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Short communication

Evidence for a novel P_{2X} purinoceptor in human placental chorionic surface arteries

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Abstract

To characterize the P_{2X} purinoceptor of arteries of human term placenta, a non-innervated organ, actions of ATP, α,β-methylene-ATP and UTP on de-endothelialized chorionic surface artery segments were compared. ATP and α,β-methylene-ATP caused reversible concentration-dependent contractions, but UTP elicited little or no contraction up to 517 μM. Concentration-effect curves to ATP and α,β-methylene-ATP were parallel, and α,β-methylene-ATP, EC_{50} 4.2 ± 1.2 μM, was 28-times as potent as ATP. At a saturating concentration, 103 μM, α,β-methylene-ATP did not desensitize the ATP receptor. Contractions to ATP and α,β-methylene-ATP were antagonized by 300 μM suramin. These findings indicate that P_{2X} purinoceptors are present in placental chorionic surface arteries and that they differ from P_{2X} purinoceptors in arteries of other tissues.

Keywords: P_{2X} purinoceptor; Chorionic surface artery; Placenta, human, term; α,β-Methylene-ATP; UTP receptor; Smooth muscle, vascular

1. Introduction

Extracellular ATP causes vasoconstriction of isolated blood vessels and vascular preparations due to its action at specific excitatory P_{2X} purinoceptors on vascular smooth muscle (for review, see Dalziel and Westfall, 1994). The original characterization of P_{2X} purinoceptors was based on the rank order of potency of ATP and two key structural analogues, α,β-methylene-ATP and 2-methylthio-ATP, in causing contraction of smooth muscle, i.e., α,β-methylene-ATP ≫ ATP = 2-methylthio-ATP (Burnstock and Kennedy, 1985). α,β-Methylene-ATP, particularly, has been crucial in the functional characterization of P_{2X} purinoceptors in blood vessels, because of its vasoconstrictor potency and its lack of vasodilator activity (due to lack of action at P_{2Y} purinoceptors), and because it antagonizes ATP-elicited vasoconstriction by desensitizing the ATP receptor (Dalziel and Westfall, 1994). Furthermore, radiolabelled α,β-methylene-ATP has been used for autoradiographic localization of P_{2X} purinoceptors in several blood vessels, demonstrating that P_{2X} purinoceptors are associated with the smooth muscle of the vessels in regions of innervation (Bo and Burnstock, 1992) in accordance with the role of neurally released ATP as a co-transmitter in many nerves (Burnstock, 1990).

We showed that ATP, 2-methylthio-ATP and α,β-methylene-ATP caused reversible dose-dependent vasoconstriction in the dual-perfused human fetoplacental vascular bed, with a rank order of potency of α,β-methylene-ATP ≫ 2-methylthio-ATP > ATP (Dobronyi and Maguire, 1994). These observations suggested that P_{2X} purinoceptors are present in the human placental vasculature, which lacks innervation (Reilly and Russell, 1977). To characterize placental P_{2X} purinoceptors, we compared the actions of ATP, α,β-methylene-ATP and UTP on endothelium-free preparations of human placental chorionic arteries. UTP, a pyrimidine nucleotide, also elicits strong concentration-dependent contractions of vascular smooth muscle, although these contractions do not appear to be mediated via P_{2X} purinoceptors (Garcia-Velasco et al., 1995).

2. Materials and methods

2.1. Chorionic surface artery ring preparation

Human term placentas from uncomplicated pregnancies were obtained within 10–30 min after caesarian section or vaginal delivery. Segments of chorionic surface arteries, approximately 1.0 mm o.d. × 8 mm, located near the pla-

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cental margin and just proximal to descent beneath the chorionic plate, were dissected and freed from adjacent tissue. Endothelium was removed by passing a fine needle and silk suture (Ethicon size 4-0, 1.5 metric) through the artery. Rings, 3–4 mm long, were cut and mounted on two vertical iridium wires (0.25 mm diameter) in 10 ml organ baths containing Krebs salt solution (KSS; composition, mM: NaCl 133, $CaCl_2$ 2.5, NaH_2PO_4 1.5, KCl 4.7, $NaHCO_3$ 16.3, dextrose 7.8) gassed with 95% O_2/5% CO_2 and maintained at 37°C. One wire was attached to a WPI Fort 10 transducer and the other to an MM33 precision micromanipulator. Changes in isometric force were recorded via a WPI Transbridge transducer amplifier, digitized via a CODAS data acquisition system, displayed on a computer and archived for analysis.

To confirm removal of endothelium and integrity of the smooth muscle, selected arteries were fixed in 4% formaldehyde and processed for paraffin embedding. Sections about 5 μm thick were stained with haematoxylin and eosin, and assessed under the light microscope.

2.2. Experimental protocol

Following application of 500 mg resting tension, preparations were allowed to equilibrate for 90 min; during this time they were rinsed every 15 min with KSS maintained at 37°C, and tension was adjusted to 350–450 mg, which was found to be optimal. Contractions to cumulative additions of KCl, final concentration 180 mM, were checked. Preparations which did not show concentration-dependent contraction to KCl were discarded. Histamine was used as a reference agonist. Responses to cumulative additions of histamine were determined at the beginning and end of each experiment. Cumulative addition of nucleotides was performed to generate concentration-response curves. After washout, 20–25 min were allowed to elapse before the next nucleotide or drug addition. When antagonism of ATP by α,β-methylene-ATP was investigated, concentration-response curves to ATP and α,β-methylene-ATP were obtained and α,β-methylene-ATP (final concentration 103 μM) was allowed to remain in the bath for 25 min prior to addition of ATP.

2.3. Analysis

Concentration-response relationships for histamine, ATP and/or α,β-methylene-ATP were obtained in each preparation. Contraction was expressed as a percentage of the contraction to 775 μM histamine, in the same preparation. Log concentration-effect curves were drawn by Slide-Write Plus 3.0 (Advanced Graphics Software, Carlsbad, CA, USA) without curve fitting. Tests of parallelism of log concentration-effect curves to ATP and α,β-methylene-ATP and relative potency of ATP and α,β-methylene-ATP were performed using PHARM/PCS Software Version 4.2 (Microcomputer Specialists, Philadelphia, PA, USA). Results are given as means ± S.E.M. Differences at $P < 0.05$ were considered significant.

2.4. Drugs

Drugs were obtained from the following sources: ATP, disodium salt, from Research Biochemicals International (Natick, MA, USA); UTP, sodium salt; α,β-methylene-ATP, lithium salt; U-46619 (9,11-dideoxy-11α,9α-epoxymethanoprostaglandin $F_{2\alpha}$) and histamine dihydrochloride from Sigma (St. Louis, MO, USA); angiotensin II from Bachem (Torrance, CA, USA); dextrose and inorganic salts (best analytical grades) from Fisher Scientific (St. Louis, MO, USA). Suramin was generously provided by the National Cancer Institute.

Solutions of nucleotides and drugs were prepared fresh

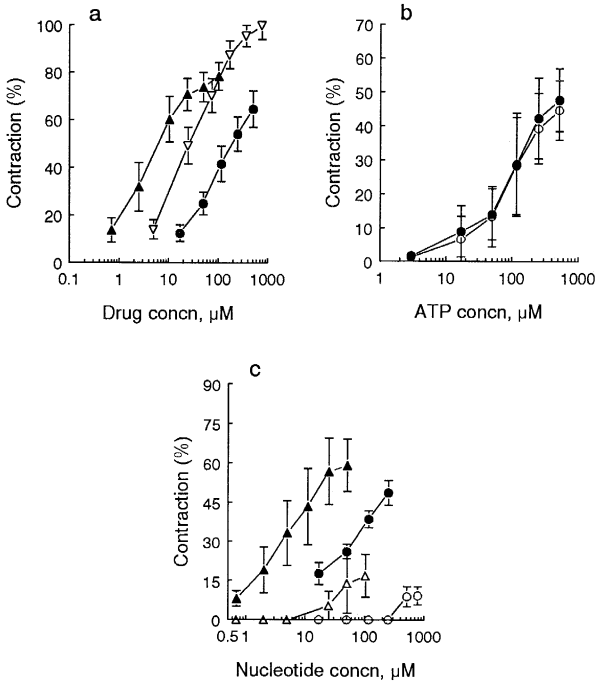

Fig. 1. (a) Log concentration–effect curves for contractions elicited by ATP (●), α,β-methylene-ATP (▲), and histamine (▽), in chorionic artery ring preparations. Contraction is expressed as a percentage of the contraction induced by 775 μM histamine in each preparation. Data points are means ± S.E.M. ($n = 5–8$). Curves are composites of individual curves, as follows: ATP, eight curves in five preparations; α,β-methylene-ATP, six curves in six preparations; histamine, five curves in five preparations. (b) ATP-induced contraction of chorionic surface artery ring preparations in the absence (●) and in the presence (○) of 103 μM α,β-methylene-ATP after 25 min exposure to 103 μM α,β-methylene-ATP. Contraction is expressed as a percentage of the contraction induced by 775 μM histamine in each preparation. Data points are means ± S.E.M., from concentration-effect curves obtained in three preparations. (c) Effect of suramin on ATP- and α,β-methylene-ATP-induced contraction of chorionic surface artery ring preparations. Concentration-effect curves were obtained to ATP and α,β-methylene-ATP in the absence (ATP ●, α,β-methylene-ATP ▲) and in the presence (ATP ○, α,β-methylene-ATP △) of 300 μM suramin. Preparations were exposed to suramin for 30 min before addition of nucleotides. Contraction is expressed as a percentage of the contraction to histamine in the same preparation. Concentration-effect curves in the absence of suramin are composites of five curves in four preparations and in the presence of suramin curves are composites of four curves in the four preparations. Data points are means ± S.E.M.

in distilled water, except U-46619, which was diluted with distilled water from a stock in ethanol.

3. Results

Light microscopy showed the preparations to be free of endothelium, to have intact muscle structure and the unilateral muscular cushion characteristic of placental chorionic surface arteries (Fujikura and Carleton, 1968) (results not shown). Concentration-dependent contractions were elicited by nM concentrations of U46619, and μM concentrations of angiotensin II and histamine. Responses to histamine were more consistent than those to U-46619 and histamine was more potent than angiotensin II. Histamine was used as the reference agonist. Fig. 1a shows the histamine log concentration–effect curve.

ATP elicited spontaneously reversible contractions, which were concentration-dependent in the majority of preparations studied (Fig. 1a; Fig. 2); in some of these preparations, repeated cumulative additions of ATP resulted in a gradual run-down of responses, suggesting that desensitization was occurring. Approximately 30% of all preparations responded weakly to ATP, although responses to histamine were in the normal range; possibly these vessels were exposed to ATP during delivery, resulting in desensitization. Nevertheless, these preparations responded normally to α,β-methylene-ATP.

α,β-Methylene-ATP elicited reversible, concentration-dependent contractions (Fig. 1a; Fig. 2). In the continued presence of a saturating concentration of α,β-methylene-ATP, contractions slowly recovered to an elevated level of basal tone as shown in Fig. 2. Cumulative concentration–effect curves to α,β-methylene-ATP could be repeated without run-down in responses, indicating resistance to desensitization to α,β-methylene-ATP. In the concentration ranges studied, α,β-methylene-ATP responses reached saturation, but responses to ATP did not, as indicated by the log concentration-effect curves for the two nucleotides (Fig. 1a). As a result, an EC_{50} for ATP could not be determined. The EC_{50} for α,β-methylene-ATP was 4.2 ± 1.2 μM. The log concentration-effect curves for ATP and α,β-methylene-ATP were parallel (difference from parallelism not significant at the 95% confidence level), and the potency ratio of α,β-methylene-ATP relative to ATP was 28 (range 20–40).

Observations in five different preparations, each from a different placenta, showed that 25 min exposure of preparations to 103 μM α,β-methylene-ATP did not antagonize responses to ATP, as indicated by the traces shown in Fig. 2, and by the superimposable log concentration–effect curves to ATP obtained without and with α,β-methylene-ATP pre-treatment (Fig. 1b).

Suramin (300 μM) reduced contractions to ATP and α,β-methylene-ATP. Responses to lower concentrations of both nucleotides were abolished by exposure of the preparations to 300 μM suramin, but the inhibition was partially overcome at higher concentrations of the nucleotides as indicated by log concentration–effect curves (Fig. 1c). Some recovery of responses to α,β-methylene-ATP was observed 30 min after washout of suramin, but recovery of ATP responses was observed in only one preparation. Suramin treatment did not affect baseline tension of the preparations or responses to histamine.

UTP, 517 μM, failed to elicit contractions in six chorionic artery rings from three placentas, although contractions to ATP and/or α,β-methylene-ATP were in the normal range. In a preparation from a different placenta, 517 μM UTP caused a weak contraction, equal to 20% of the response to 517 μM ATP.

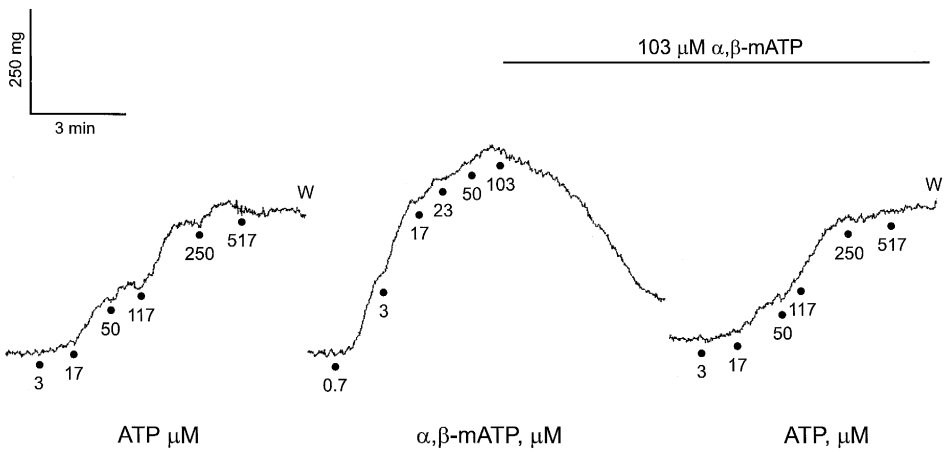

Fig. 2. Typical responses of chorionic surface artery rings to sequential cumulative additions of ATP, α,β-methylene-ATP (α,β-mATP) and ATP in the presence of 103 μM α,β-methylene-ATP after 25 min exposure to 103 μM α,β-methylene-ATP. Dots indicate drug or nucleotide additions to give indicated bath concentrations. Observations in a single preparation. Bar indicates the presence of 103 μM α,β-methylene-ATP. 'W' signifies wash-out. For further details, see text.

4. Discussion

ATP and α,β-methylene-ATP elicited spontaneously reversible contractions of chorionic surface artery preparations, and responses to both nucleotides were concentration-dependent. About 30% of preparations studied showed signs of desensitization to ATP, evidenced by weak or diminishing responses to increasing concentrations of ATP, but there were no similar signs of desensitization to α,β-methylene-ATP in these preparations, or in any of the preparations studied, although contraction to α,β-methylene-ATP faded in the continued presence of the analogue. These observations contrast with findings in other vessels, e.g., rat aorta, where contractions to both ATP and α,β-methylene-ATP rapidly desensitize (Garcia-Velasco et al., 1995).

Log concentration–effect curves to ATP and α,β-methylene-ATP were parallel, supporting the concept that the nucleotides act at the same receptor. Moreover, suramin, a non-selective P_2 purinoceptor antagonist (Hoyle et al., 1990), antagonized both nucleotides, and in each case antagonism was overcome at higher nucleotide concentrations. The α,β-methylene-ATP EC_{50} of 4.2 μM and the 28-fold greater potency of α,β-methylene-ATP compared to ATP fit the functional criteria for a P_{2X} purinoceptor (Burnstock and Kennedy, 1985). Another criterion for functional characterization of vascular smooth muscle P_{2X} purinoceptors has been the inhibition of ATP responses that results from desensitizing the receptor with α,β-methylene-ATP (Dalziel and Westfall, 1994). Exposure of chorionic artery rings to a saturating concentration of α,β-methylene-ATP did not inhibit responses to ATP. This finding suggests that ATP and α,β-methylene-ATP may act at different receptors. Similar observations were reported for P_{2X} purinoceptors in rabbit ear arteries, in which a significant desensitization-resistant component of the ATP response remained after exposure to 30 μM α,β-methylene-ATP; it was thought that this could be due to an effect of ATP at UTP-recognizing receptors (O'Connor et al., 1990). This explanation is not pertinent to the desensitization resistance of chorionic arteries, because they do not contract significantly to UTP.

P_{2X} purinoceptors have been cloned from smooth muscle and nervous tissue, and shown to be structurally related ATP-gated ion channels (Surprenant et al., 1995). Heterologously expressed P_{2X} receptor sub-types vary in their responses to α,β-methylene-ATP (Collo et al., 1996). α,β-Methylene-ATP activated $P2X_4$, albeit weakly, but at 100 μM only partly desensitized the ATP response (Bo et al., 1995), thus resembling its actions in the chorionic arteries. Heteropolymerisation of sub-types $P2X_2$ and $P2X_3$ yielded a channel with new phenotypic responses to α,β-methylene-ATP and ATP (Lewis et al., 1995), indicating a means of generating pharmacological diversity in native P_{2X} purinoceptors, although whether native P_{2X} purinoceptors are homomultimers or heteromultimers is not presently known. There may be additional P_{2X} purinoceptor sub-types not yet cloned. This emerging new information suggests that the unusual nature of responses to ATP and α,β-methylene-ATP in non-innervated chorionic arteries may ultimately be explained in terms of the component P_{2X} purinoceptor sub-types present. Failure of UTP to elicit significant contraction of chorionic surface artery preparations implies that receptors at which UTP acts are not present in chorionic surface artery smooth muscle, in contrast to findings in other, innervated vascular preparations.

Acknowledgements

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