicals International, Natick, MA) and 10 mM α,β -me ATP. The preload (PBS or APV) was followed by the α,β -me ATP injection 5 min later. In addition, to test for any possible APV systemic effects, 0.5 μ M APV (10 μ L) or PBS (10 μ L) was administered intravenously 5 min before 10 mM α,β -me ATP (10 μ L) was injected into the TMJ (n=7 per group). A contralateral injection of 1 μ M TNP-ATP (10 μ L) followed 5 min later with an ipsilateral injection of 10 mM α,β -me ATP (10 μ L) was also carried out in 7 rats to test if any observed antagonistic effects in the third series of experiments could be attributed to local or systemic effects. All drugs were slowly injected into the TMJ over a period of ~5 sec.

Stimulation and recording techniques

The bilateral EMG activities of DIG (jaw-opening) and MASS (jaw-closing) muscles were recorded (Cairns et al., 1998; Yu et al., 1996) before and after the TMJ injections. However, since evoked contralateral EMG activities were usually small and less frequently evoked than ipsilateral responses, and also failed to reach significant levels ($>$ mean plus 2 S.D. of baseline, see **Data analysis and statistics** and AUC in Table 1), we only report the ipsilateral EMG results in the second, third and fourth series of experiments. EMG activity was amplified (gain, 500x; bandwidth, 30–1000 Hz) and fed into a computer equipped with a CED 1401 Plus board and analysis software (Spike2; Cambridge Electronics; signal sampling rate was 2000 Hz). Recorded EMG activity was stored electronically and analyzed off-line. The EMG activities with the jaw in its resting position were first recorded for 10 min to establish a baseline and the resulting changes in EMG activity with either antagonist or agonist were continuously recorded for another 35 min: 5 min prior to the agonist administration of α,β -me ATP into the TMJ region, either antagonist (TNP-ATP or APV) or PBS was administered into the TMJ region.

Data analysis and statistics

Recorded EMG data were rectified off-line, and EMG area bins (micro-volts per minute) were calculated. Baseline EMG activity was calculated as a mean of EMG area bins recorded over the first 10 min before injection of agents into the TMJ region. Agents applied to the TMJ were considered to have evoked jaw muscle activity if the value of the first EMG bin after TMJ application was 2 S.D. above the baseline (Cairns et al., 1998). The value of the baseline plus 2 S.D. was chosen as the signal-to-noise limit because it represents an approximation of the 95% confidence interval for the mean baseline activity (Cairns et al., 1998). The relative area under the EMG response curve (AUC), was calculated by summing the value of the first and all subsequent EMG area bins greater than 2 S.D. above the mean baseline (10 min before injection). Similarly, the onset latency was calculated by noting the first bin greater than 2 S.D. and the duration was calculated by noting the first bin and the last bin greater than 2 S.D. EMG activity and defined as the overall response, and presented as median and interquartile range values (median [25%-75%]). ANOVA on ranks and post-hoc, Dunnett's, Dunn's, Mann-Whitney U or Fisher Exact tests were used as appropriate; $p < 0.05$ was considered to reflect statistically significant differences.

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Fig. 1.

The effect of vehicle (PBS) and three doses of the P2X receptor agonist α,β -me-ATP on the ipsilateral (Ipsi.) DIG (in A) and MASS (in B) integrated EMG activity (calculated as AUC) at different time points; examples of representative raw EMG traces are shown above the graphs in A and B. In A and B, 10 min after the EMG recording, a pre-load of PBS was injected into the TMJ region and then α,β -me-ATP was injected at 15 min. *, +: represent the time points when AUC values are 2 S.D. above the baseline for 10 mM and 100 mM of α,β -me-ATP, respectively. Note the co-activation of both DIG and MASS. In C and D, AUC values of ipsilateral DIG and MASS EMG activity evoked by different doses of α,β -me-ATP are shown in box-plots. Below the x-axis, the top row of labeling refers to the first injection (Pre-load, at 10 min) and the bottom row indicates the second injection (Load, at 15 min). * represents $p < 0.05$, ANOVA on rank and Dunn tests. At the far right of C and D are shown the desensitizing effect when two consecutive α,β -me-ATP injections were made into the same TMJ site **: $p < 0.01$, Mann-Whitney U-tests).

Fig. 2.

The effect of TNP-ATP on the α,β -me-ATP-evoked ipsilateral (Ipsi.) DIG (in A) and MASS (in B) integrated EMG activity at different time points. Ten min after the EMG recording, a pre-load of PBS, 1 μ M or 100 nM TNP-ATP was injected into TMJ region and then 10 or 100 mM α,β -me-ATP was injected at 15 min. +, *: represent the time points when AUC values are 2 S.D. above the baseline between PBS vs 10 mM α,β -me-ATP or 1 μ M TNP-ATP vs 10 mM α,β -me-ATP, respectively. DIG (in C) and MASS (in D) EMG activity evoked by the lower dose (10 mM) of the α,β -me-ATP was significantly modulated (Pre-load) by the higher dose of the TNP-ATP (1 μ M) as shown in the box-plots. Below the x-axis, the top row of labeling refers to the first injection (Pre-load, at 10 min) and the bottom row to the second injection (Load, at 15 min). * represents $p < 0.05$, ** represents $p < 0.01$, ANOVA on rank and Dunn tests.

Fig. 3.

The effect of APV on the α,β -me-ATP-evoked ipsilateral (Ipsi.) DIG (in A) and MASS (in B) integrated EMG activity (calculated as AUC, $\mu V \cdot \text{min}$) at different time points. Ten minutes after the EMG recording, a pre-load of PBS, 0.05 or 0.5 μM APV was injected into the TMJ region and α,β -me-ATP was injected at 15 min. +, *, #: represent the time points when AUC values are 2 S.D. above the baseline for PBS, 0.05 μM or 0.5 μM APV and 10 mM α,β -me-ATP. Ipsilateral EMG activity in DIG (in C) but not in MASS (in D) evoked by 10 mM α,β -me-ATP was significantly modulated by the higher dose of APV (0.5 μM) as shown in the box-plots. On the x-axis labeling, the top row refers to the first injection (Pre-Load, at 10 min) and the bottom row to the second injection (Load, at 15 min). * represents $p < 0.05$, ANOVA on rank and Dunn tests.

Table 1

Incidence, mean onset latency, mean duration and median of area under the curve (AUC) of EMG responses to PBS or different dose of α,β -me-ATP applied to the TMJ

		α,β -me-ATP		
		PBS	1 mM	10 mM
Ipsi.	Number of responses	1/9	12/12 a, b, c	12/12 a, b, c
	Onset latency (s)	8.7	14.3 $\pm$ 2.2	6.6 $\pm$ 1.3 a
	Duration (s)	16.9	55.6 $\pm$ 13.5	142.8 $\pm$ 31.0
	AUC (μ V $\cdot$ min)	0.000 [0.000, 0.000]	0.022 [0.002, 0.049]	0.202 [0.0075, 0.0755]
	Number of responses	1/9	7/12 c	11/12 b, C*
MASS	Onset latency (s)	7.7	13.3 $\pm$ 2.7	6.7 $\pm$ 0.9
	Duration (s)	14.9	80.2 $\pm$ 19.5	265.1 $\pm$ 65.3 a
	AUC (μ V $\cdot$ min)	0.000 [0.000, 0.00025]	0.002 [0.000, 0.007]	0.018 [0.0045, 0.0535]
	Number of responses	1/9	5/12 b	5/12 b
	Onset latency (s)	5.9	9.4 $\pm$ 3.9	7.7 $\pm$ 0.9
DIG	Duration (s)	0.52	21.3 $\pm$ 5.2	26.9 $\pm$ 12.6
	AUC (μ V $\cdot$ min)	0.000 [0.000, 0.00125]	0.000 [0.000, 0.006]	0.000 [0.000, 0.085]
	Number of responses	1/9	4/12	4/12 b
	Onset latency (s)	26.6	11.9 $\pm$ 5.1	12.3 $\pm$ 2.6
	Duration (s)	3.4	30.7 $\pm$ 7.5	39.7 $\pm$ 7.5
Cont.	AUC (μ V $\cdot$ min)	0.000 [0.000, 0.000]	0.0005 [0.000, 0.003]	0.000 [0.000, 0.008]
	Number of responses	1/9	4/12	4/12 b
	Onset latency (s)	26.6	11.9 $\pm$ 5.1	12.3 $\pm$ 2.6
	Duration (s)	3.4	30.7 $\pm$ 7.5	39.7 $\pm$ 7.5
	AUC (μ V $\cdot$ min)	0.000 [0.000, 0.000]	0.0005 [0.000, 0.003]	0.000 [0.000, 0.008]

mean $\pm$ S.E. for Latency and Duration, median [25%, 75%] for AUC, a: vs PBS, b: Ipsi. vs Cont., c: DIG vs MASS

Ipsi.: Side ipsilateral to injection, Cont.: Side contralateral to injection

*
p<0.05

100>d
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