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$P2Y_1$ purinergic receptors in sensory neurons: Contribution to touch-induced impulse generation

(somatic sensation/mechanosensitivity/ATP receptors/mechanoelectric transduction/dorsal root ganglion neurons)

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ABSTRACT **Somatic sensation requires the conversion of physical stimuli into the depolarization of distal nerve endings. A single cRNA derived from sensory neurons renders *Xenopus laevis* oocytes mechanosensitive and is found to encode a $P2Y_1$ purinergic receptor. $P2Y_1$ mRNA is concentrated in large-fiber dorsal root ganglion neurons. In contrast, $P2X_3$ mRNA is localized to small-fiber sensory neurons and produces less mechanosensitivity in oocytes. The frequency of touch-induced action potentials from frog sensory nerve fibers is increased by the presence of P2 receptor agonists at the peripheral nerve ending and is decreased by the presence of P2 antagonists. P2X-selective agents do not have these effects. The release of ATP into the extracellular space and the activation of peripheral $P2Y_1$ receptors appear to participate in the generation of sensory action potentials by light touch.**

Tactile sensation depends on the primary afferent neuron with its cell body located in the dorsal root ganglion (DRG). Various mechanical, thermal, or chemical events depolarize the membrane of the peripheral nerve ending and generate the action potentials that are conducted centrally to the spinal cord and brain stem. The neuronal proteins that transduce external mechanical stimuli into altered membrane conductance are not known. Stretch-activated channel proteins have been demonstrated in a number of tissues (1–6), but their role in sensory neuron mechanoelectric coupling is not proven.

Strong genetic evidence implicates nematode *mec* genes in sensory neuron function (7, 8). The structural homology of *mec-4* and *mec-10* gene products with amiloride-sensitive sodium channels has led to the hypothesis that they may be primary components of a mechanoelectric transduction system (7). Likewise, the homology of *mec-2* with stomatin, an erythrocyte cytoskeleton-associated protein, has raised the possibility that this protein links cytoskeletal deformations to changes in membrane ion channel function (8). There is no direct evidence that these proteins can mediate mechanoelectric transduction.

Noxious stimuli, such as deep pressure, initiate nerve impulses in part via the local generation of compounds such as prostaglandins (prostaglandin E_2 in particular), leukotrienes, bradykinin, substance P, and serotonin. These compounds can reproduce at least in part the sensations associated with noxious stimuli. Their actions are the basis for the use of capsacin, cyclooxygenase inhibitors, and related compounds as analgesics.

The ability of extracellular ATP to depolarize isolated dorsal root ganglion neurons and dorsal horn neurons has been appreciated for a number of years and has suggested that ATP functions as a transmitter or cotransmitter at central synapses of sensory fibers (9, 10). There is evidence that intrathecal administration of compounds that interact with P2 purinergic receptors can modulate the transmission of sensory information in the spinal cord (11). P2 purinoceptors for ATP are composed of two large classes: ionotropic P2X receptors and G-protein-linked P2Y receptors (12). Several P2X receptor RNAs have recently been localized to DRG neurons, with the $P2X_3$ receptor expressed exclusively in those small neurons that mediate responses to noxious stimuli (13, 14). Thus, P2X receptors appear to have an important role in pain sensation. There is no information as to whether these receptors function at central synapses or at distal nerve endings. It has recently been suggested that noxious stimuli might injure cells, release ATP, and stimulate peripheral $P2X_{2/3}$ receptors (13, 14). P2Y receptors have not been studied in DRG neurons.

We have developed an *Xenopus* oocyte expression cloning system to identify molecules that may be involved in transducing mechanical stimuli into electrical signals. This system involves the expression of dorsal root ganglion-derived mRNAs in oocytes and electrophysiologic analysis of the oocyte response to mechanical effects. We find that oocytes expressing $P2Y_1$ receptors exhibit inward currents in response to membrane deformation. *In vivo*, this receptor is expressed by large-fiber sensory neurons. Compounds that modify $P2Y_1$ receptor activity alter the sensitivity of the skin to light touch. Thus, a metabotropic ATP receptor appears to have a role in the generation of sensory potentials by innocuous somatic stimuli.

MATERIALS AND METHODS

Oocytes were injected with cRNA, incubated, and voltage-clamped as described (15, 16). Two different stimuli were presented after the flow of buffer in the bath was discontinued. In one experiment, 4 μl of buffer was ejected over 1 sec from a Gilson P200 pipette tip located 1 mm from the oocyte at the time indicated by the arrows. In the second experiment, oocytes were injected with 50 nl of 100 mM KCl/2 mM Hepes·NaOH, pH 7.4, over 100 msec at the time of the arrows. A single clone (A2.1) was isolated from an embryonic day 7 chicken DRG cDNA library by sib selection of clones mediating a puff-induced current. The DNA sequence of A2.1 is identical to that of the chicken $P2Y_1$ receptor (17).

To measure puff-induced ATP release, 15 oocytes were incubated in ND-96 with no $CaCl_2$, no $MgCl_2$, and 1 mM EDTA (20 μl per oocyte). Two buffer puffs were delivered to each oocyte by the same procedure as in electrophysiologic studies. The medium was removed and incubated with the luciferin/luciferase assay mixture (Sigma) after the addition of $MgCl_2$ to 1 mM, $MgSO_4$ to 1 mM, and $CaCl_2$ to 0.3 mM (18). Luminescence was monitored over 30 sec, and results were standardized over the range of 10 fmol to 10 pmol of ATP per assay.

A cDNA clone for the rat $P2Y_1$ receptor was amplified from a rat adult brain cDNA library by polymerase chain reaction

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Abbreviation: DRG, dorsal root ganglion.

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using primer sequences derived from the known sequence (GenBank accession no. U22830) and ligated into pCRII vector. The $P2X_2$ cDNA was a gift of D. Julius (University of California, San Francisco) and the $P2X_3$ cDNA was a gift of J. N. Wood (University College, London). For purified receptor RNAs, 0.5–5 ng of RNA was routinely injected per oocyte.

Sense and antisense digoxegenin-labeled RNA probes were synthesized and hybridized to cryostat sections as described (16). Bound digoxegenin was detected with an anti-digoxegenin antibody-alkaline phosphatase conjugate. Sense probe yielded no reaction product. After *in situ* hybridization of rat DRG, tissue sections were stained for peripherin by using anti-peripherin (Sigma) and fluorescein isothiocyanate (FITC)-labeled goat anti-rabbit IgG or for neurofilament with RT-97 monoclonal antibody and FITC-conjugated goat anti-mouse IgG (13).

Xenopus laevis female frogs were immersed in 2°C water and then euthanized by pithing. The hindleg with the sciatic nerve and IX and X spinal roots attached were dissected free. The leg was placed in a 22°C bath of frog Ringer's (120 mM NaCl/3 mM KCl/3 mM $NaHCO_3$/3 mM $CaCl_2$), and the nerve was led through a vaseline-sealed hole into a grounded recording chamber of the same buffer. Fibers were teased from the posterior root, looped over a chloridated silver wire, and lifted into mineral oil. Potentials were amplified, filtered, digitized, and recorded as the skin of the leg was stimulated. The skin was stimulated by to-and-fro movement with a small paint brush (RA fibers) or by light (1–3 g/mm^2) constant pressure (SA fibers). The receptive field was mapped by testing numerous areas of the skin with both stimuli. No pharmacologic differences were detected between the two fiber types, and approximately half of the data was derived from RA fibers and half was from SA fibers. The optimal stimulus was repeated for 3–15 sec every 30 sec to 2 min and yielded stable responses for up to 3 h. Drugs (100 μl in Ringer's buffered to pH 7.0) were injected subcutaneously into the center of the receptive field at the following concentrations: ATP, 5 mM; adenosine 5′-[α,β-methylene]triphosphate, 5 mM; suramin, 3 mM; apyrase, 1 mg/ml from a preparation at 600 units/mg (Sigma, type VIII); pyridoxal-phosphate-6-azophenyl-2′,4′-disulphonic acid 4-sodium, 3 mM. Baseline responses were recorded on 6–12 occasions and then experimental responses were recorded 6–12 times during the 3–15 min after drug injection.

RESULTS

Isolation of a cDNA Mediating Mechanosensitivity in *X. laevis* Oocytes. To explore the molecular basis of sensory neuron mechanoelectric transduction, we assessed the mechanosensitivity of *X. laevis* oocytes expressing DRG-derived mRNAs. A puff of buffer directed at the oocyte surface induces an inward current of 50 nA in the majority of oocytes injected 2 days previously with poly(A)$^+$ RNA from DRG neurons and voltage-clamped at −60 mV. We screened 5000 clones from a DRG cDNA library and isolated a single clone termed A2.1 capable of mediating this response. Oocytes injected with 50 pg of the cRNA derived from the purified plasmid yield inward currents at 350 ± 45 nA in this paradigm (Fig. 1). Buffer puffs did not induce a current response in more than 100 H_2O-injected control oocytes. As another measure of the whole cell to pressure gradients, we injected buffer into the oocyte. In A2.1-injected cells, an oscillating current of 100–500 nA was observed after a rapid increase in oocyte volume (Fig. 1). In control cells, buffer injection over 100 msec occasionally produces a 1- to 2-sec inward current that reflects a loss of a membrane seal around the three glass pipettes in the oocyte. This was never followed by an oscillating inward current in more than 30 control oocytes.

The complete coding sequence of the A2.1 cDNA clone is identical to a metabotropic $P2Y_1$ ATP receptor previously cloned from chicken brain (17). Indeed, oocytes expressing the A2.1-derived protein generate 1000- to 4000-nA inward oscillating currents when 20 μM ATP is present in the bath. The puff-induced current of A2.1-injected cells has several characteristics similar to that typically induced by agonist binding

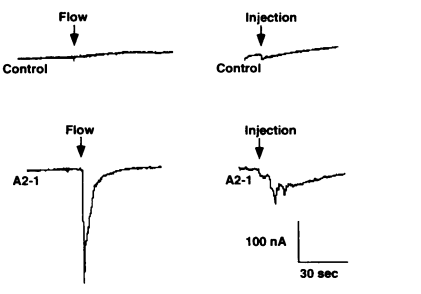

FIG. 1. Clone A2.1 expression induces mechanical sensitivity in oocytes. Puffs of buffer (*Left*) (Flow) elicit no alteration of current in the control oocyte, but the oocyte injected with A2.1 cRNA (50 pg) exhibits a 300-nA inward current after buffer puff. In 50 control oocytes, no current fluctuation greater than 5 nA was observed, whereas the puff-induced current in A2.1-oocytes was 350 ± 45 nA. The injection of buffer into oocytes (*Right*) (Injection) occasionally induced a small nonspecific conductance in control oocytes lasting several seconds. In A2.1-injected cells, this injection artifact was followed by 10–100 sec of oscillating inward current. The example is representative of 15 oocytes injected in this fashion.

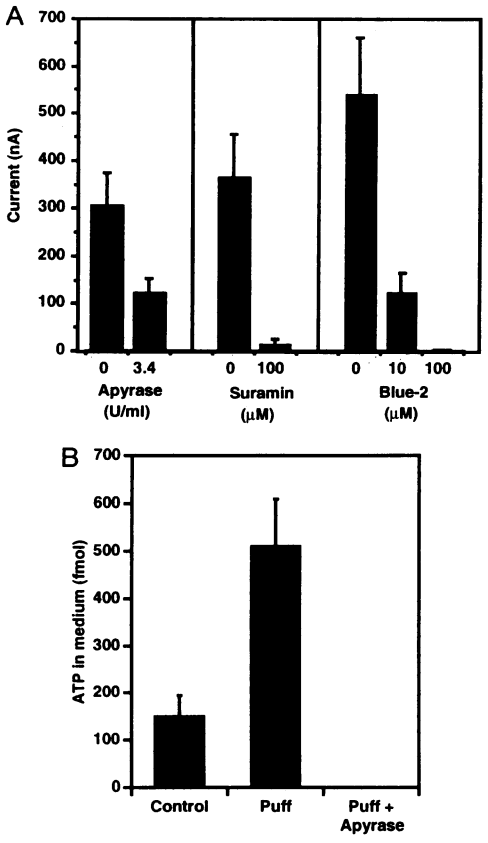

FIG. 2. The pressure-induced current is mediated by extracellular ATP. (*A*) A puff of buffer was presented to $P2Y_1$ cRNA-injected oocytes as in Fig. 1. The bath consisted of ND-96 with 0.3 mM Ca^{2+} with the indicated concentrations of apyrase (Sigma, grade VIII), suramin, or reactive blue 2. The data from 6 to 15 oocytes are presented as the mean ± SEM. (*B*) The quantity of ATP in oocyte bath medium was quantitated with or without buffer puffs, in the presence or absence of apyrase (10 units/ml). Each assay contained medium from 15 oocytes receiving two puffs each. The data are the mean ± SEM for 7–12 determinations.

to G-protein-coupled receptors in oocytes. The reversal potential of the induced current is −23 mV, similar to that for chloride in the oocyte (data not shown). The current has an oscillating pattern lasting 10–300 sec.

$P2Y_1$ Receptor Mediates Mechanosensitivity by an Autocrine ATP Mechanism. The response of oocytes expressing the $P2Y_1$ receptor to mechanical events might be due to a heretofore unrecognized sensitivity of the $P2Y_1$ receptor protein to membrane deformation or to stimulation of the receptor by the release of intracellular ATP. To distinguish these mechanisms, we added several ATP antagonists to the bath prior to puffing buffer onto A2.1-injected oocytes (Fig. 2*A*). Apyrase is an enzyme that will degrade ATP and ADP in the extracellular space. Suramin and reactive blue 2 are competitive antagonists of ATP at P2 receptors (19, 20). Apyrase significantly decreases the puff-induced current in A2.1-injected cells while suramin and reactive blue 2 abolish these puff-induced currents. These compounds do not have general toxic effects because the response to stimulation of the coexpressed $5HT_{1c}$ receptor or the endogenous LPA receptor is not altered (data not shown). Thus, a sensory neuron $P2Y_1$ receptor appears to induce mechanosensitivity in oocytes by an autocrine ATP mechanism.

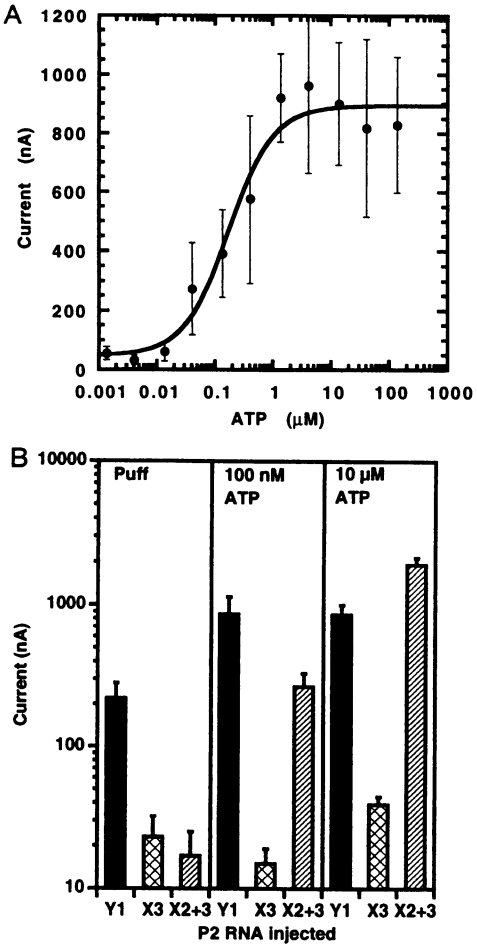

FIG. 3. Relative sensitivity of P2 receptors to puff and ATP. (*A*) Oocytes were injected with 50 pg of $P2Y_1$ receptor RNA and then exposed to the indicated bath concentrations of ATP. The peak inward current for 3–7 oocytes is plotted as mean ± SEM. The apparent K_d for ATP is 140 ± 25 nM. (*B*) Oocytes were injected with equal amounts of RNA encoding the $P2Y_1$, the $P2X_3$, or the $P2X_2$ plus $P2X_3$ receptors and then stimulated by buffer puff, 100 nM ATP, or 10 μM ATP. The peak inward current for 9–23 oocytes in each group is plotted as mean ± SEM.

This conclusion implies the release of intracellular ATP from oocytes by buffer puffs. ATP release from intact oocytes was quantitated directly by luciferase assay of oocyte medium. For each puff similar to that delivered in the electrophysiologic experiments, 12 fmol of ATP is released (Fig. 2*B*). No ATP is detected in the presence of apyrase. If this amount of ATP were distributed in the 1 μl immediately surrounding the oocyte, then a concentration of 12 nM ATP would be achieved.

The sensitivity of oocytes expressing various P2 receptors to low levels of ATP was examined. Defolliculated oocytes expressing the $P2Y_1$ receptor respond to ATP with an apparent K_d of 140 nM (Fig. 3*A*). This is much lower than the affinity for ATP measured in folliculated $P2Y_1$-expressing oocytes (17) and significantly lower than that of defolliculated oocytes expressing the $P2X_3$ receptor (13). The puff-induced response is equal in magnitude to that initiated by 25 nM ATP, in agreement with the concentration predicted from the ATP release experiments (Fig. 2*B*). Under these conditions, oocytes expressing the $P2X_3$ or the $P2X_2$ plus $P2X_3$ receptors generate inward currents in response to 100 nM ATP or puff that are of lesser magnitude than those generated by oocytes injected with $P2Y_1$ receptor RNA (Fig. 3*B*). In oocytes, the $P2Y_1$ receptor provides a high-affinity signal amplification system for detecting minute amounts of ATP released by gentle mechanical stimuli. This occurs in the absence of any detectable damage to the cell or its membrane potential.

$P2Y_1$ Receptor RNA Is Localized to Large-Fiber DRG Neurons. The distribution of $P2Y_1$ receptor mRNA is consistent with a role for the protein in transducing mechanical

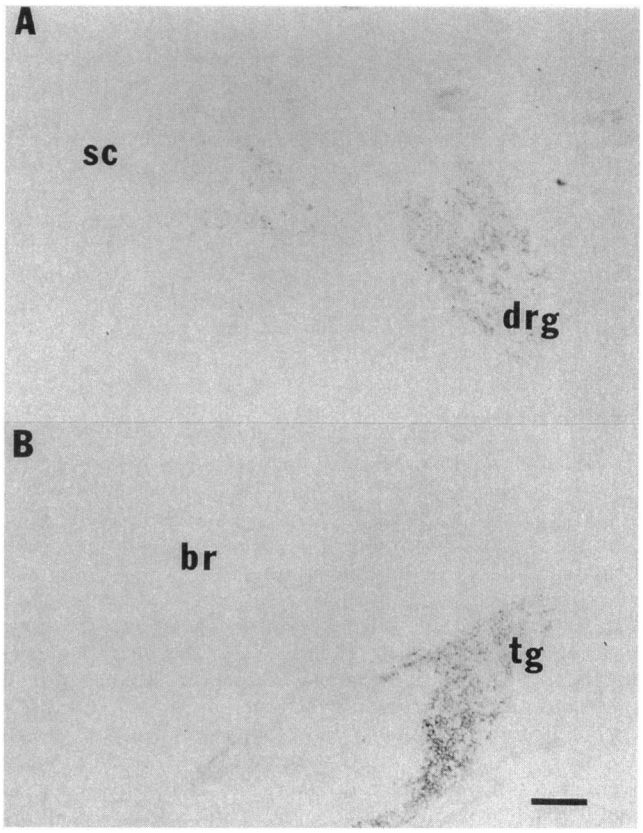

FIG. 4. $P2Y_1$ receptor RNA in chicken sensory ganglia. Cross sections of midthorax (*A*) and coronal sections of the head containing the base of the skull (*B*) from embryonic day 18 chicken embryos were hybridized with a digoxegenin-labeled antisense RNA probe containing the entire coding region of the chicken $P2Y_1$ receptor. Note the staining of most neurons in the DRG (drg) and trigeminal ganglion (tg). Staining is less intense in the spinal cord (sc), although a few cell bodies are detectable in the anterior horn. Labeling of the brain (br) in the hypothalamus and thalamus is minimal. (Bar = 100 μm.)

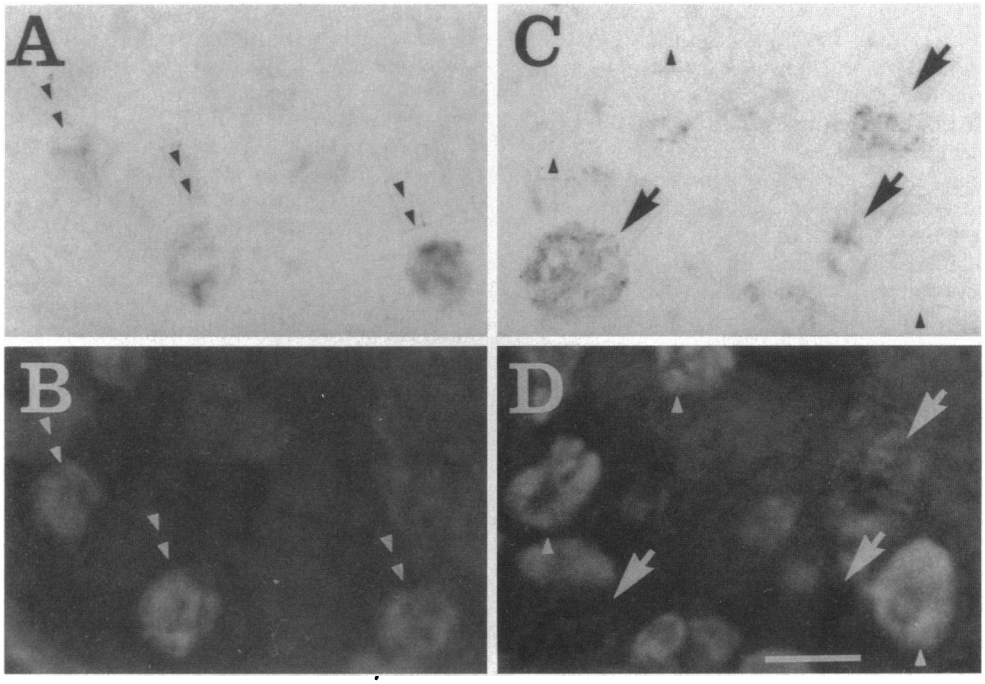

FIG. 5. Rat $P2Y_1$ mRNA is localized to large fiber sensory neurons. Sections of adult rat DRG were processed for rat $P2Y_1$ RNA *in situ* hybridization (*A* and *C*) and then for immunofluorescent detection of neurofilament (*B*) or peripherin (*D*). The section double-labeled in *A* and *B* demonstrates several cells expressing both $P2Y_1$ RNA and RT-97 antigenicity are shown (double arrowheads). (*C* and *D*) Several cells that express the $P2Y_1$ receptor but not peripherin (large arrows) and several cells containing peripherin staining but no $P2Y_1$ receptor RNA (small arrowheads). (Bar = 25 μm.)

stimuli into sensory fiber action potentials. Northern blot analysis has demonstrated RNA in brain, spinal cord, gastrointestinal tract, and skeletal muscle but not in other peripheral tissues (17). *In situ* hybridization demonstrates very high levels of $P2Y_1$ receptor mRNA signal in DRG and trigeminal ganglia neurons (Fig. 4). Anterior horn cells and cerebral cortex exhibit weak staining. Other neuronal structures, the gastrointestinal tract and muscle have very much less prominent reaction product. A comparison of the level of $P2Y_1$ receptor RNA in different cells indicates that there is heterogeneity among DRG and trigeminal ganglia neurons.

To determine which subset of sensory neurons express $P2Y_1$ receptor, we compared the distribution of staining for peripherin and a neurofilament epitope recognized by the antibody RT-97. The RT-97 antibody selectively stains large-fiber neurons and is absent from cells expressing the $P2X_3$ receptor (13). $P2Y_1$ receptor and RT-97 staining are colocalized in DRG neurons (Fig. 5 *A* and *B*). Peripherin is a marker for small-fiber neurons and has been shown to colocalize with the $P2X_3$ receptor RNA (13). Peripherin staining and $P2Y_1$ *in situ* hybridization are concentrated in different cell populations (Fig. 5 *C* and *D*). P2Y1 mRNA staining is faint but detectable in peripherin-positive cells. Thus, the $P2Y_1$ receptor appears to function primarily in those large-fiber neurons mediating light touch and proprioceptive information.

$P2Y_1$ Receptor Agents Modulate the Generation of Touch Potentials. To directly assess the role of ATP in transducing touch into peripheral nerve electrical activity, we recorded impulses in teased single fibers of the posterior roots from the IX nerve of frogs. Fibers with discrete responses to light brushing or constant pressure on the skin were analyzed (Fig. 6), and minimal spontaneous firing was observed. After obtaining a baseline stimulus-induced firing rate, ATP was injected subcutaneously in the receptive field of these neurons. Injection of ATP, but not buffer, enhances action potential frequency from about half of fibers. This increased spontaneous firing was detectable during the period from 30 sec to 3 min after ATP injection. Several minutes after ATP injection, when spontaneous activity has returned to baseline, touch-induced sensory impulse frequency is 3-fold higher than baseline. The increased sensitivity to light touch persists for at least 10 min but is not detected 20 min after injection. Injection of P2 receptor antagonist suramin or the ATP-degrading enzyme apyrase within the receptive field suppresses touch-induced firing rates (Fig. 6). Suramin also decreases spontaneous firing rates.

ATP, suramin, and apyrase are not specific for a subtype of P2 receptor, so these agents cannot distinguish which ATP receptors play a role in light touch. There are no high-potency $P2Y_1$-selective agents now available (12). However, adenosine 5′-[α,β-methylene]triphosphate (AMP-C-PP) stimulates rat P2X receptors containing $P2X_1$ or $P2X_3$ subunits (13, 14) but not chicken $P2Y_1$ receptors (17), and pyridoxal-phosphate-6-azophenyl-2′,4′-disulphonic acid 4-sodium selectively blocks most P2X receptors without affecting $P2Y_1$ receptor activation (13, 14, 21). Neither of these relatively P2X-selective agents alters spontaneous or touch-induced firing (Fig. 6 *D* and *E*), suggesting that activation of P2Y receptors is necessary for the observed modulation of touch-induced discharges.

DISCUSSION

We present three lines of evidence implicating ATP binding to $P2Y_1$ receptors in the peripheral generation of light touch-induced sensory potentials. (*i*) From 5000 DRG-derived cDNAs expressed in oocytes, a clone encoding this receptor was isolated by selecting for mechanosensitivity. (*ii*) The RNA for this receptor is expressed in large fiber sensory neurons. (*iii*) Peripheral application of agents which modulate the activity of this receptor alter the generation of touch-induced potentials. To our knowledge, these experiments provide the first data directly demonstrating a role for P2 purinergic receptors in the initiation of sensory potentials at distal nerve terminals.

In oocytes, it is clear that this receptor responds to low levels of ATP released from the oocyte, in an autocrine fashion. If

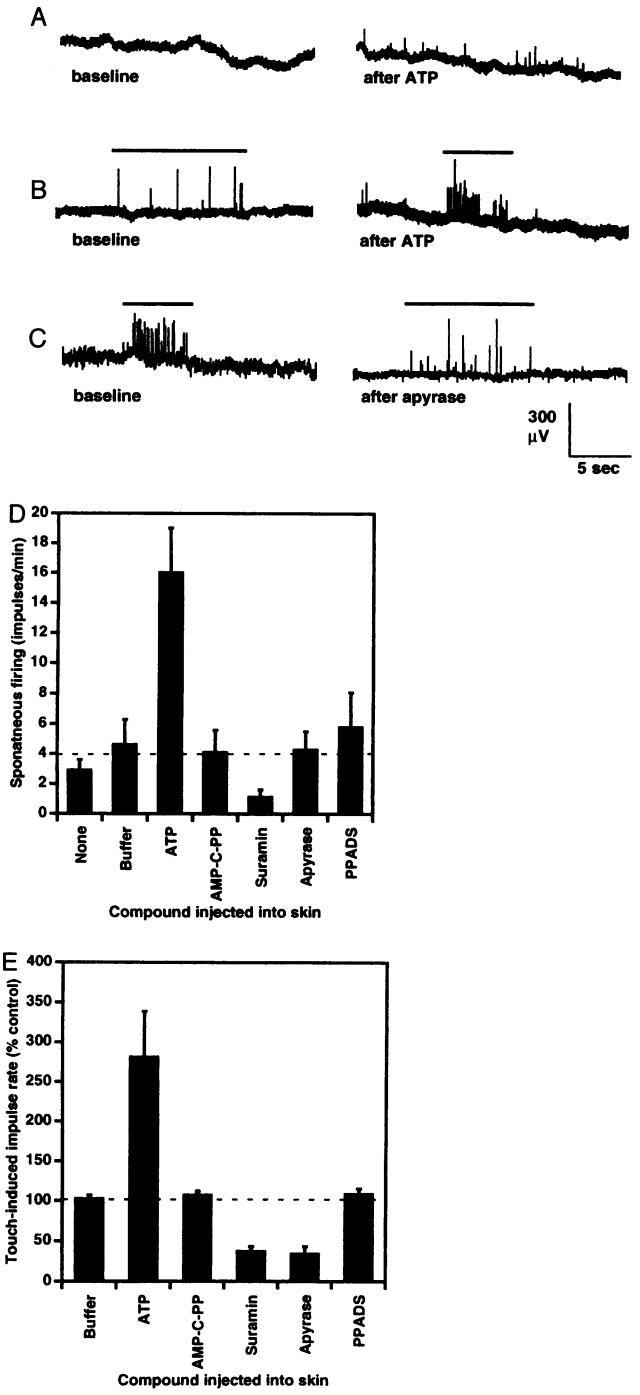

FIG. 6. ATP receptors and the generation of sensory impulses from skin. (*A*) There was no spontaneous firing in a frog sensory fiber prior to ATP injection (*Left*), but during the period 1–3 min after injection, spontaneous discharges were observed (*Right*). Nonetheless, the spontaneous discharge rate (0.7 sec^{-1} in this example) was much less than the touch-induced rate (5 sec^{-1} in this example). (*B*) Impulses from a sensory nerve fiber were stimulated by continuous to-and-fro movement of a small paint brush over the medial superior aspect of the lower leg as indicated by the bar. The baseline response is shown at the left. Subcutaneous injection of ATP within the receptive field of this fiber increased the frequency of touch-induced spikes. (*C*) A sensory fiber responded to brushing the dorsal aspect of the skin between the second and third toes, as indicated by the bar. Subcutaneous injection of apyrase within the receptive field of this fiber decreased stimulus-induced impulse rates to 17% of control levels. (*D*) The spontaneous firing rate for touch-sensitive fibers is illustrated during the period 30 sec to 3 min after the injection of various compounds subcutaneously within the receptive field of the fiber. The values are the mean rate ± SEM for 5–23 fibers under each condition. (*E*) The touch-induced firing rate for sensory fibers is illustrated after the injection of various compounds subcutaneously within the receptive field of the fiber. The values are expressed as a percentage of the mean preinjection rate ± SEM for 5–23 fibers under each condition.

the intracellular ATP concentration is 2 mM, then the oocyte produces large fluctuations in membrane conductance when the extracellular concentration of ATP rises to 1/100,000th of the intracellular concentration for a brief period of time. In the area surrounding a peripheral nerve ending the source of extracellular ATP might be the nerve itself or those cells that immediately surround the nerve ending.

We have no data to indicate a mechanism for this ATP release. It is plausible that this small amount of ATP could be released by a nonspecific mechanical disruption of the plasma membrane, even though no changes in conductance are detectable during these minor mechanical stimuli. It is also possible that oocytes and/or nerve terminals contain specific proteins that mediate the extracellular transport of ATP in response to mechanical stimuli. Proteins of the ABC transporter family, including the CFTR and the sulfonylurea receptor, can permeate ATP in other cell types (22, 23).

P2X purinergic receptors have also been localized to primary sensory neurons (13, 14). One subtype, $P2X_3$, is expressed exclusively in small-fiber sensory neurons (13). As the $P2Y_1$ receptor functions in the peripheral generation of sensory potentials by light touch, the P2X receptor may contribute to the formation of nerve impulses by noxious stimuli (13). To our knowledge, we provide the first data that P2X receptors can mediate small inward current fluxes in oocytes subjected to minor mechanical stimuli. Although extrapolation from oocytes to DRG neurons *in vivo* is tenuous, the data suggest that the $P2Y_1$ receptor may provide a high-sensitivity system to respond to minor touch-induced fluctuations in extracellular ATP whereas the $P2X_{2/3}$ receptor registers larger ATP concentration changes initiated by noxious stimuli.

Although the RNA localization studies provide a clue as to the involvement of specific ATP receptors in particular sensory modalities, the use of subtype-specific pharmacologic agents would greatly advance this assignment. As yet the number of compounds known to interact with high affinity at these receptors is extremely limited, and none are highly selective (12). The development of subtype-specific function-blocking antibodies may provide a means to further correlate ATP receptor subtypes with specific sensory fiber types. For example, it is unclear whether $P2Y_1$ and/or other P2 purinergic receptors have a role in proprioceptive impulse generation.

The mechanism of $P2Y_1$ receptor action in oocytes is well defined (refs. 17 and 24 and this study). Mechanical stimuli release small quantities of ATP from the cell that bind to the receptor. The ATP-bound receptor then undergoes a conformational change that activates a heterotrimeric G protein, leading to phospholipase C stimulation. Increased inositol trisphosphate stimulates the release of stored intracellular calcium and the opening of a plasma membrane chloride channel. It is highly unlikely that all of these steps occur in sensory nerve endings. Release of ATP, receptor binding, and G-protein activation are probable events, but downstream effector events are unclear (25). Numerous second messenger pathways regulated by G proteins could lead to membrane depolarization and the initiation of action potentials (26).

Touch-induced depolarization in nerve endings occurs rapidly, and some have argued that for this reason diffusible second messenger systems are unlikely to be involved (27). Thus, the direct regulation of ion channels by $P2Y_1$-activated heterotrimeric G proteins may be a more plausible mechanism. It has been shown that amiloride-sensitive sodium channels open in the presence of activated G_{i3} (28–30). Because the

sensory neuron-specific genes *mec-4* and *mec-10* are related to this sodium channel (7), it is tempting to suggest that ATP-activated $P2Y_1$ receptors might regulate the opening of Na^+ channels formed by proteins related to the products *mec-4* and *mec-10*.

Although responses to light touch are terminated abruptly after the stimuli is removed, ATP injection sensitizes the skin to light touch for at least 10 min. We suggest that the ATP enhancement of touch responses is long-lasting because a large amount of the compound suffuses a significant volume of the interstitial space and requires this period of time to be degraded. During this period, the concentration of ATP present at the nerve terminal is not high enough to induce spontaneous firing but does sensitize the nerve ending to touch-induced firing (Fig. 6). In contrast, mechanical stimuli may release a smaller quantity of ATP but a focally higher concentration at the nerve terminal, and the action of this ATP may be rapidly terminated by a combination of diffusion and degradation.

In oocytes, the $P2Y_1$ receptor can directly support mechanoelectric transduction. In the distal endings of touch-sensitive neurons, a transduction system based on this receptor may form the sole basis for the generation of nerve impulses or may be one of several mechanisms for creating such potentials. Thus, activation of the $P2Y_1$ receptor might be an essential early step in touch-sensitivity, or its activation might have a later modulatory effect on other pathways. The ability of peripheral ATP injection to create spontaneous neural activity argues for the former possibility. Regardless, it is clear from the studies herein that this receptor participates in the conversion of touch into nerve impulses.

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