Purinergic Signalling (2012) 8:199–206
DOI 10.1007/s11302-011-9264-5

ORIGINAL ARTICLE

Extracellular ATP induces fast and transient non-selective cationic currents and cytosolic Ca^{2+} changes in human umbilical artery smooth muscle cells

N. Enrique · A. Rebolledo · P. Martín · A. R. Roldán Palomo · F. Tanzi · V. Milesi

Received: 5 July 2011 / Accepted: 22 September 2011 / Published online: 4 October 2011

Abstract Ionotropic purinergic receptors (P2X) are expressed in endothelial and smooth muscle cells of blood vessels. ATP acting on smooth muscle P2X receptors is able to induce vasoconstriction in different kind of vessels. However, to our knowledge, there are no reports that directly show the activity of these purinergic receptors in native human vascular smooth muscle cells. In this work, we describe for the first time an ATP-induced current in freshly isolated human umbilical artery (HUA) smooth muscle cells. The current was measured by patch-clamp technique in whole-cell condition on cells clamped at −50 mV. At 100 μM of ATP the current showed a rapid activation and desensitization, and was carried by both Na^+ and Ca^{2+}. The current was completely blocked by suramin (300 μM) and partially blocked by 100 μM of Zn^{2+} without affecting the kinetic of desensitization. All these properties suggest that the ATP-induced ionic currents are mediated through $P2X_1$-like receptors. Moreover, we show that ATP transiently increased cytosolic Ca^{2+} in "in situ" smooth muscle cells of intact HUA segments and that this response is dependent of extracellular and intracellular Ca^{2+}. These data expand the knowledge of purinergic receptors properties in vascular smooth muscle cells and the probable role of ATP as a paracrine modulator of contractile tone in a human artery which is fundamental for feto-placental blood flow.

Keywords ATP · Human umbilical artery · Patch clamp · Microfluorimetry

N. Enrique · A. Rebolledo (✉) · P. Martín · A. R. Roldán Palomo · V. Milesi
Departamento de Ciencias Biológicas, GINFIV—Grupo de Investigación en Fisiología Vascular, Facultad de Ciencias Exactas, Universidad Nacional de La Plata,
Calles 47 y 115,
La Plata (1900), Argentina
e-mail: ar@biol.unlp.edu.ar

F. Tanzi
Dipartimento Di Fisiologia, Sezione Di Fisiologia Generale,
Via Forlanini 6,
Pavia (27100), Italy

Introduction

Ionotropic purinergic receptors (P2X) are expressed in different types of cells. In blood vessels, in particular, they are present in endothelial and smooth muscle cells, where they are subject to ATP coming from different sources. ATP is released by sympathetic varicosities as a co-transmitter together with noradrenaline [1], acting on smooth muscle P2X receptors which favor vasoconstriction [2]. When faced with hypoxia or due to shear stress, the endothelium also releases ATP [3] which can reach smooth muscle by a paracrine route. Alternatively, ATP may act on endothelial cells to induce the production of the vasorelaxant agent NO [2, 4]. Moreover, ATP coming from all these sources may be transformed by ectoenzymes to adenosine, which can now act on P1 purinergic receptors on smooth muscle cells promoting vasodilation [2].

Umbilical cord vessels are essential for feto-placental blood flow, and the control of their degree of contraction is of fundamental importance. Since the umbilical cord is not innervated, smooth muscle cells are under exclusive control of mechanical stretch and vasoactive substances secreted by endocrine glands or locally produced by endothelial cells, platelets and blood cells. Umbilical cord and chorionic vessels respond to extracellular ATP. Results obtained by the RT-PCR technique showed that human umbilical artery (HUA) smooth muscle cells express mRNAs for different

P2X receptors, namely, $P2X_1$, $P2X_2$, $P2X_4$, $P2X_5$, $P2X_6$, and $P2X_7$, being the mRNA of $P2X_2$ only present in the proximal segment of the umbilical artery, and the $P2X_5$ subtype present in the middle and distal portions of it [5]. In the same work, by measuring the force development produced by exogenous ATP or by an agonist of $P2X_1$ receptors, the authors show that stimulation of $P2X_1$ is able to contract HUA segments. These results are in accordance with those obtained by Bo et al. [6] indicating the presence of $P2X_1$ receptors in the smooth muscle cells of human umbilical artery using in vitro organ bath recording, radioligand binding assays, autoradiography, and immunohistochemistry.

However, up to now, there are no reports that directly show the activity of these purinergic receptors in native human vascular smooth muscle cells. In this work, an ATP-induced current in freshly isolated HUA smooth muscle cells is described for the first time. This current was carried by Na^+ and Ca^{2+}, presented a transient temporal course and was inhibited by Zn^{2+} and suramin. All these properties are characteristic of ionic currents through $P2X_1$-like receptors. Moreover, we show that at the same concentration, ATP transiently increased cytosolic Ca^{2+} in "in situ" smooth muscle cells of intact HUA segments.

Methods

Umbilical cords (n=32) were obtained after vaginal and caesarean deliveries performed in the Instituto Central de Medicina, La Plata, Argentina and in the Policlinico San Matteo, Pavia, Italy. The middle portions of these umbilical cords were placed in a Krebs solution (KS) of the following composition (in mM): 130 NaCl, 4.7 KCl, 1.17 KH_2PO_4, 1.16 $MgSO_4$, 24 $NaCO_3H$, 2.5 $CaCl_2$, pH 7.4 at 4°C and immediately taken to the laboratory where they were stored at 4°C and used within the next 24 h.

Patch-clamp recordings

The arteries were dissected from the Wharton's jelly just before the cell isolation procedure in a Petri dish containing KS. Endothelial cells were eliminated by gently rubbing the luminal vessel surface with a cotton swab. Previous experience shows that this maneuver does not damage the smooth muscle layer. Afterwards, HUA smooth muscle cells were obtained by a method based on the one described by Klockner [7] and later modified in our laboratory [8] in order to diminish the enzyme content in the dissociation medium (DM). Briefly, segments of HUA were cleaned of any residual connective tissue, cut in small strips and placed for 15 min in a DM containing (in mM): 140 NaCl, 5 KH_2PO_4, 5 $MgCl_2$, 6 glucose, 5 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), pH was adjusted to 7.4 with NaOH. The strips were then placed in DM with 2 mg/ml collagenase type I during 25 min, with gentle agitation, at 35°C. After the incubation period the strips were washed with DM and single HUA smooth muscle cells were obtained by a gentle dispersion of the treated tissue using a Pasteur pipette. The remaining tissue and the supernatant containing isolated cells were stored at room temperature (20–23°C) until used. HUA smooth muscle cells were allowed to settle onto the coverglass bottom of a 3 ml experimental chamber. The cells were observed with a mechanically stabilized, inverted microscope (Zeiss-Telaval3) equipped with a ×40 objective lens. The chamber was perfused for 15 min, at 1 ml min^{-1} by gravity, with the extracellular saline solution (ES, see composition later) before the patch-clamp experiment was started. After each experiment on a single cell, the experimental chamber was replaced by another one containing a new sample of cells. Only well-relaxed, spindle-shaped smooth muscle cells were used for electrophysiological recordings. Data were collected within 4–6 h after cell isolation. All experiments were performed at room temperature (20–23°C).

The standard tight-seal whole-cell configuration of the patch-clamp technique [9] was used to record ionic currents. Glass pipettes were drawn from WPI PG52165-4 glass on a two-stage vertical micropipette puller (PP-83, Narishige Scientific Instrument Laboratories, Tokyo, Japan) and pipette resistance ranged from 2 to 4 MΩ measured in ES. Ionic currents were measured with an Axopatch 200A amplifier (Axon Instruments, Foster City, CA).

Whole-cell currents activated by ATP were recorded on cells clamped at −50 mV. Application of ATP solution was performed through a multibarreled pipette positioned immediately adjacent to the cell investigated, so the temporal delay of ATP reaching the cell was negligible. The currents were filtered at 2 kHz, digitized at a sample frequency of 100 kHz (Digidata 1200 Axon Instruments, Foster City, CA), and stored on a computer hard disk for later analysis. The currents were analyzed by measuring the peak amplitude and the kinetic of desensitization by fitting them with an exponential function and obtaining their tau value.

Composition of patch-clamp solutions

Extracellular solution (ES) in mM: 150 NaCl, 5.4 KCl, 2.5 $CaCl_2$, 1.2 $MgCl_2$, 6 glucose, 5 HEPES, pH=7.4.

Intracellular pipette solution (IPS) in mM: 130 KCl, 0.1 ethylene glycol-bis(β-aminoethyl ether) *N*,*N*,*N*′,*N*′,-tetraacetic acid (EGTA), 20 HEPES, pH=7.4.

For some of the experiments two modified ES were used: an ES without Ca^{2+} (no $CaCl_2$ plus 1 mM EGTA) or an ES without Na^+ (NaCl entirely replaced by *N*-methyl-glucamine chloride).

Intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) measurement by microfluorimetry in HUA smooth muscle cells in situ

The HUAs were dissected from the Wharton's jelly from the umbilical cord and placed in a Petri dish filled with KS, cleaned of adherent connective tissue, and cut in small rectangular strips. Endothelial cells were eliminated by gently rubbing the luminal vessel surface.

The strips were then incubated for 180 min at room temperature in an extracellular saline solution (ES) of the same composition already described containing 16 μM fura-2 AM (1 mM fura-2 AM in DMSO stock solution). They were afterwards washed with ES without fura-2 AM and anchored to the bottom of a Petri dish. In situ smooth muscle cells were visualized by an upright epifluorescence microscope (Zeiss, Axiolab) equipped with a 100-W Hg lamp and a Zeiss ×63 Achroplan water immersion objective (0.75 numerical aperture). The cells were excited alternately at 340 or 380 nm and the emitted light was detected at 510 nm. A neutral density filter (0.3 optical density) reduced the overall intensity of the exciting light and a second neutral density filter (optical density=0.3) was coupled to the 380 nm filter to approach the intensity of the 340 nm light. The exciting filters were mounted on a filter wheel (Lambda 10, Sutter Instrument, Novato, CA, USA). Custom software, working in the LINUX environment, was used to drive the camera (Extended-ISIS Camera, Photonic Science, Millham, UK), the filter wheel, and to measure and plot on-line the fluorescence from about five rectangular regions of interest (ROI), which enclosed one to five single cells each. Variations in the $[Ca^{2+}]_i$ were monitored by evaluating for each ROI the ratio of the fluorescence signal emitted at 510 nm when exciting at 340 and 380 nm. Experiments were carried out at room temperature (20–23°C) and ratio measurements were performed every 2.0 s.

Reagents

ATP (disodium salt), suramin, EGTA, HEPES, αβ-methylene ATP, cyclopiazonic acid, and collagenase type I used for cell isolation were purchased from Sigma Chemical Company (St Louis, MS, USA). Fura-2 AM was obtained from Molecular Probes (Eugene, OR, USA). All other reagents were of analytical grade and purchased from local vendors.

Statistical analysis

All data are presented as mean±SEM. Comparisons between groups were made by ANOVA followed by an appropriate test. The figure legends indicate which test was used for each comparison.

Results

Patch-clamp recordings

The application of extracellular ATP on freshly isolated HUA smooth muscle cells evoked a transient inward current, characterized by a fast activation and a peak current followed by a decrease toward the baseline value, which represents the desensitization of ATP receptors (Fig. 1a, b, and c). After a first stimulus, it took more than 15 min of washing to achieve a second response to a new ATP application. Nevertheless, this second response was significantly lower than the first one (not shown).

The peak amplitude of ATP-induced current was dependent on ATP concentration (Fig. 1a, b, c, and d). The kinetics of desensitization was analyzed fitting the current decay with a first order exponential function, which accurately fits the time course of current decrease. The mean time constants of the exponential decay (tau) for each ATP concentration are shown in Fig. 1e. As it is possible to observe, the kinetics of desensitization is also dependent on ATP concentration. By comparing these results with published properties for different types of P2X receptor, it seems that in HUA smooth muscle cells the ATP-induced current is carried through $P2X_1$ receptors or through heteromeric $P2X_1$-like receptors.

The response to 100 μM extracellular ATP in normal ES was considered as the control condition for the next experiments. Suramin (100 μM), a blocker of P2X receptors, inhibited most of the whole-cell current induced by ATP, leaving a small component which had a slower activation and desensitization (Fig. 2b). A higher concentration of suramin (300 μM) induced a complete block of the current (Fig. 2c). Zn^{2+} is a modulator of ATP-induced currents through P2X receptors [10], so the effects of different concentrations of Zn^{2+} were tested. While 20 μM of Zn^{2+} did not affect the current (data not shown), 100 μM produced a significant decrease in the peak current amplitude without affecting the kinetic of receptor desensitization (Fig. 2d). Figure 2e and f summarize data obtained with Zn^{2+} and suramin.

As it is known that, in physiological conditions, currents through P2X receptors are mainly carried by Na^+ and Ca^{2+}, the next series of experiments were directed to study the effects of extracellular Na^+ and Ca^{2+} substitution on ATP-induced current. When extracellular Na^+ ions were completely replaced by *N*-methyl-glucamine, the peak amplitude of the ATP-induced current was significantly reduced (Fig. 3b and d), conserving its desensitization kinetics (Fig. 3b and e). When it was recorded without extracellular Ca^{2+} (plus EGTA 1 mM) the ATP-induced current was also significantly reduced (Fig. 3c and d) but not as much as when Na^+ was absent

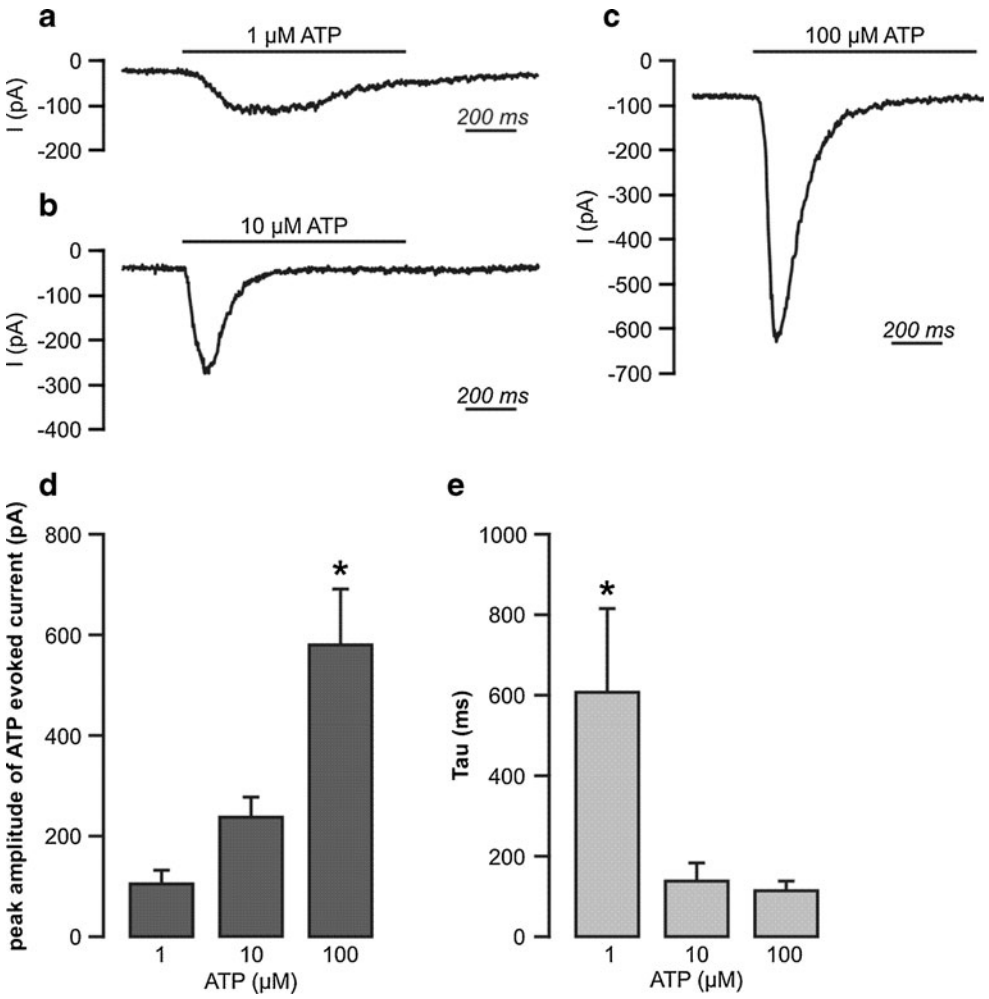

Fig. 1 Currents evoked by ATP in human umbilical artery smooth muscle cells. Typical recordings of whole-cell currents induced by **a** 1 μM (n=5), **b** 10 μM (n=7), and **c** 100 μM ATP (n=5) in cells clamped at −50 mV. **d** Mean ±SEM peak amplitude values of the currents shown in **a**, **b**, and **c**. **e** Mean±SEM time constant of desensitization (tau) obtained by fitting the currents shown in **a**, **b**, and **c** with an exponential function. The symbol * indicates a statistically significant difference by all pairwise multiple comparison (one-way analysis of variance, Holm–Sidak test)

(Fig. 3d). The desensitization kinetics in Ca^{2+}-free ES was not significantly affected (Fig. 3c and e).

Measurements of $[Ca^{2+}]_i$ changes induced by ATP

Stimulation with 100 μM ATP induced a transient $[Ca^{2+}]_i$ elevation characterized by a rapid increase and return to baseline levels (about 2 min after initial increase, Fig. 4a and e). A similar response was obtained when the cells were stimulated with 3 μM of αβ-methylene ATP, an activator of $P2X_1$ receptors (Fig. 4c and e), suggesting that the observed Ca^{2+} response to ATP is evoked by activation of $P2X_1$ receptors or heteromeric $P2X_1$-like receptors.

Both extracellular Ca^{2+} and sarcoplasmic reticulum release contribute to the ATP-induced changes in $[Ca^{2+}]_i$. The response was completely abolished in a Ca^{2+}-free ES (plus 1 mM EGTA), suggesting that Ca^{2+} influx is a necessary signal (Fig. 4b and e) to evoke it. After sarcoplasmic reticulum depletion with 10 μM cyclopiazonic acid (CPA), ATP-induced Ca^{2+} increase was less than in control conditions, meaning that this Ca^{2+} reservoir also participates in the response to ATP (Fig. 4d and e).

Discussion

ATP activates $P2X_{1-7}$ receptors, which are non-selective cationic channels through which Na^+ and Ca^{2+} can permeate [11–13]. Among the members of ATP receptors, $P2X_1$ subtype is the more frequently found in vascular smooth muscle cells.

The ionic currents induced by ATP in freshly isolated HUA smooth muscle cells have never been investigated before. Our data showed that ATP induces a cationic current carried by both Na^+ and Ca^{2+}, where Na^+ influx is quantitatively more relevant. These data regarding the current carriers, plus the inhibitory effect of suramin and the kinetics of desensitization, point to the $P2X_1$ subtype as the main structure responsible for the current activated by 100 μM ATP. However, since HUA smooth muscle also expresses mRNA for other types of purinergic receptors [5],

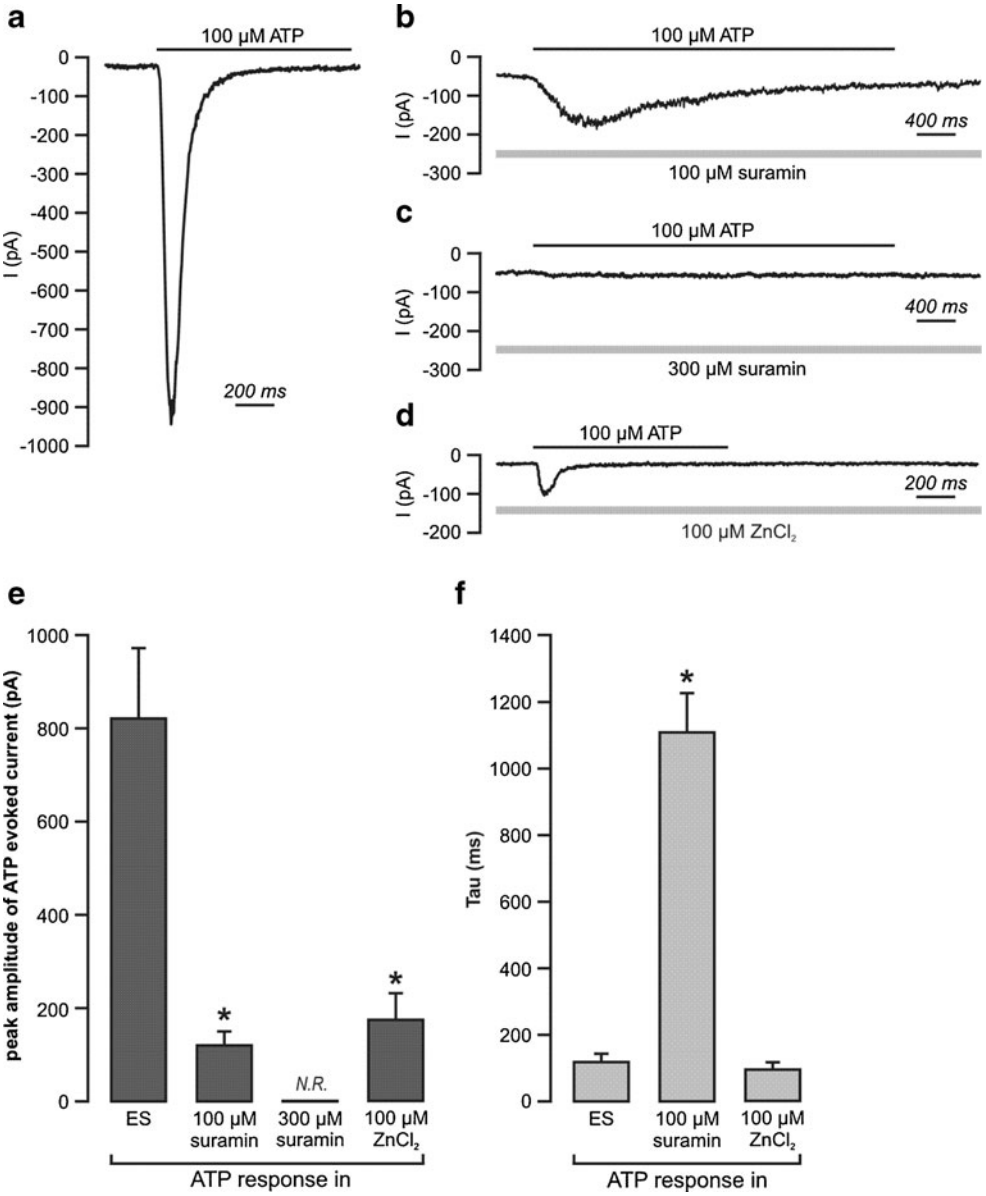

Fig. 2 Inhibition by suramin and Zn^{2+} of currents evoked by ATP in human umbilical artery smooth muscle cells. Typical recording of whole-cell currents induced by 100 μM ATP in cells clamped at −50 mV **a** in control conditions (ES) (n=16), **b** in presence of 100 μM suramin (n=9), **c** in presence of 300 μM suramin (n=4), **d** in presence of 100 μM $ZnCl_2$ (n=6). **e** Mean ±SEM peak amplitude values of the currents shown in **a**, **b**, **c**, and **d**. **f** Mean±SEM time constant of desensitization (tau) obtained by fitting the currents shown in **a**, **b**, and **d** with an exponential function. The symbol * indicates a statistically significant difference by multiple comparison versus control (100 μM ATP in ES) group (one-way analysis of variance, Holm–Sidak test)

we cannot exclude as current carriers $P2X_1$-like heteromers formed by combinations of different subunits (like P2X1/4, P2X1/5 or others).

In good agreement with these results, we found that in "in situ" HUA smooth muscle cells ATP increases $[Ca^{2+}]_i$. Moreover, αβ-methylene ATP, produced a $[Ca^{2+}]_i$ change displaying a similar temporal course as that of ATP (Fig. 4d). This agonist stimulates mainly homomeric $P2X_1$ or heteromeric $P2X_1$-like receptors, although it can also be effective on $P2X_3$ and $P2X_5$. However, $P2X_3$ mRNA was not found to be expressed by HUA smooth muscle [5], and currents through $P2X_5$ receptors do not show fast desensitization [13]. Hence, we think that combining the evidence obtained from the whole-cell currents plus the effects of αβ-methylene ATP gives $P2X_1$, or a closely related heteromer, as the main structure responsible for both the current and the initiation of the $[Ca^{2+}]_i$ transient.

Using cultured HUA smooth muscle cells, Meng et al. [14] found that αβ-methylene ATP induced a $[Ca^{2+}]_i$ response similar to the one we observed. However, in their case ATP, at a concentration similar to ours, induced Ca^{2+} oscillations. The authors suggested that these oscillations were produced by ATP stimulation of P2Y receptors. Our results did not show oscillations in $[Ca^{2+}]_i$ and, as discussed, do not suggest involvement of P2Y receptors. There could be many causes for this discrepancy, especially because in the microfluorimetry experiments we used intact tissue, while Meng et al. used cultured cells, which do not have cell-to-cell interactions and attachment to the extracellular matrix. Additionally, several authors have reported

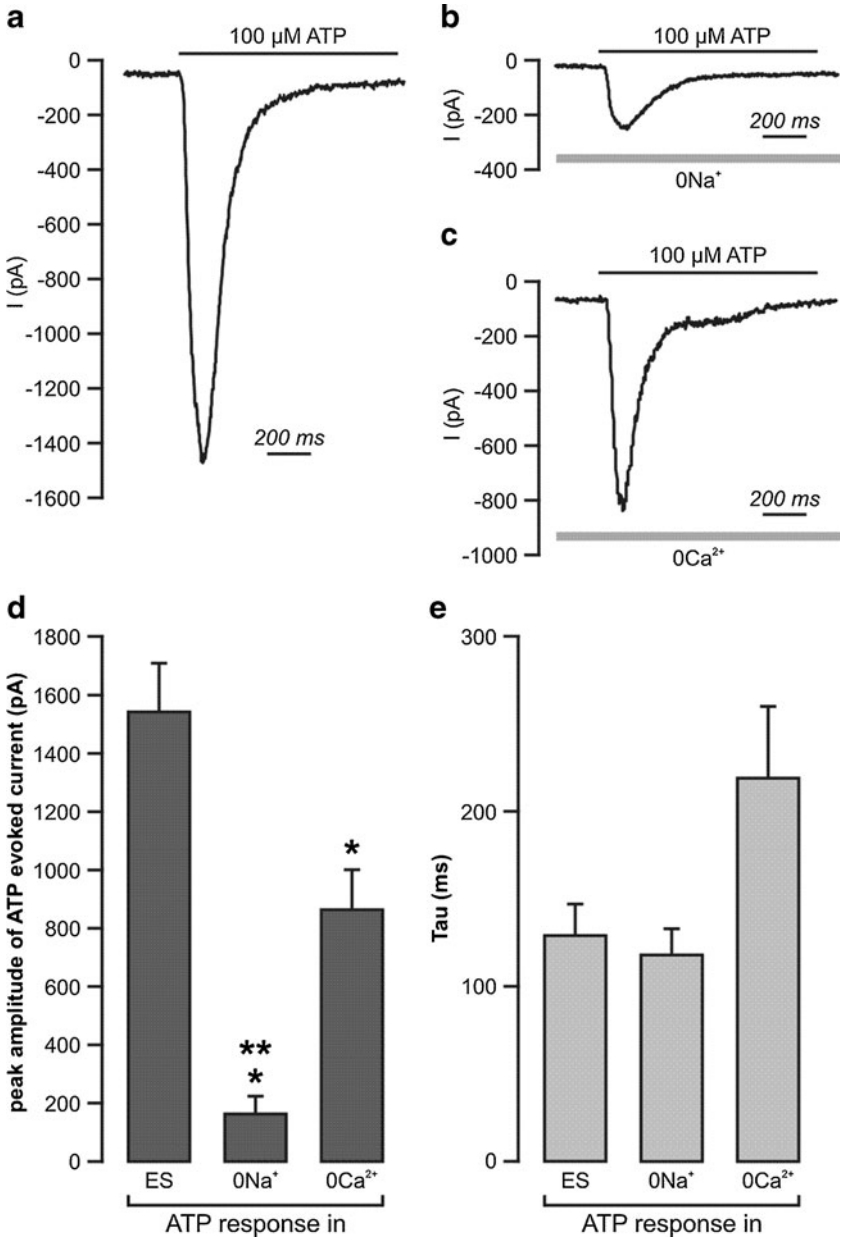

Fig. 3 Inhibition by extracellular Ca^{2+} and Na^{+} deprivation of currents evoked by ATP in human umbilical artery smooth muscle cells. Typical recording of whole-cell currents induced by 100 µM ATP in cells clamped at −50 mV **a** in control conditions (ES; n=13), **b** in a Na^{+}-free extracellular solution ($0Na^{+}$; n=12), **c** in a Ca^{2+}-free extracellular solution with 1 mM EGTA ($0Ca^{2+}$) (n=12). **d** Mean±SEM peak amplitude values of the currents shown in **a**, **b**, and **c**. **e** Mean±SEM time constant of desensitization (tau) obtained by fitting the currents shown in **a**, **b**, and **c** with an exponential function. The symbol * indicates a statistically significant difference by multiple comparison versus control (100 µM ATP in ES) group and the symbol ** indicates a statistically significant difference by multiple comparison versus $0Ca^{2+}$ group (one-way analysis of variance, Holm–Sidak test)

significant differences in receptors and channels expression between freshly isolated cells or intact tissue and culture cells [15, 16], so the difference between what Meng et al. reported and our results could also be interpreted in this light. We think that it will be interesting to explore this subject in the future in order to understand the different phenotypes (proliferative, secretor, contractile) that these smooth muscle cells could present in different conditions and if ATP receptors could have different functional roles in each case, as suggested by Erlinge et al. [17].

Our results also show that extracellular Ca^{2+} entry is a necessary step to produce intracellular Ca^{2+} increase, likely inducing further Ca^{2+} release from the sarcoplasmic reticulum. Hence, we can conclude that in in situ smooth muscle cells of excised HUA 100 µM ATP mainly activates $P2X_1$-like receptors. This is a quite novel smooth muscle cell model, avoiding enzymatic treatment and dissociation, and allows investigations in a more relevant physiological condition than cultured systems. The expression of $P2X_1$ receptors had been demonstrated in HUA as mRNA [5] and protein [6]. It has been shown that ATP-binding sites in HUA are restricted to the smooth muscle cells, with radioactive probes not attaching to the endothelial cells [6]. Both groups concluded that in this vessel ATP contractions could be evoked by $P2X_1$ receptor activation [5, 6].

Zinc is known to increase the current through $P2X_2$ and $P2X_4$ purinergic receptors [13], and inhibit $P2X_1$ [10, 18],

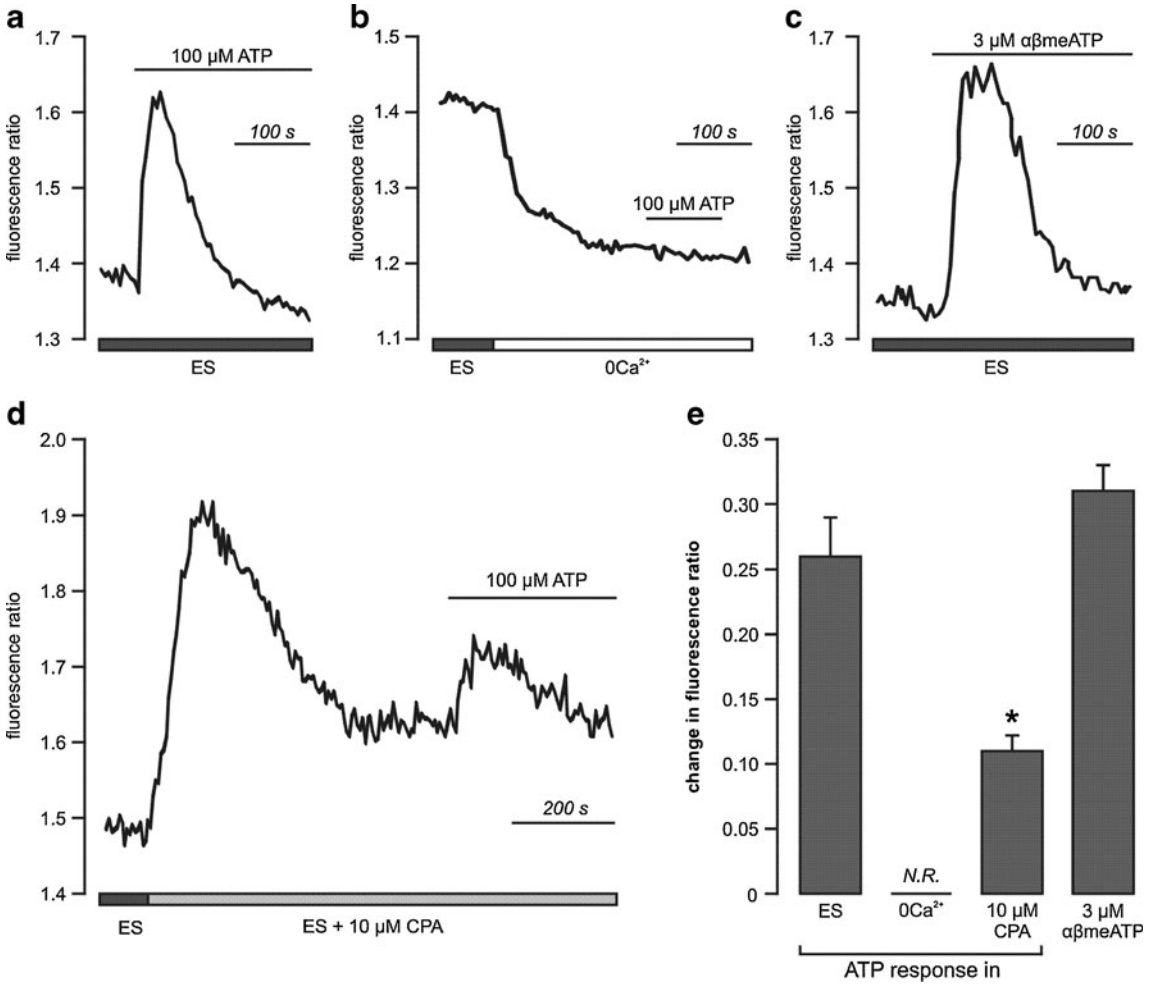

Fig. 4 ATP induces transitory increases in cytosolic Ca^{2+} in human umbilical artery smooth muscle cells loaded with fura-2 AM which depend on extracellular Ca^{2+} influx and sarcoplasmic reticulum Ca^{2+} release. Typical recording of cytosolic Ca^{2+} changes (measured as fluorescence ratio changes) induced by **a** 100 μM ATP in control conditions (ES); **b** 100 μM ATP in a Ca^{2+}-free extracellular solution with 1 mM EGTA ($0Ca^{2+}$); **c** 3 μM αβ-methylene ATP; and **d** 100 μM ATP after incubation with 10 μM cyclopiazonic acid (CPA) to induce sarcoplasmic reticulum depletion. **e** Mean±SEM values of the responses shown in **a** (n=31), **b** (n=41), **c** (n=21), and **d** (n=42). The symbol * indicates a statistically significant difference by multiple comparison versus control (100 μM ATP in ES) group (one-way analysis of variance, Holm–Sidak test)

so we used this ion to further characterize the current. We found that 100 μM Zn^{2+} reduced the current by about 80%, contrary to what could be expected if any of the subtypes 2 or 4 of P2X receptors participated in the ATP-induced current. Hence, we think that despite their mRNAs being expressed in this artery, these receptors have a minor role in ATP signaling.

Since umbilical vessels are not innervated, local regulation of the degree of contraction surely plays an important role. ATP produces the contraction of umbilical cord vessels [5]. This nucleotide can be released from endothelial cells [3] and also from red blood cells in conditions of hypoxia [19], so it is conceivable that ATP plays a role in the regulation of HUA tone in vivo, where it would be one of the many factors promoting contraction of HUA smooth muscle and, hence, vessel contraction.

In summary, we describe for the first time the ionic currents induced by extracellular ATP in human vascular smooth muscle cells, we show that these are carried by extracellular Ca^{2+} and Na^{+} and that pharmacological and kinetic evidence point to $P2X_1$-like type purinergic receptors. Additionally, we characterized cytosolic Ca^{2+} transients produced by ATP and showed that they are fully dependent on Ca^{2+} entry from the extracellular space. These data expand the knowledge of purinergic receptor properties in vascular smooth muscle cells and its probable role as a paracrine modulator of contractile tone in a human artery which is fundamental for feto-placental blood flow.

Acknowledgments The authors gratefully acknowledge excellent technical assistance by Mr. Luciano Piccinini and Mr. Matías Vilche. They also wish to thank Mr. Pablo Urdampilleta, Ms. Anabel Poch, and the staff of the Instituto Central de Medicina for collection of umbilical cords. This work was financially supported by the grant PIP 0202 from the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina, and by PICT 14415 from the Agencia Nacional de Promoción Científica y Tecnológica (ANPCYT), Argentina.

References

1. Kennedy C, McLaren GJ, Westfall TD, Sneddon P (1996) ATP as a co-transmitter with noradrenaline in sympathetic nerves – function and fate. In: Chadwick DJ, Goode J (eds) P2 purinoceptors: localization, function and transduction mechanisms. Wiley, New York, pp 223–235
2. Burnstock G (2009) Purinergic regulation of vascular tone and remodelling. Auton Autacoid Pharmacol 29(3):63–72
3. Yamamoto K, Korenaga R, Kamiya A, Qi Z, Sokabe M, Ando J (2000) P2X4 receptors mediate ATPinduced calcium influx in human vascular endothelial cells. Am J Physiol 27:H285–H292
4. Yamamoto K, Sokabe T, Matsumoto T, Yoshimura K, Shibata M, Ohura N, Fukuda T, Sato T, Sekine K, Kato S, Isshiki M, Fujita T, Kobayashi M, Kawamura K, Masuda H, Kamiya A, Ando J (2006) Impaired flow-dependent control of vascular tone and remodeling in P2X4-deficient mice. Nat Med 12(1):133–137
5. Valdecantos P, Briones R, Moya P, Germain A, Huidobro-Toro JP (2003) Pharmacological identification of P2X1, P2X4 and P2X7 nucleotide receptors in the smooth muscles of human umbilical cord and chorionic blood vessels. Placenta 24(1):17–26
6. Bo X, Sexton A, Xiang Z, Nori SL, Burnstock G (1998) Pharmacological and histochemical evidence for P2X receptors in human umbilical vessels. Eur J Pharmacol 353(1):59–65
7. Klockner U (1993) Intracellular calcium ions activate a low-conductance chloride channel in smooth-muscle cells isolated from human mesenteric artery. Pflügers Arch 424:231–237
8. Iveli MF, Rebolledo A, Martín P, Enrique N, Roldán Palomo AR, Rimorini L, Salemme S, Milesi V (2010) Risperidone inhibits contractions induced by serotonin and histamine and reduces K^+ currents in smooth muscle of human umbilical artery. Reprod Sci 17(9):854–860
9. Hamill OP, Marty A, Neher E, Sackmann B, Sigworth FJ (1981) Improved patch clamp techniques for high-resolution current readings from cells and cell-free membrane patches. Pflügers Arch 391:85–100
10. Wildman SS, King BF, Burnstock G (1999) Modulatory activity of extracellular H^+ and Zn^{2+} on ATP-responses at rP2X1 and rP2X3 receptors. Br J Pharmacol 128(2):486–492
11. Benham CD, Tsien RW (1987) A novel receptor-operated Ca^{2+}-permeable channel activated by ATP in smooth muscle. Nature 328(6127):275–278
12. Egan TM, Samways DS, Li Z (2006) Biophysics of P2X receptors. Pflugers Arch 452(5):501–512
13. North RA (2002) Molecular physiology of P2X receptors. Physiol Rev 82(4):1013–1067
14. Meng F, To W, Kirkman-Brown J, Kumar P, Gu Y (2007) Calcium oscillations induced by ATP in human umbilical cord smooth muscle cells. J Cell Physiol 213(1):79–87
15. Berra-Romani R, Mazzocco-Spezzia A, Pulina MV, Golovina VA (2008) Ca^{2+} handling is altered when arterial myocytes progress from a contractile to a proliferative phenotype in culture. Am J Physiol Cell Physiol 295(3):C779–C790
16. Sandow SL, Grayson TH (2009) Limits of isolation and culture: intact vascular endothelium and BKCa. Am J Physiol Heart Circ Physiol 297(1):H1–H7
17. Erlinge D (2004) Extracellular ATP: a central player in the regulation of vascular smooth muscle phenotype. Focus on "Dual role of PKA in phenotype modulation of vascular smooth muscle cells by extracellular ATP". Am J Physiol Cell Physiol 287:C260–C262
18. Gever JR, Cockayne DA, Dillon MP, Burnstock G, Ford AP (2006) Pharmacology of P2X channels. Pflugers Arch 452(5):513–537
19. Ellsworth ML, Forrester T, Ellis CG, Dietrich HH (1995) The erythrocyte as a regulator of vascular tone. Am J Physiol 269(6 Pt 2):H2155–H2161