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P2X3 and P2X2/3 receptors inhibition produces a consistent analgesic efficacy: A systematic review and meta-analysis of preclinical studies

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ABSTRACT

Background: P2X3 and P2X2/3 receptors are promising therapeutic targets for pain treatment and selective inhibitors are under evaluation in ongoing clinical trials. Here we aim to consolidate and quantitatively evaluate the preclinical evidence on P2X3 and P2X2/3 receptors inhibitors for pain treatment.
Methods: A literature search was conducted in PubMed, Scopus and Web-of-Science on August 5, 2023. Data was extracted and meta-analyzed using a random-effects model to estimate the analgesic efficacy of the intervention; then several subgroup analyses were performed.
Results: 67 articles were included. The intervention induced a consistent pain reduction (66.5 [CI95% = 58.5, 74.5]; $p < 0.0001$), which was highest for visceral pain (114.3), followed by muscle (79.8) and neuropathic pain (71.1), but lower for cancer (64.1), joint (57.5) and inflammatory pain (49.0). Further analysis showed a greater effect for mechanical hypersensitivity (70.4) compared to heat hypersensitivity (64.5) and pain-related behavior (54.1). Sex (male or female) or interspecies (mice or rats) differences were not appreciated ($p > 0.05$). The most used molecule was A-317491, but other such as gefapixant or eliapixant were also effective ($p < 0.0001$ for all). The analgesic effect was higher for systemic or peripheral administration than for intrathecal administration. Conversely, intracerebroventricular administration was not analgesic, but potentiated pain.
Conclusion: P2X3 and P2X2/3 receptor inhibitors showed a good analgesic efficacy in preclinical studies, which was dependent on the pain etiology, pain outcome measured, the drug used and its route of administration. Further research is needed to assess the clinical utility of these preclinical findings.
Protocol registration: PROSPERO ID CRD42023450685.

1. Introduction

Pain management remains a significant challenge in modern medicine, with millions of people worldwide suffering from various forms of chronic pain (Dubois et al., 2009). Despite the availability of several pharmacological treatments—such as nonsteroidal anti-inflammatory drugs, amine reuptake inhibitors, antiepileptic drugs, and opioids—the efficacy of these analgesics is often limited, particularly for certain types of chronic pain (Finnerup et al., 2015). Moreover, these treatments are frequently associated with serious safety concerns, including tolerance, dependence, and toxicity. In Europe and USA, approximately 30–40% of the population experiences pain (Fayaz et al., 2016; Johannes et al., 2010; Leadley et al., 2012), highlighting the substantial socioeconomic impact of this condition. Consequently, there is a pressing need for new medications that offer improved efficacy with fewer side effects (Yekkirala et al., 2017).

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In this context, several lines of evidence suggest that P2X3-containing receptors are a promising target for treating certain types of chronic pain (Krajewski, 2020). Since ATP was proposed as a neurotransmitter and a peripheral algogen in the early 1970s by (Burnstock, 1972), the modulation of purinergic receptors has been broadly explored in the context of pain signaling (Chizh and Illes, 2001; Donnelly-Roberts et al., 2008; Kennedy, 2005). The P2X-purinoceptors are ligand-gated cation channels that upon activation with ATP in sensory nerves evoke fast excitatory responses (Abbracchio and Burnstock, 1994). In particular, homomeric P2X3 and heteromeric P2X2/3 receptors (hereafter referred to as P2X3-P2X2/3R) expression is highly restricted to small-size neurons in the dorsal root ganglia that are also positive for IB4+ (non-peptidergic C-nociceptors) (Brederson and Jarvis, 2008; Lewis et al., 1995; Mo et al., 2009), making them prime targets for reducing nociceptor excitability. Other P2X-purinoceptors, such as P2X7 and P2X4 receptors, mainly expressed in microglia and macrophages (but also in other immune cell types), are also implicated in pain signaling (Hua et al., 2022; Trang and Salter, 2012). However, there is currently less preclinical evidence supporting their role in pain, and their clinical development as therapeutic targets for pain management remains less advanced (Bernier et al., 2018; Krajewski, 2020). ATP and its analogs can elicit a painful response through direct excitation of P2X3-P2X2/3R in nociceptive fibers (including humans) and can potentiate activity in these fibers under inflamed or sensitized conditions (Donnelly-Roberts et al., 2008; Shieh et al., 2006; Vulchanova et al., 1998). Concomitantly, pain-related behaviors are reduced by P2X3-P2X2/3R antagonists (Ford, 2012), P2X3-P2X2/3R antisense oligodeoxynucleotides (Honore et al., 2002), and P2X3-P2X2/3R gene knockout (Cockayne et al., 2005).

Recently, several clinical trials have been initiated to evaluate specific P2X3-P2X2/3R inhibitors for the treatment of pain associated with conditions such as endometriosis-related pain (NCT04614246; NCT03654326), osteoarthritis-related knee pain (NCT01554579), and diabetic neuropathic pain (NCT04641273). Moreover, gefapixant was recently approved in Japan for the treatment of refractory or unexplained chronic cough (Markham, 2022), and other P2X3-P2X2/3R inhibitors (such as eliapixant, filapixant, camlipixant, sivopixant, and others) are being tested in advanced clinical trials for treating cough (Sykes et al., 2022). The human safety of P2X3-P2X2/3R inhibition is well established, with only a few mild adverse events reported, such as hypogeusia, ageusia, and dysgeusia (Krajewski, 2020; Ramadan et al., 2023).

The analgesic properties of P2X3-P2X2/3R inhibition may vary depending on the type of pain, as each pain condition involves distinct physiological pathways that may be modulated differently by P2X3-P2X2/3R inhibition (e.g., neuropathic (Arribas-Blázquez et al., 2019; Dong et al., 2022; Lu et al., 2021; Yu et al., 2013) or visceral pain (Burnstock, 2009)). Moreover, differences in efficacy may arise between species and sexes due to inherent variations in purinergic physiology (High et al., 2023; Serrano et al., 2012; Zhong et al., 2000). Additionally, variations in pain assessment methods, the specific P2X3-P2X2/3R inhibitors used, and their routes of administration could also impact analgesic outcomes. Therefore, all these factors should be carefully considered as potential determinants of the effectiveness of P2X3-P2X2/3R inhibition.

Given this background, the aim of the present article is to perform a systematic review and meta-analysis to assess the efficacy of P2X3-P2X2/3R inhibitors in animal models of pain. Our study goes beyond a broad analysis of the overall analgesic effects of P2X3-P2X2/3R inhibitors by conducting subgroup analyses that consider all the variables mentioned above, including the pain model used, the species and sexes involved, the pain outcomes measured, the specific drugs tested, and their administration routes. This comprehensive analysis aims to identify the conditions under which P2X3-P2X2/3R inhibitors have the most significant effect, which may be crucial for the design of future clinical studies.

2. Methods

2.1. Protocol and registration

The methodology used in this review was specified in advance and documented in a protocol that was registered in the CRD (Centre for Reviews and Dissemination) York website PROSPERO (International Prospective Register of Systematic Reviews) under the registration ID CRD42023450685. The study was performed adhering to the last version (2020) of PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines on systematic reviews and meta-analyses (Page et al., 2021).

2.2. Review question statement and PICOS elements

What impact does inhibit P2X3-P2X2/3R have on pain in animal models?

(P) Population: animal models of pain (e.g., intraplantar injection of proinflammatory substances)
(I) Intervention: selective inhibitors of P2X3-P2X2/3R -e.g., A-317493, gefapixant and others (Müller and Namasivayam, 2021)-
(C) Control: vehicle-treated group
(O) Outcome: pain assessed by different standardized tests
(S) Studies: original research studies

2.3. Information sources and search strategy

A comprehensive systematic search was performed up to August 2023 in three databases: PubMed, Web of Science, and Scopus, without restriction in year or language. Complete search strategy per database can be found in Supplementary Table 1.

2.4. Inclusion and exclusion criteria

Inclusion Criteria: original research studies in which the effect on pain of pharmacological P2X3-P2X2/3R inhibition is evaluated in animal models of pain.

Exclusion Criteria: review articles, systematic reviews, in vitro experiments, human studies, studies including no relevant information and violation of any of the above inclusion criteria.

2.5. Article selection

The selection of the studies retrieved by the search strategy was conducted using the software Rayyan (Rayyan Systems Inc., Cambridge, MA, USA) as previously described (M. Huerta et al., 2023). Two reviewers (MAH and DMF) independently screened the titles and abstracts (in a blinded manner) to identify studies that could potentially met the inclusion criteria. Then, the eligibility of these studies was assessed by the same team members (MAH and DMF) by accessing the full texts of the articles. Disagreements were resolved through discussion with a third reviewer (MAT).

2.6. Data extraction

Extracted information included: study setting, study population (animal model of pain used including species, strains, age and sexes) and baseline characteristics, details of the P2X3-P2X2/3R inhibitor used and its administration route, the timing, the dose used and control conditions, study methodology, pain-related outcomes (pain assessment test used) and the times of measurement, main results of the intervention and information for risk of bias assessment. Numerical data necessary for the meta-analysis was extracted manually from the graphs using a digital ruler software (web plot digitizer) and included in a standardized, pre-piloted form. Two authors (MAH and DMF) extracted data

independently in a blind manner. Discrepancies were resolved through discussion with a third author where necessary (MAT).

2.7. Risk of bias assessment

We followed the SYRCLE's Risk of Bias tool for animal studies (Hooijmans et al., 2014), which uses ten question domains based on the reporting of six methodological quality criteria: selection bias (with three question domains: sequence generation, baseline characteristics and allocation concealment), performance bias (with two question domains: random housing and blinding), detection bias (with two question domains: random outcome assessment and blinding), attrition bias, reporting bias and other biases (with one question domain each: incomplete outcome data, elective outcome reporting and other sources of bias, respectively). Two reviewers (D.M.F. and J.D.L.N.) independently stated whether each criterion was reported within the methods sections of the studies reviewed here. The final score for each article was obtained by adding all the domains classified as low risk and subtracting the number of domains classified as high risk. If the final value was higher than four the article was considered as low risk of bias, if the value was between two and four (both included) it was considered as unclear risk and if the value was two or less it was considered as high risk of bias. A summary chart was done using *robvis*, an R package for visualizing risk-of-bias assessments (McGuinness and Higgins, 2021).

2.8. Data synthesis and analysis

Meta-analyses were conducted using the *metafor* package in R, version 4.1.2 (Harrer et al., 2021; Viechtbauer, 2010) Since different pain outcomes were measured with different scales in the included studies, outcome data were normalized using the normalized mean difference (NMD), which is a useful approach in meta-analysis of preclinical data because it relates the magnitude of effect in the treatment group to a group of healthy animals (Vesterinen et al., 2014). Then, a 95% confidence interval (CI95%) was computed. The inverse variance statistical analysis method was used to summarize the effect sizes of the treatment, and the combined results were analyzed using the random effect model, which accounted for the variance within and between studies (Cumpston et al., 2019). The results of the analysis were represented in forest plots, which display the NMD for each evaluation, indicating the effect size of the intervention (P2X3-P2X2/3R inhibition). A larger positive NMD suggests a greater analgesic effect. Each study is represented by a square and line, with a diamond at the bottom summarizing the overall effect using a random effects model, which accounts for variability across studies. This visualization helps interpret how consistently the treatment outperforms the control across different studies. Effects were considered statistically significant when the p value was less than 0.05.

2.8.1. Assessment of heterogeneity

The Cochrane's Q test (with $P < 0.10$ indicating asymmetry) and the Higgins-Thompson I^2 values (null or low, 0–30%; medium, 30–50%; moderate, 50–75%; and high heterogeneity, >75%) were used to assess the heterogeneity within the pooled studies (Higgins et al., 2003).

2.8.2. Subgroup analysis

After the overall analysis, several subgroup analyses were performed to focus on the influence of specific variables on the efficacy of the intervention. At least four independent cohort-level effect sizes (k) were required for each meta-analysis. An analysis was performed to test the influence of the type of pain (inflammatory, neuropathic, joint, cancer, visceral and muscle pain). Also, the pain outcome assessed was considered: mechanical hypersensitivity, heat hypersensitivity, and pain-related behavior. Mechanical hypersensitivity included both mechanical allodynia (punctate stimulus such as von Frey test) and mechanical hyperalgesia (blunt stimulation such as Randall-Selitto test). Heat hypersensitivity included both radiant (Hargreaves test) or contact (hot plate) heat stimulus. The pain-related behavior group included diverse pain-related behaviors that were performed in the absence of further cutaneous stimulations (e.g., weight bearing differences, paw lifting or licking and others). A subgroup analysis was performed to test whether there were differences between sex (male or female) and rodent species (mouse or rat). Another analysis was performed to evaluate differences between the different P2X3-P2X2/3R inhibitors (A-317493, gefapixant and others). Finally, an analysis was conducted to evaluate the influence of the administration route (intraplantar, subcutaneous, intraperitoneal, intramuscular, intravenous, oral and intrathecal).

2.8.3. Sensitivity analyses

The leave-one-out method was used to identify studies with potentially disproportionate impact on the effect size of the overall meta-analysis. Studies were sorted based on effect size magnitude, and each study was sequentially removed to evaluate its influence on the overall effect estimate. Then, the effect size value obtained after each omission was compared with the overall effect size including all studies (Harrer et al., 2021).

2.8.4. Publication bias

Funnel plot was performed for the overall meta-analysis using the *funnel* function (*metaphor* package) of R (Harrer et al., 2021). Asymmetrical distribution of studies around the estimated effect size can be interpreted as an indicator of publication bias (Egger et al., 1997). Additionally, the funnel plot was complemented with the trim-and-fill method, which was used to estimate and quantify the number of missing studies and adjust for potential bias in sensitivity analyses, providing a theoretical pooled estimate (Peters et al., 2007).

3. Results

3.1. Study selection

The summary flowchart of studies identified in the search and the process of selection is given in Fig. 1. The search in PubMed, Scopus and Web of Science retrieved a total of 560 studies, of which 84 were removed because they were duplicated. Out of the remaining 476 studies screened by title and abstract, 338 were excluded, primarily because they were not original research articles (e.g., review articles) or did not focus on pain (instead, they centered on cough or cough-related conditions). The full text of the remaining 138 articles was retrieved and carefully assessed for eligibility, resulting in the exclusion of 71 articles for various reasons: 29 articles did not use a selective P2X3-P2X2/3R inhibitor (mainly TNP-ATP or PPADS), 26 articles did not measure pain, 10 articles did not utilize an animal model of pain, 3 articles involved the administration of a P2X3-P2X2/3R inhibitor in combination with another intervention, and the remaining 3 articles were reviews. Finally, a total of 67 articles were included in the analysis.

3.2. Study characteristics

The most relevant information about the 67 studies that met the inclusion criteria is summarized in Table 1. Further information such as the animal strain, the timing of drug administration and pain evaluation, the dose of the drugs used and the number of animals per group can be found in Supplementary Table 2. All the studies were controlled, the majority used only male animals (n = 50), and a small proportion used female (n = 7) or both sexes (n = 8), in the remaining 2 studies the sex of the animals was not stated. Most of the experiments were performed in young/adult animals (3–15 weeks), with a weight range of 25–40 g for mice and 120–350 g for rats. Experimental models with similar etiology were classified under general well-established categories. For inflammatory pain, 17 articles were included where soft tissue inflammation was induced through intraplantar injection of inflammatory substances

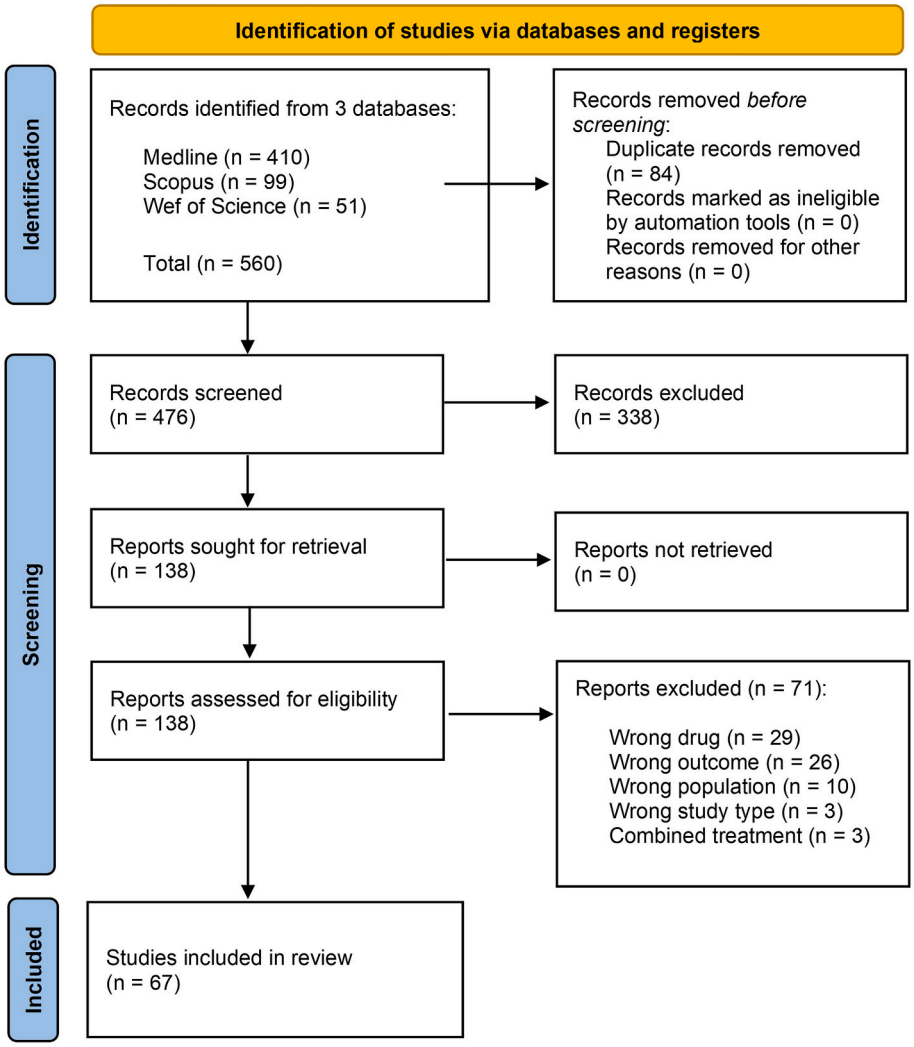

Fig. 1. Study selection flow diagram.

(CFA or carrageenan), or by incision. 19 articles focused on neuropathic pain, primarily employing models of traumatic nerve injury (e.g., spared nerve injury), as well as neuropathic pain caused by chemotherapy or diabetes. Additionally, 4 studies investigated joint pain, using osteoarthritis models or intraarticular administration of inflammatory substances, and 8 studies used a model of cancer pain (bone cancer in all cases). Visceral pain was explored in 8 studies, where the pain was induced by damage (mainly by irritants such as TNBS) to various visceral organs to generate models of endometriosis, colitis or pancreatitis or by ovariectomy. Finally, 6 studies investigated muscle pain, caused by excessive muscle contraction or intramuscular administration of inflammatory substances (CFA, α,β-meATP and others).

3.3. Analgesic efficacy of a single dose of P2X3 and P2X2/3 receptors inhibitors in pathological pain models

The first meta-analysis evaluated the analgesic efficacy of P2X3-P2X2/3R inhibitors in animal models of pain in which the treatment was given as a single administration and at the dose that produced the greatest effect (Fig. 2). The overall result of the analysis suggested that P2X3-P2X2/3R inhibition produced a clear decrease in pain (ES = 66.52 [CI95% = 58.54, 74.50]), which was statistically significant ($p < 0.0001$). The heterogeneity in this meta-analysis was high ($I^2 = 99.2\%$), indicating a high variability in the results when all types of pain were considered together. In this regard, it was convenient to perform a subgroup analysis based on the type of pain to evaluate whether the high heterogeneity can be explained by the differences between the types of pain. This subgroup analysis, concerning the different types of pain, suggested a different efficacy of the intervention ($p < 0.0001$), so the intervention worked better in some types of pain than in others, but heterogeneity remained high in all the subgroups (I^2 ranged between 93.7 and 99.3%). Also, for all the subgroups (types of pain), the analgesic effect of the intervention remained significant ($p < 0.0001$), despite differences between them were found in the effect size (analgesic efficacy).

Most studies used models of inflammatory pain ($k = 28$), where the intervention produced the lowest analgesic efficacy (ES = 49.0 [CI95%: 38.0, 60.0]). In some cases, the treatment even worsened the pain instead of relieving it, contributing to the high heterogeneity in this subgroup ($I^2 = 99.1\%$). Neuropathic pain, the second more studied group ($k = 22$), showed a notably higher effect size (ES = 71.1 [CI95% = 54.1, 88.1]) compared to the overall effect size. Joint and cancer pain presented 6 determinations each ($k = 6$) and showed similar effect sizes (ES = 57.5 [CI95% = 46.5, 68.6]; ES = 64.1 [CI95% = 42.2, 86.0], respectively), slightly lower than the overall effect. Visceral pain had 7 determinations ($k = 7$) and showed the highest effect among all pain types (ES = 114.3 [CI95% = 89.6, 139.1]). Finally, muscle pain had 10 determinations ($k = 10$) and its effect size (ES = 114.3 [CI95% = 89.6, 139.1]) was higher than the overall effect.

Table 1
Detailed information of the studies.

Pain type	Ref	[a]Species[Sex]	[e]Pain model	Intervention		Test[Outcome]
				Drug	Route & Schedule[f]	
Inflammatory	Deng et al. (2023)	Rat	CFA	A-317491	i.t.	vFrey[Mech], Hargreaves[Heat], Cold plate[Cold]
	Xiang et al. (2019)	Rat	CFA	A-317491	i.pl. & i.t.	vFrey[Mech]
	Viatchenko-Karpinski et al. (2016)	Rat	CFA	AppCH2ppA[1] AppNHppA[2]	i.pl. & i.t.[1]; i.pl.[2]	Hargreaves[Heat]
	Prado et al. (2013)	Rat	Carrag	A-317491	s.c.	Randall–Selitto[Mech]
	Cantin et al. (2012)	Rat	CFA	15h	i.p.; i.t. & s.c.	Randall–Selitto[Mech]
	Ballini et al. (2011)	Rat	CFA	Compound B	s.c.	Weight bearing[PRB]
	Wu et al. (2004)	Rat	CFA	A-317491	s.c.	e-vFrey[Mech]
	Yasuda et al. (2012)	Mice	CFA[Upper lip]	A-317493	s.c.	vFrey[Mech]
	Oliveira et al. (2009)	Rat	Carrag	A-317491[1] O. antisense[2]	s.c.[1].; i.t.[2] (S & R)	Randall–Selitto[Mech], grip force[Mech]
	Davenport et al. (2021)	Rat Mice[b]	CFA	Eliapixant (BAY817080)	p.o. (R[mice])	vFrey[mice Mech], Randall-Shelitto[1Mech]
	Grishin et al. (2010)	Rat[d]	CFA, Carrag	PT-1	s.c.	Hargreaves[Heat]
	McGaraughty et al. (2003)	Rat	CFA	A-317491	i.t. & i.p.	Hargreaves[Heat]
	McGaraughty et al. (2005)	Rat	CFA	A-317491	s.c.	Hargreaves[Heat]
	Barclay et al. (2002)	Rat	CFA	O. antisense	i.t. (S & R)	e-vFrey[Mech], vFrey[Mech]
	Shcherbatko et al. (2016)	Rat[b]	CFA	Monoclonal antibody 12D4	s.c.	Plantar test[Heat]
	Richards et al. (2019)	Rat	CFA	Gefapixant (MK-7264)	p.o.	vFrey[Mech], Weight bearing[PRB]
	Jarvis et al. (2002)	Rat[1] Mice[2]	CFA, Carrag, Incision	A-317491	s.c.	e-vFrey[Mech], vFrey[Mech] Hargreaves[1] [Heat], Hot plate[2] [Heat]
	Honore et al. (2002)	Rat	CFA, Carrag	O. antisense	i.t. (R)	vFrey[Mech], Hargreaves[Heat]
Neuropathic	Bae et al. (2022)	Rat	SNL, CIPN	14h (3,4-difluorobenzoyl)	i.v.	vFrey[Mech]
	Koizumi et al. (2021)	Rat	TNC	A-317491	s.c.[Cheek]	vFrey[Mech]
	Fei et al. (2020)	Rat	DNP	A-317491	i.pl.	e-vFrey[Mech], Hargreaves[Heat]
	Zhang et al. (2015)	Rat[b]	DNP	A-317490	i.t.	vFrey[Mech], Hargreaves[Heat]
	(Q.Wang et al., 2015)	Rat	Disc herniation	A-317491	i.t.	VFrey[Mech], Weight bearing[PRB]
	Li et al. (2014)	Rat	CCI	A-317491	PAG	e-vFrey[Mech]
	Li et al. (2017)	Rat	CCI	MRAK00973 siRNA	i.t.	e-vFrey[Mech], Hargreaves [Heat]
	Jung et al. (2017)	Rat	SNL	AF-353	i.t.	vFrey[Mech]
	Wang et al. (2014)	Rat	CCI	A-317491	i.t.	e-vFrey[Mech], Hargreaves[Heat]
	Hsieh et al. (2012)	Mice	RTX	A-317491	i.pl.	vFrey[Mech]
	Hori et al. (2010)	Rat	CIPN	A-317491	s.c.	vFrey[Mech], Randall-Selitto[Mech], Grip Force[Mech]
	Kiso et al. (2008)	Mice	SNL	A-317491	i.t.	vFrey[Mech]
	McGaraughty et al. (2003)	Rat	SNL	A-317491	i.t. & i.p.	vFrey[Mech]
	McGaraughty et al. (2005)	Rat	SNL	A-317491	s.c.	vFrey[Mech]
	Jarvis et al. (2002)	Rat[1] Mice[2]	CCI, SNL	A-317491	s.c.	e-vFrey[Mech], vFrey[Mech] Hargreaves[1Heat], Hot plate[2Heat]
	Dorn et al. (2004)	Rat[M]	SNL	siRNA O. antisense	i.t. (R)	vFrey[Mech], e-vFrey[Mech]
	Richards et al. (2019)	Rat	SNI	Gefapixant (MK-7264)	p.o. (R)	vFrey[Mech], Weight bearing[PRB]
	Barclay et al. (2002)	Rat	PSNL	O. antisense	i.t. (R)	e-vFrey[Mech], vFrey[Mech]
	Yi et al. (2017)	Rat	HIV-NP	A-317491	i.t. (R)	vFrey[Mech], Hargreaves[Heat]
	Xiong et al. (2017)	Rat[M]	CCI-ION	A-317491	i.p. (R)	e-vFrey[Mech]
Joint	Teixeira et al. (2020)	Rat	αβ-meATP[i.a.]	A-317491	i.a.	Gait disturbance[PRB]
	Teixeira et al. (2017)	Rat[c]	Carrag[i.a.]	A-317488	i.a.	Gait disturbance[PRB]
	Qi et al. (2016)	Rat	OITM	A-317488	i.m & i.a	vFrey[Mech]
	Richards et al. (2019)	Rat	MIA	Gefapixant (MK-7264)	p.o. (S & R)	vFrey[Mech], Weight bearing[PRB]
Cancer	He et al. (2020)	Mice	Bone cancer	A-317491	i.t.	vFrey[Mech], Hargreaves[Heat]
	González-Rodríguez et al. (2017)	Mice[c]	Bone cancer	A-317491	Peritumor	Hot plate[Heat]
	Liu et al. (2013)	Rat[d]	Bone cancer	A-317491	i.t.	vFrey[Mech], Hargreaves[Heat]
	González-Rodríguez et al. (2009)	Rat	Osteosarcoma	A-317491	s.c.	Hargreaves[Heat]
	Hansen et al. (2012)	Mice	Bone cancer	A-317491	s.c. (S & R)	Limb use[PRB], vFrey [Mech], weight bearing[PRB], nº of flinches[PRB]
	Kaan et al. (2010)	Rat[c]	Bone cancer	AF-353	p.o. (R)	vFrey[Mech], Weight bearing[PRB]
	Tian et al. (2023)	Rat[b]	Bone cancer	A-317491	i.pl. (R)	vFrey[Mech]
	Wu et al. (2012)	Rat[b]	Bone cancer	A-317491	i.t. (R)	e-vFrey[Mech], Spontaneous ambulation[PRB]
Visceral	Yuan et al. (2017)	Rat[b]	Endometriosis	A-317491	i.v.	vFrey[Mech], Hargreaves[Heat]
	Deiteren et al. (2015)	Rat	TNBS-Colitis	A-317491	i.p.	Colon distension[Mech]
	(S. Wang et al., 2015)	Rat	TNBS- Pancreatitis	A-317491	i.t.	vFrey[Mech]
	Ma et al. (2011)	Rat[b]	Ovariectomy	A-317491	s.c.[Hindpaw]	vFrey[Mech]
	Shcherbatko et al. (2016)	Rat[b]	TNBS-Colitis	Monoclonal antibody 12D4	s.c.	Colon distension[Mech]

(*continued on next page*)

Table 1 (*continued*)

Pain type	Ref	[a]Species[Sex]	[e]Pain model	Intervention		Test[Outcome]
				Drug	Route & Schedule[f]	
	Zhang et al. (2007)	Rat[c]	Myocardic ischemia	A-317491	i.p. (R)	Grooming, exploring and sleeping[PRB]
Muscle	Schiavuzzo et al. (2015)	Rat	α,β-meATP[i.m.]	A-317491	i.m.	Randall-Selitto[Mech]
	Noma et al. (2013)	Rat	CMMC	A-317491	i.m.	vFrey[Mech], Randall–Selitto[Mech]
	Knežević et al. (2016)	Rat	CFA[i.m. masseter]	A-317491	i.m.	vFrey[Mech]
	Hanaka et al. (2018)	Mice	Immobilization	A-317491	s.c.	e-vFrey[Mech], Hargreaves[Heat] Paw lifting[PRB]
	Jorge et al. (2020)	Mice	Carrag[i.m.]	A-317491	i.m. & i.t.	Randall-Selitto[Mech]
	de Melo Aquino et al. (2019)	Rat	GSC	A-317491	i.m. & i.t. (S & R)	Randall-Selitto[Mech]
Nociceptive	Ohyama et al. (2022)	Rat	Dentin Exposure	A-317491	i.v.	Cold Water[Cold]
	Jin et al. (2021)	Rat	TNF-α	A-317491	s.c.[Hindpaw]	vFrey [Mech]
	Jin et al. (2020)	Rat	ET-1	A-317491	s.c.	vFrey[Mech]
	Ferrari et al. (2016)	Rat[c]	Intradermal GTN or 48/80	A-317490	s.c.[Hindpaw]	Randall-Selitto[Mech]
	Wieskopf et al. (2015)	Mice[c]	αβ-meATP[i.t.]	A-317491	i.pl.	e-vFrey[Mech]
	Krimon et al. (2013)	Rat	αβ-meATP Formalin	A-317491[1] O. antisense[2]	s.c[1]; i.t.[2]	nº of flinches[PRB]
	Kunori et al. (2009)	Mice	α,β-meATP[i.t.]	A-317491	i.t.	Paintbrush[Mech]
	Hansen et al. (2012)	Mice	Formalin	A-317491	s.c.	nº of flinches[PRB]
	McGaraughty et al. (2005)	Rat	Formalin	A-317491	s.c.	nº of flinches[PRB]
	McGaraughty et al. (2003)	Rat	Formalin αβ-meATPP	A-317491	i.t. & i.p.	nº of flinches[PRB]
	de Oliveira Fusaro et al. (2010)	Rat	BK, TNF, IL-1β, IL-6, CINC-1, PGE_2, dopamine	A-317491	i.pl.	Randall–Selitto[Mech]
	Grishin et al. (2010)	Rat[d]	Capsaicin, Formalin	PT-1	s.c.	Hargreaves[Heat]
	Kakimoto et al. (2008)	Mice	α,β-meATP Formalin	Minodronic acid	s.c.	Paw lifting, shaking or licking
	Fukui et al. (2006)	Rat	α,β-meATP [1 i.c.v.] Formalin [2]	A-317492	i.c.v.	Randall–Selitto [1 Mech], Paw lift/shake/lick [2]
	Jarvis et al. (2002)	Rat [1] Mice[2]	Formalin, Capsaicin	A-317491	s.c.	e-vFrey[Mech], vFrey[Mech] Hargreaves[Heat], Hot plate[2 Heat]
	Browe et al. (2020)	Mice [c] NMR	Formalin	A-317491	i.pl.	nº of flinches[PRB]
	Barclay et al. (2002)	Rat	αβ-meATP	O. antisense	i.t. (R)	e-vFrey[Mech], vFrey[Mech]
	Dorn et al. (2004)	Rat	αβ-meATP	siRNA O. antisense	i.t (R)	VFrey[Mech], e-vFrey[Mech]

Abbreviations: α,β-meATP, α,β-methyleneATP; BK, bradikinin; Carrag, carrageenan; CCI, chronic constriction injury; CFA, Complete Freund's Adjuvant; CINC-1, cytokine-induced neutrophil chemoattractant 1; CIPN, chemotherapy-induced pain; CMMC, chronic masseter muscle contraction; DNP, diabetic neuropathic pain; GSC, gastrocnemius static contraction; Heat, heat hypersensitivity; HIV-NP, human immunodeficiency virus associated neuropathic pain; i.a., intraarticular; IL-1β, interleukin 1β; IL-6, interleukin 6, i.m., intramuscular; ION, infraorbital nerve; i.p., intraperitoneal; i.pl., intraplantar; i.t., intrathecal; i.v., intravenous; Mech, mechanical hypersensitivity; MIA, mono-iodoacetate-induced arthritis; NMR, naked mole-rat; O. antisense, antisense oligonucleotides; OITM, occlusal interference temporomandibular joint; PAG, periaqueductal grey; PGE2, prostaglandin E2; p.o., oral; PRB, pain-related behavior; PSNL, partial sciatic nerve ligation; RTX, resiniferatoxin; s.c., subcutaneous; SNI, spared nerve injury; SNL, spinal nerve ligation; TNC, trigeminal nerve compression; TNBS, 2,4,6-Trinitrobenzenesulfonic acid; TNF, tumor necrosis factor.

Subheadings [1, 2] indicates variables within the same row.

[a] Sex is male unless otherwise indicated as.

[b] for female.

[c] when both sexes were used and.

[d] when not stated.

[e] In these studies, the administration route was intraplantar if other not specified.

[f] Schedule for drug administration is single unless indicated in the table as R (repetitive) or R & S (repetitive and single).

3.3.1. The analgesic effects of P2X3 and P2X2/3 receptors inhibitors were independent on the sex and species

A subgroup analysis was performed to evaluate the influence of sex (Fig. S1). Most of the experiments were performed in male animals ($k = 59$), which effect size was 65.1 (CI95% = 56.5, 73.8). A smaller number involved female animals ($k = 12$), showing a slightly higher effect size of 74.9 (CI95% = 48.9, 100.9). The remaining used both male and female animals ($k = 7$) showing a similar effect size of 76.73 (CI95% = 44.1, 109.3). The subgroup analysis by sex suggested no significant differences between studies evaluating male, female and both sexes animals ($p > 0.05$).

Another subgroup analysis evaluated the influence of species (Fig. S2). All studies employed rats or mice. Rats were used more frequently ($k = 68$), while fewer evaluations involved mice ($k = 11$). The effect sizes were similar between rats (ES = 65.7 [CI95% = 56.8, 74.7]) and mice (ES = 71.5 [CI95% = 56.4, 86.7), with no significant differences found in the subgroup analysis ($p > 0.05$).

3.3.2. The analgesic effects of P2X3 and P2X2/3 receptors inhibitors were dependent on the pain outcome assessed

P2X3-P2X2/3R inhibitors significantly reduced pain regardless of the outcome assessed ($p < 0.0001$; see Fig. 3). However, effect sizes varied depending on the pain-related outcome ($p < 0.0001$ for subgroup analysis). Mechanical ($k = 45$; ES = 70.4 [CI95% = 59.6, 81.2]) and heat hypersensitivity ($k = 24$; ES = 64.5 [CI95% = 48.4, 80.6]) exhibited similar levels of pain relief and were more effective than pain-related behavior ($k = 9$; ES = 54.1 [CI95% = 42.3, 65.9]). Heterogeneity remained high across all subgroups ($I^2 = 96.0–99.5\%$).

3.3.3. The analgesic effects of P2X3 and P2X2/3 receptors inhibitors are dependent on the drug

All the P2X3-P2X2/3R inhibitors produced a significant analgesic effect ($p < 0.0001$; Fig. 4). However, the extent of the effect varied depending on the drug used ($p < 0.0001$ for subgroup analysis). A-317491, which was by far the most used drug ($k = 58$), showed the highest efficacy (ES = 71.95 [CI95% = 62.4, 81.5]). In contrast, gefapixant showed a lower effect size ($k = 6$; ES = 44.2 [CI95% = 32.9,

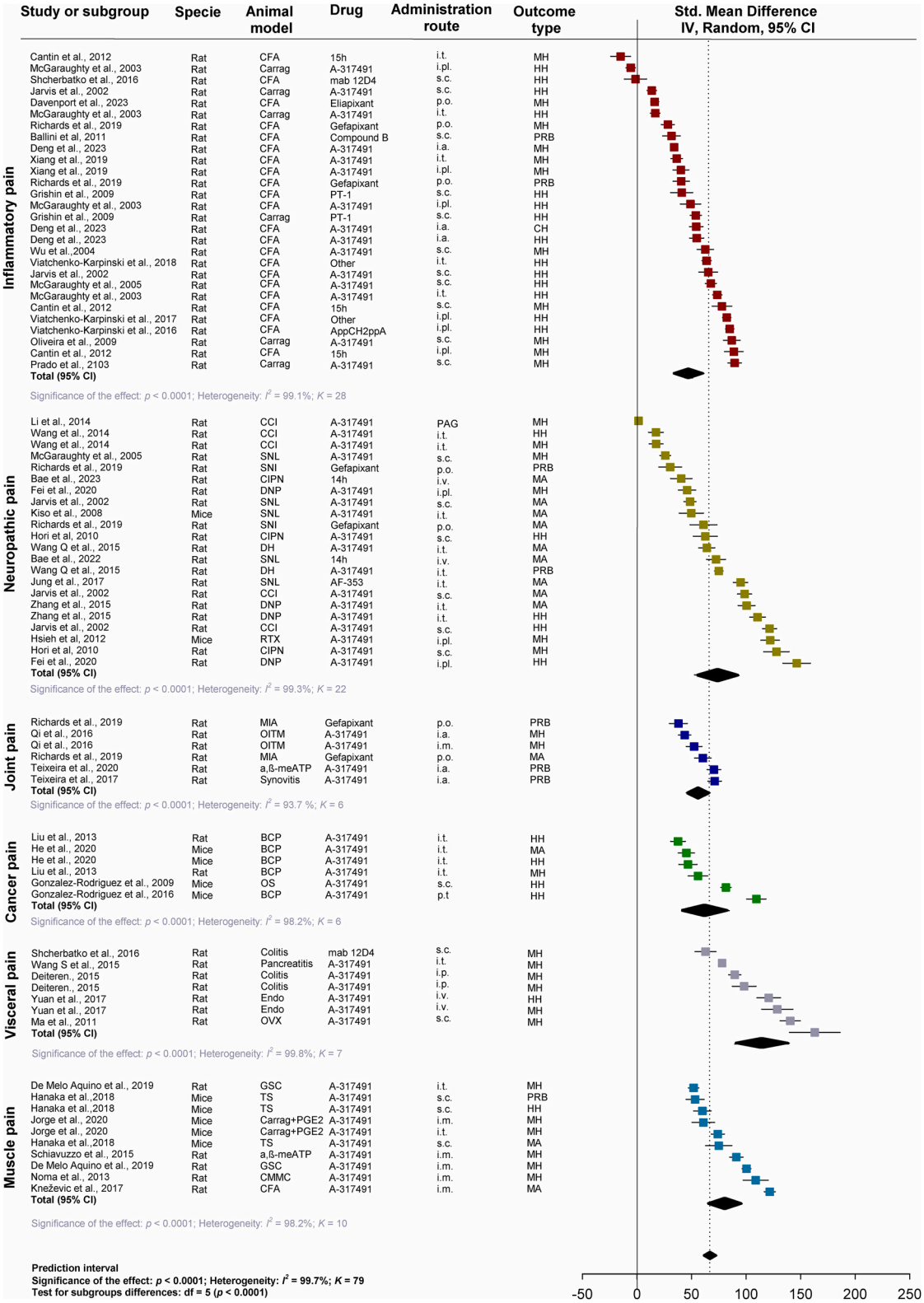

Fig. 2. Subgroup analysis of the analgesic effect of the intervention stratified by the type of pain. Forest plot of the analgesic effect of P2X3-P2X2/3R inhibition in inflammatory, neuropathic, joint, cancer, visceral and muscle. Abbreviations: α,β-meATP, α,β-methyleneATP; BCP, bone cancer pain; Carrag, carrageenan; CCI, chronic constriction injury; CFA, Complete Freund's Adjuvant; CIPN, chemotherapy-induced pain; CMMC, chronic masseter muscle contraction; DH, disc herniation; DNP, diabetic neuropathic pain; endo, endometriosis; GSC, gastrocnemius static contraction; HH, heat hypersensitivity; i.a., intraarticular; i.m., intramuscular; i.p., intraperitoneal; i.pl., intraplantar; i.t., intrathecal; i.v., intravenous; MH, mechanical hypersensitivity; MIA, mono-iodoacetate-induced arthritis; OITM, occlusal interference temporomandibular joint; OS, osteosarcoma; OVX, ovariectomy; PAG, periaqueductal grey; PGE2, prostaglandin E2; p.o., oral; PRB, pain-related behavior; p.t., peritumoral; RTX, resiniferatoxin; s.c., subcutaneous; SNI, spared nerve injury; SNL, spinal nerve ligation; TS, tail suspension.

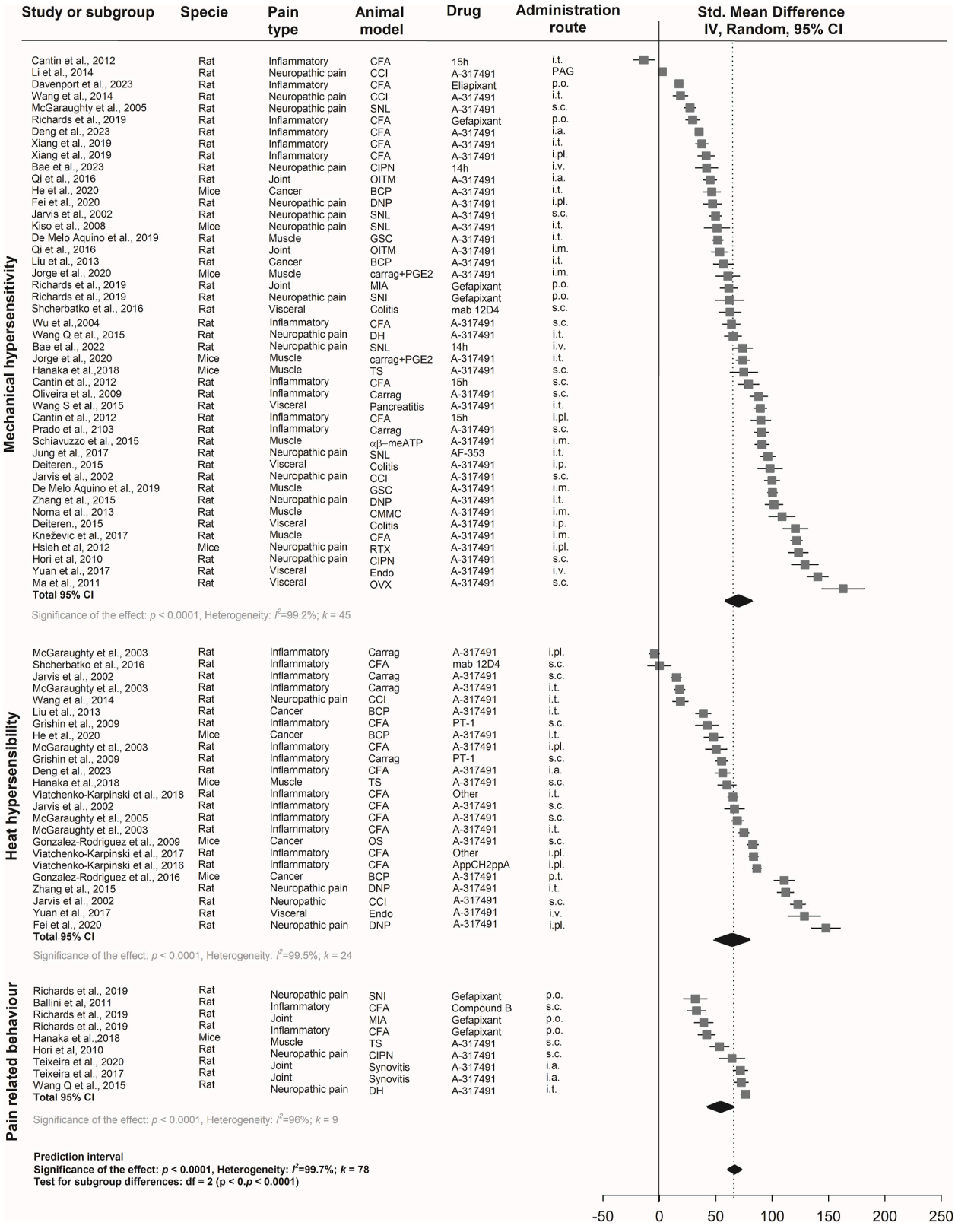

Fig. 3. Subgroup analysis of the effect of the intervention stratified by the pain outcome assessed. Forest plot of the effect of P2X3-P2X2/3R inhibition in mechanical hypersensitivity, heat hypersensitivity and pain related behavior. Abbreviations: α,β-meATP, α,β-methyleneATP; BCP, bone cancer pain; Carrag, carrageenan; CCI, chronic constriction injury; CFA, Complete Freund's Adjuvant; CIPN, chemotherapy-induced pain; CMMC, chronic masseter muscle contraction; DH, disc herniation; DNP, diabetic neuropathic pain; endo, endometriosis; GSC, gastrocnemius static contraction; HH, heat hypersensitivity; i.a., intraarticular; i.m., intramuscular; i.p., intraperitoneal; i.pl., intraplantar; i.t., intrathecal; i.v., intravenous; MH, mechanical hypersensitivity; MIA, mono-iodoacetate-induced arthritis; OITM, occlusal interference temporomandibular joint; OS, osteosarcoma; OVX, ovariectomy; PAG, periaqueductal grey; PGE2, prostaglandin E2; p.o., oral; PRB, pain-related behavior; p.t., peritumoral; RTX, resiniferatoxin; s.c., subcutaneous; SNI, spared nerve injury; SNL, spinal nerve ligation; TS, tail suspension.

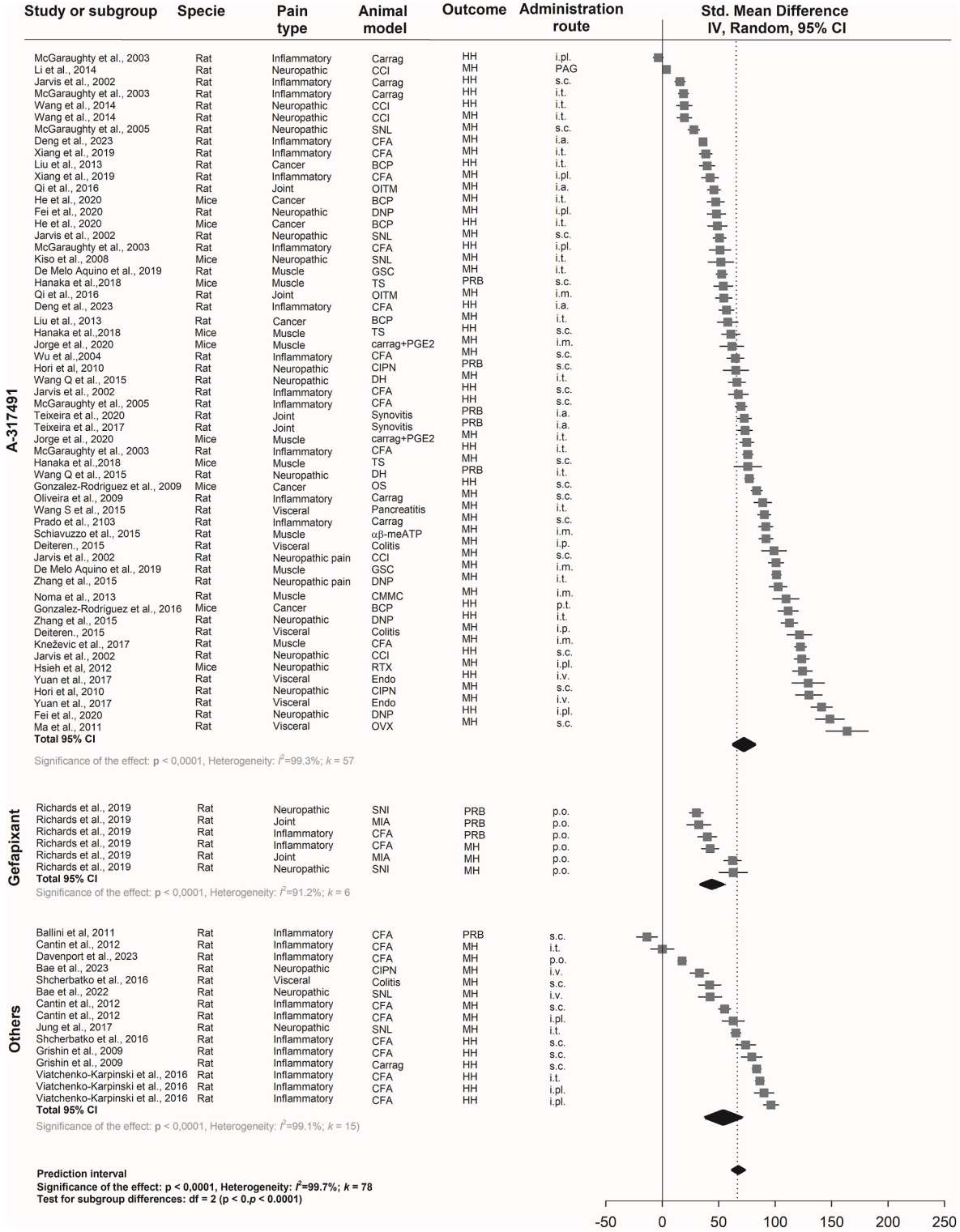

Fig. 4. Subgroup analysis of the effect of the intervention stratified by the drug used. Forest plot of the effect of P2X3-P2X2/3R inhibition by A-317491, gefapixant and others (eliapixant, AF-353, including monoclonal antibodies (12D4), compound B, 14h, 15h, PT-1, AppCH2ppA and AppNHppA). Abbreviations: α,β-meATP, α,β-methyleneATP; BCP, bone cancer pain; Carrag, carrageenan; CCI, chronic constriction injury; CFA, Complete Freund's Adjuvant; CIPN, chemotherapy-induced pain; CMMC, chronic masseter muscle contraction; DH, disc herniation; DNP, diabetic neuropathic pain; endo, endometriosis; GSC, gastrocnemius static contraction; HH, heat hypersensitivity; i.a., intraarticular; i.m., intramuscular; i.p., intraperitoneal; i.pl., intraplantar; i.t., intrathecal; i.v., intravenous; MH, mechanical hypersensitivity; MIA, mono-iodoacetate-induced arthritis; OITM, occlusal interference temporomandibular joint; OS, osteosarcoma; OVX, ovariectomy; PAG, periaqueductal grey; PGE2, prostaglandin E2; p.o., oral; PRB, pain-related behavior; p.t., peritumoral; RTX, resiniferatoxin; s.c., subcutaneous; SNI, spared nerve injury; SNL, spinal nerve ligation; TS, tail suspension.

55.6]), estimated from a single study. The "Others" category comprised a heterogeneous number of compounds (eliapixant, AF-353, monoclonal antibodies, compound B, 14h, 15h, PT-1, AppCH2ppA and AppNHppA) and showed a lower effect size ($k = 15$; ES = 54.4 [CI95% = 37.4, 71.4]) when compared to the standard drug (A-317491). High heterogeneity was observed across all subgroups ($I^2 = 91.2–99.3\%$).

3.3.4. The analgesic effects of P2X3 and P2X2/3 receptors inhibitors are dependent on the route of administration of the drug

The analgesic effect of P2X3-P2X2/3R inhibition was significant across all the routes of administration ($p < 0.0001$, for all of them; Fig. 5). However, according to the subgroup analysis, the effect was dependent on this variable ($p < 0.0001$). Intravenous injection showed the highest effect size (ES = 96.2 [CI95% = 50.7, 141.6]), but was based in only 4 evaluations ($k = 4$). Intramuscular injection had a slightly lower effect size ($k = 6$; ES = 89.6 [CI95% = 68.0, 111.1]). Subcutaneous injection, the most common route ($k = 23$), had an effect size of 69.1 (CI95% = 54.3, 84.0), similar to the one obtained with intraplantar administration ($k = 9$; ES = 73.9 [CI95% = 44.0, 103.8]), both similar to the overall effect (66.5 [CI95% = 58.5, 74.5]). On the contrary, intrathecal and intraarticular administration showed a lower effect size (ES = 56.5 [CI95% = 42.7, 70.4] and ES = 56.01 [CI95% = 44.2, 67.8], respectively), while the lowest effect size was obtained with oral administration (ES = 40.3 [CI95% = 28.0, 52.6]). All the subgroups analyzed presented a high heterogeneity ($I^2 > 95\%$).

3.4. Risk of bias

The overall results of the SYRCLE's Risk of Bias tool are summarized in Fig. 6. For most of the domains (excepting domains 1, 2, 7, 9 and 10), almost all the studies were classified as unclear risk. The allocation sequence adequately generated and applied in the half of the studies and in the rest was unclear (domain 1). The baseline between groups were similar in a big part of studies (≈80%) suggesting low risk of bias in the domain 2. In slightly more than half of the studies, the outcome assessor was blind (domain 7). Only a few articles suggested selective outcome reporting and other problems (domain 9 and 10, respectively), while the majority showed low risk of bias in these domains. In the overall results, near 27% of the included articles were classified as low risk of bias, around 48% were classified as unclear risk of bias and 25% were classified as high risk of bias. The individual full analysis of the SYRCLE's Risk of Bias tool can be found in Fig. S3.

3.5. Publication bias

A graphical representation of publication bias for the overall meta-analysis is shown in Fig. 7. Values on the right of the pyramid had a bigger effect size of the intervention while the ones on the left had a lower effect size. The trim and fill method performed in the funnel plot did not find any missing study (0 studies were filled). The absence of missing studies and the apparent symmetry of the funnel plot suggests that there is no publication bias.

3.6. Sensitivity analysis

The leave-one-out method was performed for sensitivity analysis in the overall meta-analysis. Pooled effect estimates ranged from 63.87 to 65.99 when excluding one study at each analysis (Fig. S. 4), indicating that no single study had a substantial influence on the pooled overall effect-size estimate. Also, heterogeneity was always maintained as at 99%, indicating that no particular studies exerted a substantial influence on the overall variability.

4. Discussion

The preclinical efficacy of P2X3-P2X2/3R inhibition on pain was evaluated considering the influence of different factors which might potentially impact the results obtained: the type of pain, the species and sexes, the pain outcome assessed, the drug used for the inhibition of P2X3-P2X2/3R and its administration route. The pooled effect resulted in a consistent analgesic efficacy of the intervention. In addition, subgroup analysis showed that the effect size was significantly different among the different pain types, but still significant for all of them.

The highest efficacy of the intervention was observed in experimental models of visceral pain, yet only a limited number (barely six) were included. These data confirmed the relevant role of purinergic signaling in visceral pain pathways, as previously suggested (Burnstock, 2001; Cockayne et al., 2000; Luo et al., 2023). Specifically, peripheral terminals of visceral nociceptors express not only mechanically activated channels, but also P2X3-P2X2/3R, which might be also mechanosensitive (Cockayne et al., 2005; Gonzalez-Cano et al., 2021). It is well-established that during mechanical deformation ATP is released from epithelial cells in the lumen of hollow internal organs like the ureter, bladder, or bowel (Burnstock, 1999, 2009). Visceral distension not only activates nociceptors, but it also promotes their sensitization through ATP (Li and Sinoway, 2002); also, P2X3 receptors can be directly activated by miR-1306-3p (Wu et al., 2023). P2X3-P2X2/3R inhibition produced a robust analgesic efficacy in experimental models of colitis (Deiteren et al., 2015; Shcherbatko et al., 2016) and endometriosis (Yuan et al., 2017), via ERK signaling pathway (Ding et al., 2017). It is known that during breakdown phase of the menstrual period, ATP is released from the endometrium but also from endometriotic foci (endometrial tissue growing outside the uterine wall) (Trapero and Martín-Satué, 2020). Additionally, in endometriosis, somewhat similar to cancer, both overexpression of P2X3 (Ding et al., 2017) and excessive cell proliferation occur (associated with high needs of ATP and its liberation after cellular damage) (Kong et al., 2021). All the above would contribute to the generation of abdominal pain by activation of P2X3-P2X2/3R expressed in uterine nociceptors (Chaban et al., 2007). From a translational perspective, several P2X3-P2X2/3R antagonists are being evaluated in phase II clinical trials for different pathologies associated with visceral pain: gefapixant for endometriosis-related pain (NCT03654326) and cystitis (NCT01569438); eliapixant for endometriosis-related pain (NCT04614246) and overactive bladder (NCT04545580).

In models of pain induced by muscle trauma, the efficacy of the intervention was higher compared to the overall effect. This aligns with the well-stablished fact that ATP is a very effective activator of muscle nociceptors, as even its isolated intramuscular injection was sufficient to elicit pain responses (Mense, 2008; Mørk et al., 2003). Moreover, ATP is found in particularly high concentration in the muscle and increases significantly during contraction (Li and Sinoway, 2002; Mense, 2010; Stewart et al., 1994). It has been proposed that ATP leakage from muscle fibers precedes trauma or cell necrosis and may be responsible for muscle-induced pain during maintained contraction (Hoheisel et al., 2004). Whilst most of the studies attributed the painful actions of ATP to peripheral purinergic innervation of the muscle (de Melo Aquino et al., 2019), central P2X3 receptors at the dorsal horn of the spinal cord have also a relevant contribution during chronic muscle pain (de Melo Aquino et al., 2019; Jorge et al., 2020).

Cancer-related pain treatment is specially challenging (Glare et al., 2022; Mercadante, 2022). In experimental models of pain induced by bone cancer, P2X3-P2X2/3R inhibitors had an effect size equivalent to the overall effect. Cell proliferation and bone destruction around the tumor area is accompanied by a massive release of signaling molecules to promote cell death (of miscellaneous types) (Kaan et al., 2010; Tian et al., 2023). This will lead to a large accumulation of ATP into the peritumoral environment that will, in turn, excite nociceptors through activation of purinergic receptors (Kaan et al., 2010). To note, in these models, the expression of P2X3 receptors is upregulated in DGR neurons (Liu et al., 2013).

Regarding neuropathic pain, a robust effect was observed, which is

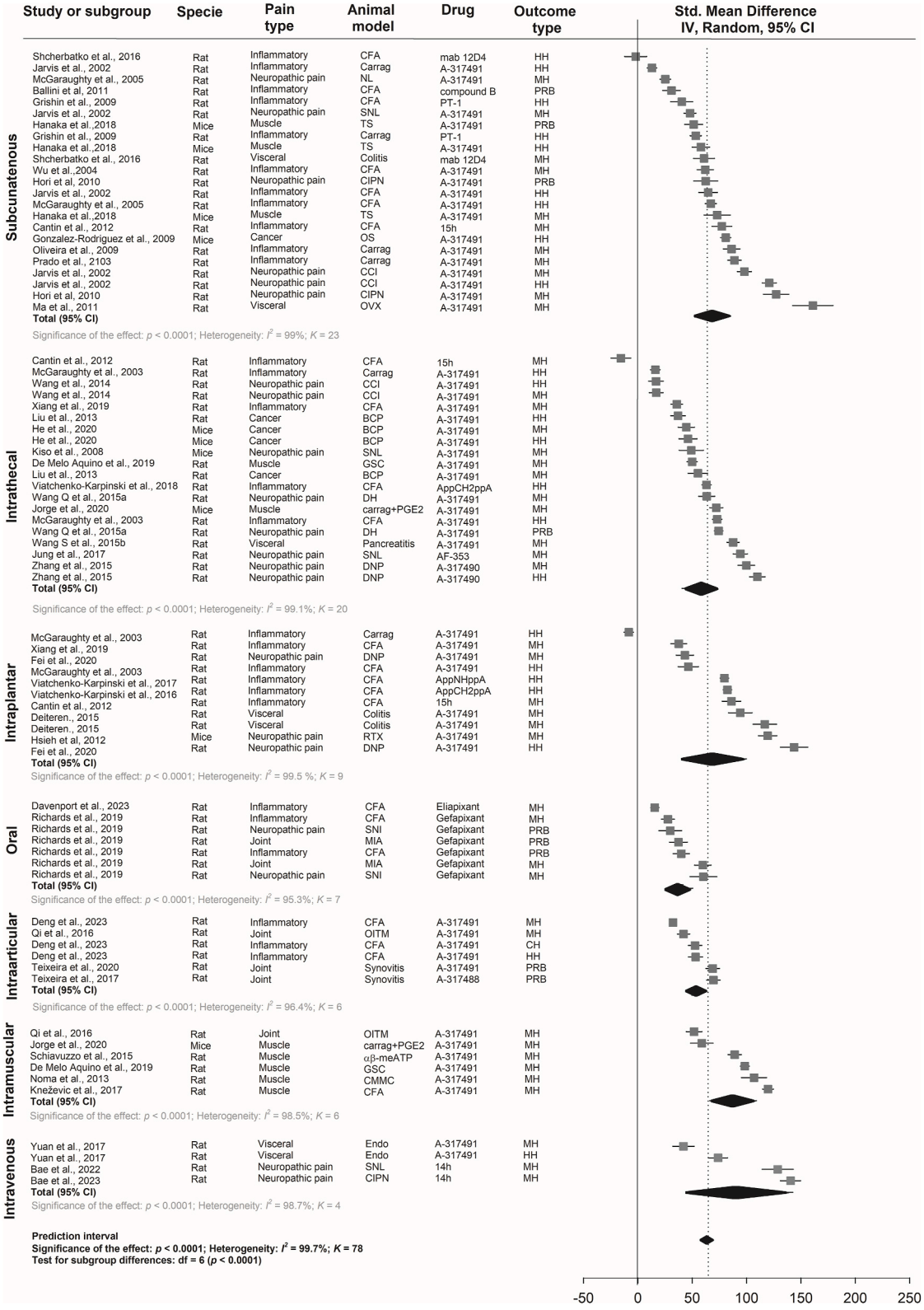

Fig. 5. Subgroup analysis of the effect of the intervention stratified by the administration route of the drug used. Forest plot of the effect of P2X3-P2X2/3R inhibition by A-317491, gefapixant and others (eliapixant, AF-353, including monoclonal antibodies (12D4), compound B, 14h, 15h, PT-1, AppCH2ppA and AppNHppA). Abbreviations: α,β-meATP, α,β-methyleneATP; BCP, bone cancer pain; Carrag, carrageenan; CCI, chronic constriction injury; CFA, Complete Freund's Adjuvant; CIPN, chemotherapy-induced pain; CMMC, chronic masseter muscle contraction; DH, disc herniation; DNP, diabetic neuropathic pain; endo, endometriosis; GSC, gastrocnemius static contraction; HH, heat hypersensitivity; i.a., intraarticular; i.m., intramuscular; i.p., intraperitoneal; i.pl., intraplantar; i.t., intrathecal; i.v., intravenous; MH, mechanical hypersensitivity; MIA, mono-iodoacetate-induced arthritis; OITM, occlusal interference temporomandibular joint; OS, osteosarcoma; OVX, ovariectomy; PGE2, prostaglandin E2; p.o., oral; PRB, pain-related behavior; p.t., peritumoral; RTX, resiniferatoxin; s.c., subcutaneous; SNI, spared nerve injury; SNL, spinal nerve ligation; TS, tail suspension.

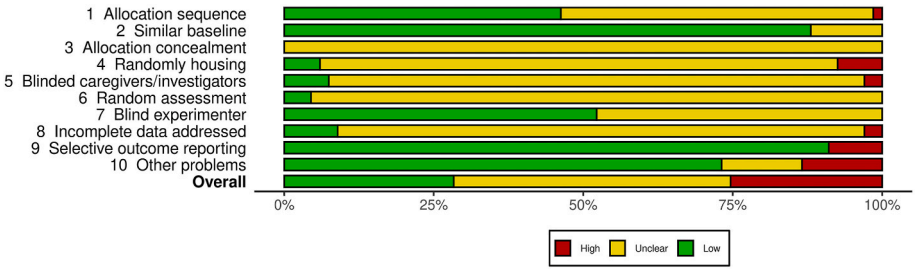

Fig. 6. Overall results of the SYRCLE's Risk of Bias tool. Green color: low risk of bias; yellow color: unclear risk of bias/not applicable to the study design; red color: high risk of bias.

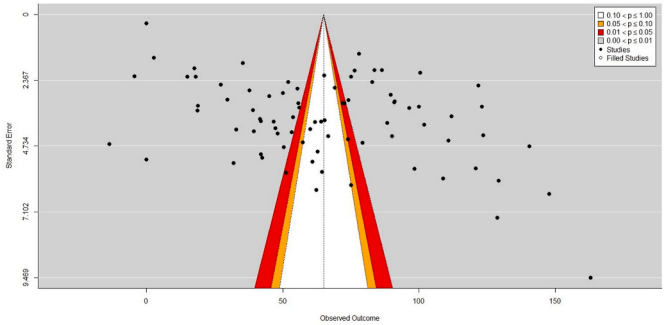

Fig. 7. Funnel plot of the overall meta-analysis including the trim and fill method. The closed dots indicate the included data (n = 79), and the open dots indicate the missing studies imputed by the trim-and-fill method (n = 0). The dashed lines that create a triangular area indicate the 90%, 95% and 99% confidence limits respectively, and the vertical dashed line represents the overall effect size.

particularly promising, since neuropathic pain is usually refractory to pharmacological treatment (Finnerup et al., 2015; M. Á. Huerta et al., 2023). Eliapixant is being tested for the treatment of diabetic neuropathic pain (NCT04641273). Damaged neurons and surrounding tissue are important sources of ATP during neuropathic pain (Hilliges et al., 2002; North, 2004). This massive ATP liberation could have a greater effect due to up-regulation of P2X3 receptors in the DRG (Xiang et al., 2008), spinal cord (Yu et al., 2013) and adrenomedullary chromaffin cells (Arribas-Blázquez et al., 2019).

The intervention showed the lowest efficacy for inflammatory pain, despite this group was relatively homogeneous, with only two experimental models (intraplantar administration of CFA or carrageenan). Even if during inflammation immune cells can cause the release of ATP, which directly activates P2X3-P2X2/3R in nociceptive terminals causing pain (Kato et al., 2017), several reasons might account for this apparent discrepancy. First, it has been reported that peripheral purinergic blockade completely reversed carrageenan induced pain when administered prophylactically (before inflammation), but not when given therapeutically, that is, once the inflammation is stablished (Oliveira et al., 2009). Furthermore, the activation of P2X3-P2X2/3R by ATP was found to specifically mediate mechanical hyperalgesia caused by bradykinin, but not by other proinflammatory mediators such as TNF, IL-1B, IL-6, CINC-1, PGE2 or dopamine (de Oliveira Fusaro et al., 2010). In addition, other purinergic receptors such as P2X7 have been shown to participate in inflammatory pain, which may explain the reduced efficacy of inhibiting only P2X3-P2X2/3R (Lopes et al., 2020).

In the case of joint pain, we found a clearly lower efficacy in comparison to the overall. This appears to contradict the promising effects of gefapixant for osteoarthritis pain in a phase II clinical trial (which showed numerically positive but non-significant; NCT01554579). However, only four studies were included, with only two specifically modeling osteoarthritis (using mono-iodoacetate). In one of these studies, the efficacy of gefapixant was moderate and equivalent to naproxen (Richards et al., 2019). To note, the largest analgesic efficacy was achieved with local drug administration (directly intra-articular), which aligns with the significant expression of P2X3-P2X2/3R in afferent fibers innervating joints (Teixeira et al., 2017, 2020).

To interpret the results of preclinical pain studies, it is crucial to consider the specific pain outcomes evaluated, as the intervention may impact the different pain parameters in distinct ways. Noxious stimuli of different nature activate distinct nociceptors (Cobos et al., 2018; Rosenbaum et al., 2022; Tavares-Ferreira et al., 2022). The expression of P2X3-P2X2/3R seems to be restricted to non-peptidergic ($IB4^+$) nociceptors (Gonzalez-Cano et al., 2021; Vulchanova et al., 1998), which are involved in mechanical hypersensitivity but not heat sensitivity (Cavanaugh et al., 2009; Ruiz-Cantero et al., 2023) and minimally co-expressed in $TRPV1^+$ nociceptors (Gonzalez-Cano et al., 2021), responsible for heat pain (Basbaum et al., 2009). Consistently, we found that the highest analgesic efficacy was achieved for mechanically induced pain, while efficacy was lower for heat-induced pain. Unfortunately, only one study evaluated responses to cold stimulus, hindering meta-analysis. The lowest efficacy of the intervention was noted when evaluating pain-related behavior (mainly non-evoked pain), which is especially relevant since it reflects more accurately the human pain experience and is therefore more translational (Cobos and Portillo-Salido, 2013; Deuis et al., 2017; González-Cano et al., 2020; Huerta et al., 2024; Mogil, 2009). The non-evoked tests used were highly heterogeneous and model-dependent, making it difficult to speculate about underlying mechanisms. Still, the significant analgesic efficacy observed is promising, especially considering that spontaneous pain is often refractory to pharmacologic treatment (Ma et al., 2022).

The analgesic efficacy was dependent on the drug employed for inhibiting P2X3-P2X2/3R, which may be explained by pharmacodynamic or pharmacokinetic variations (Müller and Namasivayam, 2021). The highest efficacy was obtained with A-317491 (previously ABT-202), a selective antagonist developed by Abbott and reported in 2002 (Jarvis et al., 2002). Despite the drug development was discontinued in Phase I (Xu et al., 2004), It has become the standard drug for experimentation. Although A-317491 exhibited competitive and reversible P2X3-P2X2/3R inhibition with good systemic bioavailability, its limited oral bioavailability and poor water solubility posed challenges. AF-353 emerged offering enhanced physicochemical properties compared to A-317491 (Gever et al., 2010), but it was only tested in two preclinical studies (Jung et al., 2017; Kaan et al., 2010). Gefapixant (AF-219 or MK-7264), the lead antagonist developed thereafter, proved to be a potent, reversible, and peripherally restricted noncompetitive antagonist of P2X3-P2X2/3R with clear clinical interest. In fact, preclinical studies demonstrated analgesic efficacy (Richards et al., 2019) and has been evaluated in several clinical trials (some of them for pain treatment); it is even approved for chronic cough treatment (Markham, 2022). An unfortunate common side effect associated with gefapixant is dysgeusia (Ramadan et al., 2023), likely resulting from P2X2/3 receptor blockade on taste buds (Finger et al., 2005). Another analog, eliapixant, with more selectivity for P2X3 homotrimeric receptor and consequently

minimal impact on taste perception, showed efficacy in the CFA model (Davenport et al., 2021); in clinical trials for cough (NCT04562155), and in pain related with several conditions (NCT04641273; NCT04614246; Fletcher, 2022). Unfortunately, the clinical development of eliapixant was discontinued (Bayer, 2022). Currently, other derivatives are in advanced clinical development (primarily for chronic cough) and may soon be evaluated in clinical trials for pain treatment (e.g., sivopixant (NCT04110054; McGarvey et al., 2023; Niimi et al., 2022), camlipixant (NCT05600777 (Garceau and Chauret, 2019; GSK, 2023); or filapixant (Friedrich et al., 2023)). Furthermore, other inhibitors were tested in animal models of pain, including monoclonal antibodies (12D4), compound B, 14h, 15h, and further derivatives of A-317491 (see Table 1), each with barely one study per compound and no further clinical development.

The route of administration of the P2X3-P2X2/3R inhibitor significantly impacted its efficacy. Although this may be influenced by drug-specific differences, this is unlikely, as the majority of experiments used the compound A-317493 (57 out of 78, >70%), and subgroups were homogeneously distributed. Systemic and local administration routes demonstrated greater analgesic efficacy compared to intrathecal (still significant). This highlights the relevance of peripheral P2X3-P2X2/3R at the injury site (previously demonstrated (McGaraughty et al., 2003)). As a general rule, P2X3-P2X2/3R inhibition demonstrated evident analgesic effects, with the exception of intracerebroventricular (i.c.v.) administration that showed consistently pro-algesic effects (Fukui et al., 2006; Li et al., 2014; Liu et al., 2017). This apparent contradiction might be explained by the presence of P2X3-P2X2/3R in endogenous descending inhibitory pathways situated specifically in the ventrolateral midbrain periaqueductal grey, to produce endogenous analgesia (Fukui et al., 2006; Li et al., 2014; Liu et al., 2017). Because of that, when administering a P2X3-P2X2/3R inhibitor directly in the brain, an increase in pain was observed associated with the inhibition of the endogenous analgesic system (effect not observed with systemic administration).

From a translational perspective, P2X3-P2X2/3R inhibition showed to reduce nociceptor excitability (preventing sensitization) and inflammation (immune cells recruitment) after pain model induction (de Melo Aquino et al., 2019; Dong et al., 2022). The modulation of these physiological mechanisms by systemic administration of P2X3-P2X2/3R inhibitors to alleviate pain may be associated with adverse effects. In this regard, several studies suggest that blocking acute inflammation due to injury could promote the chronification of pain (Parisien et al., 2022). However, this adverse effect is less likely with these drugs compared to other more potent anti-inflammatory agents such as NSAIDs. Moreover, the neuronal mechanisms of P2X3-P2X2/3R inhibitors appear to be more relevant for the efficacy, being associated with several adverse effects that have been observed in clinical trials (hypogeusia, ageusia, and dysgeusia) (Krajewski, 2020). However, P2X3-P2X2/3R inhibitors showed a favorable safety profile in clinical trials (low incidence of mild adverse events) (Krajewski, 2020; McGarvey et al., 2022), which is one of the key advantages of these drugs. This stands in sharp contrast to many existing analgesic drugs, which are often associated with frequent and significant side effects (e.g., opioid tolerance and dependence, or sedation and dizziness caused by gabapentinoids) (Di Stefano et al., 2021). In contrast, a potential drawback of P2X3-P2X2/3R inhibitors is that their analgesic efficacy may not be as strong as that of opioids, which requires further investigation through larger clinical trials. Another potential concern of these drugs is that purinergic receptors share significant structural similarities, leading many of their ligands to act across multiple receptor types (Burnstock, 2018). In our analysis, these off-target effects are minimized as selective compounds (such as A-317491, gefapixant and eliapixant) were used (Müller and Namasivayam, 2021).

5. Conclusions

Pharmacologic inhibition of P2X3-P2X2/3R showed a robust overall analgesic effect in animal models of pain. The effect size was dependent on the type of pain: higher for visceral, muscle and neuropathic pain, but lower for inflammatory pain. The efficacy was also dependent on the pain outcome evaluated, the drug used and its administration route. This study predicts which are the more favorable situations for the highest analgesic effect of P2X3-P2X2/3R inhibition, which should be confirmed with the pertinent clinical trials.

Declaration of Generative AI and AI-assisted

During the preparation of this work the author(s) used ChatGPT3.5 in order to enhance the quality and clarity of the writing. After using this tool, the author(s) reviewed and edited the content as needed and take (s) full responsibility for the content of the publication.

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CRediT authorship contribution statement

Miguel Á. Huerta: Writing – original draft, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Daniel Marcos-Frutos:** Writing – review & editing, Data curation, Conceptualization. **Javier de la Nava:** Writing – review & editing, Data curation, Conceptualization. **Amador García-Ramos:** Writing – review & editing, Supervision, Conceptualization. **Miguel Ángel Tejada:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization. **Carolina Roza:** Writing – review & editing, Supervision, Project administration, Investigation, Funding acquisition, Formal analysis, Conceptualization.

Declaration of competing interest

None.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejphar.2024.177052.

Data availability

Data will be made available on request.

References

Abbracchio, M.P., Burnstock, G., 1994. Purinoceptors: are there families of P2X and P2Y purinoceptors? Pharmacol. Ther. 64, 445–475. https://doi.org/10.1016/0163-7258(94)00048-4.

Arribas-Blázquez, M., Olivos-Oré, L.A., Barahona, M.V., de la Muela, M.S., Solar, V., Jiménez, E., Gualix, J., McIntosh, J.M., Ferrer-Montiel, A., Miras-Portugal, M.T., Artalejo, A.R., 2019. Overexpression of P2X3 and P2X7 receptors and TRPV1 channels in adrenomedullary chromaffin cells in a rat model of neuropathic pain. Int. J. Mol. Sci. 20. https://doi.org/10.3390/IJMS20010155.

Bae, J., Kim, Y.O., Han, X., Yoon, M.H., Kim, W.M., Kim, Y.C., 2022. Synthesis and structure-activity relationship studies of benzimidazole-4,7-dione-based P2X3 receptor antagonists as novel anti-nociceptive agents. Molecules 27. https://doi.org/10.3390/MOLECULES27041337.

Ballini, E., Virginio, C., Medhurst, S.J., Summerfield, S.G., Aldegheri, L., Buson, A., Carignani, C., Chen, Y.H., Giacometti, A., Lago, I., Powell, A.J., Jarolimek, W., 2011. Characterization of three diaminopyrimidines as potent and selective antagonists of P2X3 and P2X2/3 receptors with in vivo efficacy in a pain model. Br. J. Pharmacol. 163, 1315–1325. https://doi.org/10.1111/J.1476-5381.2011.01322.X.

Barclay, J., Patel, S., Dorn, G., Wotherspoon, G., Moffatt, S., Eunson, L., Abdel'Al, S., Natt, F., Hall, J., Winter, J., Bevan, S., Wishart, W., Fox, A., Ganju, P., 2002. Functional downregulation of P2X3 receptor subunit in rat sensory neurons reveals a significant role in chronic neuropathic and inflammatory pain. J. Neurosci. 22, 8139–8147. https://doi.org/10.1523/JNEUROSCI.22-18-08139.2002.

Basbaum, A.I., Bautista, D.M., Scherrer, G., Julius, D., 2009. Cellular and molecular mechanisms of pain. Cell 139, 267. https://doi.org/10.1016/J.CELL.2009.09.028.

Bayer will discontinue phase II development candidate eliapixant [WWW Document], n. d. URL https://www.bayer.com/media/en-us/bayer-will-discontinue-phase-ii-development-candidate-eliapixant (accessed 12.24.23).

Bernier, L.P., Ase, A.R., Séguéla, P., 2018. P2X receptor channels in chronic pain pathways. Br. J. Pharmacol. 175, 2219. https://doi.org/10.1111/BPH.13957.

Brederson, J.D., Jarvis, M.F., 2008. Homomeric and heteromeric P2X3 receptors in peripheral sensory neurons. Curr Opin Investig Drugs 9, 716–725.

Browe, B.M., Olsen, A.R., Ramirez, C., Rickman, R.H., Smith, E.S.J., Park, T.J., 2020. The naked mole-rat has a functional purinergic pain pathway despite having a non-functional peptidergic pain pathway. Neurobiol Pain 8. https://doi.org/10.1016/J.YNPAI.2020.100047.

Burnstock, G., 2018. Purine and purinergic receptors. Brain Neurosci Adv 2, 239821281881749. https://doi.org/10.1177/2398212818817494.

Burnstock, G., 2009. Purinergic mechanosensory transduction and visceral pain. Mol. Pain 5, 69. https://doi.org/10.1186/1744-8069-5-69.

Burnstock, G., 2001. Purine-mediated signalling in pain and visceral perception. Trends Pharmacol. Sci. 22, 182–188. https://doi.org/10.1016/S0165-6147(00)01643-6.

Burnstock, G., 1999. Release of vasoactive substances from endothelial cells by shear stress and purinergic mechanosensory transduction. J. Anat. 194 (Pt 3), 335–342. https://doi.org/10.1046/J.1469-7580.1999.19430335.X.

Burnstock, G., 1972. Purinergic nerves. Pharmacol. Rev. 24.

Cantin, L.D., Bayrakdarian, M., Buon, C., Grazzini, E., Hu, Y.J., Labrecque, J., Leung, C., Luo, X., Martino, G., Paré, M., Payza, K., Popovic, N., Projean, D., Santhakumar, V., Walpole, C., Yu, X.H., Tomaszewski, M.J., 2012. Discovery of P2X3 selective antagonists for the treatment of chronic pain. Bioorg. Med. Chem. Lett. 22, 2565–2571. https://doi.org/10.1016/J.BMCL.2012.01.124.

Cavanaugh, D.J., Lee, H., Lo, L., Shields, S.D., Zylka, M.J., Basbaum, A.I., Anderson, D.J., 2009. Distinct subsets of unmyelinated primary sensory fibers mediate behavioral responses to noxious thermal and mechanical stimuli. Proc. Natl. Acad. Sci. U.S.A. 106, 9075–9080. https://doi.org/10.1073/PNAS.0901507106.

Chaban, V., Christensen, A., Wakamatsu, M., McDonald, M., Rapkin, A., McDonald, J., Micevych, P., 2007. The same dorsal root ganglion neurons innervate uterus and colon in the rat. Neuroreport 18, 209–212. https://doi.org/10.1097/WNR.0B013E32801231BF.

Chizh, B.A., Illes, P., 2001. P2X receptors and nociception. Pharmacol. Rev. 53, 553–568. https://doi.org/10.1016/s0021-5198(19)47612-9.

Cobos, E., Portillo-Salido, E., 2013. "Bedside-to-Bench" behavioral outcomes in animal models of pain: beyond the evaluation of reflexes. Curr. Neuropharmacol. 11, 560–591. https://doi.org/10.2174/1570159X113119990041.

Cobos, E.J., Nickerson, C.A., Gao, F., Chandran, V., Bravo-Caparrós, I., González-Cano, R., Riva, P., Andrews, N.A., Latremoliere, A., Seehus, C.R., Perazzoli, G., Nieto, F.R., Joller, N., Painter, M.W., Ma, C.H.E., Omura, T., Chesler, E.J., Geschwind, D.H., Coppola, G., Rangachari, M., Woolf, C.J., Costigan, M., 2018. Mechanistic differences in neuropathic pain modalities revealed by correlating behavior with global expression profiling. Cell Rep. 22, 1301–1312. https://doi.org/10.1016/J.CELREP.2018.01.006.

Cockayne, D.A., Dunn, P.M., Zhong, Y., Rong, W., Hamilton, S.G., Knight, G.E., Ruan, H. Z., Ma, B., Yip, P., Nunn, P., McMahon, S.B., Burnstock, G., Ford, A.P.D.W., 2005. P2X2 knockout mice and P2X2/P2X3 double knockout mice reveal a role for the P2X2 receptor subunit in mediating multiple sensory effects of ATP. J. Physiol. 567, 621–639. https://doi.org/10.1113/JPHYSIOL.2005.088435.

Cockayne, D.A., Hamilton, S.G., Zhu, Q.M., Dunn, P.M., Zhong, Y., Novakovic, S., Malmberg, A.B., Cain, G., Berson, A., Kassotakis, L., Hedley, L., Lachnit, W.G., Burnstock, G., McMahon, S.B., Ford, A.P.D.W., 2000. Urinary bladder hyporeflexia and reduced pain-related behaviour in P2X3-deficient mice. Nature 407, 1011–1015. https://doi.org/10.1038/35039519.

Cumpston, M., Li, T., Page, M.J., Chandler, J., Welch, V.A., Higgins, J.P., Thomas, J., 2019. Updated guidance for trusted systematic reviews: a new edition of the Cochrane Handbook for Systematic Reviews of Interventions. Cochrane Database Syst. Rev. 10, ED000142. https://doi.org/10.1002/14651858.ED000142.

Davenport, A.J., Neagoe, I., Bräuer, N., Koch, M., Rotgeri, A., Nagel, J., Laux-Biehlmann, A., Machet, F., Coelho, A.M., Boyce, S., Carty, N., Gemkow, M.J., Hess, S. D., Zollner, T.M., Fischer, O.M., 2021. Eliapixant is a selective P2X3 receptor antagonist for the treatment of disorders associated with hypersensitive nerve fibers. Sci. Rep. 11. https://doi.org/10.1038/S41598-021-99177-0.

de Melo Aquino, B., da Silva dos Santos, D.F., Jorge, C.O., Marques, A.C.S., Teixeira, J. M., Parada, C.A., Oliveira-Fusaro, M.C.G., 2019. P2X3 receptors contribute to muscle pain induced by static contraction by a mechanism dependent on neutrophil migration. Purinergic Signal. 15, 167–175. https://doi.org/10.1007/S11302-019-09659-0.

de Oliveira Fusaro, M.C.G., Pelegrini-da-Silva, A., Araldi, D., Parada, C.A., Tambeli, C.H., 2010. P2X3 and P2X2/3 receptors mediate mechanical hyperalgesia induced by bradykinin, but not by pro-inflammatory cytokines, PGE_2 or dopamine. Eur. J. Pharmacol. 649, 177–182. https://doi.org/10.1016/J.EJPHAR.2010.09.037.

Deiteren, A., Van Der Linden, L., De Wit, A., Ceuleers, H., Buckinx, R., Timmermans, J.P., Moreels, T.G., Pelckmans, P.A., De Man, J.G., De Winter, B.Y., 2015. P2X3 receptors mediate visceral hypersensitivity during acute chemically-induced colitis and in the post-inflammatory phase via different mechanisms of sensitization. PLoS One 10. https://doi.org/10.1371/JOURNAL.PONE.0123810.

Deng, Y., Yuan, D., Deng, Z., Liang, J., Zhang, Z., Hei, Z., Li, X., 2023. Bone marrow-derived mesenchymal stem cells attenuate complete Freund's adjuvant-induced inflammatory pain by inhibiting the expression of P2X3. Cell Prolif. 56. https://doi.org/10.1111/CPR.13461.

Deuis, J.R., Dvorakova, L.S., Vetter, I., 2017. Methods used to evaluate pain behaviors in rodents. Front. Mol. Neurosci. 10, 271711. https://doi.org/10.3389/FNMOL.2017.00284/BIBTEX.

Di Stefano, G., Di Lionardo, A., Di Pietro, G., Cruccu, G., Truini, A., 2021. Pharmacotherapeutic options for managing neuropathic pain: a systematic review and meta-analysis. Pain Res. Manag. 2021, 6656863. https://doi.org/10.1155/2021/6656863.

Ding, S., Zhu, L., Tian, Y., Zhu, T., Huang, X., Zhang, X., 2017. P2X3 receptor involvement in endometriosis pain via ERK signaling pathway. PLoS One 12. https://doi.org/10.1371/JOURNAL.PONE.0184647.

Dong, C.R., Zhang, W.J., Luo, H.L., 2022. Association between P2X3 receptors and neuropathic pain: as a potential therapeutic target for therapy. Biomed. Pharmacother. 150. https://doi.org/10.1016/J.BIOPHA.2022.113029.

Donnelly-Roberts, D., McGaraughty, S., Shieh, C.C., Honore, P., Jarvis, M.F., 2008. Painful purinergic receptors. J. Pharmacol. Exp. Therapeut. 324, 409–415. https://doi.org/10.1124/JPET.106.105890.

Dorn, G., Patel, S., Wotherspoon, G., Hemmings-Mieszczak, M., Barclay, J., Natt, F.J.C., Martin, P., Bevan, S., Fox, A., Ganju, P., Wishart, W., Hall, J., 2004. siRNA relieves chronic neuropathic pain. Nucleic Acids Res. 32. https://doi.org/10.1093/NAR/GNH044.

Dubois, M.Y., Gallagher, R.M., Lippe, P.M., 2009. Pain medicine position paper. Pain Med. 10, 972–1000. https://doi.org/10.1111/J.1526-4637.2009.00696.X.

Egger, M., Smith, G.D., Schneider, M., Minder, C., 1997. Bias in meta-analysis detected by a simple, graphical test. BMJ 315, 629–634. https://doi.org/10.1136/BMJ.315.7109.629.

Fayaz, A., Croft, P., Langford, R.M., Donaldson, L.J., Jones, G.T., 2016. Prevalence of chronic pain in the UK: a systematic review and meta-analysis of population studies. BMJ Open 6. https://doi.org/10.1136/BMJOPEN-2015-010364.

Fei, X., He, X., Tai, Z., Wang, H., Qu, S., Chen, L., Hu, Q., Fang, J., Jiang, Y., 2020. Electroacupuncture alleviates diabetic neuropathic pain in rats by suppressing P2X3 receptor expression in dorsal root ganglia. Purinergic Signal. 16, 491–502. https://doi.org/10.1007/S11302-020-09728-9.

Ferrari, L.F., Levine, J.D., Green, P.G., 2016. Mechanisms mediating nitroglycerin-induced delayed-onset hyperalgesia in the rat. Neuroscience 317, 121–129. https://doi.org/10.1016/J.NEUROSCIENCE.2016.01.005.

Finger, T.E., Danilova, V., Barrows, J., Bartel, D.L., Vigers, A.J., Stone, L., Hellekant, G., Kinnamon, S.C., 2005. ATP signaling is crucial for communication from taste buds to gustatory nerves. Science 310, 1495–1499. https://doi.org/10.1126/SCIENCE.1118435.

Finnerup, N.B., Attal, N., Haroutounian, S., McNicol, E., Baron, R., Dworkin, R.H., Gilron, I., Haanpää, M., Hansson, P., Jensen, T.S., Kamerman, P.R., Lund, K., Moore, A., Raja, S.N., Rice, A.S.C., Rowbotham, M., Sena, E., Siddall, P., Smith, B.H., Wallace, M., 2015. Pharmacotherapy for neuropathic pain in adults: a systematic review and meta-analysis. Lancet Neurol. 14, 162–173. https://doi.org/10.1016/S1474-4422(14)70251-0.

Fletcher, M.C., 2022. Selectivity of the P2X3 receptor antagonist Eliapixant, and its potential use in the treatment of endometriosis. Purinergic Signal. 18. https://doi.org/10.1007/S11302-021-09831-5.

Ford, A.P., 2012. In pursuit of P2X3 antagonists: novel therapeutics for chronic pain and afferent sensitization. Purinergic Signal. 8, 3–26. https://doi.org/10.1007/S11302-011-9271-6.

Friedrich, C., Francke, K., Birring, S.S., van den Berg, J.W.K., Marsden, P.A., McGarvey, L., Turner, A.M., Wielders, P., Gashaw, I., Klein, S., Morice, A.H., 2023. The P2X3 receptor antagonist filapixant in patients with refractory chronic cough: a randomized controlled trial. Respir. Res. 24, 1–12. https://doi.org/10.1186/S12931-023-02384-8/TABLES/5.

Fukui, M., Nakagawa, T., Minami, M., Satoh, M., Kaneko, S., 2006. Inhibitory role of supraspinal P2X3/P2X2/3 subtypes on nociception in rats. Mol. Pain 2. https://doi.org/10.1186/1744-8069-2-19/ASSET/IMAGES/LARGE/10.1186_1744-8069-2-19-FIG2.JPEG.

Garceau, D., Chauret, N., 2019. BLU-5937: a selective P2X3 antagonist with potent anti-tussive effect and no taste alteration. Pulm. Pharmacol. Ther. 56, 56–62. https://doi.org/10.1016/J.PUPT.2019.03.007.

Gever, J.R., Soto, R., Henningsen, R.A., Martin, R.S., Hackos, D.H., Panicker, S., Rubas, W., Oglesby, I.B., Dillon, M.P., Milla, M.E., Burnstock, G., Ford, A.P.D.W., 2010. AF-353, a novel, potent and orally bioavailable P2X3/P2X2/3 receptor antagonist. Br. J. Pharmacol. 160, 1387–1398. https://doi.org/10.1111/J.1476-5381.2010.00796.X.

Glare, P., Aubrey, K., Gulati, A., Lee, Y.C., Moryl, N., Overton, S., 2022. Pharmacologic management of persistent pain in cancer survivors. Drugs 82, 275–291. https://doi.org/10.1007/S40265-022-01675-6/TABLES/5.

Gonzalez-Cano, R., Montilla-García, Á., Perazzoli, G., Torres, J.M., Cañizares, F.J., Fernández-Segura, E., Costigan, M., Baeyens, J.M., Cobos, E.J., 2021. Intracolonic mustard oil induces visceral pain in mice by TRPA1-dependent and -independent mechanisms: role of tissue injury and P2X receptors. Front. Pharmacol. 11. https://doi.org/10.3389/FPHAR.2020.613068/FULL.

González-Cano, R., Montilla-García, Á., Ruiz-Cantero, M.C., Bravo-Caparrós, I., Tejada, M., Nieto, F.R., Cobos, E.J., 2020. The search for translational pain outcomes to refine analgesic development: where did we come from and where are we going? Neurosci. Biobehav. Rev. 113, 238–261. https://doi.org/10.1016/J.NEUBIOREV.2020.03.004.

González-Rodríguez, S., Pevida, M., Roques, B.P., Fournié-Zaluski, M.C., Hidalgo, A., Menéndez, L., Baamonde, A., 2009. Involvement of enkephalins in the inhibition of osteosarcoma-induced thermal hyperalgesia evoked by the blockade of peripheral P2X3 receptors. Neurosci. Lett. 465, 285–289. https://doi.org/10.1016/J.NEULET.2009.09.015.

González-Rodríguez, S., Poras, H., Menéndez, L., Lastra, A., Ouimet, T., Fournié-Zaluski, M.C., Roques, B.P., Baamonde, A., 2017. Synergistic combinations of the dual enkephalinase inhibitor PL265 given orally with various analgesic compounds acting on different targets, in a murine model of cancer-induced bone pain. Scand J Pain 14, 25–38. https://doi.org/10.1016/J.SJPAIN.2016.09.011.

Grishin, E.V., Savchenko, G.A., Vassilevski, A.A., Korolkova, Y.V., Boychuk, Y.A., Viatchenko-Karpinski, V.Y., Nadezhdin, K.D., Arseniev, A.S., Pluzhnikov, K.A., Kulyk, V.B., Voitenko, N.V., Krishtal, O.O., 2010. Novel peptide from spider venom inhibits P2X3 receptors and inflammatory pain. Ann. Neurol. 67, 680–683. https://doi.org/10.1002/ANA.21949.

GSK reaches agreement to acquire late-stage biopharmaceutical company BELLUS Health | GSK [WWW Document], n.d. URL https://www.gsk.com/en-gb/media/press-releases/gsk-reaches-agreement-to-acquire-late-stage-biopharmaceutical-company-bellus-health/#_BRA1 (accessed 12.24.23).

Hanaka, M., Iba, K., Dohke, T., Kanaya, K., Okazaki, S., Yamashita, T., 2018. Antagonists to TRPV1, ASICs and P2X have a potential role to prevent the triggering of regional bone metabolic disorder and pain-like behavior in tail-suspended mice. Bone 110, 284–294. https://doi.org/10.1016/J.BONE.2018.02.006.

Hansen, R.R., Nasser, A., Falk, S., Baldvinsson, S.B., Ohlsson, P.H., Bahl, J.M.C., Jarvis, M.F., Ding, M., Heegaard, A.M., 2012. Chronic administration of the selective P2X3, P2X2/3 receptor antagonist, A-317491, transiently attenuates cancer-induced bone pain in mice. Eur. J. Pharmacol. 688, 27–34. https://doi.org/10.1016/J.EJPHAR.2012.05.008.

Harrer, M., Cuijpers, P., Furukawa, T., Ebert, D., 2021. Doing meta-analysis with R: a hands-on guide. https://doi.org/10.1201/9781003107347.

He, J.J., Wang, X., Liang, C., Yao, X., Zhang, Z.S., Yang, R.H., Fang, D., 2020. Wnt5b/Ryk-mediated membrane trafficking of P2X3 receptors contributes to bone cancer pain. Exp. Neurol. 334. https://doi.org/10.1016/J.EXPNEUROL.2020.113482.

Higgins, J.P.T., Thompson, S.G., Deeks, J.J., Altman, D.G., 2003. Measuring inconsistency in meta-analyses. BMJ 327, 557–560. https://doi.org/10.1136/BMJ.327.7414.557.

High, B., Jetté, M.E., Li, M., Ramakrishnan, V.R., Clary, M., Prager, J., Draf, J., Hummel, T., Finger, T.E., 2023. Variability in P2X receptor composition in human taste nerves: implications for treatment of chronic cough. ERJ Open Res 9. https://doi.org/10.1183/23120541.00007-2023.

Hilliges, M., Weidner, C., Schmelz, M., Schmidt, R., Ørstavik, K., Torebjörk, E., Handwerker, H., 2002. ATP responses in human C nociceptors. Pain 98, 59–68. https://doi.org/10.1016/S0304-3959(01)00469-9.

Hoheisel, U., Reinöhl, J., Unger, T., Mense, S., 2004. Acidic pH and capsaicin activate mechanosensitive group IV muscle receptors in the rat. Pain 110, 149–157. https://doi.org/10.1016/j.pain.2004.03.043.

Honore, P., Kage, K., Mikusa, J., Watt, A.T., Johnston, J.F., Wyatt, J.R., Faltynek, C.R., Jarvis, M.F., Lynch, K., 2002. Analgesic profile of intrathecal P2X3 antisense oligonucleotide treatment in chronic inflammatory and neuropathic pain states in rats. Pain 99, 11–19. https://doi.org/10.1016/S0304-3959(02)00032-5.

Hooijmans, C.R., Rovers, M.M., De Vries, R.B.M., Leenaars, M., Ritskes-Hoitinga, M., Langendam, M.W., 2014. SYRCLE's risk of bias tool for animal studies. BMC Med. Res. Methodol. 14. https://doi.org/10.1186/1471-2288-14-43.

Hori, K., Ozaki, N., Suzuki, S., Sugiura, Y., 2010. Upregulations of P2X(3) and ASIC3 involve in hyperalgesia induced by cisplatin administration in rats. Pain 149, 393–405. https://doi.org/10.1016/J.PAIN.2010.03.005.

Hsieh, Y.L., Chiang, H., Lue, J.H., Hsieh, S.T., 2012. P2X3-mediated peripheral sensitization of neuropathic pain in resiniferatoxin-induced neuropathy. Exp. Neurol. 235, 316–325. https://doi.org/10.1016/J.EXPNEUROL.2012.02.013.

Hua, S. qi, Hu, J. ling, Zou, F. long, Liu, J. peng, Luo, H. liang, Hu, D. xia, Wu, L. dong, Zhang, W. jun, 2022. P2X7 receptor in inflammation and pain. Brain Res. Bull. 187, 199–209. https://doi.org/10.1016/J.BRAINRESBULL.2022.07.006.

Huerta, M., Garcia, M.M., García-Parra, B., Serrano-Afonso, A., Paniagua, N., 2023. Investigational drugs for the treatment of postherpetic neuralgia: systematic review of randomized controlled trials. Int. J. Mol. Sci. 24. https://doi.org/10.3390/IJMS241612987.

Huerta, M.Á., Cisneros, E., Alique, M., Roza, C., 2024. Strategies for measuring non-evoked pain in preclinical models of neuropathic pain: systematic review. Neurosci. Biobehav. Rev. 163, 105761. https://doi.org/10.1016/J.NEUBIOREV.2024.105761.

Huerta, M.Á., de la Nava, J., Artacho-Cordón, A., Nieto, F.R., 2023. Efficacy and security of tetrodotoxin in the treatment of cancer-related pain: systematic review and meta-analysis. Mar. Drugs 21, 316. https://doi.org/10.3390/MD21050316/S1.

Jarvis, M.F., Burgard, E.C., McGaraughty, S., Honore, P., Lynch, K., Brennan, T.J., Subieta, A., Van Biesen, T., Cartmell, J., Bianchi, B., Niforatos, W., Kage, K., Yu, H., Mikusa, J., Wismer, C.T., Zhu, C.Z., Chu, K., Lee, C.H., Stewart, A.O., Polakowski, J., Cox, B.F., Kowaluk, E., Williams, M., Sullivan, J., Faltynek, C., 2002. A-317491, a novel potent and selective non-nucleotide antagonist of P2X3 and P2X2/3 receptors, reduces chronic inflammatory and neuropathic pain in the rat. Proc. Natl. Acad. Sci. U.S.A. 99, 17179–17184. https://doi.org/10.1073/PNAS.252537299.

Jin, Y., Qiu, C.Y., Wei, S., Han, L., Liu, T.T., Hu, W.P., 2020. Potentiation of P2X3 receptor mediated currents by endothelin-1 in rat dorsal root ganglion neurons. Neuropharmacology 181. https://doi.org/10.1016/J.NEUROPHARM.2020.108356.

Jin, Y., Wei, S., Liu, T.T., Qiu, C.Y., Hu, W.P., 2021. Acute P38-mediated enhancement of P2X3 receptor currents by TNF-α in rat dorsal root ganglion neurons. J. Inflamm. Res. 14, 2841–2850. https://doi.org/10.2147/JIR.S315774.

Johannes, C.B., Le, T.K., Zhou, X., Johnston, J.A., Dworkin, R.H., 2010. The prevalence of chronic pain in United States adults: results of an Internet-based survey. J. Pain 11, 1230–1239. https://doi.org/10.1016/J.JPAIN.2010.07.002.

Jorge, C.O., de Azambuja, G., Gomes, B.B., Rodrigues, H.L., Luchessi, A.D., de Oliveira-Fusaro, M.C.G., 2020. P2X3 receptors contribute to transition from acute to chronic muscle pain. Purinergic Signal. 16, 403–414. https://doi.org/10.1007/S11302-020-09718-X.

Jung, Y.H., Kim, Y.O., Han, J.H., Kim, Y.C., Yoon, M.H., 2017. Isobolographic analysis of drug combinations with intrathecal BRL52537 (κ-opioid agonist), pregabalin (calcium channel modulator), AF 353 (P2X3 receptor antagonist), and A804598 (P2X7 receptor antagonist) in neuropathic rats. Anesth. Analg. 125, 670–677. https://doi.org/10.1213/ANE.0000000000001883.

Kaan, T.K.Y., Yip, P.K., Patel, S., Davies, M., Marchand, F., Cockayne, D.A., Nunn, P.A., Dickenson, A.H., Ford, A.P.D.W., Zhong, Y., Malcangio, M., McMahon, S.B., 2010. Systemic blockade of P2X3 and P2X2/3 receptors attenuates bone cancer pain behaviour in rats. Brain 133, 2549–2564. https://doi.org/10.1093/BRAIN/AWQ194.

Kakimoto, S., Nagakura, Y., Tamura, S., Watabiki, T., Shibasaki, K., Tanaka, S., Mori, M., Sasamata, M., Okada, M., 2008. Minodronic acid, a third-generation bisphosphonate, antagonizes purinergic P2X(2/3) receptor function and exerts an analgesic effect in pain models. Eur. J. Pharmacol. 589, 98–101. https://doi.org/10.1016/J.EJPHAR.2008.05.011.

Kato, Y., Hiasa, M., Ichikawa, R., Hasuzawa, N., Kadowaki, A., Iwatsuki, K., Shima, K., Endo, Y., Kitahara, Y., Inoue, T., Nomura, M., Omote, H., Moriyama, Y., Miyaji, T., 2017. Identification of a vesicular ATP release inhibitor for the treatment of neuropathic and inflammatory pain. Proc. Natl. Acad. Sci. U.S.A. 114, E6297–E6305. https://doi.org/10.1073/PNAS.1704847114.

Kennedy, C., 2005. P2X receptors: targets for novel analgesics? Neuroscientist 11, 345–356. https://doi.org/10.1177/1073858404274063.

Kiso, T., Watabiki, T., Tsukamoto, M., Okabe, M., Kagami, M., Nishimura, K., Aoki, T., Matsuoka, N., 2008. Pharmacological characterization and gene expression profiling of an L5/L6 spinal nerve ligation model for neuropathic pain in mice. Neuroscience 153, 492–500. https://doi.org/10.1016/J.NEUROSCIENCE.2008.02.031.

Knežević, P.T., Vukman, R., Antonić, R., Kovač, Z., Uhač, I., Simonić-Kocijan, S., 2016. The role of P2X3 receptors in bilateral masseter muscle allodynia in rats. Croat. Med. J. 57, 530–539. https://doi.org/10.3325/CMJ.2016.57.530.

Koizumi, M., Asano, S., Furukawa, A., Hayashi, Y., Hitomi, S., Shibuta, I., Hayashi, K., Kato, F., Iwata, K., Shinoda, M., 2021. P2X3 receptor upregulation in trigeminal ganglion neurons through TNFα production in macrophages contributes to trigeminal neuropathic pain in rats. J. Headache Pain 22. https://doi.org/10.1186/S10194-021-01244-4.

Kong, Y., Shao, Y., Ren, C., Yang, G., 2021. Endometrial stem/progenitor cells and their roles in immunity, clinical application, and endometriosis. Stem Cell Res. Ther. 12. https://doi.org/10.1186/S13287-021-02526-Z.

Krajewski, J.L., 2020. P2X3-Containing receptors as targets for the treatment of chronic pain. Neurotherapeutics 17, 826–838. https://doi.org/10.1007/S13311-020-00934-2/TABLES/1.

Krimon, S., Araldi, D., Do Prado, F.C., Tambeli, C.H., Oliveira-Fusaro, M.C.G., Parada, C. A., 2013. P2X3 receptors induced inflammatory nociception modulated by TRPA1, 5-HT3 and 5-HT1A receptors. Pharmacol. Biochem. Behav. 112, 49–55. https://doi.org/10.1016/J.PBB.2013.09.017.

Kunori, S., Matsumura, S., Mabuchi, T., Tatsumi, S., Sugimoto, Y., Minami, T., Ito, S., 2009. Involvement of prostaglandin F 2 alpha receptor in ATP-induced mechanical allodynia. Neuroscience 163, 362–371. https://doi.org/10.1016/J.NEUROSCIENCE.2009.05.069.

Leadley, R.M., Armstrong, N., Lee, Y.C., Allen, A., Kleijnen, J., 2012. Chronic diseases in the European union: the prevalence and health cost implications of chronic pain. J. Pain Palliat. Care Pharmacother. 26, 310–325. https://doi.org/10.3109/15360288.2012.736933.

Lewis, C., Neidhart, S., Holy, C., North, R.A., Buell, G., Surprenant, A., 1995. Coexpression of P2X2 and P2X3 receptor subunits can account for ATP-gated currents in sensory neurons. Nature 377, 432–435. https://doi.org/10.1038/377432A0.

Li, G., Jiang, H., Zheng, C., Zhu, G., Xu, Y., Sheng, X., Wu, B., Guo, J., Zhu, S., Zhan, Y., Lin, W., Ding, R., Zhang, C., Liu, S., Zou, L., Yi, Z., Liang, S., 2017. Long noncoding RNA MRAK009713 is a novel regulator of neuropathic pain in rats. Pain 158, 2042–2052. https://doi.org/10.1097/J.PAIN.0000000000001013.

Li, J., Sinoway, L.I., 2002. ATP stimulates chemically sensitive and sensitizes mechanically sensitive afferents. Am. J. Physiol. Heart Circ. Physiol. 283. https://doi.org/10.1152/AJPHEART.00395.2002.

Li, S.F., Zhang, Y.Y., Li, Y.Y., Wen, S., Xiao, Z., 2014. Antihyperalgesic effect of 5-HT7 receptor activation on the midbrain periaqueductal gray in a rat model of neuropathic pain. Pharmacol. Biochem. Behav. 127, 49–55. https://doi.org/10.1016/J.PBB.2014.10.007.

Liu, M., Yang, H., Fang, D., Yang, J.J., Cai, J., Wan, Y., Chui, D.H., Han, J.S., Xing, G.G., 2013. Upregulation of P2X3 receptors by neuronal calcium sensor protein VILIP-1 in dorsal root ganglions contributes to the bone cancer pain in rats. Pain 154, 1551–1568. https://doi.org/10.1016/J.PAIN.2013.04.022.

Liu, P.F., Fang, H.Z., Yang, Y., Zhang, Q.Q., Zhou, Q.Q., Chen, S.S., Zhou, F., Zhang, L.C., 2017. Activation of P2X3 receptors in the cerebrospinal fluid-contacting nucleus

neurons reduces formalin-induced pain behavior via PAG in a rat model. Neuroscience 358, 93–102. https://doi.org/10.1016/j.neuroscience.2017.06.036.
Lopes, A.H., Silva, R.L., Fonseca, M.D., Gomes, F.I., Maganin, A.G., Ribeiro, L.S., Marques, L.M.M., Cunha, F.Q., Alves-Filho, J.C., Zamboni, D.S., Lopes, N.P., Franklin, B.S., Gombault, A., Ramalho, F.S., Quesniaux, V.F.J., Couillin, I., Ryffel, B., Cunha, T.M., 2020. Molecular basis of carrageenan-induced cytokines production in macrophages. Cell Commun. Signal. 18. https://doi.org/10.1186/S12964-020-00621-X.
Lu, R., Metzner, K., Zhou, F., Flauaus, C., Balzulat, A., Engel, P., Petersen, J., Ehinger, R., Bausch, A., Ruth, P., Lukowski, R., Schmidtko, A., 2021. Functional coupling of slack channels and P2X3 receptors contributes to neuropathic pain processing. Int. J. Mol. Sci. 22, 1–18. https://doi.org/10.3390/IJMS22010405.
Luo, H. mei, Ye, J. rong, Pu, F. qin, Luo, H. liang, Zhang, W. jun, 2023. Role and therapeutic target of P2X2/3 receptors in visceral pain. Neuropeptides 101. https://doi.org/10.1016/J.NPEP.2023.102355.
Ma, B., Yu, L. hua, Fan, J., Cong, B., He, P., Ni, X., Burnstock, G., 2011. Estrogen modulation of peripheral pain signal transduction: involvement of P2X(3) receptors. Purinergic Signal. 7, 73–83. https://doi.org/10.1007/S11302-010-9212-9.
Ma, L., Liu, S., Yi, M., Wan, Y., 2022. Spontaneous pain as a challenge of research and management in chronic pain. Medical Review 2, 308–319. https://doi.org/10.1515/MR-2022-0007/ASSET/GRAPHIC/J_MR-2022-0007_FIG_001.JPG.
Markham, A., 2022. Gefapixant: first approval. Drugs 82, 691–695. https://doi.org/10.1007/S40265-022-01700-8.
McGaraughty, S., Honore, P., Wismer, C.T., Mikusa, J., Zhu, C.Z., McDonald, H.A., Bianchi, B., Faltynek, C.R., Jarvis, M.F., 2005. Endogenous opioid mechanisms partially mediate P2X3/P2X2/3-related antinociception in rat models of inflammatory and chemogenic pain but not neuropathic pain. Br. J. Pharmacol. 146, 180–188. https://doi.org/10.1038/SJ.BJP.0706346.
McGaraughty, S., Wismer, C.T., Zhu, C.Z., Mikusa, J., Honore, P., Chu, K.L., Lee, C.H., Faltynek, C.R., Jarvis, M.F., 2003. Effects of A-317491, a novel and selective P2X3/P2X2/3 receptor antagonist, on neuropathic, inflammatory and chemogenic nociception following intrathecal and intraplantar administration. Br. J. Pharmacol. 140, 1381–1388. https://doi.org/10.1038/SJ.BJP.0705574.
McGarvey, L., Smith, J.A., Morice, A., Birring, S.S., Chung, K.F., Dicpinigaitis, P.V., Niimi, A., Benninger, M.S., Sher, M., Matsunaga, Y., Miyazaki, S., Machida, M., Ishihara, H., Mahmood, A., Gomez, J.C., 2023. A randomized, double-blind, placebo-controlled, parallel-group phase 2b trial of P2X3 receptor antagonist sivopixant for refractory or unexplained chronic cough. Lung 201, 25–35. https://doi.org/10.1007/S00408-022-00592-5.
McGarvey, L.P., Birring, S.S., Morice, A.H., Dicpinigaitis, P.V., Pavord, I.D., Schelfhout, J., Nguyen, A.M., Li, Q., Tzontcheva, A., Iskold, B., Green, S.A., Rosa, C. La, Muccino, D.R., Smith, J.A., 2022. Efficacy and safety of gefapixant, a P2X3 receptor antagonist, in refractory chronic cough and unexplained chronic cough (COUGH-1 and COUGH-2): results from two double-blind, randomised, parallel-group, placebo-controlled, phase 3 trials. Lancet 399, 909–923. https://doi.org/10.1016/S0140-6736(21)02348-5.
McGuinness, L.A., Higgins, J.P.T., 2021. Risk-of-bias VISualization (robvis): an R package and Shiny web app for visualizing risk-of-bias assessments. Res. Synth. Methods 12, 55–61. https://doi.org/10.1002/JRSM.1411.
Mense, S., 2010. Functional anatomy of muscle: muscle, nociceptors and afferent fibers. Muscle Pain: Understanding the Mechanisms 17–48. https://doi.org/10.1007/978-3-540-85021-2_2.
Mense, S., 2008. Muscle pain: mechanisms and clinical significance. Dtsch Arztebl Int 105, 214–219. https://doi.org/10.3238/ARTZEBL.2008.0214.
Mercadante, S., 2022. Cancer pain treatment strategies in patients with cancer. Drugs 82, 1357–1366. https://doi.org/10.1007/S40265-022-01780-6/TABLES/2.
Mo, G., Bernier, L.P., Zhao, Q., Chabot-Doré, A.J., Ase, A.R., Logothetis, D., Cao, C.Q., Séguéla, P., 2009. Subtype-specific regulation of P2X3 and P2X2/3 receptors by phosphoinositides in peripheral nociceptors. Mol. Pain 5. https://doi.org/10.1186/1744-8069-5-47.
Mogil, J.S., 2009. Animal models of pain: progress and challenges. Nat. Rev. Neurosci. 10, 283–294. https://doi.org/10.1038/NRN2606.
Mørk, H., Ashina, M., Bendtsen, L., Olesen, J., Jensen, R., 2003. Experimental muscle pain and tenderness following infusion of endogenous substances in humans. Eur. J. Pain 7, 145–153. https://doi.org/10.1016/S1090-3801(02)00096-4.
Müller, C.E., Namasivayam, V., 2021. Recommended tool compounds and drugs for blocking P2X and P2Y receptors. Purinergic Signal. 17, 633–648. https://doi.org/10.1007/S11302-021-09813-7.
Niimi, A., Saito, J., Kamei, T., Shinkai, M., Ishihara, H., Machida, M., Miyazaki, S., 2022. Randomised trial of the P2X3 receptor antagonist sivopixant for refractory chronic cough. Eur. Respir. J. 59. https://doi.org/10.1183/13993003.00725-2021.
Noma, N., Shinoda, M., Honda, K., Kiyomoto, M., Dezawa, K., Nakaya, Y., Komiyama, O., Imamura, Y., Iwata, K., 2013. Interaction of IL-1β and P2X(3) receptor in pathologic masseter muscle pain. J. Dent. Res. 92, 456–460. https://doi.org/10.1177/0022034513483770.
North, R.A., 2004. P2X3 receptors and peripheral pain mechanisms. J. Physiol. 554, 301–308. https://doi.org/10.1113/JPHYSIOL.2003.048587.
Ohyama, S., Ouchi, T., Kimura, M., Kurashima, R., Yasumatsu, K., Nishida, D., Hitomi, S., Ubaidus, S., Kuroda, H., Ito, S., Takano, M., Ono, K., Mizoguchi, T., Katakura, A., Shibukawa, Y., 2022. Piezo1-pannexin-1-P2X3 axis in odontoblasts and neurons mediates sensory transduction in dentinal sensitivity. Front. Physiol. 13. https://doi.org/10.3389/FPHYS.2022.891759.
Oliveira, M.C.G., Pelegrini-da-Silva, A., Tambeli, C.H., Parada, C.A., 2009. Peripheral mechanisms underlying the essential role of P2X3,2/3 receptors in the development of inflammatory hyperalgesia. Pain 141, 127–134. https://doi.org/10.1016/J.PAIN.2008.10.024.
Page, M.J., McKenzie, J.E., Bossuyt, P.M., Boutron, I., Hoffmann, T.C., Mulrow, C.D., Shamseer, L., Tetzlaff, J.M., Akl, E.A., Brennan, S.E., Chou, R., Glanville, J., Grimshaw, J.M., Hróbjartsson, A., Lalu, M.M., Li, T., Loder, E.W., Mayo-Wilson, E., McDonald, S., McGuinness, L.A., Stewart, L.A., Thomas, J., Tricco, A.C., Welch, V.A., Whiting, P., Moher, D., 2021. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ 372, n71. https://doi.org/10.1136/bmj.n71.
Parisien, M., Lima, L.V., Dagostino, C., El-Hachem, N., Drury, G.L., Grant, A.V., Huising, J., Verma, V., Meloto, C.B., Silva, J.R., Dutra, G.G.S., Markova, T., Dang, H., Tessier, P.A., Slade, G.D., Nackley, A.G., Ghasemlou, N., Mogil, J.S., Allegri, M., Diatchenko, L., 2022. Acute inflammatory response via neutrophil activation protects against the development of chronic pain. Sci. Transl. Med. 14. https://doi.org/10.1126/SCITRANSLMED.ABJ9954.
Peters, J.L., Sutton, A.J., Jones, D.R., Abrams, K.R., Rushton, L., 2007. Performance of the trim and fill method in the presence of publication bias and between-study heterogeneity. Stat. Med. 26, 4544–4562. https://doi.org/10.1002/SIM.2889.
Prado, F.C., Araldi, D., Vieira, A.S., Oliveira-Fusaro, M.C.G., Tambeli, C.H., Parada, C.A., 2013. Neuronal P2X3 receptor activation is essential to the hyperalgesia induced by prostaglandins and sympathomimetic amines released during inflammation. Neuropharmacology 67, 252–258. https://doi.org/10.1016/J.NEUROPHARM.2012.11.011.
Qi, D., Yang, Y., Ji, P., Kong, J., Wu, Q., Si, H., 2016. Upregulation of the purinergic receptor subtype P2X3 in the trigeminal ganglion is involved in orofacial pain induced by occlusal interference in rats. J Oral Facial Pain Headache 30, 51–60. https://doi.org/10.11607/OFPH.1459.
Ramadan, A., El-Samahy, M., Elrosasy, A., Al-Tawil, M., Abdelaziz, A., Soliman, M.A., Abouzid, M., 2023. Safety and efficacy of P2X3 receptor antagonist for the treatment of refractory or unexplained chronic cough: a systematic review and meta-analysis of 11 randomized controlled trials. Pulm. Pharmacol. Ther. 83, 102252. https://doi.org/10.1016/J.PUPT.2023.102252.
Richards, D., Gever, J.R., Ford, A.P., Fountain, S.J., 2019. Action of MK-7264 (gefapixant) at human P2X3 and P2X2/3 receptors and in vivo efficacy in models of sensitisation. Br. J. Pharmacol. 176, 2279–2291. https://doi.org/10.1111/BPH.14677.
Rosenbaum, T., Morales-Lázaro, S.L., Islas, L.D., 2022. TRP channels: a journey towards a molecular understanding of pain. Nat. Rev. Neurosci. 23, 596–610. https://doi.org/10.1038/S41583-022-00611-7.
Ruiz-Cantero, M.C., Cortés-Montero, E., Jain, A., Montilla-García, Á., Bravo-Caparrós, I., Shim, J., Sánchez-Blázquez, P., Woolf, C.J., Baeyens, J.M., Cobos, E.J., 2023. The sigma-1 receptor curtails endogenous opioid analgesia during sensitization of TRPV1 nociceptors. Br. J. Pharmacol. 180, 1148–1167. https://doi.org/10.1111/BPH.16003.
Schiavuzzo, J.G., Teixeira, J.M., Melo, B., da Silva dos Santos, D.F., Jorge, C.O., Oliveira-Fusaro, M.C.G., Parada, C.A., 2015. Muscle hyperalgesia induced by peripheral P2X3 receptors is modulated by inflammatory mediators. Neuroscience 285, 24–33. https://doi.org/10.1016/J.NEUROSCIENCE.2014.11.020.
Serrano, A., Mo, G., Grant, R., Paré, M., O'Donnell, D., Yu, X.H., Tomaszewski, M.J., Perkins, M.N., Séguéla, P., Cao, C.Q., 2012. Differential expression and pharmacology of native P2X receptors in rat and primate sensory neurons. J. Neurosci. 32, 11890–11896. https://doi.org/10.1523/JNEUROSCI.0698-12.2012.
Shcherbatko, A., Foletti, D., Poulsen, K., Strop, P., Zhu, G., Hasa-Moreno, A., Witt, J.M., Loo, C., Krimm, S., Pios, A., Yu, J., Brown, C., Lee, J.K., Stroud, R., Rajpal, A., Shelton, D., 2016. Modulation of P2X3 and P2X2/3 receptors by monoclonal antibodies. J. Biol. Chem. 291, 12254–12270. https://doi.org/10.1074/JBC.M116.722330.
Shieh, C.C., Jarvis, M.F., Lee, C.H., Perner, R.J., 2006. P2X receptor ligands and pain. Expert Opin. Ther. Pat. 16, 1113–1127. https://doi.org/10.1517/13543776.16.8.1113.
Stewart, L.C., Deslauriers, R., Kupriyanov, V.V., 1994. Relationships between cytosolic [ATP], [ATP]/[ADP] and ionic fluxes in the perfused rat heart: a 31P, 23Na and 87Rb NMR study. J. Mol. Cell. Cardiol. 26, 1377–1392. https://doi.org/10.1006/JMCC.1994.1156.
Sykes, D.L., Zhang, M., Morice, A.H., 2022. Treatment of chronic cough: P2X3 receptor antagonists and beyond. Pharmacol. Ther. 237. https://doi.org/10.1016/J.PHARMTHERA.2022.108166.
Tavares-Ferreira, D., Shiers, S., Ray, P.R., Wangzhou, A., Jeevakumar, V., Sankaranarayanan, I., Cervantes, A.M., Reese, J.C., Chamessian, A., Copits, B.A., Dougherty, P.M., Gereau IV, R.W., Burton, M.D., Dussor, G., Price, T.J., 2022. Spatial transcriptomics of dorsal root ganglia identifies molecular signatures of human nociceptors. Sci. Transl. Med. 14. https://doi.org/10.1126/SCITRANSLMED.ABJ8186.
Teixeira, J.M., Bobinski, F., Parada, C.A., Sluka, K.A., Tambeli, C.H., 2017. P2X3 and P2X2/3 receptors play a crucial role in articular hyperalgesia development through inflammatory mechanisms in the knee joint experimental synovitis. Mol. Neurobiol. 54, 6174–6186. https://doi.org/10.1007/S12035-016-0146-2.
Teixeira, J.M., Parada, C.A., Tambeli, C.H., 2020. P2X3 and P2X2/3 receptors activation induces articular hyperalgesia by an indirect sensitization of the primary afferent nociceptor in the rats' knee joint. Eur. J. Pharmacol. 879. https://doi.org/10.1016/J.EJPHAR.2020.173054.
Tian, S. xin, Xu, T., Shi, R. yi, Cai, Y. qian, Wu, M. hui, Zhen, S. jia, Wang, W., Zhou, Y., Du, J. ying, Fang, Jun fan, Shao, X. mei, Liu, B. yi, Jiang, Y. liang, He, X. fen, Fang, Jian qiao, Liang, Y., 2023. Analgesic effect of electroacupuncture on bone cancer pain in rat model: the role of peripheral P2X3 receptor. Purinergic Signal. 19, 13–27. https://doi.org/10.1007/S11302-022-09861-7.
Trang, T., Salter, M.W., 2012. P2X4 purinoceptor signaling in chronic pain. Purinergic Signal. 8, 621–628. https://doi.org/10.1007/S11302-012-9306-7.

Trapero, C., Martín-Satué, M., 2020. Purinergic signaling in endometriosis-associated pain. Int. J. Mol. Sci. 21, 1–28. https://doi.org/10.3390/IJMS21228512.
Vesterinen, H.M., Sena, E.S., Egan, K.J., Hirst, T.C., Churolov, L., Currie, G.L., Antonic, A., Howells, D.W., Macleod, M.R., 2014. Meta-analysis of data from animal studies: a practical guide. J. Neurosci. Methods 221, 92–102. https://doi.org/10.1016/J.JNEUMETH.2013.09.010.
Viatchenko-Karpinski, V., Novosolova, N., Ishchenko, Y., Azhar, M.A., Wright, M., Tsintsadze, V., Kamal, A., Burnashev, N., Miller, A.D., Voitenko, N., Giniatullin, R., Lozovaya, N., 2016. Stable, synthetic analogs of diadenosine tetraphosphate inhibit rat and human P2X3 receptors and inflammatory pain. Mol. Pain 12. https://doi.org/10.1177/1744806916637704.
Viechtbauer, W., 2010. Conducting meta-analyses in R with the metafor package. J. Stat. Software 36, 1–48. https://doi.org/10.18637/jss.v036.i03.
Vulchanova, L., Reidl, M.S., Shuster, S.J., Stone, L.S., Hargreaves, K.M., Buell, G., Surprenant, A., North, R.A., Elde, R., 1998. P2X3 is expressed by DRG neurons that terminate in inner lamina II. Eur. J. Neurosci. 10, 3470–3478. https://doi.org/10.1046/J.1460-9568.1998.00355.X.
Wang, Q., Zhu, H., Zou, K., Yuan, B., Zhou, Y.L., Jiang, X., Yan, J., Xu, G.Y., 2015. Sensitization of P2X3 receptors by cystathionine β-synthetase mediates persistent pain hypersensitivity in a rat model of lumbar disc herniation. Mol. Pain 11. https://doi.org/10.1186/S12990-015-0012-7.
Wang, S., Zhu, H.Y., Jin, Y., Zhou, Y., Hu, S., Liu, T., Jiang, X., Xu, G.Y., 2015. Adrenergic signaling mediates mechanical hyperalgesia through activation of P2X3 receptors in primary sensory neurons of rats with chronic pancreatitis. Am. J. Physiol. Gastrointest. Liver Physiol. 308, G710–G719. https://doi.org/10.1152/AJPGI.00395.2014.
Wang, W.S., Tu, W.Z., Cheng, R.D., He, R., Ruan, L.H., Zhang, L., Gong, Y.S., Fan, X.F., Hu, J., Cheng, B., Lai, Y.P., Zou, E.M., Jiang, S.H., 2014. Electroacupuncture and A-317491 depress the transmission of pain on primary afferent mediated by the P2X3 receptor in rats with chronic neuropathic pain states. J. Neurosci. Res. 92, 1703–1713. https://doi.org/10.1002/JNR.23451.
Wieskopf, J.S., Mathur, J., Limapichat, W., Post, M.R., Al-Qazzaz, M., Sorge, R.E., Martin, L.J., Zaykin, D.V., Smith, S.B., Freitas, K., Austin, J.S., Dai, F., Zhang, J., Marcovitz, J., Tuttle, A.H., Slepian, P.M., Clarke, S., Drenan, R.M., Janes, J., Al Sharari, S., Segall, S.K., Aasvang, E.K., Lai, W., Bittner, R., Richards, C.I., Slade, G.D., Kehlet, H., Walker, J., Maskos, U., Changeux, J.P., Devor, M., Maixner, W., Diatchenko, L., Belfer, I., Dougherty, D.A., Su, A.I., Lummis, S.C.R., Damaj, M.I., Lester, H.A., Patapoutian, A., Mogil, J.S., 2015. The nicotinic α6 subunit gene determines variability in chronic pain sensitivity via cross-inhibition of P2X2/3 receptors. Sci. Transl. Med. 7. https://doi.org/10.1126/SCITRANSLMED.3009986.
Wu, G., Whiteside, G.T., Lee, G., Nolan, S., Niosi, M., Pearson, M.S., Ilyin, V.I., 2004. A-317491, a selective P2X3/P2X2/3 receptor antagonist, reverses inflammatory mechanical hyperalgesia through action at peripheral receptors in rats. Eur. J. Pharmacol. 504, 45–53. https://doi.org/10.1016/J.EJPHAR.2004.09.056.
Wu, J.X., Xu, M.Y., Miao, X.R., Lu, Z.J., Yuan, X.M., Li, X.Q., Yu, W.F., 2012. Functional up-regulation of P2X3 receptors in dorsal root ganglion in a rat model of bone cancer pain. Eur. J. Pain 16, 1378–1388. https://doi.org/10.1002/J.1532-2149.2012.00149.X.
Wu, Y.Y., Wang, Q., Zhang, P.A., Zhu, C., Xu, G.Y., 2023. MiR-1306-3p directly activates P2X3 receptors in primary sensory neurons to induce visceral pain in rats. Pain 164, 1555–1565. https://doi.org/10.1097/J.PAIN.0000000000002853.
Xiang, X., Wang, S., Shao, F., Fang, Junfan, Xu, Y., Wang, W., Sun, H., Liu, X., Du, J., Fang, Jianqiao, 2019. Electroacupuncture stimulation alleviates CFA-induced inflammatory pain via suppressing P2X3 expression. Int. J. Mol. Sci. 20. https://doi.org/10.3390/IJMS20133248.
Xiang, Z., Xiong, Y., Yan, N., Li, X., Mao, Y., Ni, X., He, C., LaMotte, R.H., Burnstock, G., Sun, J., 2008. Functional up-regulation of P2X 3 receptors in the chronically compressed dorsal root ganglion. Pain 140, 23–34. https://doi.org/10.1016/J.PAIN.2008.07.006.
Xiong, W., Tan, M., He, L., Ou, X., Jin, Y., Yang, G., Huang, L., Shen, Y., Guan, S., Xu, C., Li, G., Liu, S., Xu, H., Liang, S., Gao, Y., 2017. Inhibitory effects of tetramethylpyrazine on pain transmission of trigeminal neuralgia in CCI-ION rats. Brain Res. Bull. 134, 72–78. https://doi.org/10.1016/J.BRAINRESBULL.2017.07.005.
Xu, N., Kim, G.E., Gregg, H., Wagdy, A., Swaine, B.A., Chang, M.S., El-Shourbagy, T.A., 2004. Automated 96-well liquid-liquid back extraction liquid chromatography-tandem mass spectrometry method for the determination of ABT-202 in human plasma. J. Pharm. Biomed. Anal. 36, 189–195. https://doi.org/10.1016/j.jpba.2004.05.013.
Yasuda, M., Shinoda, M., Kiyomoto, M., Honda, K., Suzuki, A., Tamagawa, T., Kaji, K., Kimoto, S., Iwata, K., 2012. P2X3 receptor mediates ectopic mechanical allodynia with inflamed lower lip in mice. Neurosci. Lett. 528, 67–72. https://doi.org/10.1016/J.NEULET.2012.08.067.
Yekkirala, A.S., Roberson, D.P., Bean, B.P., Woolf, C.J., 2017. Breaking barriers to novel analgesic drug development. Nat. Rev. Drug Discov. 16, 545–564. https://doi.org/10.1038/NRD.2017.87.
Yi, Z., Rao, S., Ouyang, S., Bai, Y., Yang, J., Ma, Y., Han, X., Wu, B., Zou, L., Jia, T., Zhao, S., Hu, X., Lei, Q., Gao, Y., Liu, S., Xu, H., Zhang, C., Liang, S., Li, G., 2017. A317491 relieved HIV gp120-associated neuropathic pain involved in P2X3 receptor in dorsal root ganglia. Brain Res. Bull. 130, 81–89. https://doi.org/10.1016/J.BRAINRESBULL.2017.01.002.
Yu, J., Fu, P., Zhang, Y., Liu, S., Cui, D., 2013. Pregabalin alters nociceptive behavior and expression level of P2X3 receptor in the spinal dorsal horn in a rat model induced by chronic compression of the dorsal root ganglion. Anat. Rec. 296, 1907–1912. https://doi.org/10.1002/AR.22816.
Yuan, M., Ding, S., Meng, T., Lu, B., Shao, S., Zhang, X., Yuan, H., Hu, F., 2017. Effect of A-317491 delivered by glycolipid-like polymer micelles on endometriosis pain. Int. J. Nanomed. 12, 8171–8183. https://doi.org/10.2147/IJN.S146569.
Zhang, C.P., Xu, C.S., Liang, S.D., Li, G.L., Gao, Y., Wang, Y.X., Zhang, A.X., Wan, F., 2007. The involvement of P2X3 receptors of rat sympathetic ganglia in cardiac nociceptive transmission. J. Physiol. Biochem. 63, 249–257. https://doi.org/10.1007/BF03165788.
Zhang, H.H., Hu, J., Zhou, Y.L., Qin, X., Song, Z.Y., Yang, P.P., Hu, S., Jiang, X., Xu, G.Y., 2015. Promoted interaction of nuclear factor-κb with demethylated purinergic P2X3 receptor gene contributes to neuropathic pain in rats with diabetes. Diabetes 64, 4272–4284. https://doi.org/10.2337/DB15-0138.
Zhong, Y., Dunn, P.M., Burnstock, G., 2000. Pharmacological comparison of P2X receptors on rat coeliac, mouse coeliac and mouse pelvic ganglion neurons. Neuropharmacology 39, 172–180. https://doi.org/10.1016/S0028-3908(99)00145-8.