Mechanisms of mechanotransduction by specialized low-threshold mechanoreceptors in the guinea pig rectum

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HISTORY

Zagorodnyuk, Vladimir P., Penny Lynn, Marcello Costa, and Simon J. H. Brookes. Mechanisms of mechanotransduction by specialized low-threshold mechanoreceptors in the guinea pig rectum. Am J Physiol Gastrointest Liver Physiol 289: G397–G406, 2005. First published June 2, 2005; doi:10.1152/ajpgi.00557.2004.—The guinea pig rectum, but not the colon, is innervated by a specialized class of distension-sensitive mechanoreceptors that have transduction sites corresponding to rectal intraganglionic laminar endings (rIGLEs). Rectal mechanoreceptors recorded in vitro had low threshold to circumferential stretch, adapted slowly, and could respond within 2 ms to mechanical stimulation by a piezo-electric probe. Antagonists to ionotropic N-methyl-d-aspartate (NMDA; CGS 19755, memantine) and non-NMDA (6,7-dinitroquinoxaline-2,3-dione) glutamate receptors did not affect mechanotransduction. In normal Krebs solution, the P2X purinoreceptor agonist α,β-methylene ATP reduced mechanoreceptor firing evoked by distension but simultaneously relaxed circular smooth muscle and inhibited stretch-induced contractions. Neither ATP nor α,β-methylene ATP affected mechanotransduction when transduction sites were directly compressed with von Frey hairs. The P2 purinoreceptor antagonist pyridoxal phosphate-6-azophenyl-2',4'-disulfonic acid did not affect stretch-induced firing but reduced the inhibitory effect of α,β-methylene ATP on stretch-induced firing. Under isometric conditions, blocking synaptic transmission in Ca2+-free solution reduced stretch-evoked firing but not when basal tension was restored to control levels. Under isotonic conditions, Ca2+-free solution did not significantly affect load-evoked firing. The blockers of mechanogated and/or transient receptor potential channels, benzamil, Gd3+, SKF 96365, and ruthenium red inhibited stretch-induced firing but, in parallel, significantly reduced stretch-induced contractions. Benzamil and SKF 96365 were able to inhibit mechanotransduction when transduction sites were compressed with von Frey hairs. The results show that mechanotransduction is rapid but does not depend on fast excitatory release of mediators. It is likely that stretch-activated ion channels on rIGLEs are involved in direct, physical mechanotransduction by rectal low-threshold mechanoreceptors.

afferents; mechanosensory transduction

DISTENSION OF THE GUT WALL activates both intrinsic and extrinsic neuronal reflex pathways and may cause conscious sensation. The mechanisms underlyng activation of mechanosensitive extrinsic afferent neurons are not well understood. Two types of mechanisms, chemical and physical, have been proposed for mechanotransduction by afferent neurons in different systems. Chemical transduction relies on mediators being released from nonneuronal cells by mechanical stimulation, which then activate afferent endings via corresponding receptors. Direct mechanotransduction is characterized by having all molecular elements (mechanogated ion channels and associated proteins) located in the afferent endings, without extracellular mediators being essential (6, 17, 23). Several recent reports have provided evidence for a role for chemical transduction in primary afferent neurons in several systems. Cutaneous slowly adapting mechanoreceptor corpuscles, Merkel cells, release glutamate in response to mechanical stimuli to activate afferent nerve fibers (18). Likewise, in the cochlea, glutamate probably mediates transmission between mechanosensitive inner hair cells and spiral ganglion neurons (40). In visceral primary afferents neurons, ionotropic glutamate receptor antagonists attenuate distension-evoked firing of spinal and vagal afferents innervating the gastrointestinal tract (30, 44). ATP has been postulated to be a key signaling molecule mediating mechanosensitivity in several regions (6), and mechanosensitive release of ATP may be a ubiquitous mechanism in eukaryotic cells (3, 24, 32). Both ionotropic P2X and metabotropic P2Y purinoreceptors are expressed on sensory neurons and their endings (9, 19, 46, 57) and purinoreceptor antagonists, such as suramin, pyridoxal phosphate-6-azophenyl-2',4'-(2-methyldiazodicarbamoyl)thiocarbonyl-1H-pyridine, and 2',3'-O-trinitrophenyl-ATP (TNP-ATP), reduce mechanosensitivity of spinal afferents innervating the lower gut and urinary bladder (39, 52).

On the other hand, mechanogated channels also appear to be ubiquitous among eukaryotic cells and are the prime candidates for transduction of mechanical stimuli into cellular electrochemical signals in some primary afferent neurons (23, 24). The most likely mechanosensitive ion channels belonging to TRP and/or ENaC/ASIC/degenerin of cationic and sodium ion channel families are involved in mechanotransduction in ciliated and nonciliated mechanoreceptors including mammalian extrinsic primary afferent neurons (21–23). A recent study has identified a role for a TRP channel family member (TRPA1) as the mechanotransducing channel in vertebrate hair cells (13).

In the gastrointestinal tract, both low- and high-threshold spinal mechanoreceptors have been documented (43); five main types of mechanoreceptors were identified in splanchic and pelvic mechanosensory pathways to the mouse colon including two stretch-sensitive types of mechanoreceptor (4). Previously, we have identified rectal intraganglionic laminar endings (rIGLEs) as the mechanotransduction sites of low-threshold rectal mechanoreceptors (28). These endings are largely restricted to the rectum, being present in only small numbers in the distal colon, indicating that they comprise a specialized class of rectal mechanoreceptors (33). It has been suggested that they behave grossly as tension receptors (28) and may correspond to the muscular mechanoreceptors classified in the mouse colorectum (4). In the esophagus and stom-
ach, morphologically similar endings, called intraganglionic laminar endings (IGLEs), are the transduction sites of vagal mechanoreceptors (54, 55). We have recently provided evidence indicating that IGLEs use direct, physical mechanisms of mechanotransduction via stretch-activated ion channels (57). The aim of this study was to investigate whether rectal mechanoreceptors in the guinea pig rectum transduce mechanical stimuli physically (via mechanosensitive ion channels) or indirectly (via release of chemicals from other cells).

**MATERIALS AND METHODS**

*Extracellular recording.* Adult guinea pigs [total no. of animals (N) = 66], weighing between 180 and 250 g, were killed by a blow to the occipital region and exsanguination, in a manner approved by the Animal Welfare Committee of Flinders University in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (National Health and Medical Research Council of Australia, 7th ed., 2004). The method of extracellular recordings from rectal mechanoreceptor afferents in guinea pig has been described previously (28). Briefly, the distal rectum was mounted on a piezoelectric element (DSC no.46-CA). Differences were considered significant if P < 0.05.

**RESULTS**

Most of rectal afferents [no. of units (n) = 84, N = 66] were electrically silent in unstretched rectal preparations and had low thresholds (−1 mm) to circumferential distension. After the onset of stretch using the tissue stretcher, mechanoreceptor firing showed an initial burst of firing followed by marked adaption that closely followed muscle tension in time course. These units were similar to previously described rIGLEs as transduction sites (29).

*Latency of mechanotransduction.* Mechanoreceptor afferents were slowly conducting with an average conduction velocity of 1.28 ± 0.13 m/s (n = 7, N = 6), within the C-fiber range. Focal compression of the tissue with light von Frey hairs (0.1–1 mN) activated rectal mechanoreceptors only at several highly localized sites (hot spots), which correspond to rIGLEs (28). The response latency at marked hot spots, determined for each unit using the piezoelectric probe, was 11.3 ± 2.2 ms (n = 7, N = 6). The latency for electrical stimulation from the same sites was 9.7 ± 2.3 ms (n = 7, N = 6; Fig. 1). The latter latency represents the delay due to conduction of action potential from the hot spot to the recording site. By subtracting the conduction latency from total latency for each unit, the mean delay due to mechanotransduction was calculated at 1.7 ± 0.4 ms (n = 7, N = 6). Latencies evoked by mechanical stimulation were more variable than those for electrical stimulation. Typically, shorter latencies were associated with a larger number of spikes evoked by mechanical stimulation. This suggests that small movements of the preparation may have moved hot spots relative to the probe, causing longer latencies and weaker responses when the probe was off center. Despite this variability, mechanotransduction by low-threshold rectal mechanoreceptors occurred on a millisecond time scale...
and must be due either to rapid chemical transmission from other cells or, alternatively, to activation via mechanogated ion channels located on rIGLEs.

Effects of glutamate ionotropic receptors antagonists on transduction of rectal mechanosensitive afferents. Glutamate can mediate fast synaptic transmission on a submillisecond time scale via ionotropic receptors (41) and has been suggested to be involved in distension-induced activation of spinal and vagal mechanoreceptors (30, 44). The competitive antagonist to ionotropic N-methyl-D-aspartate (NMDA) receptors, CGS 19755 (30 μM), did not affect either stretch-induced firing (107 ± 19% of control for 3-mm stretch, n = 4, N = 3; not significant (NS)) or stretch-induced contraction (108 ± 5%, N = 3, NS). Similarly, the selective antagonist to non-NMDA ionotropic receptors, DNQX (100 μM), did not inhibit stretch-induced firing (91 ± 8% of control at 3-mm stretch, n = 5, N = 4, NS) or contractions (118 ± 3%, N = 4, NS). The open channel blocker of NMDA receptors, memantine (30 μM), affected neither stretch-evoked firing nor contraction (106 ± 23%, n = 5, N = 4, and 96 ± 4%, N = 4, of control 3-mm stretch, respectively). However, at 100 μM, memantine decreased stretch-induced firing slightly (to 74 ± 16% of 3-mm control stretch, NS, n = 6, N = 5) but also significantly decreased stretch-induced tension (to 62 ± 6% of control 3-mm stretch, N = 5, P < 0.005 by two-way ANOVA; Fig. 2). These results indicate that activation of ionotrophic receptors by endogenous glutamate is not involved in mechanotransduction by spinal low-threshold mechanoreceptors in the guinea pig rectum.

ATP and mechanotransduction. ATP, acting via P2X2/3 receptors, may be involved in mechanotransduction by some spinal afferents to the rat colorectum and mouse urinary bladder (39, 52). In the guinea pig rectum, the P2X-prefering agonist α,β-me ATP (30 μM for 1 min, n = 9, N = 6) evoked relaxation of circular muscle and inhibited muscle contractile responses to distension (to 15 ± 3% of control, 3-mm stretch, N = 6, P < 0.005 by paired t-test). In parallel, α,β-me ATP inhibited stretch-evoked firing (to 26 ± 6% of control, n = 9, N = 6, P < 0.005 by paired t-test) of rectal afferents (Fig. 3A). To determine whether the effect on the muscle could explain the reduced firing, studies were carried out in Ca2+-free Krebs solution. Under these conditions, basal muscle tension and stretch-evoked tension were reduced, presumably due to blockade of active muscle responses. The inhibitory effect of α,β-me ATP on muscle tension was abolished. However, even under these conditions, α,β-me ATP (30 μM) did not excite mechanosensitive endings. The nonselective P2 purinoreceptor antagonist PPADS (30 μM) did not affect stretch-induced firing of rectal mechanoreceptors in normal Krebs solution (91 ± 12% of control 3-mm stretch, n = 6, N = 4, NS by 2-way ANOVA; Fig. 3C). This concentration of PPADS (30 μM for 20 min) was effective, because in its presence, α,β-me ATP (30 μM) no longer inhibited stretch (3 mm)-evoked firing (77 ± 24% of control, n = 6, N = 4, NS by 2-way ANOVA).

![Fig. 1. Latency of responses to mechanical and electrical stimulation. A: the latency of an afferent action potential in response to electrical stimulation (black trace) is shorter than the response to mechanical stimulation (gray trace); in this example, the difference in latency, reflecting the mechanotransduction delay, is 2.7 ms (shown by double-headed arrow). B: histogram of mechanotransduction delay calculated following mechanical and electrical stimulation from the same hot spots [no. of units (n) = 7, no. of animals (N) = 6].](http://ajpgi.physiology.org/)

![Fig. 2. Memantine reduced stretch-evoked firing and contraction in parallel.](http://ajpgi.physiology.org/)
receptors are unlikely to be involved in mechanotransduction by the majority of low-threshold mechanoreceptors.

Effects of Ca\(^{2+}\)-free Krebs solution on mechanotransduction. We investigated the effect of blocking all rapid synaptic neurotransmitter release mechanisms on transduction using Ca\(^{2+}\)-free and high-Mg\(^{2+}\) Krebs solution. Under isometric conditions, transduction of mechanical stimuli by rectal mechanoreceptors in the guinea pig rectum persisted at a reduced level in Ca\(^{2+}\)-free (1 mM EDTA, 6 mM Mg\(^{2+}\)) Krebs solution (Fig. 4). The number of action potentials evoked by 3-mm stretch was significantly reduced (to 40 ± 14% of control Krebs solution, \(n = 9, N = 7, P < 0.05\) by 2-way ANOVA).

This may have been due to the significant reduction of both resting tension and stretch-evoked tension (to 12 ± 3% of control, \(N = 7, P < 0.001\) by 2-way ANOVA; Fig. 4, E and F). After resting tension was adjusted back to the control level, 3-mm stretch evoked an increase in passive tension that was still less than that in normal Krebs solution (30 ± 32% of control, \(N = 7, P < 0.001\) by 2-way ANOVA). However, under these conditions, stretch-induced firing was not significantly different from control (100 ± 21%, \(n = 9, N = 7, NS\); Fig. 4, E and F). To exclude the confounding effect of changes in muscle tension, we also investigated the effects of Ca\(^{2+}\)-free Krebs solution under isotonic conditions. Application of Ca\(^{2+}\)-free (with 3.6 mM Mg\(^{2+}\)) Krebs solution for 1 h did not affect significantly either firing (\(n = 8, N = 6, NS\) by 2-way ANOVA) evoked by isotonic stretching of the tissue with imposed load (10–50 mN) or the length of the preparations (\(N = 6, NS\) by 2-way ANOVA; Fig. 4, G and H). These findings indicate that Ca\(^{2+}\)-dependent fast synaptic release is not required for mechanosensory transduction by rIGLEs in the guinea pig rectum.

Effects of stretch-activated and cationic channel blockers on transduction by rectal afferents. We have previously showed that benzamil (a potent analog of amiloride) inhibited stretch-induced firing in the guinea pig esophagus at high concentrations (100 \(\mu M\)) (57). In the guinea pig rectum, benzamil (10–30 \(\mu M\) for 20–25 min) significantly decreased stretch-induced mechanoreceptor firing (Fig. 5A). However, it also reduced stretch-evoked increases in wall tension (Fig. 5B). Thus benzamil (30 \(\mu M\)) reduced firing to 22 ± 6% of control (\(n = 4, N = 4, P < 0.001\) by 2-way ANOVA), whereas it reduced integrated tension to 33 ± 8% of control (\(N = 4, P < 0.001\) by 2-way ANOVA). From these results, it was not possible to determine whether the inhibition of stretch-induced firing by benzamil was due to an effect on mechanogated ion channels on rIGLEs or alteration of the mechanical properties of muscle. Therefore, we studied the effects of benzamil on firing evoked by focal compression of hot spots (rIGLEs) with a von Frey hair. Benzamil (30 \(\mu M\) for 30–35 min) significantly reduced firing evoked by a von Frey hair (≈1 mN) probing of hot spots (63 ± 9% of control, \(n = 5, N = 5, P \leq 0.01\) by 1-way ANOVA). The effect was reversible by washing for 60 min with normal Krebs solution (Fig. 5C).

Gadolinium ion (Gd\(^{3+}\); 100 \(\mu M\) for 20 min) inhibited stretch-induced firing to 13 ± 8% of control (3 mm stretch, \(n = 4, N = 3, P < 0.01\) by 1-way ANOVA) but simultaneously reduced stretch-induced muscle tone (to 24 ± 6% of control, \(N = 3, P < 0.01\) by 1-way ANOVA; Fig. 6A). The effect of Gd\(^{3+}\) on stretch-induced firing developed with a time course.

Fig. 3. Involvement of purinoreceptors in mechanotransduction. A: averaged data (from 9 units, \(N = 6\)) of the inhibitory effects of the P2X-prefering agonist α,β-methylene ATP (α,β-me ATP; 30 \(\mu M\)) on stretch (3 mm)-induced firing (left) and stretch induced contractions (\(N = 6\); right). Note the reduction of stretch-induced firing by α,β-me ATP was paralleled by a similar reduction in stretch-induced contraction of the circular muscle. B: averaged data of the effects of α,β-me ATP (30 \(\mu M\), \(n = 5, N = 4\); left) and ATP (1 mM, \(n = 6, N = 5\); right) on firing evoked by compression of transduction sites with a von Frey hair (≈1 mN). C: pyridoxal phosphate-6-azophenyl-2′,4′-disulfonic acid (PPADS; 30 \(\mu M\)) did not affect stretch-induced firing of rectal mechanoreceptors. Each value in C is the mean ± SE from 6 units in 4 preparations. *\(P < 0.005\).

To exclude the indirect effect of purines on firing due to their influence on muscle tension, the actions of ATP and α,β-me ATP were examined on firing evoked by focal compression with a von Frey hair (≈1 mN) on a previously marked hot spot. Neither α,β-me ATP (30 \(\mu M\) for 1 min) nor ATP (1 mM for 1 min) affected firing evoked by von Frey hair probing (91 ± 19% of control, \(n = 5, N = 4, NS\); and 127 ± 15% of control, \(n = 6, N = 5, NS\), respectively). Neither agonist activated low-threshold, stretch-sensitive units (Fig. 3B). However, two spontaneously active units (for which hot spots were not found) of 14 recorded units in 5 guinea pigs were slightly excited by application of 1 mM ATP. Overall, these data suggest that P2X
that paralleled the inhibition of stretch-induced intramural tension (Fig. 6B). Gd3+ (100 µM for 20 min) did not affect firing evoked by von Frey hair (~1 mN) compression of hot spots (112 ± 12% of control, n = 4, N = 4, NS by 1-way ANOVA; Fig. 6C). This suggests that the effect of Gd3+ on stretch-induced firing was probably largely due to reduction of muscle tension.

The nonselective cation channel blocker SKF 96365 (30–50 µM for 10–15 min) also inhibited stretch-induced firing. Applied at 50 µM, stretch (3 mm)-induced firing was reduced to 16 ± 15% of control (n = 3, N = 3, P < 0.001 by 2-way ANOVA). Again, this followed a parallel reduction of stretch-induced contraction of smooth muscle (to 38 ± 3% of control, N = 3, P < 0.001 by 2-way ANOVA; Fig. 7, A and B). SKF 96365 (50 µM for 15–20 min) significantly inhibited firing evoked by von Frey hair (~1 mN) compression of hot spots (48 ± 11% of control, n = 6, N = 6, P ≤ 0.05 by 1-way ANOVA). The effect was washable within 45 min (Fig. 7C).

The nonselective TRP channel blocker ruthenium red concentration-dependently inhibited stretch-induced firing. At 30 µM for 20 min, stretch (3 mm)-induced firing was 30 ± 14% of control (n = 5, N = 4, P < 0.01 by 2-way ANOVA), but, at the same time, stretch-induced tone was reduced to 22 ± 12% of control (N = 4, P < 0.001 by 2-way ANOVA) (Fig. 8, A and B). However, ruthenium red (30 µM for 35–40 min) significantly increased the firing evoked by von Frey hair (~1 mN) probing of hot spots (193 ± 22% of control, n = 6, N = 5, P ≤ 0.05 by 1-way ANOVA). The effect was washable for ≥60 min (Fig. 8C).
DISCUSSION

The present study has shown that mechanotransduction by low-threshold mechanoreceptors in the guinea pig rectum does not require synaptic transmission or endogenous transmitters ATP and glutamate, suggesting that stretch-activated ion channels on rIGLEs are involved in direct physical mechanotransduction by rectal low-threshold mechanoreceptors. By using a piezoelectrical probe to measure accurately responses to mechanical stimulation and subtracting conduction delays, the mechanotransduction delay was shown to be 110 ms. A similar short mechanotransduction delay of 6 ms was reported for vagal mechanoreceptors (57) and of 8 ms for low-threshold cutaneous D-hair receptors (45). In contrast, the mechanical latency for cutaneous high-threshold mechanoreceptors, in particular slowly adapting AM mechanoreceptors and polymodal nociceptors, is many times longer: up to 80 ms (45). Slowly adapting cutaneous mechanoreceptors supplying Merkel cell complexes in touch domes may rely on glutamate, released from Merkel cells, to activate afferent nerve fibers (18). Nevertheless, on its own, rapid mechanotransduction by rectal afferents is not sufficient proof that direct (nonchemical) mechanisms are responsible. At some glutamatergic central nervous system synapses, the total synaptic delay is only 150 μs (41). However, the observation that transduction persists in 0 mM Ca²⁺, when rapid exocytotic transmitter release is blocked, is strong evidence that chemical transduction is unlikely to be involved.

It has been reported from in vivo studies that glutamate acting at NMDA receptors may be involved in responses to noxious colonic distension in the rat. Recordings from three afferent units in pelvic nerves were reported to show that memantine, an open channel blocker of NMDA ionotropic receptors, reduced the increase in firing evoked by constant pressure distension (30). In the present study, memantine at 30 μM did not affect mechanotransduction by rectal afferents. This concentration is well above the IC₅₀ for NMDA receptors.

Fig. 5. Effects of benzamil on stretch- and compression-evoked firing and intramural tension. Averaged data (5 units in 4 guinea pigs) of the effect of benzamil (10 and 30 μM) on stretch-induced firing (A) and on stretch-induced contraction (B). Note that inhibition of stretch-induced firing was accompanied by a similar reduction of stretch-induced contraction. *P < 0.001; #P < 0.01; @P < 0.05. C: averaged data of the firing evoked by compression of transduction sites with a von Frey hair (~1 mN) in control solution, in the presence of benzamil (30 μM, n = 5, N = 5), and after wash out (Wash) for 60 min. *P ≤ 0.01.

Fig. 6. Effects of Gd³⁺ on stretch- and compression-evoked firing and intramural tension. Averaged data (4 units in 3 guinea pigs) of the effect of Gd³⁺ (100 μM) on stretch-induced firing (A) and on stretch-induced contraction (B). Note that effect of Gd³⁺ on stretch-induced firing occurred in parallel with inhibition of stretch-induced intramural tension. *@P < 0.01; #P < 0.05. C: averaged data (n = 4, N = 4) of the effect of Gd³⁺ (100 μM) on the firing evoked by compression of transduction sites with a von Frey hair (~1 mN).
In our hands, at 100 μM, memantine slightly reduced firing but strongly and significantly inhibited stretch-induced contractility. In the study of McRoberts et al. (30), balloon volume was not monitored during the study so that effects of memantine on smooth muscle contractility were not measured. It has been reported for vagal afferents in the intact rat stomach in vivo that distension-evoked firing was reduced by CNQX, memantine, and MK-801 (44). In our hands, other ionotropic glutamate antagonists for both NMDA and non-NMDA receptors (CGS 19755 and DNQX) did not affect mechanotransduction of spinal low-threshold mechanoreceptors in the guinea pig rectum. Similarly, ionotropic glutamate receptor antagonists did not inhibit stretch-induced firing in an in vitro study of vagal mechanoreceptors in the guinea pig esophagus (57).

The apparent discrepancies between these findings may be due to experimental differences. First, there may be differences between species. Second, our study was restricted to a highly defined, relatively homogenous class of low-threshold mechanoreceptors, with well-characterized, specialized endings in myenteric ganglia (28). In the other studies, it is not possible to be sure of the homogeneity of recorded receptors or the sites of their sensitive endings within the gut wall. Third, in the present study, high-resolution recordings were made of muscle length and tension within a small area surrounding the receptive field; in other studies, large balloons have been used for distension with or without recordings of contractile responses. A fourth potential explanation is the difference between in vivo and in vitro preparations. It is possible that higher levels of endogenous glutamate may access visceral afferent endings in an intact, perfused organ. Despite these differences in experimental approach, our results suggest that for rIGLE-bearing low-threshold rectal mechanoreceptors, glutamatergic transmission is not required for mechanotransduction. Whether it may contribute to modulating excitability of endings, which could affect the amplitude of responses to distension, remains to be determined. It is worth pointing out that in neither of the

Fig. 7. Effects of SKF 96365 on stretch- and compression-evoked firing and intramural tension. Averaged data (3 units in 3 guinea pigs) of the effect of SKF 96365 (30 and 50 μM) on stretch-induced firing (A) and on stretch-induced contractions (B). Note that the inhibitory effects of SKF 96365 on stretch-induced firing was associated with a significant reduction of stretch-induced intramural tension. *P < 0.001. C: averaged data (n = 5, N = 5) of the effect of SKF 96365 (50 μM) on the firing evoked by compression of transduction sites with a von Frey hair (−1 mN). *P ≤ 0.05.

Fig. 8. Effects of ruthenium red (RR) on stretch- and compression-evoked firing and intramural tension. Averaged data (5 units in 4 guinea pigs) of the effect of RR (3, 10, and 30 μM) on stretch-induced firing (A) and on stretch-induced contraction (B). Note that the inhibitory effects of RR on stretch-induced firing were similar to the reduction in stretch-induced intramural tension. *P < 0.001; #P < 0.01. C: averaged data of the effect of RR (30 μM, n = 6, N = 5) on the firing evoked by compression of the transduction sites with a von Frey hair (−1 mN). *P ≤ 0.05.
published studies cited above did antagonists entirely block responses. In our opinion, this is consistent with a modulatory, rather than transductive role for glutamate. Alternatively, multiple transduction mechanisms, using one or more chemical messenger such as ATP, 5-HT, and glutamate, may be involved.

The purinergic hypothesis for chemical mechanotransduction by spinal afferents suggests that ATP, released from epithelium or urothelium, activates afferent nerve endings via P2X2/3 receptors during gut or bladder distension (6). ATP is well established now as a neurotransmitter in the central and peripheral nervous systems but is also released from other tissues by touch, stretch, or tissue injury (2, 3, 6, 32, 38, 52). Both ionotropic P2X and metabotropic P2Y purinoreceptors are widely expressed on sensory neurons (9, 19, 46). In the current study, neither α,β-me ATP nor ATP activated low-threshold rectal mechanoreceptors. In addition, P2X2 receptor immunoreactivity was not detected on rectal IGLEs (V. P. Zagórodnyuk and S. J. H. Brookes, unpublished observations). In contrast, in the guinea pig esophagus and stomach, IGLES of vagal low-threshold mechanoreceptors were distinctively labeled by P2X2 receptor antibodies, and nearly all of afferent units were excited by α,β-me ATP with EC50 of ~20 µM (8, 57). The effects of purine receptor agonists on rectal mechanoreceptors were complicated by their direct inhibitory effects on smooth muscle; however, these were blocked in Ca2+/Mg2+-free Krebs solution (53, 59). Under identical conditions, a recent report (57) demonstrated that excitatory effects of α,β-me ATP were actually increased for vagal mechanoreceptors. In the rectum, however, we were still unable to detect the excitatory effect of α,β-me ATP on mechanoreceptors, even in Ca2+/Mg2+-free Krebs. The inability of purines to affect firing directly was supported by their lack of effect on firing evoked by compression of hot spots (rIGLES) with a von Frey hair. In the rat colorectum, it was reported that α,β-me ATP at high concentrations (100 µM–1 mM) activated ~80% of mechanosensitive fibers (50). In contrast, fewer than 50% of esophageal mechanoreceptors are activated by the P2X agonist in the mouse and none in normal ferret esophagus (35, 36). These data support significant species differences in receptor expression in visceral afferents as reported previously (58). It has been suggested that ATP, acting on autocrine P2Y1 receptors, may be involved in some vertebrate touch sensitivity (32). However, in the guinea pig esophagus, the P2Y1 receptor agonist adenosine 5′-O-(2-thiodiphosphate) (38) activated vagal tension receptors with much less efficiency than α,β-me ATP (57). In the present study, the P2 antagonist PPADS did not affect mechanotransduction but antagonized the inhibitory effect of α,β-me ATP on stretch-induced firing. In previously published studies (39, 52), PPADS, suramin, and TNP-ATP modestly inhibited the peak of stretch-induced firing of a subset of low-threshold mechanoreceptors in the rat colorectum and mouse urinary bladder. In our opinion, all these data are compatible with a modulatory, rather than transductive role for purines in low-threshold mechanoreceptors. Alternatively, multiple transductive mechanism, species, and experimental condition differences may be involved explaining apparently discrepant observations between previously published reports and the present study. Purines are released from nonneuronal cells in damaged tissue or during inflammation (2, 6). After acid-induced inflammation, vagal mechanoreceptors in the ferret esophagus become sensitive to α,β-me ATP (36). Thus purinoreceptors and purine release may play an important role in setting the excitability of mechanoreceptor endings, particularly in the mucosa and especially in the context of tissue damage. Consistent with this is the observation that high-threshold mechanoreceptors are more sensitive to ATP and purinergic antagonists than low-threshold mechanoreceptors (39, 52).

The latency of mechanotransduction in rectal mechanoreceptors in the guinea pig was <2 ms. In contrast, mechanically activated ATP release typically occurs over a time scale of seconds (2, 42), even when this is mediated by “burst release” (1). ATP and other transmitters can be released very rapidly, on a submillisecond timescale, by calcium-sensitive exocytotic release mechanisms at synapses (41, 60). The use of Ca2+/Mg2+-free and raised Mg2+ Krebs solution is a well-established procedure to block fast exocytotic synaptic release. In our hands, Ca2+-free solution significantly reduced stretch-induced firing. However, when basal tension was readjusted to control levels, the stretch-induced firing was also restored together with a partial recovery of peak tension during the stretch. In addition, in isotonic conditions, where confounding changes in muscle tension were prevented, Ca2+-free solution did not affect load-induced firing. Thus, mechanotransduction appears to be independent of exocytotic release, as reported previously for vagal esophageal low-threshold mechanoreceptors (57). This makes it very unlikely that chemical transduction is significant for rectal mechanoreceptors.

The most likely candidates for mechanogated channels of extrinsic primary afferent neurons in mammals belong probably to TRP and/or ENaC/ASIC/degenerin families of cation and sodium ion channels (14, 15, 20, 34, 37, 51). Recent studies have provided strong evidence for a role in TRP family members in mechanosensitivity of cochlear hair cells (13) and TRPC1 channels in stretch responsiveness of many cell types (29). Nonselective blockers of mechanogated channels, Gd3+, amiloride, and benzamil, can block both families of ionic channels (15, 24, 26, 48). In the present study, benzamil (10–30 µM) inhibited stretch-induced firing of rectal mechanoreceptors, but this may have been due to its effects on wall tension. The inhibitory action of benzamil on firing evoked by von Frey hair compression of transduction sites suggests that mechanogated channels are present on rIGLE-bearing, low-threshold rectal mechanoreceptors. Similar to benzamil, Gd3+ inhibited both stretch-induced firing and contraction in the rectum. The latter effect may be due to blockade of stretch-activated cation channels in smooth muscle (47, 49, 50) because Gd3+ (100 µM) did not affect the firing evoked by von Frey hair compression of hot spots. This is in agreement with previous observations for vagal mechanoreceptors, in which Gd3+ did not inhibit mechanotransduction in concentrations up to 300 µM (55, 57).

Nonselective blockers of cation TRP channels, SKF 96365 and ruthenium red (12, 26), reduced in parallel both stretch-induced firing and muscle contractility. It is likely that the latter effects were due to inhibition of cation and/or voltage-operated L-type Ca2+-channels on smooth muscle cells (11, 25, 31). When the effects of these drugs were studied on firing evoked by von Frey hair compression of transduction sites, SKF 96365 still significantly inhibited mechanotransduction, suggesting an involvement of mechanogated channels (possi-
bly belonging to the TRP family). In contrast to SKF 96365, ruthenium red also inhibits with similar potency a range of K⁺ channels (11, 25) that could be responsible for the increased excitability of rectal afferents. It has been previously reported (56) that several types of K⁺ channels are involved in control of spontaneous and stretch-induced firing of vagal esophageal 1GLE-bearing mechanoreceptors. It should be noted that, although the drugs used to block mechanogated channels (benzamil, Gd³⁺, SKF 96365, and ruthenium red) are currently the best available pharmacological tools, all of them are known to affect other classes of ion channels (7, 16, 24, 27, 31). We cannot exclude the possibility that such nonspecific action may have contributed to their effects on firing. Our results show that care must be taken when interpreting the responses to drugs that affect mechanoreceptor firing; it is important to monitor their effects on smooth muscle tone, particularly when afferents behave grossly as tension receptors.

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