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1	Article Title	<b>Investigation of the functional expression of purine and pyrimidine receptors in porcine-isolated pancreatic arteries</b>	
2	Article Sub- Title		
3	Article Copyright - Year	<b>Springer Science+Business Media Dordrecht 2013 (This will be the copyright line in the final PDF)</b>	
4	Journal Name	Purinergic Signalling	
5		Family Name	<b>Ralevic</b>
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29	Schedule	Received	2 July 2013
30		Revised	

31	Accepted	5 November 2013
32	Abstract	<p>Receptors for purines and pyrimidines are expressed throughout the cardiovascular system. This study investigated their functional expression in porcine-isolated pancreatic arteries. Pancreatic arteries (endothelium intact or denuded) were prepared for isometric tension recording and precontracted with U46619, a thromboxane A<sub>2</sub> mimetic; adenosine-5'-diphosphate (ADP), uridine-5'-triphosphate (UTP) and MRS2768, a selective P<sub>2</sub>Y<sub>2</sub> agonist, were applied cumulatively, while adenosine-5'-triphosphate (ATP) and <math>\alpha\beta</math>-methylene-ATP (<math>\alpha\beta</math>-meATP) response curves were generated from single concentrations per tissue segment. Antagonists/enzyme inhibitors were applied prior to U46619 addition. ATP, <math>\alpha\beta</math>-meATP, UTP and MRS2768 induced vasoconstriction, with a potency order of <math>\alpha\beta</math>-meATP &gt; MRS2768 &gt; ATP <math>\geq</math> UTP. Contractions to ATP and <math>\alpha\beta</math>-meATP were blocked by NF449, a selective P<sub>2</sub>X<sub>1</sub> receptor antagonist. The contraction induced by ATP, but not UTP, was followed by vasorelaxation. Endothelium removal and DUP 697, a cyclooxygenase-2 inhibitor, had no significant effect on contraction to ATP but attenuated that to UTP, indicating actions at distinct receptors. MRS2578, a selective P<sub>2</sub>Y<sub>6</sub> receptor antagonist, had no effect on contractions to UTP. ADP induced endothelium-dependent vasorelaxation which was inhibited by MRS2179, a selective P<sub>2</sub>Y<sub>1</sub> receptor antagonist, or SCH58261, a selective adenosine A<sub>2A</sub> receptor antagonist. The contractions to ATP and <math>\alpha\beta</math>-meATP were attributed to actions at P<sub>2</sub>X<sub>1</sub> receptors on the vascular smooth muscle, whereas it was shown for the first time that UTP induced an endothelium-dependent vasoconstriction which may involve P<sub>2</sub>Y<sub>2</sub> and/or P<sub>2</sub>Y<sub>4</sub> receptors. The relaxation induced by ADP is mediated by P<sub>2</sub>Y<sub>1</sub> and A<sub>2A</sub> adenosine receptors. Porcine pancreatic arteries appear to lack vasorelaxant P<sub>2</sub>Y<sub>2</sub> and P<sub>2</sub>Y<sub>4</sub> receptors.</p>
33	Keywords separated by ' - '	<p><math>\alpha\beta</math>-meATP - ATP - UTP - ADP - MRS2578 - P<sub>2</sub>Y<sub>1</sub> - P<sub>2</sub>Y<sub>2</sub> - P<sub>2</sub>X<sub>1</sub> - A<sub>2A</sub> adenosine receptors - Vasoconstriction - Relaxation - Endothelium</p>
34	Foot note information	

# Investigation of the functional expression of purine and pyrimidine receptors in porcine-isolated pancreatic arteries

M. Alsaqati · S. L. F. Chan · V. Ralevic

Received: 2 July 2013 / Accepted: 5 November 2013  
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**Abstract** Receptors for purines and pyrimidines are expressed throughout the cardiovascular system. This study investigated their functional expression in porcine-isolated pancreatic arteries. Pancreatic arteries (endothelium intact or denuded) were prepared for isometric tension recording and precontracted with U46619, a thromboxane A<sub>2</sub> mimetic; adenosine-5'-diphosphate (ADP), uridine-5'-triphosphate (UTP) and MRS2768, a selective P2Y<sub>2</sub> agonist, were applied cumulatively, while adenosine-5'-triphosphate (ATP) and αβ-methylene-ATP (αβ-meATP) response curves were generated from single concentrations per tissue segment. Antagonists/enzyme inhibitors were applied prior to U46619 addition. ATP, αβ-meATP, UTP and MRS2768 induced vasoconstriction, with a potency order of αβ-meATP > MRS2768 > ATP ≥ UTP. Contractions to ATP and αβ-meATP were blocked by NF449, a selective P2X1 receptor antagonist. The contraction induced by ATP, but not UTP, was followed by vasorelaxation. Endothelium removal and DUP 697, a cyclooxygenase-2 inhibitor, had no significant effect on contraction to ATP but attenuated that to UTP, indicating actions at distinct receptors. MRS2578, a selective P2Y<sub>6</sub> receptor antagonist, had no effect on contractions to UTP. ADP induced endothelium-dependent vasorelaxation which was inhibited by MRS2179, a selective P2Y<sub>1</sub> receptor antagonist, or SCH58261, a selective adenosine A<sub>2A</sub> receptor antagonist. The contractions to ATP and αβ-meATP were attributed to actions at P2X1 receptors on the vascular smooth muscle, whereas it was shown for the first time that UTP induced an endothelium-dependent vasoconstriction which may involve P2Y<sub>2</sub> and/or P2Y<sub>4</sub> receptors. The relaxation induced by ADP is mediated by P2Y<sub>1</sub> and A<sub>2A</sub>

adenosine receptors. Porcine pancreatic arteries appear to lack vasorelaxant P2Y<sub>2</sub> and P2Y<sub>4</sub> receptors.

**Keywords** αβ-meATP · ATP · UTP · ADP · MRS2578 · P2Y<sub>1</sub> · P2Y<sub>2</sub> · P2X1 · A<sub>2A</sub> adenosine receptors · Vasoconstriction · Relaxation · Endothelium

**Abbreviations**

αβ-meATP	αβ-Methylene-adenosine-5'-triphosphate	50
ADP	Adenosine-5'-diphosphate	53
ATP	Adenosine-5'-triphosphate	53
EDCFs	Endothelium-derived contractile factors	56
ENTPDase	Ecto-nucleotidase 5'-triphosphate diphosphohydrolase	58
PPADS	Pyridoxal phosphate-6-azo(benzene-2,4-disulfonic acid)	62
UTP	Uridine-5'-triphosphate	63
VSMCs	Vascular smooth muscle cells	66
XAC	Xanthine amine congener	68

**Introduction**

The activities of both exocrine and endocrine cells of the pancreas are regulated by autonomic nerves (parasympathetic and sympathetic) as well as by hormones and autocrine and paracrine mediators. Although the exact mechanisms remain to be established, it is generally agreed that an increase in endocrine cell activity during hormone secretion corresponds with an increase in blood flow, to meet the metabolic demand. The role of exogenous purine and pyrimidine nucleotides in controlling the functions of endocrine and exocrine components of the pancreas are well described [1, 2], but little is known about their effects on pancreatic arterial vasocontractility.

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84 There are two main families of P2 purine and pyrimidine  
 85 receptors, ionotropic P2X and G protein-coupled P2Y recep-  
 86 tors. Molecular cloning has identified seven mammalian P2X  
 87 receptor subunits: P2X1, P2X2, P2X3, P2X4, P2X5, P2X6  
 88 and P2X7 [3], while eight mammalian P2Y receptors have  
 89 been identified: P2Y<sub>1</sub>, P2Y<sub>2</sub>, P2Y<sub>4</sub>, P2Y<sub>6</sub>, P2Y<sub>11</sub>, P2Y<sub>12</sub>,  
 90 P2Y<sub>13</sub> and P2Y<sub>14</sub> [4]. P2X receptors are activated by  
 91 adenosine-5'-triphosphate (ATP) and its stable, and conse-  
 92 quently more potent, analogue  $\alpha\beta$ -methylene-ATP ( $\alpha\beta$ -  
 93 meATP) [5, 6]. P2Y receptors can be divided on the basis of  
 94 their endogenous agonists into adenine nucleotide-preferring  
 95 (P2Y<sub>1</sub>, P2Y<sub>11</sub>, P2Y<sub>12</sub> and P2Y<sub>13</sub>) receptors and uracil nucle-  
 96 otide or UDP-sugar-preferring (P2Y<sub>2</sub>, P2Y<sub>4</sub>, P2Y<sub>6</sub> and  
 97 P2Y<sub>14</sub>) receptors [7]. Among the adenine nucleotide group,  
 98 the human P2Y<sub>11</sub> receptor is selectively activated by ATP and  
 99 fails to respond to adenosine-5'-diphosphate (ADP) [8], al-  
 100 though the dog orthologue responds to both ADP and ATP  
 101 [9]. P2Y<sub>1</sub>, P2Y<sub>12</sub> and P2Y<sub>13</sub> receptors are activated by ADP,  
 102 with lower potency by ATP [10–13]. Among the uracil nucle-  
 103 otide or UDP-sugar receptors, P2Y<sub>2</sub> is equally activated by  
 104 ATP and uridine-5'-triphosphate (UTP), while P2Y<sub>4</sub> receptor  
 105 is highly selective for UTP over ATP [14]. The P2Y<sub>6</sub> receptor  
 106 is activated by UDP and UTP, while the P2Y<sub>14</sub> receptor is  
 107 activated by UDP and UDP-sugars [6, 15].  
 108 Within the pancreatic vasculature, P2X1, P2X2, P2Y<sub>1</sub> and  
 109 P2Y<sub>2</sub> receptors were detected by immunohistochemistry [16].  
 110 More than two decades ago, it was shown that P2X receptors  
 111 mediate pancreatic vasoconstriction, and P2Y receptors medi-  
 112 ate vasodilatation in response to ATP [17], and subsequent  
 113 studies showed an additional involvement of contractile recep-  
 114 tors sensitive to UTP (named P2U receptors) [18]. Purine  
 115 receptor subclassification has advanced significantly since  
 116 that time. A re-evaluation of purine receptors in the pancreatic  
 117 vasculature is clearly warranted. In the current study, we  
 118 describe the pharmacological characterisation of P2Y<sub>1</sub> and  
 119 A<sub>2A</sub> receptor-mediated relaxatory responses, in addition to  
 120 P2X1, P2Y<sub>2</sub> and P2Y<sub>4</sub> receptor-mediated contractile re-  
 121 sponses of porcine-isolated pancreatic artery preparations.  
 122 P2Y<sub>2</sub> and/or P2Y<sub>4</sub> receptors appear to be expressed mainly  
 123 in endothelial cells, while P2X1 and A<sub>2A</sub> receptors appear to  
 124 be expressed in smooth muscle cells of the pancreatic arteries.  
 125 A preliminary account of some of these data has previously  
 126 been presented to the British Pharmacological Society [19].

127 **Materials and methods**

128 Tissue preparation

129 Pancreases from pigs (either sex, age less than 6 months, wt  
 130 ~50 kg) were obtained on ice from a local abattoir (G Wood &  
 131 Sons Ltd., Mansfield). A crude dissection was conducted to  
 132 isolate the porcine pancreatic arteries (greater pancreatic

artery) which were located in the body of the pancreas. The  
 vessels were dissected out and placed in Krebs–Henseleit  
 buffer containing 2 % (w/v) Ficoll (hydrophilic polysaccha-  
 ride, type 70) and were refrigerated overnight at 4 °C. The  
 next day, a fine dissection was performed on arteries, and the  
 artery segments were cut into rings of about 0.5 cm in length  
 and suspended in Krebs–Henseleit buffer (gassed, 95 % O<sub>2</sub>,  
 5 % CO<sub>2</sub>).

The endothelium of some arteries was removed by gently  
 rubbing the innermost surface of the artery with forceps on a  
 paper tissue before attaching it to the set-up [20]. Successful  
 removal of the endothelium was tested using substance P  
 (10 nM). Endothelium-denuded arteries relaxed in response  
 to substance P to less than 10 % of the U46619-induced  
 contraction, while in endothelium-intact arteries, the relaxa-  
 tion to substance P was 36 %±8 (n=7, data not shown).

Responses in the porcine-isolated pancreatic artery 149

Arterial rings were mounted onto wires in tissue baths con-  
 taining warm (37 °C), oxygenated Krebs–Henseleit solution  
 and were connected via isometric force transducers  
 (mechanotransducer MLT 050/D; ADInstruments, Sydney,  
 Australia) to a PC running the computer programme,  
 LabChart (ADInstruments, Sydney, Australia). Rings were  
 put under tension (15 g) and allowed to equilibrate for  
 60 min before assessing the viability with two challenges of  
 75 mM potassium chloride (KCl). The tissues were then  
 allowed to equilibrate for 60 min, after which U46619 (10–  
 100 nM), a thromboxane A<sub>2</sub> mimetic, was used to contract the  
 tissues to between 40 and 80 % of the second KCl response.  
 This ensured that if there was a vasodilator component to the  
 response, this could be detected. Once an appropriate level of  
 U46619 response had been achieved, ATP,  $\alpha\beta$ -meATP, UTP,  
 ADP or MRS2768 were added. Antagonists or enzyme inhib-  
 itors were applied 10 min prior to the addition of U46619,  
 allowing them to be incubated with the tissues for a minimal  
 contact time of 30 min prior to the application of agonists.  
 Some arteries were incubated with 0.1 % (v/v) DMSO (vehi-  
 cle control).

Reagents and drugs 171

Krebs–Henseleit buffer was composed of the following (mM):  
 NaCl, 118; KCl, 4.8; CaCl<sub>2</sub>·H<sub>2</sub>O, 1.3; NaHCO<sub>3</sub>, 25.0;  
 KH<sub>2</sub>PO<sub>4</sub>, 1.2; MgSO<sub>4</sub>·7H<sub>2</sub>O, 1.2; and glucose, 11.1. Suramin,  
 UTP, ATP,  $\alpha\beta$ -meATP, ADP, U46619, xanthine amine con-  
 gener (XAC) and SCH58261 (7-(2-phenylethyl)-5-amino-  
 2-(2-furyl)-pyrazolo-[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidine)  
 were purchased from Sigma (Poole, Dorset, UK), while DUP  
 6 9 7 ( 5 - b r o m o - 2 - ( 4 - f l u o r o p h e n y l ) -  
 3-[4-(methylsulfonyl)phenyl]-thiophene), pyridoxal  
 phosphate-6-azo(benzene-2,4-disulfonic acid) (PPADS),

182 MRS2578 (*N,N''*-1,4-butanediyl bis(*N'*-[3-  
183 isothiocyanatophenyl] thiourea)), MRS2179 (2'-deoxy-*N*<sup>6</sup>-  
184 methyladenosine 3',5'-bisphosphate tetrasodium salt),  
185 MRS2768 (uridine-5'-tetrphosphate  $\delta$ -phenyl ester  
186 tetrasodium salt) and substance P were purchased from Tocris  
187 Biosciences Ltd. (Bristol, UK). NF449 (4,4',4'',4'  
188 ''-[carbonylbis(imino-5,1,3-benzenetriyl-bis(carbonylimino))]  
189 tetrakis-1,3-benzenedisulfonic acid) was purchased from  
190 Calbiochem-Merck4Biosciences. U46619 was dissolved in  
191 ethanol at 10 mM stock concentration. PPADS, suramin,  
192  $\alpha\beta$ -meATP, ATP, ADP, UTP, NF449, MRS2179, MRS2768  
193 and substance P were dissolved in distilled water. DUP 697,  
194 XAC, MRS2578 and SCH58261 were dissolved in DMSO at  
195 10 mM stock concentration.

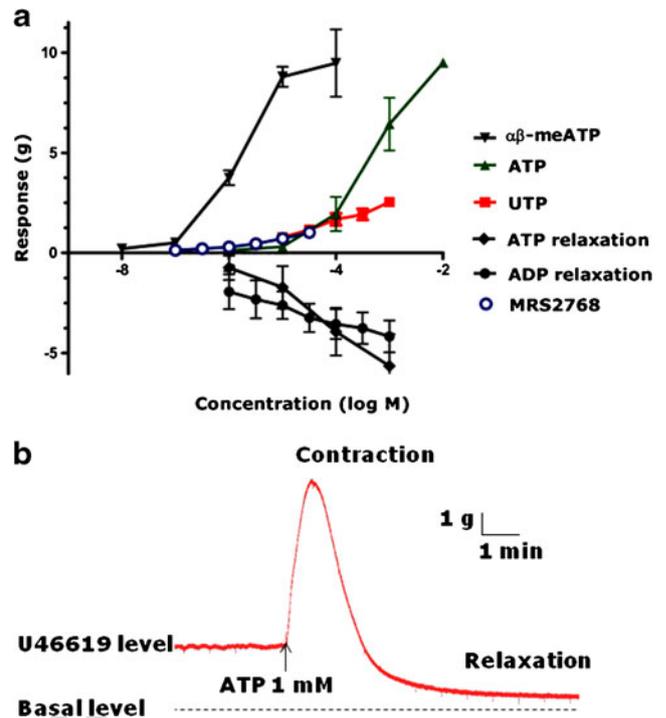
196 **Data analysis**

197 The contractions to ATP,  $\alpha\beta$ -meATP and UTP were measured  
198 from the stabilised U46619-induced response and were  
199 expressed in grams, while the relaxations to ATP and ADP  
200 were expressed as a percentage of the U46619-induced con-  
201 traction. Data were expressed as log concentration–response  
202 plots. Values for all figures refer to mean  $\pm$  standard error of  
203 the mean (SEM) with 95 % confidence. Results were com-  
204 pared by one- or two-way ANOVA with Bonferroni's post hoc  
205 test or unpaired Student's *t* test (Prism, GraphPad, San Diego,  
206 CA, USA). Differences were considered to be significant  
207 when the *P* value was <0.05. *N* expresses the number of  
208 animals.

209 **Results**

210 **Effect of purine and pyrimidine nucleotides on vascular tone**  
211 **in porcine-isolated pancreatic arteries**

212 To investigate the effect of purine and pyrimidine nucleotide  
213 agonists on porcine pancreatic arteries,  $\alpha\beta$ -meATP (10 nM to  
214 100  $\mu$ M), ATP (1  $\mu$ M to 10 mM), UTP (10  $\mu$ M to 1 mM),  
215 ADP (1  $\mu$ M to 1 mM) and MRS2768 (100 nM to 30  $\mu$ M)  
216 were applied after precontraction with U46619. The res-  
217 sponses to ATP and  $\alpha\beta$ -meATP were found to be desensitised  
218 rapidly. Therefore, they were applied at single concentrations  
219 (one concentration per tissue segment). The responses to UTP,  
220 ADP and MRS2768 did not desensitise rapidly; thus, cumu-  
221 lative concentration–response curves were generated. ATP,  
222  $\alpha\beta$ -meATP, UTP and MRS2768 induced concentration-  
223 dependent contraction with a potency order of  $\alpha\beta$ -meATP >  
224 MRS2768 > ATP  $\geq$  UTP (*P*<0.001, two-way ANOVA;  
225 Fig. 1a). The response to ATP was biphasic, since its contrac-  
226 tion was followed by a relaxation (Fig. 1b) which was  
227 equipotent to the concentration-dependent relaxation pro-  
228 duced by ADP (Fig. 1a). The efficacies of ATP and  $\alpha\beta$ -



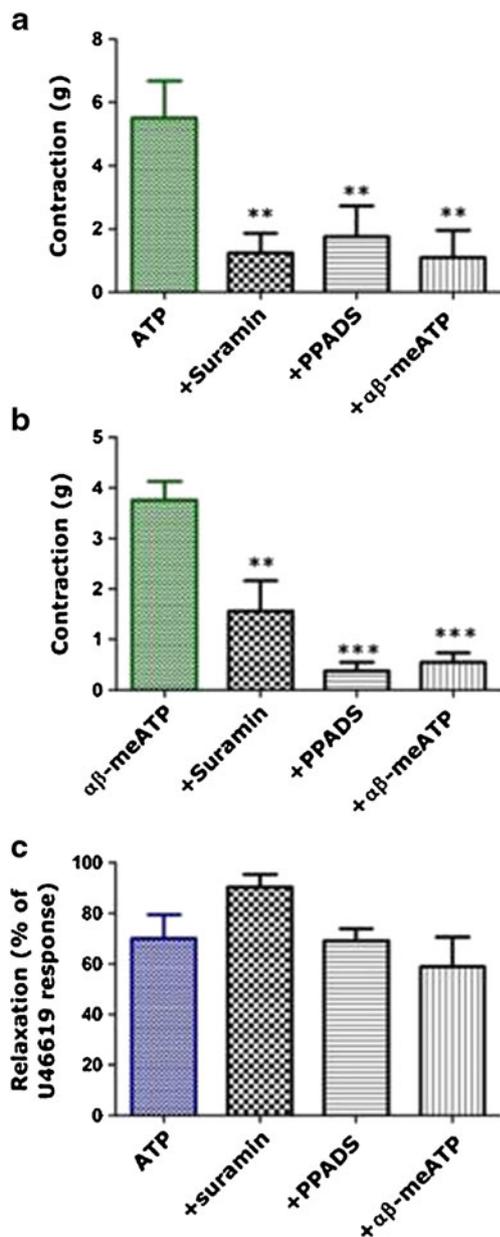
**Fig. 1** a Concentration-dependent contraction of ATP,  $\alpha\beta$ -meATP, UTP and MRS2768, a selective P2Y<sub>2</sub> agonist, and relaxation of ADP and ATP in U46619-precontracted porcine pancreatic arteries (*n*=7–12). b Typical trace showing the biphasic response to ATP (contraction followed by relaxation). Data are presented as mean  $\pm$  SEM

meATP in inducing contraction were similar and greater than those of UTP or MRS2768. The relaxation to ADP and ATP at the highest concentration of the agonists used (1 mM) was similar at 4.5 $\pm$ 0.5 g (*n*=10) and 5.5 $\pm$ 0.2 g (*n*=7), respectively; there was no significant difference between these responses (Fig. 1a). UTP, MRS2768 and  $\alpha\beta$ -meATP did not elicit vasorelaxation.

*Characterisation of responses to ATP and  $\alpha\beta$ -meATP in U46619-precontracted porcine-isolated pancreatic arteries*

- Effect of suramin, PPADS and  $\alpha\beta$ -meATP  
Responses to ATP and  $\alpha\beta$ -meATP were characterised using the non-selective P2 receptor antagonists, suramin (100  $\mu$ M) and PPADS (10  $\mu$ M). Both suramin and PPADS significantly attenuated the contractions evoked by ATP (1 mM) and  $\alpha\beta$ -meATP (1  $\mu$ M) (Fig. 2a, b). These concentrations of ATP and  $\alpha\beta$ -meATP were chosen since they produced robust and submaximal responses, and for  $\alpha\beta$ -meATP, the concentration was close to the half maximal effective concentration (EC<sub>50</sub>) value [mean EC<sub>50</sub> value was 1.6  $\mu$ M (95 % confidence interval (CI), 1.05 to 2.53  $\mu$ M; *n*=8; Fig. 1a)]. The relaxation to ATP was not affected by suramin or PPADS (Fig. 2c). Since  $\alpha\beta$ -meATP induces desensitisation of P2X receptors more

253 readily than ATP because it is broken down more slowly  
 254 than ATP [5], the responses to ATP and  $\alpha\beta$ -meATP were  
 255 studied in the presence of  $\alpha\beta$ -meATP, in which  $\alpha\beta$ -  
 256 meATP (1  $\mu$ M) was added 10 min prior the addition of  
 257 U46619. As seen in Fig. 2a, b, the contractions to ATP and  
 258  $\alpha\beta$ -meATP were reduced in the presence of the

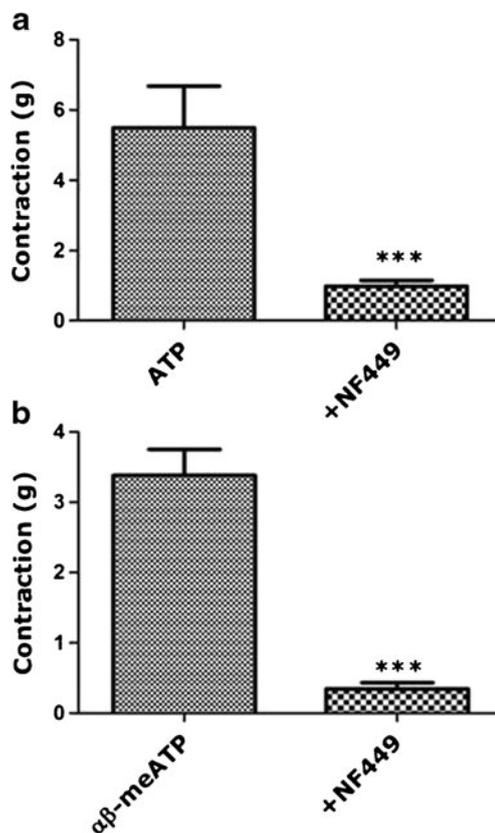


**Fig. 2** Effect of suramin (100  $\mu$ M), PPADS (10  $\mu$ M) and desensitisation by  $\alpha\beta$ -meATP (1  $\mu$ M) on contractions to **a** ATP (1 mM) and **b**  $\alpha\beta$ -meATP (1  $\mu$ M) and **c** on the relaxation to ATP in U46619-precontracted porcine pancreatic arteries. PPADS, suramin and  $\alpha\beta$ -meATP reduced the contractions of **a** ATP and **b**  $\alpha\beta$ -meATP (\*\* $P$ <0.01; \*\*\* $P$ <0.001, one-way ANOVA with Bonferroni's post hoc test, responses of ATP or  $\alpha\beta$ -meATP vs their responses in the presence of PPADS, suramin or  $\alpha\beta$ -meATP,  $n$ =6–9). **c** The relaxation to ATP was not significantly different in the absence or presence of PPADS, suramin or  $\alpha\beta$ -meATP ( $n$ =7). Data are presented as mean  $\pm$  SEM

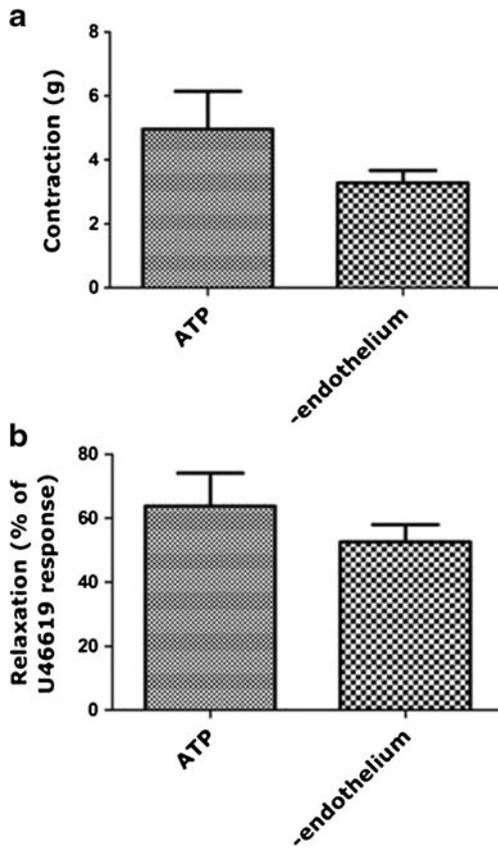
desensitising agent, while the relaxation to ATP was not  
 affected (Fig. 2c).

- Effect of NF449, a selective P2X1 receptor antagonist  
 Contractile responses to  $\alpha\beta$ -meATP suggest an expres-  
 sion of P2X1 receptors in porcine pancreatic arteries  
 (Fig. 2b). In turn, the involvement of P2X1 receptors in  
 contraction to ATP seems likely because contraction was  
 significantly blocked by  $\alpha\beta$ -meATP (Fig. 2a). The res-  
 ponses to ATP and  $\alpha\beta$ -meATP were studied further in  
 the presence of NF449 (10  $\mu$ M), a P2X1 receptor-  
 selective antagonist. The contractions to ATP and  $\alpha\beta$ -  
 meATP were inhibited in the presence of NF449 (Fig. 3).
- Effect of endothelium removal

The response to ATP was tested after the endothelium  
 had been removed. The contraction and the relaxation  
 induced by ATP (Fig. 4) were statistically not significantly  
 different in the absence or presence of the endothelium.  
 Similarly, removal of the endothelium had no effects on  
 the contractions to KCl, U46619 or  $\alpha\beta$ -meATP; for ex-  
 ample, the contraction to 75 mM KCl was  $9.5 \pm 0.5$  g in  
 endothelium-intact arteries, while it was  $9 \pm 0.5$  g in  
 endothelium-denuded arteries ( $n$ =7–9). The contraction



**Fig. 3** Effect of NF449 (10  $\mu$ M), a selective P2X1 receptor antagonist, on contractions to **a** ATP (1 mM) and **b**  $\alpha\beta$ -meATP (1  $\mu$ M), in U46619-precontracted porcine pancreatic arteries. NF449 reduced the effects of **a** ATP and **b**  $\alpha\beta$ -meATP (\*\* $P$ <0.001, unpaired Student's  $t$  test,  $n$ =10–13). Data are presented as mean  $\pm$  SEM



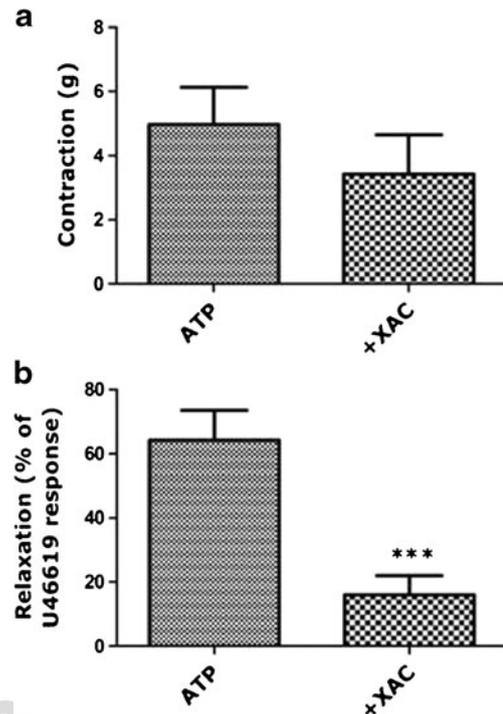
**Fig. 4** Effect of removal of the endothelium on **a** contraction and **b** relaxation to ATP (1 mM) in U46619-precontracted porcine pancreatic arteries. The effect of the removal of endothelium on the contraction or relaxation of ATP was not significantly different ( $n=9-11$ ). Data are presented as mean  $\pm$  SEM

281 to 10–100 nM U46619 was  $5.5 \pm 0.5$  g in endothelium-  
 282 intact arteries, while it was  $5.8 \pm 0.6$  g in endothelium-  
 283 denuded arteries ( $n=12-14$ ). The contraction to 1  $\mu$ M  
 284  $\alpha\beta$ -meATP was  $3.2 \pm 0.6$  g in endothelium-intact arteries,  
 285 while it was  $3 \pm 0.6$  g in endothelium-denuded arteries ( $n=$   
 286 6); there was no significant difference between these  
 287 responses.

- 288 • Effect of XAC, an adenosine receptor antagonist  
 289 The relaxation to ATP was investigated in the presence  
 290 of a non-selective adenosine receptor antagonist; XAC  
 291 (10  $\mu$ M) had no effect on the contraction evoked by  
 292 ATP (Fig. 5a), while it reduced significantly the relaxation  
 293 to ATP (Fig. 5b).

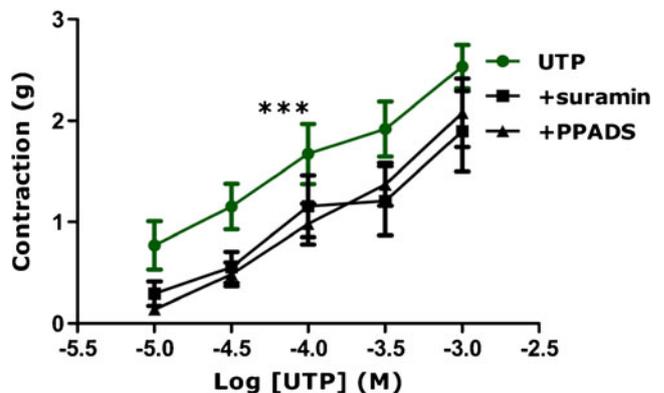
294 *Characterisation of response to UTP in U46619-*  
 295 *precontracted porcine-isolated pancreatic arteries*

- 296 • Effect of suramin, PPADS,  $\alpha\beta$ -meATP and MRS2578, a  
 297 selective P2Y<sub>6</sub> receptor antagonist  
 298 The contraction to UTP was examined in the presence  
 299 of suramin (100  $\mu$ M), PPADS (10  $\mu$ M),  $\alpha\beta$ -meATP



**Fig. 5** Effect of XAC (10  $\mu$ M) on **a** contraction and **b** relaxation to ATP (1 mM) in U46619-precontracted porcine pancreatic arteries. **a** XAC had no effect on the contraction to ATP ( $n=8-10$ ), and **b** XAC reduced the relaxation to ATP (\*\*\*)  $P < 0.001$ , unpaired Student's *t* test,  $n=8-10$ ). Data are presented as mean  $\pm$  SEM

(1  $\mu$ M) and MRS2578 (10  $\mu$ M). Suramin and PPADS 300  
 significantly reduced the contraction to UTP (Fig. 6), 301  
 while the UTP responses were not affected after P2X 302  
 receptor desensitisation in the presence of  $\alpha\beta$ -meATP 303  
 (1  $\mu$ M) or in the presence of a selective P2Y<sub>6</sub> receptor 304  
 antagonist (MRS2578); for example, the contraction to 305  
 1 mM UTP was  $1.8 \pm 0.2$  g in the absence of MRS2578 306  
 ( $n=7$ ), while it was  $2.1 \pm 0.2$  g in the presence of 307



**Fig. 6** Effect of suramin (100  $\mu$ M) and PPADS (10  $\mu$ M) on contraction to UTP in U46619-precontracted porcine pancreatic arteries. With suramin and PPADS, effect of UTP concentration ( $F=16.77$  and  $F=12.38$ , respectively,  $***P < 0.001$ ), suramin and PPADS reduced the contraction evoked by UTP ( $F=14.47$  and  $F=12.48$ , respectively,  $***P < 0.001$ , two-way ANOVA;  $n=9-12$ ). Data are presented as mean  $\pm$  SEM

308 MRS2578 ( $n=6$ ); there was no significant difference be-  
 309 tween these responses.

310 • Effect of endothelium removal

311 The effects of UTP were studied after the endothelium  
 312 had been removed. The contraction induced by UTP was  
 313 significantly attenuated in the endothelium-denuded ar-  
 314 teries (Fig. 7).

315 • Effect of DUP 697, a cyclooxygenase-2 inhibitor

316 Because the contraction to UTP was largely  
 317 endothelium-dependent, the contraction was studied in  
 318 the presence of DUP 697, a cyclooxygenase-2 (COX-2)  
 319 inhibitor, since COX-2 facilitates the release of agents  
 320 which are responsible for endothelium-dependent con-  
 321 traction. DUP 697 (3  $\mu\text{M}$ ) diminished the response to UTP  
 322 (Fig. 8) to a similar extent as removal of the endothelium  
 323 (Fig. 7), while DUP 697 did not alter the contraction to  
 324 U46619 (the precontraction agent) or the contraction to  
 325 ATP (data not shown).

326 *Characterisation of response to ADP in U46619-*  
 327 *precontracted porcine-isolated pancreatic arteries*

328 • Effect of MRS2179, a P2Y<sub>1</sub> receptor selective antagonist,  
 329 and of endothelium removal

330 The relaxation to ADP in pancreatic arteries was stud-  
 331 ied in the presence of MRS2179 (10  $\mu\text{M}$ ) and after the  
 332 endothelium had been removed. The relaxation to ADP  
 333 was reduced slightly but significantly in the presence of  
 334 MRS2179 (Fig. 9a) and in the endothelium-denuded ar-  
 335 teries (Fig. 9b), which indicates the involvement of P2Y<sub>1</sub>  
 336 receptors and the endothelium in ADP-mediated relaxa-  
 337 tion of porcine pancreatic arteries.

338 • Effect of XAC, an adenosine receptor antagonist, and  
 339 SCH58261, a selective adenosine A<sub>2A</sub> receptor antagonist

340 The relaxation to ADP was investigated in the presence  
 341 of XAC (10  $\mu\text{M}$ ). The relaxation to ADP was largely

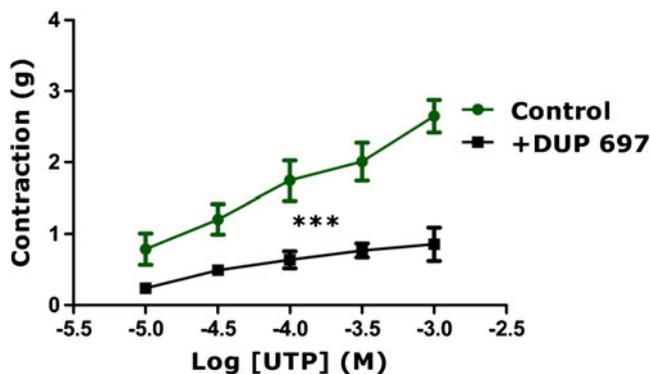


Fig. 8 Effect of DUP 697 (3  $\mu\text{M}$ ), a cyclooxygenase-2 inhibitor, on contraction to UTP in U46619-precontracted porcine pancreatic arteries. Effect of UTP concentration ( $F=8.48$ ,  $***P<0.001$ ) and DUP 697 reduced the contraction evoked by UTP ( $F=50.8$ ,  $***P<0.001$ , two-way ANOVA;  $n=8-12$ ). Data are presented as mean  $\pm$  SEM

342 reduced in the presence of this inhibitor which indicates  
 343 the involvement of adenosine receptors (Fig. 10). To find  
 344 out about the adenosine subtype involved in the relaxation  
 345 to ADP, the response to ADP was investigated in the  
 346 presence of SCH58261, a selective adenosine A<sub>2A</sub> recep-  
 347 tor antagonist. This antagonist significantly inhibited the  
 348 relaxation to ADP, to a similar extent as seen with XAC

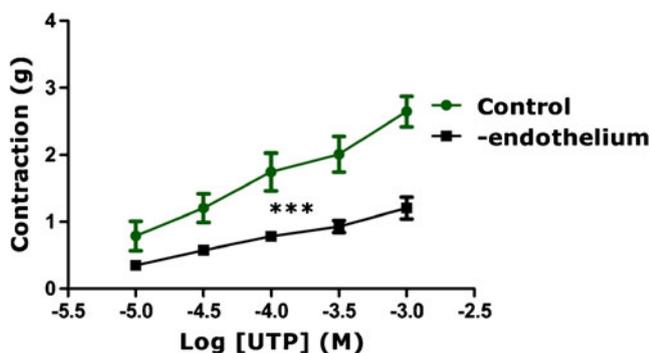


Fig. 7 Effect of removal of the endothelium on contraction to UTP in U46619-precontracted porcine pancreatic arteries. Effect of UTP concentration ( $F=11.91$ ,  $***P<0.001$ ) and removal of endothelium reduced the contraction evoked by UTP ( $F=43$ ,  $***P<0.001$ , two-way ANOVA;  $n=10-12$ ). Data are presented as mean  $\pm$  SEM

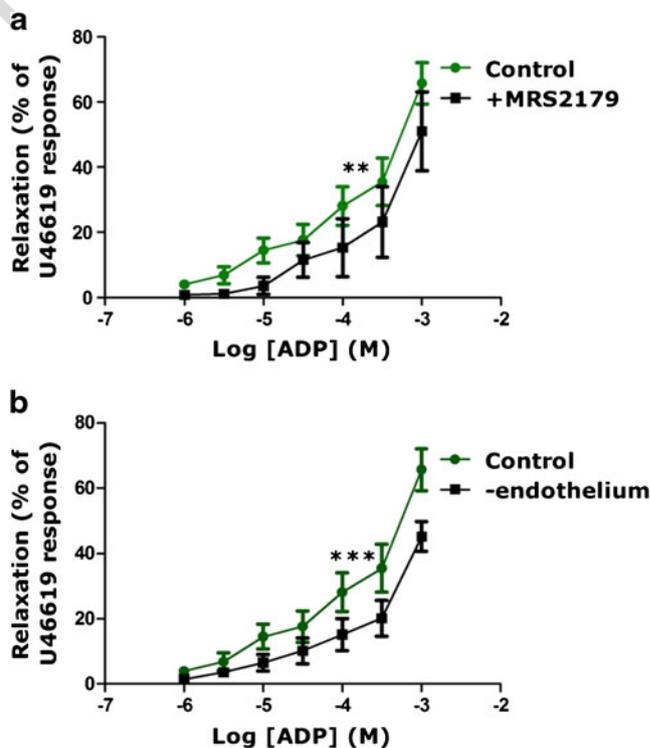
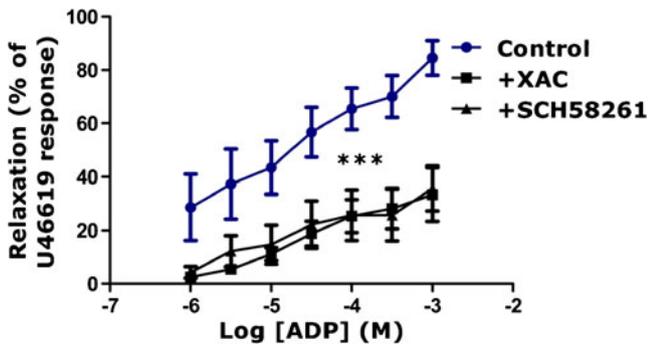


Fig. 9 Effect of a MRS2179 (10  $\mu\text{M}$ ) and b the removal of the endothelium on relaxation to ADP in U46619-precontracted porcine pancreatic arteries. With MRS2179 and in endothelium-denuded arteries, effect of ADP concentration ( $F=21.42$  and  $16.77$ , respectively,  $***P<0.001$ ) and MRS2179 and removal of endothelium reduced the contraction evoked by ADP ( $F=21.42$  and  $F=32.04$ , respectively,  $***P<0.001$ , two-way ANOVA;  $n=10-12$ ). Data are presented as mean  $\pm$  SEM



**Fig. 10** Effect of XAC (10  $\mu$ M), a non-selective adenosine receptor antagonist, and SCH58261 (1  $\mu$ M), a selective adenosine  $A_{2A}$  receptor antagonist, on relaxation to ADP in U46619-precontracted porcine pancreatic arteries. With XAC and SCH58261, effect of ADP concentrations ( $F=7.14$  and  $F=6.08$ , respectively,  $***P<0.001$ ), XAC and SCH58261 reduced the relaxation evoked by ADP ( $F=71.19$  and  $F=58.16$ , respectively,  $***P<0.001$ , two-way ANOVA;  $n=9-14$ ). Data are presented as mean  $\pm$  SEM

349 (Fig. 10). This showed that the relaxation to ADP involved  
350  $A_{2A}$  adenosine receptors.

351 **Discussion**

352 The current report has provided evidence for the functional  
353 expression of contractile P2X<sub>1</sub>, P2Y<sub>2</sub> and P2Y<sub>4</sub> receptors and  
354 vasorelaxant P2Y<sub>1</sub> and  $A_{2A}$  adenosine receptors in porcine  
355 pancreatic arteries. These receptors are sensitive to the extra-  
356 cellular nucleotides ATP (P2X<sub>1</sub>), UTP (P2Y<sub>2</sub> and P2Y<sub>4</sub>) and  
357 ADP (P2Y<sub>1</sub> and  $A_{2A}$ ). The contraction to ATP was endothe-  
358 lium independent, while UTP induced an endothelium-  
359 dependent contraction which may involve P2Y<sub>2</sub> and/or  
360 P2Y<sub>4</sub> receptors. The relaxation to ADP involved the endothe-  
361 lium and P2Y<sub>1</sub> receptors and  $A_{2A}$  adenosine receptors.

362 A vasoconstrictor response elicited by ATP has been re-  
363 ported in a number of different arteries [21–23]. ATP may also  
364 induce vasorelaxation depending on the experimental condi-  
365 tions (level of pre-tone) and relative expression of relevant  
366 vasocontractile and vasorelaxant receptors [24, 25]. In porcine  
367 pancreatic arteries, ATP induced a biphasic response  
368 consisting of a contraction followed by a relaxation  
369 (Fig. 1b). Since the contraction to ATP was rapidly  
370 desensitising, non-cumulative concentration–response curves  
371 were investigated. The contractions to ATP and  $\alpha\beta$ -meATP  
372 were reduced in the presence of suramin, PPADS,  $\alpha\beta$ -meATP  
373 (a desensitiser of P2X<sub>1</sub> receptors) and NF449 (a P2X<sub>1</sub> selec-  
374 tive antagonist) (Figs. 2a, b and 3a, b), which indicates that a  
375 large part of the contraction to ATP could be attributed to the  
376 activation of P2X<sub>1</sub> receptors. Moreover, the contractile effect  
377 of  $\alpha\beta$ -meATP is consistent with the expression of P2X<sub>1</sub>  
378 receptors in porcine pancreatic arteries.  $\alpha\beta$ -meATP was more  
379 potent than ATP in eliciting vasoconstriction most likely due

to its greater stability [5]. Since the contraction to ATP was not  
380 changed after the endothelium had been removed (Fig. 4a),  
381 the expression of P2X<sub>1</sub> receptors was shown to be on the  
382 vascular smooth muscle cells (VSMCs). This is consistent  
383 with the abundant expression of P2X<sub>1</sub> receptors on VSMCs  
384 of most tissues [7].  
385

ATP-induced vasorelaxation was not affected after the  
386 endothelium had been removed or in the presence of suramin  
387 or PPADS, which suggests that the relaxation to ATP was not  
388 due to its action at P2Y receptors. However, the relaxation to  
389 ATP was significantly inhibited in the presence of XAC,  
390 which suggested an involvement of adenosine receptors  
391 expressed on VSMCs of the pancreatic arteries; it is likely  
392 that this is due to the activity of adenosine derived from ATP  
393 metabolism by ecto-nucleotidase 5'-triphosphate  
394 diphosphohydrolase (ENTPDase) enzymes followed by the  
395 activity of CD37 and ecto-5'-nucleotidase enzymes [26]. Sim-  
396 ilarly, in rat coronary arteries, the relaxation to ATP involved  
397 P1 receptors, although there was an additional involvement of  
398 P2Y receptors [24]. In the current study, further investigation  
399 of the adenosine receptor subtypes involved in the relaxation  
400 to ATP is required. We and others have shown previously a  
401 slow relaxation in response to  $\alpha\beta$ -meATP in rat mesenteric  
402 arteries, subsequent to contraction [27–29], but we did not  
403 observe this in the present study in the porcine pancreatic  
404 arteries.  
405

The vasoconstriction to UTP did not desensitise quickly;  
406 therefore, cumulative concentration–response curves were  
407 used to study the effect of UTP on pancreatic arteries. This  
408 contraction was significantly inhibited by suramin and  
409 PPADS (Fig. 6), and there was a reduction of the response  
410 after the removal of the endothelium (Fig. 7). That would  
411 indicate for the first time an endothelium-dependent vasocon-  
412 striction evoked by UTP. UTP is known to be active at P2Y<sub>2</sub>,  
413 P2Y<sub>4</sub> and P2Y<sub>6</sub> receptors [30]. The expression of these recep-  
414 tors in the endothelium and smooth muscle of vessels has been  
415 reported [31]. Since MRS2578 was not able to alter the  
416 contraction to UTP, this indicates that UTP had no action at  
417 P2Y<sub>6</sub> receptors. There are currently no commercially available  
418 selective antagonists for either P2Y<sub>2</sub> or P2Y<sub>4</sub> receptors. How-  
419 ever, we believe that UTP acted at P2Y<sub>4</sub> receptors since the  
420 contraction to UTP was significantly inhibited by both endo-  
421 thelium removal and in the presence of DUP 697, but res-  
422 sponses to ATP were unaffected. UTP-induced contraction  
423 may also be mediated by P2Y<sub>2</sub> receptors, since MRS2768  
424 which is a selective agonist at P2Y<sub>2</sub> receptors and displays no  
425 affinity for P2Y<sub>4</sub> or P2Y<sub>6</sub> receptors was able to evoke a  
426 contraction in pancreatic arteries [32] (Fig. 1a).  
427

UTP-induced vasoconstriction has been documented in a  
428 number of arteries including rat pulmonary arteries in which  
429 the contraction was attributed to P2Y<sub>2</sub> receptors, and in rabbit  
430 basilar arteries in which the contraction to UTP was due to  
431 action of P2Y<sub>4</sub> receptors [33, 34]. UTP produced an  
432

433 endothelium-dependent relaxation in rabbit pulmonary arteries and in rat mesenteric arterial bed, but the receptor subtypes  
 434 were undefined [22, 35]. In bovine middle cerebral arterial  
 435 strips, UTP had a dual response, and it induced a contraction  
 436 in endothelium-denuded arteries, but a relaxation in  
 437 endothelium-intact arteries [36]. The absence of  
 438 endothelium-dependent or endothelium-independent relaxa-  
 439 tion to UTP and some other nucleotides in rat renal arteries  
 440 was reported [37], which is consistent with the current study  
 441 since there was no evidence of a UTP-mediated relaxation in  
 442 porcine pancreatic arteries. Hence, porcine pancreatic arteries  
 443 appear not to express relaxant P2Y<sub>2</sub> and/or P2Y<sub>4</sub> receptors.  
 444

445 To investigate the mechanism underlying the contraction  
 446 mediated by UTP in pancreatic arteries, the response to UTP  
 447 was examined in the presence of DUP 697. As seen in Fig. 8,  
 448 the endothelium-dependent contraction was attenuated in the  
 449 presence of the selective COX-2 inhibitor. Endothelial cells  
 450 can release endothelium-derived contractile factors (EDCFs),  
 451 which may include thromboxane A<sub>2</sub>, prostaglandin F<sub>2α</sub>, leu-  
 452 kotrienes and endothelin-1. Thromboxane A<sub>2</sub> and prostaglan-  
 453 din F<sub>2α</sub> are released from the endothelium due to the activity  
 454 of COX-2 [38, 39]. The reduction of the contraction to UTP  
 455 in the presence of DUP 697 indicated the involvement of throm-  
 456 boxane A<sub>2</sub> and prostaglandins in the contraction to UTP.  
 457 These agents, after being released from the endothelium,  
 458 may act on their receptors on VSMCs to cause contraction  
 459 [39]. The different effects of DUP 697 on responses to UTP  
 460 and ATP further suggest that they are acting on different  
 461 receptors.

462 The relaxation to ADP did not desensitise rapidly; there-  
 463 fore, cumulative concentration–response curves were used to  
 464 study the effect of ADP on pancreatic arteries. The relaxation  
 465 was significantly attenuated by MRS2179, a selective P2Y<sub>1</sub>  
 466 receptor antagonist (Fig. 9a). In addition, the relaxation to  
 467 ADP was reduced after the endothelium had been removed,  
 468 by a similar extent as observed in the presence of the  
 469 MRS2179 (Fig. 9b). This may suggest that P2Y<sub>1</sub> receptors  
 470 are expressed on the endothelium. Indeed, a number of reports  
 471 show that P2Y<sub>1</sub> receptors are expressed on the endothelium  
 472 and are responsible for the relaxation of arteries, including rat  
 473 thoracic aortic and porcine mesenteric arteries [40, 41]. The  
 474 relaxation to ADP in our study was largely reduced in the  
 475 presence of XAC and SCH58261 (adenosine receptor antag-  
 476 onists). Adenosine receptors may be expressed on the endo-  
 477 thelium or the vascular smooth muscle [42]. Since XAC and  
 478 SCH58261 produced a greater reduction in the relaxation to  
 479 ADP than the inhibition induced by removal of the endothe-  
 480 lium (Fig. 10), this suggests that relaxation to ADP involves  
 481 A<sub>2A</sub> adenosine receptors expressed, at least in part, on  
 482 VSMCs. The mechanism by which ADP would produce  
 483 adenosine to act at the adenosine receptors is still to be  
 484 elucidated. The simplest explanation is that it is broken down  
 485 by ENTPDases and by CD37 enzymes to adenosine [26].

Alternatively, as suggested in porcine coronary arteries, 486  
 ADP mediates a relaxation via a mechanism that involves 487  
 ADP-evoked adenosine release and the subsequent activation 488  
 of A<sub>2A</sub> receptors [20]. In contrast to the porcine pancreatic 489  
 vessels, ADP in rat pancreatic arteries induced a contraction at 490  
 a high concentration (1 mM); this contraction was similar to 491  
 that produced by ATP and was much lower than the contrac- 492  
 tion induced by αβ-meATP [43]. Further investigation is 493  
 required to determine the involvement of endothelium- 494  
 derived relaxing factors or endothelium-derived 495  
 hyperpolarising factors released from the endothelium in the 496  
 ADP-induced relaxation. 497

Reduction in pancreatic blood flow has been observed in 498  
 acute and chronic pancreatitis and some other pancreatic 499  
 diseases [44, 45], implicating pancreatic tissue perfusion as 500  
 an important factor in the pathogenesis of pancreatic diseases 501  
 and symptoms. There is increasing evidence for the role of 502  
 purinergic signalling in the pathophysiology of the pancreas 503  
 [2]. Hence, drugs designed to target specific components of 504  
 purinergic system may be of relevance to the management of 505  
 pancreatitis, cystic fibrosis, pancreatic cancer and diabetes. 506

In summary, the functional expression of P2X1 and A<sub>2A</sub> 507  
 adenosine receptors on VSMCs and P2Y<sub>2</sub> and/or P2Y<sub>4</sub> recep- 508  
 tors on the endothelium of porcine pancreatic arteries was 509  
 indicated in the current study. Activation of P2X1 receptors 510  
 by ATP or αβ-meATP induced a vasoconstriction, and UTP 511  
 acts at P2Y<sub>2</sub> and/or P2Y<sub>4</sub> receptors to induce a contraction. 512  
 ADP and ATP activate A<sub>2A</sub> adenosine receptors to induce 513  
 relaxation, together with an action of ADP on P2Y<sub>1</sub> receptors. 514  
 Pancreatic arteries appear to lack vasorelaxant P2Y<sub>2</sub> and/or 515  
 P2Y<sub>4</sub> receptors. 516

**Acknowledgments** We would like to thank the Damascus University in 517  
 Syria for funding the project. 518

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## AUTHOR QUERIES

### AUTHOR PLEASE ANSWER ALL QUERIES.

- Q1. Kindly check the inserted spelled out form 'standard error of the mean' of the abbreviation 'SEM' if correct.
- Q2. Kindly check the inserted spelled out form 'half maximal effective concentration' of the abbreviation 'EC<sub>50</sub>' if correct.
- Q3. Kindly provide access date for references 'Alsaqati et al. [19]' and 'Alefishat et al. [41]' in the list.

UNCORRECTED PROOF