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Positive Inotropic Effects by Uridine Triphosphate (UTP) and Uridine Diphosphate (UDP) via $P2Y_2$ and $P2Y_6$ Receptors on Cardiomyocytes and Release of UTP in Man During Myocardial Infarction

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Abstract

The aim of this study was to examine a possible role for extracellular pyrimidines as inotropic factors for the heart. First, nucleotide plasma levels were measured to evaluate whether UTP is released in patients with coronary heart disease. Then, inotropic effects of pyrimidines were examined in isolated mouse cardiomyocytes. Finally, expression of pyrimidine-selective receptors (a subgroup of the P2 receptors) was studied in human and mouse heart, using real time polymerase chain reaction, Western blot, and immunohistochemistry. Venous plasma levels of UTP were increased (57%) in patients with myocardial infarction. In electrically stimulated cardiomyocytes the stable $P2Y_{2/4}$ agonist UTPγS increased contraction by 52%, similar to β_1-adrenergic stimulation with isoproterenol (65%). The $P2Y_6$-agonist UDPγS also increased cardiomyocyte contraction (35%), an effect abolished by the $P2Y_6$-blocker MRS2578. The phospholipase C inhibitor U73122 inhibited both the UDPγS and the UTPγS-induced inotropic effect, indicating an IP_3-mediated effect via $P2Y_6$ receptors. The $P2Y_{14}$ agonist UDP-glucose was without effect. Quantification of mRNA with real time polymerase chain reaction revealed $P2Y_2$ as the most abundant pyrimidine receptor expressed in cardiomyocytes from man. Presence of $P2Y_6$ receptor mRNA was detected in both species and confirmed at protein level with Western blot and immunohistochemistry in man. In conclusion, UTP levels are increased in humans during myocardial infarction, giving the first evidence for UTP release in man. UTP is a cardiac inotropic factor most likely by activation of $P2Y_2$ receptors in man. For the first time we demonstrate inotropic effects of UDP, mediated by $P2Y_6$ receptors via an IP_3-dependent pathway. Thus, the extracellular pyrimidines (UTP and UDP) could be important inotropic factors involved in the development of cardiac disease.

Keywords

P2-receptors; inotropy; heart; UTP; UDP

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The cardiac effects of purines such as adenosine, adenosine triphosphate (ATP), and adenosine diphosphate (ADP) have been extensively studied since the first functional findings by Drury and Szent-Györgi in 1929.[1] Purines were subsequently found to have specific receptors in the surface membrane of the cardiac myocytes. However, only a few studies have focused on the effect of pyrimidines, such as uridine triphosphate (UTP) and uridine diphosphate (UDP), in the heart.

UTP alone has been shown to have inotropic effects in the heart, but previous studies have been performed with unstable nonselective agonists, and receptor characterization has not been possible. Furthermore, even though the release of purines has been extensively studied in different models, the release of pyrimidines has never been examined in human patients.

The extracellular nucleotides, ATP and ADP, are released from sympathetic nerves, activated platelets, erythrocytes, cardiac tissue, inflammatory, endothelial, and smooth muscle cells.[4–6] In the heart, hypoxic conditions are prominent activators of ATP release.[6, 7] There is limited knowledge considering release of pyrimidines. Continuous release of UDP-glucose from endothelial cells has been detected, however no correlation to ATP release has been observed.[8] Using a pig model we recently demonstrated that UTP is released from the heart during ischemia.[9] However, the release of pyrimidines from the human heart under ischemic conditions has not been studied.

The extracellular nucleotides activate membrane-bound purine receptors (P2 receptors), which is one of the largest known receptor families.[10] P2 receptors are divided into two classes: ligand-gated intrinsic ion channels, called P2X receptors, and G protein– coupled P2Y receptors.[10] New P2Y receptor subtypes activated not only by purines but also pyrimidines have been identified. UTP, UDP, and UDP glucose activates at least four subtypes of P2Y receptors: $P2Y_2$, $P2Y_4$, $P2Y_6$, and $P2Y_{14}$.[2, 3] UTP is an agonist for $P2Y_2$ [13] and $P2Y_4$ receptors.[14] $P2Y_6$ is activated by UDP[13, 15] and UTP glucose activates $P2Y_{14}$ receptors.[16] $P2Y_2$ is activated by both UTP and ATP.

The P2Y family is divided into two structurally-distinct subfamilies. The first is composed of $P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, and $P2Y_{11}$ receptors, all coupled to G_q, which promotes phospholipase C (PLC) and subsequent mobilization of intracellular calcium.[11, 12] The members of the second subfamily $P2Y_{12}$, $P2Y_{13}$, and $P2Y_{14}$ are coupled to G_i, inhibiting adenylate cyclase.

Extracellular ATP is known to increase cytosolic calcium in cardiac myocytes and to have inotropic effects.[5, 17, 18] We recently examined the effects of purines in cardiomyocytes and found potent inotropic ATP effects mediated via a $P2Y_{11}$-like receptor.[18] Regarding pyrimidines, the unstable agonist UTP has been shown to induce a positive inotropic effect in rat atria and in rat and guinea pig ventricular cardiomyocytes,[19–22] but the effects of UDP and UDP-glucose has not been investigated. Furthermore, there is a need to characterize which receptor mediates the inotropic effect of UTP.

We wanted to examine whether pyrimidines are released in man, evaluate their inotropic effects, and investigate which pyrimidine selective receptors are expressed in the human heart. The first objective was to quantify the plasma level of uridine nucleotides in patients with acute ischemic heart disease. The second objective was to examine the inotropic effects of selective pyrimidine receptor agonists. Because of the limited availability of human cardiomyocytes, pharmacological characterization of pyrimidine selective P2Y receptor function was done in cardiomyocytes from mice. The third objective was to analyze expression of P2-receptors in the heart using quantitative real-time polymerase chain reaction (PCR), Western blot, and immunohistochemistry.

Materials and Methods

Patients Enrolled for UTP Measurements

Sixty-four patients were enrolled from the patients admitted for chest pain to the emergency ward, Lund University Hospital 2001 to 2003. Patients with chest pain within the last hour before admittance were eligible for inclusion in this study. Exclusion criteria were: patients treated with aspirin, clopidogrel, dipyridamole, nonsteroidal antiinflammatory drugs, heparin, low molecular heparin, or warfarin. Even patients who received a bolus dose of aspirin in the ambulance on their way to hospital were excluded. Furthermore, patients were excluded if they had a platelet count $<140\times10^9$/L, hemoglobin <90 g/L, renal failure (creatinine >140 μmol/L), or hemolysis in blood samples. Based on the diagnosis at discharge, three prespecified subgroups were compared: chest pain with no sign of cardiac disease (NCD), non–ST elevation myocardial infarction (NSTEMI), and ST elevation myocardial infarction (STEMI). The study included 43 men and 21 women in the mean age of 68±12 years. The NCD group was composed of patients with normal ECGs (or no new changes compared with a previous ECG), normal values of cardiac markers (TroponinT), and when appropriate, a negative exercise test. This UTP measurement study was a substudy of a larger study on ATP release during ischemic heart disease.[23] For more methodological details see below.

Quantification of Nucleotides

UTP was measured in all 64 patients. Based on diagnosis 64 patients were grouped into STEMI (16 patients), NSTEMI (16 patients), and NCD (32 patients). Sampling was done at admission. Blood (5 mL) was added to tubes containing citrate and immediately centrifuged for 10 minutes at 1200*g*4°C. Platelet contamination was excluded by Bürker chamber examination. The plasma was aspirated and mixed with an equal amount of 10% trichloroacetic acid (TCA) to precipitate all proteins and inactivate ectonucleotidases. Samples were treated and UTP was quantified as previously described.[18, 24]

Animals

Adult female NMRI mice (B&K AB, Sollentuna, Sweden), 5 to 6 months old, were used for isolation of cardiomyocytes. The animals were kept in the university animal facilities with free access to food and water according to regulations of the local animal ethics committee.

Isolation and Contraction of Cardiomyocytes

The mice were euthanized by cervical dislocation. The cardiomyocytes were isolated as previously described.[18]

Cardiomyocyte Contraction

The cells could be kept with unaltered contractile properties for up to 4 hours in a Hepes-buffered saline solution (1.8 mmol/L Ca^{2+}) in room temperature.[25] To observe the cell shortening we used an inverted microscope and a video recording system. The cells were analyzed in a Krebs-Henseleit solution (1.8 mmol/L Ca^{2+}) composition adjusted to give pH 7.4 when oxygenated with O_2/CO_2 (95%/5%) at room temperature. Stimulation voltage was set to 20% above threshold and impulse duration was 4 ms at 0.5Hz. The cell length during shortening was analyzed using an optical edge tracking method. The shortening, measured in pixels, was compared before and after addition of drug to the electrically stimulated cardiomyocyte in the cuvette. The shortening responses were expressed relative to the amplitude of responses determined in the cells before addition of drug. The extent of shortening was approximately 5% of total cell length. The cells were stimulated with additions of the agonists UTPγS or UDPβS. To further investigate the signaling pathway of

the response to stimulation with UDPβS the contracting cardiomyocytes were incubated with the $P2Y_6$ blocker MRS2578. The signaling pathway mediating the effect of UDPβS was also studied by exposure to a PLC inhibitor, U73133, and an adenylyl cyclase inhibitor, SQ22563. The cells were preincubated for 5 minutes with inhibitors before addition of agonist.

RT-PCR Assay and Real-Time PCR

Total RNA was prepared from the mouse cardiomyocytes using the RNAeasy column (Qiagen) and the RNA was reverse-transcribed using Multiscribe RT Kit (Qiagen). Real time-PCR reactions on mouse RNA were performed with primers designed using the VectorNTI software (Invitrogen). The transcribed cDNA was amplified in a LightCycler using 1×LightCycler DNA Master SYBR Green I mix (Roche Diagnostics). The procedure has previously been described.[18] The amount of receptor was expressed relative to the housekeeping gene GAPDH. To amplify the receptors the following primers were used; $P2Y_2$ forward (fw) GCTTCAACGAGGACTTCAAG, reversed (rw) GTAATAAACCAACAGCGGCA $P2Y_4$ fw TCACTTGCCATGACACCTCGG, rw AATGGTGCGCACAGACTTGC $P2Y_6$: fw TGACCCGTTCCGCTGTGTAC, rw GCGCTGGAAGCTAATGCAGG, GAPDH fw GGTCATCCCAGAGCTGAACG, rw TTGCTGTTGAAGTCGCAGGA.

Human Heart Tissue Preparation

Hearts were explanted in the process of heart transplantation from 5 patients who were between 52 and 64 years of age and experienced ischemic heart disease or dilated cardiomyopathy. Samples were not taken from infarcted areas. The hearts were immediately examined in the operating room and myocardial tissue samples from the wall of the left and right ventricles and the left and right atria were removed gently and immersed in cold oxygenated Hepes-buffered saline solution. The epicardial and endocardial parts were removed from the pieces from the ventricles. Tissue samples were snap-frozen in liquid nitrogen or fixed in formalin immediately after acquisition. Total cellular RNA was extracted as above. The real-time PCR reactions on human RNA were performed as described earlier.[26]

Western Blot

Protein electrophoresis was performed on 10% Tris-HCl polyacrylamide ready gels (Bio-Rad Laboratories) and electroblotted onto Hybond-C nitrocellulose membranes (0.45 μm; Amersham Pharmacia Biotech). Protein loading of 15 μg for each well was diluted with 4×SDS-reducing sample buffer. The membranes were incubated with rabbit antihuman $P2Y_6$ (1:250; GlaxoSmithKline); thereafter, they were incubated with a secondary antibody (anti-rabbit Ig, horseradish peroxidase-linked, 1:1500; Amersham Life Science). The proteins were visualized by chemiluminescence using the ECLTM Western blotting RPN 2108 system (Amersham Pharmacia Biotech).

Immunocytochemistry

Human heart tissue samples were fixed in formalin, embedded in paraffin, and cut in 5-μm sections. The avidin-biotin-peroxidase complex method for antibody detection was used. After incubation with normal serum, sections were incubated with anti-$P2Y_6$ antibodies. Anti-$P2Y_6$ antibody was produced by GlaxoSmithKline. Bound primary antibody was detected using VECTASTAIN Elite ABC kit and developed with DAB substrate kit for peroxidase. After counter-staining with VECTOR hematoxylin QS nuclear counterstain (Modified Mayer Formula), the slides were examined microscopically.

Drugs

Isoproterenol, SQ22563, and U73122 were purchased from Sigma. UTPγS and UDPγS were gifts from Inspire Pharmaceuticals (Chapel Hill, NC); MRS2578 was a gift from KA Jacobson (National Institutes of Health, Bethesda, Md). All the drugs were dissolved in 0.9% saline.

Ethics

The Ethics Committee of Lund University approved the project involving human subjects. The project complies with the Declaration of Helsinki and all patients gave written consent to participation in the study. The animal study conforms to the *Guide for the Care and Use of Laboratory Animals* published by the National Institutes of Health (NIH Publication No. 85-23, revised 1996). The animal experiments were approved by the local animal ethics committee.

Calculation and Statistics

Calculations and statistics were performed using the Graph-Pad Prism 3.02 software. n denotes the number of cells if otherwise not stated. Statistical significance was accepted when $P<0.05$. Raw data from cardiomyocyte experiments were analyzed with paired Student *t* test, and cardiomyocyte shortening after drug addition was compared with cardiomyocyte shortening before addition of the drug, referred to as the control. Values are presented as mean±SEM. UTP levels were compared with Kruskal–Wallis test followed by Dunnett multiple comparisons. Spearman rank correlation coefficient test was used for regression analysis.

Results

Human venous plasma levels of UTP were significantly increased in STEMI (22±3, n=17 nmol/L, $P<0.05$) compared with NCD (14±3nmol/L, n=32) (Figure 1a). There was no difference between patients with NSTEMI compared with controls (NCD). There was no difference between patients with diabetes mellitus or on aspirin treatment compared with controls. No difference in nucleotide concentration was found between males and females. Regression analysis of the whole material revealed a correlation between UTP and ATP ($r^2=0.44$, $P<0.001$). UTP was present at ≈10% of the ATP concentration (Figure 1b).

Effects of β-adrenergic and uridine nucleotide stimulation on shortening responses of mouse cardiomyocytes are shown in Figure 2. Isoproterenol (1 μmol/L) caused a 65±17% (n=24) increase in the cardiomyocyte contraction. The $P2Y_{2/4}$ receptor agonist UTPγS (1 μmol/L) caused an increase of 52±20% (n=20; Figure 2). The specific $P2Y_6$ receptor agonist UDPγS (1 μmol/L) caused a 37±15% (n=19) increase in the myocyte contraction (Figure 2). The specific $P2Y_{14}$ receptor agonist UDP glucose had no inotropic effects (Figure 2). The inotropic response to UDPβS was abolished by the selective $P2Y_6$ blocker MRS2578 (10 μmol/L) and by the PLC inhibitor U73122 (10 μmol/L) (Figure 3). The UTPγS-induced response was unaffected by the presence of the adenylyl cyclase inhibitor, SQ22563, but the effect was inhibited by U73122 (Figure 3). No effects were observed on cardiomyocyte shortening when MRS2578, U73122, and SQ22563 were administered separately (data not shown). Absolute values of cell contractions were (% cell shortening of total cell length): control (5.0±0.4%), isoproterenol (8.8±1.2%), UTPγS (8.1±1.4%), UDPβS (7.7±0.8), U73122 (5.2±0.8%), UDPβS+U73122 (4.4±0.8%), and SQ22563 (5.1±0.6%); data presented as mean±SEM.

The expression of P2 receptor mRNA in mouse cardiomyocytes was quantified using real-time PCR. $P2Y_2$ and $P2Y_4$ were the most abundant pyrimidine selective receptors ($P2Y_4$

133±19% expressed as percent of $P2Y_2$ receptor mRNA) whereas expression of the $P2Y_6$ receptor mRNA was lower (25±5%). The ADP responsive $P2Y_1$ receptor had the highest expression among the P2Y receptors in the mouse heart. The results are shown in Figure 4.

The expression of P2 receptor mRNA was also quantified in the human heart (Figure 5). In the left ventricle $P2Y_2$ was by far the highest expressed receptor followed by $P2Y_1$ (52±11% expressed as percent of $P2Y_2$ receptor mRNA). $P2Y_6$ (8.2±0.3%) and $P2Y_{11}$ (l7±2%) were also present in significant amounts, but the $P2Y_4$ receptor had very low expression. The expression pattern was similar in the right ventricle and in the atria.

$P2Y_6$ was detected using specific antibodies in immunohistochemistry and Western blot. The receptor was detected in both ventricles and atria of the human heart with a band of approximately 45 kD, which is in agreement with the expected size (Figure 6a). No band was found in the control membrane, indicating that the band represents the $P2Y_6$ receptor. The same antibody was used in immunohistochemistry of human left ventricular heart tissue obtained from the explant heart at transplantation. Positive staining was seen for $P2Y_6$ that was absent when control peptide was added or in controls without the primary antibody (Figure 6b). $P2Y_6$ expression was only seen in cardiomyocytes and not in interstitial cells. The $P2Y_6$ receptor expression was seen in the whole cardiomyocyte, with increased expression at the intercalated discs.

Discussion

In our study we demonstrate that the plasma levels of UTP are significantly elevated in patients with acute myocardial infarction. A combination of pharmacology and mRNA quantification indicates that UTP is probably acting via $P2Y_2$ receptors in man, and via both $P2Y_2$ and $P2Y_4$ receptors on cardiomyocytes from mice. Our results indicate that UDP is a novel inotropic factor acting via $P2Y_6$ receptors. The first demonstration of UTP release used [^{3}H]uridinelabeled endothelial cells. In the study, [^{3}H]UTP release occurred in response to increased flow.[27] Since the development of the first sensitive quantitative assay for UTP, its release has been measured from a variety of cells including platelets, leukocytes, primary airway epithelial cells, rat astrocytes, and several other cell lines.[24] To our knowledge, we are now the first to quantify UTP levels in human plasma. UTP levels correlated significantly with ATP, indicating corelease of the nucleotides. The UTP levels were ≈1:10 of the ATP levels, which is in the vicinity of the relationship between UTP and ATP in most cell types (usually approximately 1:5).[24] Patients with acute myocardial infarction had significantly higher UTP levels, indicating release during ischemia. It was only possible to measure UTP from venous samples and thus it is not possible to establish the source of the released UTP. However, we recently performed animal studies in which the release was measured in blood from the cardiac vein, demonstrating UTP release from the heart during cardiac ischemia.[9] UTP is therefore likely to be released from the heart by cardiomyocytes, but endothelial and red blood cells may also contribute. Platelets are less likely as a source because they contain minor amounts of UTP.[24]

On the surface of cardiac cells there are ectonucleotidases rapidly hydrolyzing nucleotides (UTP to UDP to UMP to uridine).[28] Because UTP is rapidly degraded to UDP, these findings of cardiac UTP release indicate that UDP will be present in the circulation with possible actions on the heart via $P2Y_6$ receptors. This rapid degradation also results in much lower plasma levels than the actual nucleotide concentrations at the cell surface. Measuring UTP release from the heart during cardiac ischemia the nucleotide concentrations in plasma are in 10^{-7} mol/L range.[9] In this experiment we therefore choose the concentration of 10^{-6} mol/L, that may represent a physiologically relevant concentration.

Extracellular pyrimidines have several effects in the cardiovascular system. In the vasculature UDP and UTP induce vasoconstriction through P2Y receptor stimulation on vascular smooth muscle cells, but also dilatation acting on P2Y receptors on endothelial cells, thereby regulating vascular tone and blood pressure.[3, 4, 29–31] Extracellular UTP and UDP have also been shown to mediate growth stimulation and cell migration in vascular smooth muscle cells and intimal hyperplasia.[32–35] In rat cardiomyocytes, UTP has also been shown to cause hypertrophic growth.[36] Furthermore, UTP is known to be a positive inotropic agent in rat atria and rat and guinea pig ventricular cardiomyocytes.[19–22]

UTP is selective to $P2Y_{2/4}$ and UDP to $P2Y_6$ in both man and mouse. Earlier studies have been performed with rapidly degradable UTP, making receptor characterization difficult. UTP is degraded to UDP and may even transfer phosphate groups to generate ATP. Experience from blood vessels and other tissues have shown that it is necessary to use stable and selective compounds.[37] To analyze the inotropic effect of pyrimidine selective receptors stimulation, without interfering with degradation products, we used the stable pyrimidines: UTPγS for selective stimulation of $P2Y_2$ and $P2Y_4$ receptors and UDPβS to selectively stimulate the $P2Y_6$ receptor.[35, 37] UDP-glucose occurs naturally but is resistant to degradation and selective for $P2Y_{14}$ receptors.

In the present study UTPγS was nearly as strong an inotropic stimulator as the same concentration of isoproterenol. The selectivity of UTPγS firmly establishes that the effect is mediated via $P2Y_2$ or $P2Y_4$ receptors. UDPβS also induced a positive inotropic effect in the cardiomyocytes, although slightly weaker. Recently, a selective $P2Y_6$ receptor blocker, MRS2578, was developed by Jacobson and coworkers. [38] Using this novel antagonist, the UDPβS response was totally blocked. This strongly indicates that UDP is a positive inotropic factor acting on the $P2Y_6$ receptor. The $P2Y_{14}$ receptor is coupled to G_i, which inhibits adenylyl cyclase resulting in a decreased cAMP level. This would theoretically result in a negative inotropic effect, but no inhibitory or stimulatory effect was observed when exposing the cardiomyocytes to UDP-glucose.

The inotropic mechanisms were studied using selective inhibitors of the intracellular pathways. The receptors $P2Y_2$, $P2Y_4$, and $P2Y_6$ are G_q-coupled receptors mediating their response via PLC, resulting in increased IP_3 and increased intracellular Ca^{2+} levels. UTP has previously been shown to be a positive inotropic agent in the heart mediating its effect via a PLC pathway.[19] There is also some evidence that UTP induce cAMP elevation.[39] The inotropic response induced by UTP was confirmed using the stable analogue, UTPγS, confirming activation of the receptors $P2Y_2$ or $P2Y_4$. The PLC dependent pathway of $P2Y_{2/4}$ was confirmed by the decreased inotropic effect of UTPγS in presence of U73122. The possible involvement of cAMP was excluded because the adenylyl cyclase inhibitor SQ22563 did not attenuate the response to UTPγS. The response to UDPβS was blocked by the PLC inhibitor U73122, confirming the involvement of a G_q-coupled receptor acting via IP_3 generation.

To help discriminating between $P2Y_2$ and $P2Y_4$ effects and to establish cardiac expression patterns in man, we used real-time PCR to quantify the mRNA expression of P2 receptors in heart tissue from man and mouse. The $P2Y_2$ receptor was abundant in both human and mouse heart tissue. The $P2Y_4$ receptor was absent in man whereas in mouse it was present to about the same extent as $P2Y_2$. This is similar to heart tissue from rat where $P2Y_2$ and $P2Y_4$ have shown to be expressed to the same extent.[40] mRNA for the $P2Y_6$ receptor was present in both mouse and man, but at a relatively low level. It is well known that the mRNA expression may not correlate directly with functional importance. Furthermore, the $P2Y_6$ receptor has previously been shown to be more potently activated in vascular smooth muscles compared with the $P2Y_2$ receptor, even though the receptors were expressed to

about the similar extent at the mRNA level.[26, 30, 40] This is probably because the $P2Y_6$ receptor is resistant to desensitization during continued agonist stimulation.[41, 42] Detecting $P2Y_6$ in human tissue by immunohistochemistry verified expression on the protein level and the functional pharmacology clearly demonstrated $P2Y_6$ receptor mediated effects.

Apart from the pyrimidine selective receptors we also detected the ATP receptor $P2Y_{11}$ in the human heart. This is consistent with recent published functional data from mouse cardiomyocytes.[18] The murine $P2Y_{11}$ receptor is not yet cloned, preventing quantification of its mRNA in mouse. $P2Y_1$ was abundantly expressed in both species. However, previous functional studies of this receptor have not revealed any inotropic function in mouse cardiomyocytes.[18]

The presence of pyrimidines in the circulation and receptors by which UTP and UDP can activate IP_3 in cardiomyocytes stimulating both inotropy and hypertrophy could indicate a similar role for pyrimidines as for angiotensin II. Angiotensin II receptor antagonists and angiotensin converting enzyme (ACE) inhibitors have been clinically successful in the treatment of hypertension and heart failure. Similar beneficial effects could possibly be found for selective UTP and UDP receptor antagonists, inhibiting peripheral resistance, inotropy, and cardiac hypertrophy. Another interesting aspect is protection during hypoxia. Yitzhaki and coworkers recently found that UTP protects rat cardiomyocytes against hypoxic injury.[43] Together with our recent finding of UTP release during preconditioning[9] it suggests that UTP could be involved in the preconditioning mechanism, ie, partly explaining why a brief period of ischemia protects against a subsequent period of hypoxia. Our receptor quantification in the human heart suggests that it is the $P2Y_2$ receptor that is the appropriate target for drug therapy aiming at cardiac protection during myocardial infarction. The parallel release of UTP together with ATP could be important both by providing additional inotropic pathways but also by having other effects on the cardiomyocyte such as hypertrophic growth or protection against hypoxic stress. Furthermore, ATP effects are often counteracted by its degradation product adenosine. This is not the case for UTP.

In conclusion, patients with myocardial infarction have higher plasma levels of UTP. UTP and UDP induce a pronounced inotropic effect on mouse cardiomyocytes. mRNA quantification indicates that the inotropic effects of UTP are mediated via $P2Y_2$ and/or $P2Y_4$ receptors in mice and probably mediated by $P2Y_2$ in man. We provide novel evidence for inotropic effects of UDP acting on the $P2Y_6$ receptor on mouse cardiomyocytes. The mechanisms are mediated via PLC-mediated signaling and independent of cAMP. The extracellular pyrimidines UTP and UDP may be inotropic factors in man acting on $P2Y_2$ and $P2Y_6$ receptors. Synthetic agonists could thus be used as inotropic agents during circulatory shock, and antagonists may have effects similar to angiotensin II receptor blockers being beneficial in the treatment of hypertension and congestive heart failure.

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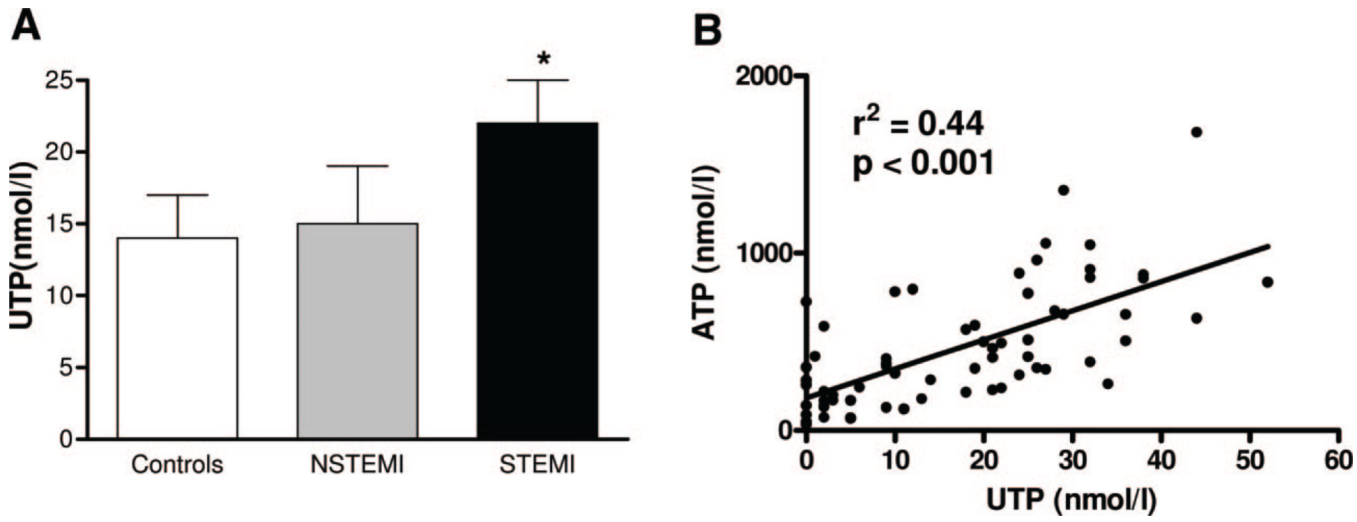

Figure 1.

a, Plasma levels of UTP in humans. Plasma concentrations of UTP in NCD (no sign of cardiac disease) (n=32), NSTEMI (non–ST elevation myocardial infarction, n=16), and STEMI (ST elevation myocardial infarction, n=16) groups expressed as mean values±SEM. **P*<0.05, compared with NCD. b, Correlation between plasma concentrations of UTP and ATP in human blood from controls and patients with acute myocardial infarction. The UTP levels were ≈1:10 of the ATP levels. n=64 patients.

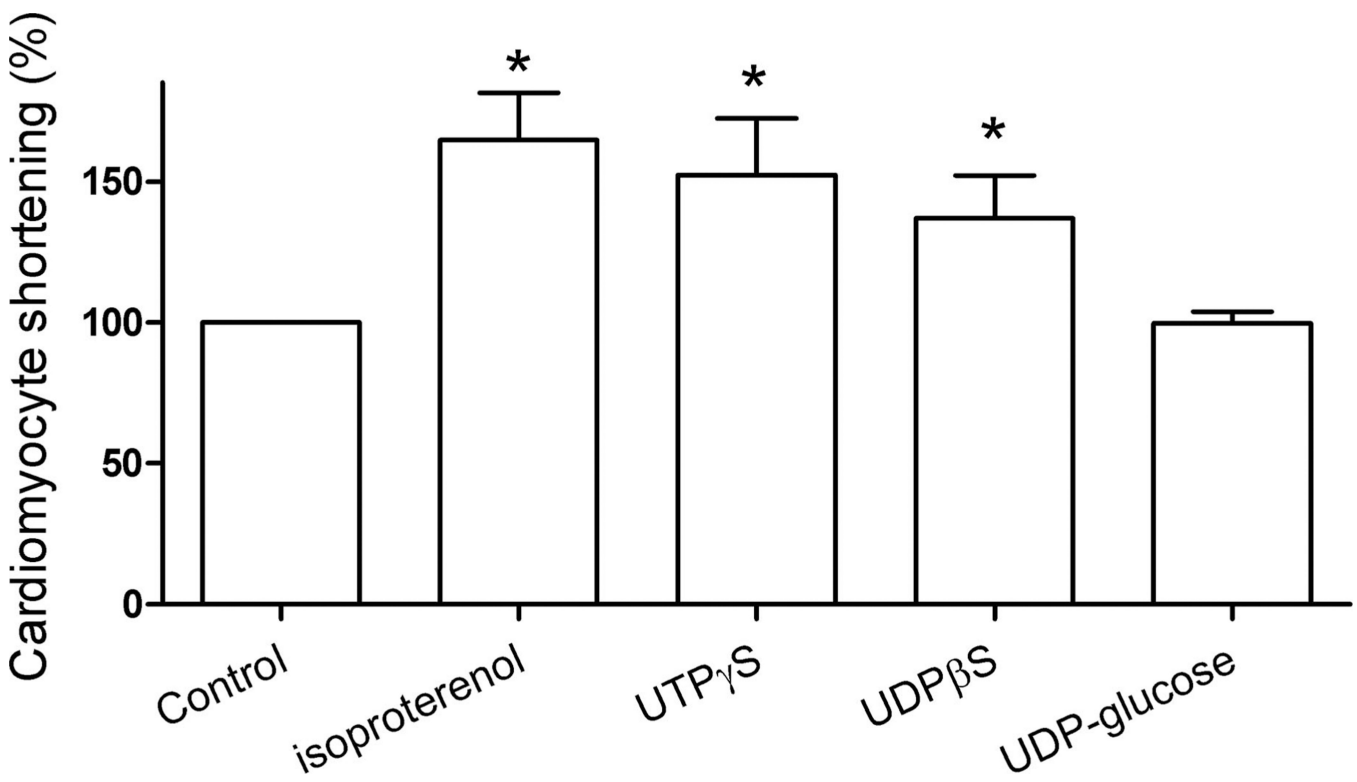

Figure 2.

Effects of β_1 agonist (isoproterenol) and stable UTP and UDP analogs on cardiomyocyte cell shortening. UTPγS (1 μmol/L) and UDPβS (1 μmol/L) induced positive inotropic effects. Contraction to isoproterenol (1 μmol/L) is shown as a control for inotropic effects. UDP glucose did not have any effect. The contractions are expressed as the cardiomyocyte shortening in percent of the control (contracting cardiomyocyte without drug addition). Data are shown as means±SEM, n=19 to 24 cells from 3 to 5 mice.

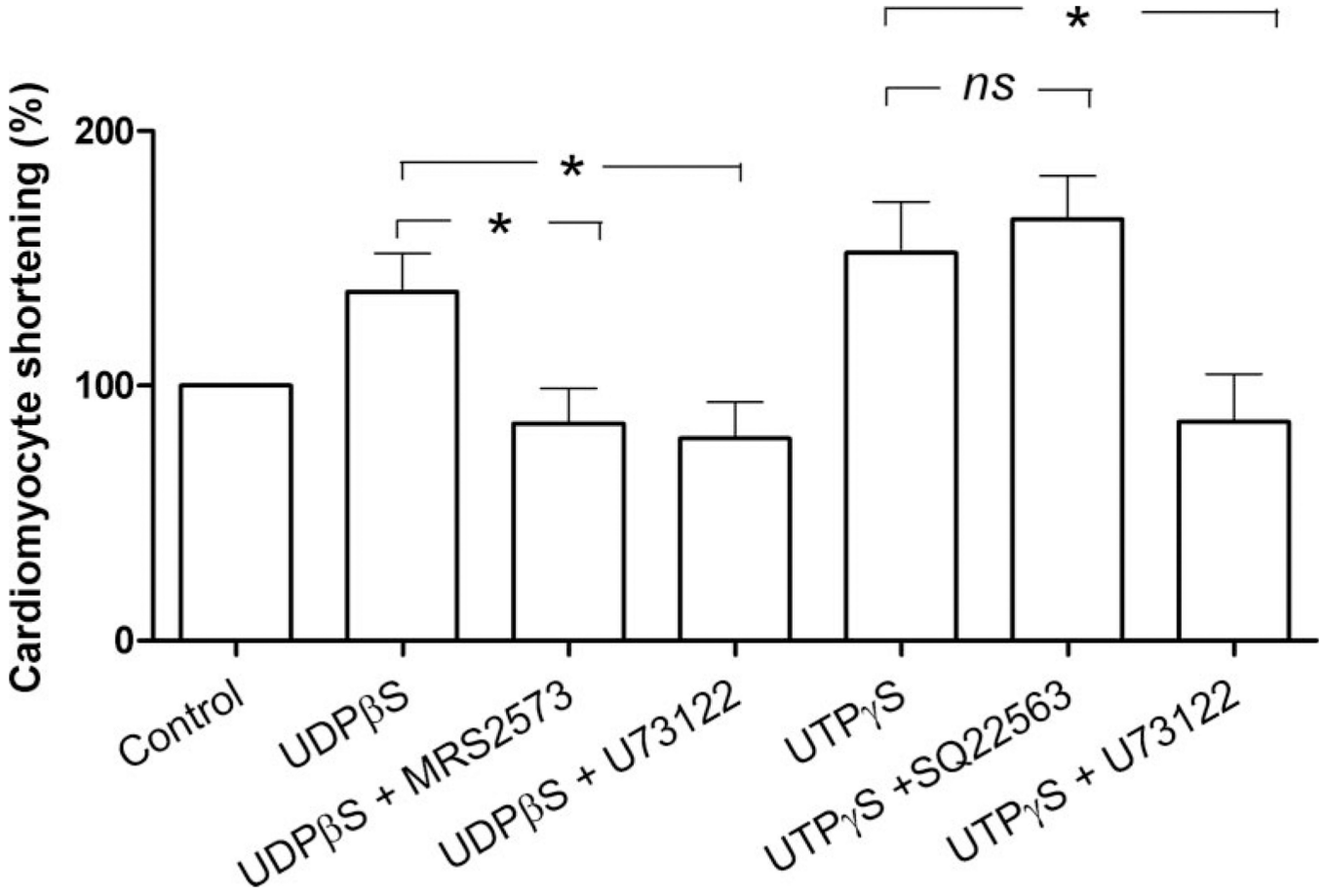

Figure 3.

Effects on the pyrimidine-induced cardiomyocyte shortening by selective inhibitors. The response to UDPβS (1 μmol/L, data also shown in Figure 2) was inhibited by the $P2Y_6$ antagonist MRS2573 (10 μmol/L), and the response to UDPβS (1 μmol/L) was inhibited by the PLC inhibitor U73122 (10 μmol/L). The response to UTPγS (1 μmol/L, data also shown in Figure 2) was not affected by the adenylyl cyclase inhibitor SQ22563. The contractions are expressed as the cardiomyocyte shortening in percent of the control. Data are shown as mean±SEM, n=6 to 19 cells from 3 mice.

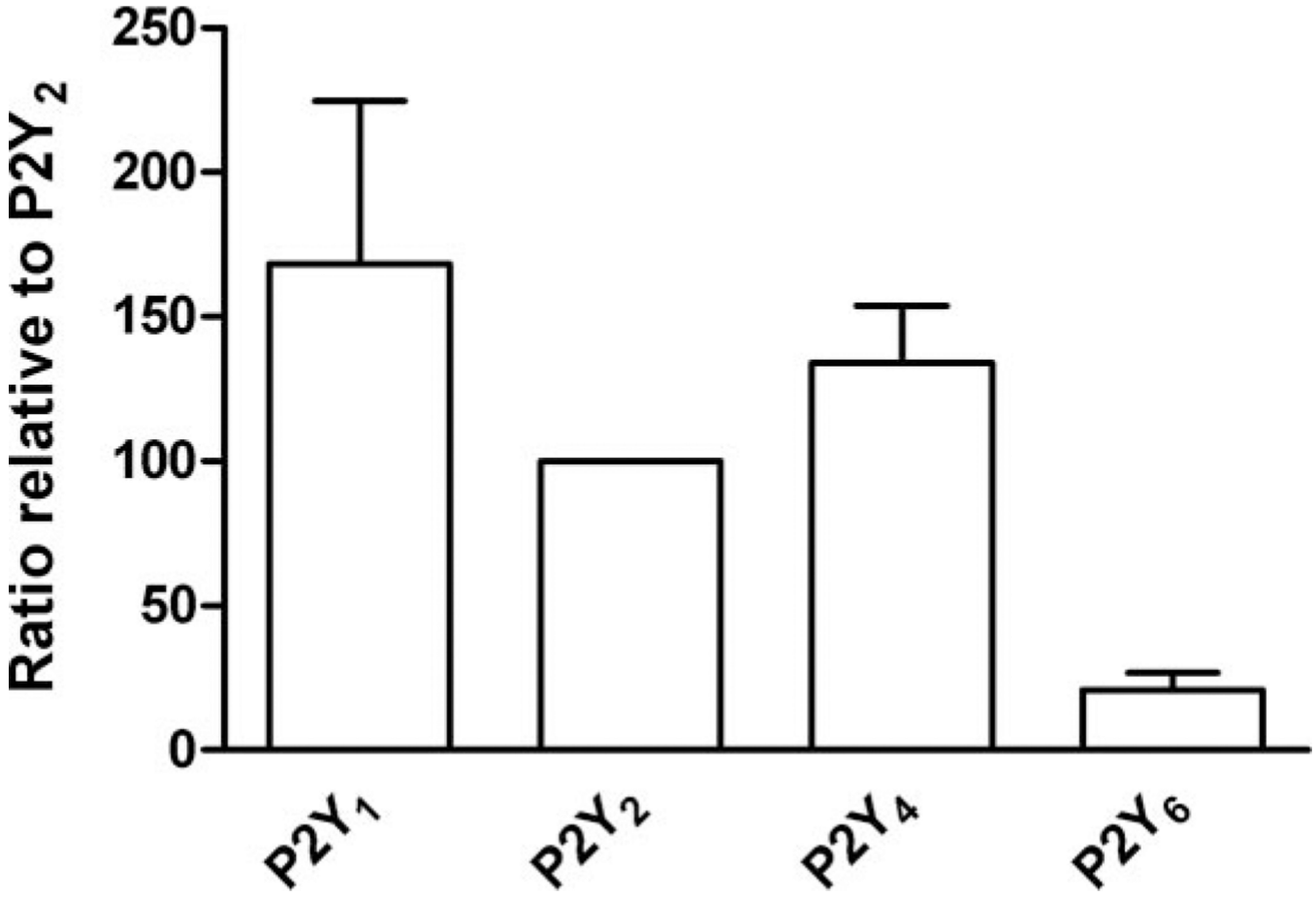

Figure 4.
The mRNA expression of P2 receptors in mouse cardiomyocytes analyzed with real-time PCR. Expression of $P2Y_1$, $P2Y_2$, $P2Y_4$, and $P2Y_6$ were detected. GAPDH was used as reference gene, and $P2Y_2$ was chosen to be the calibrator. n=5 mice.

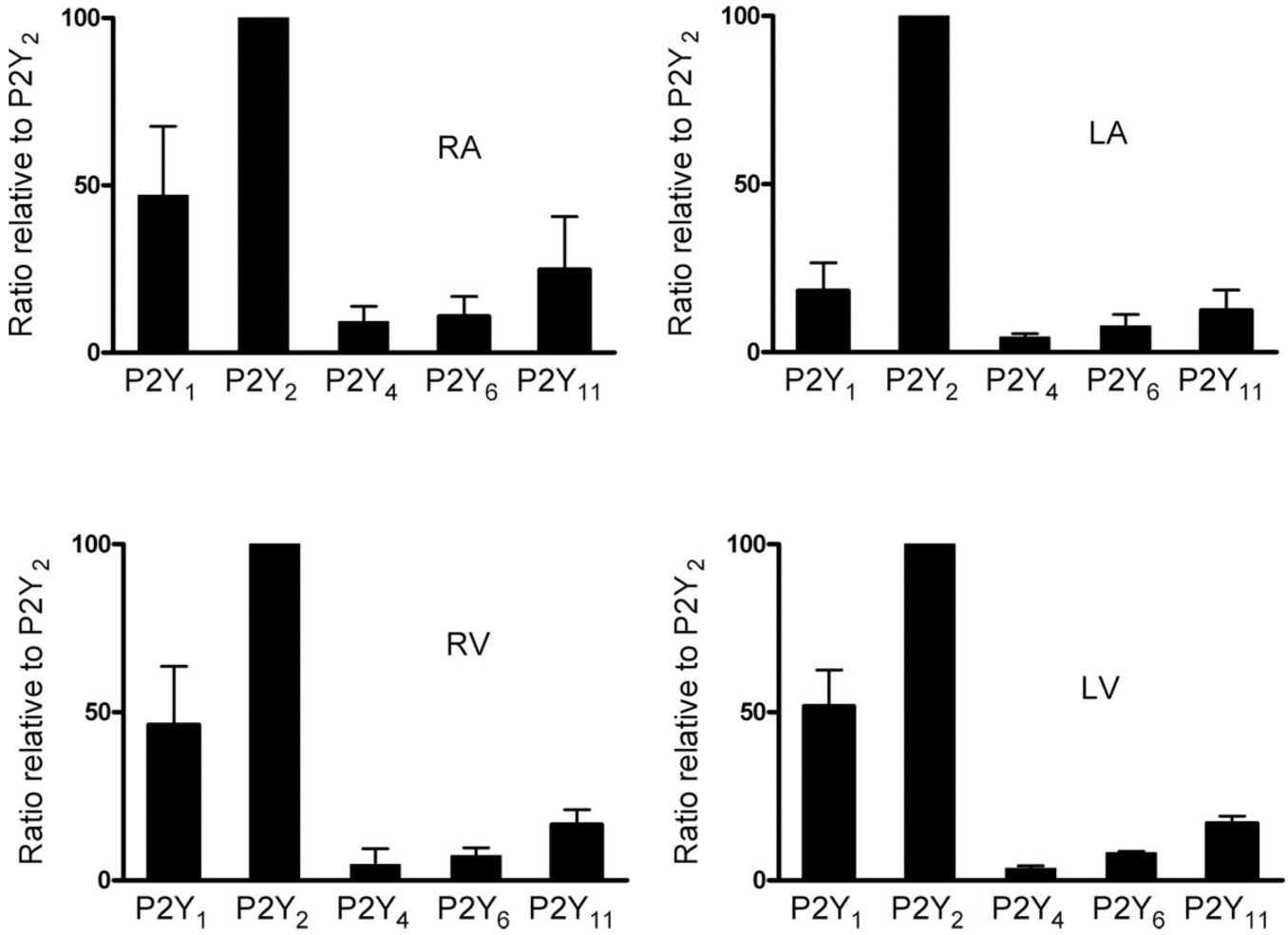

Figure 5.

Quantification of P2Y receptor mRNA in left and right ventricles (LV and RV) and atria (LA and RA) of human hearts analyzed with real-time PCR. Expression of $P2Y_1$, $P2Y_2$, $P2Y_6$, and $P2Y_{11}$ were detected. $P2Y_2$ was most abundant. Similar patterns were observed in right ventricle and both atria. Tissue from 5 patients was analyzed.

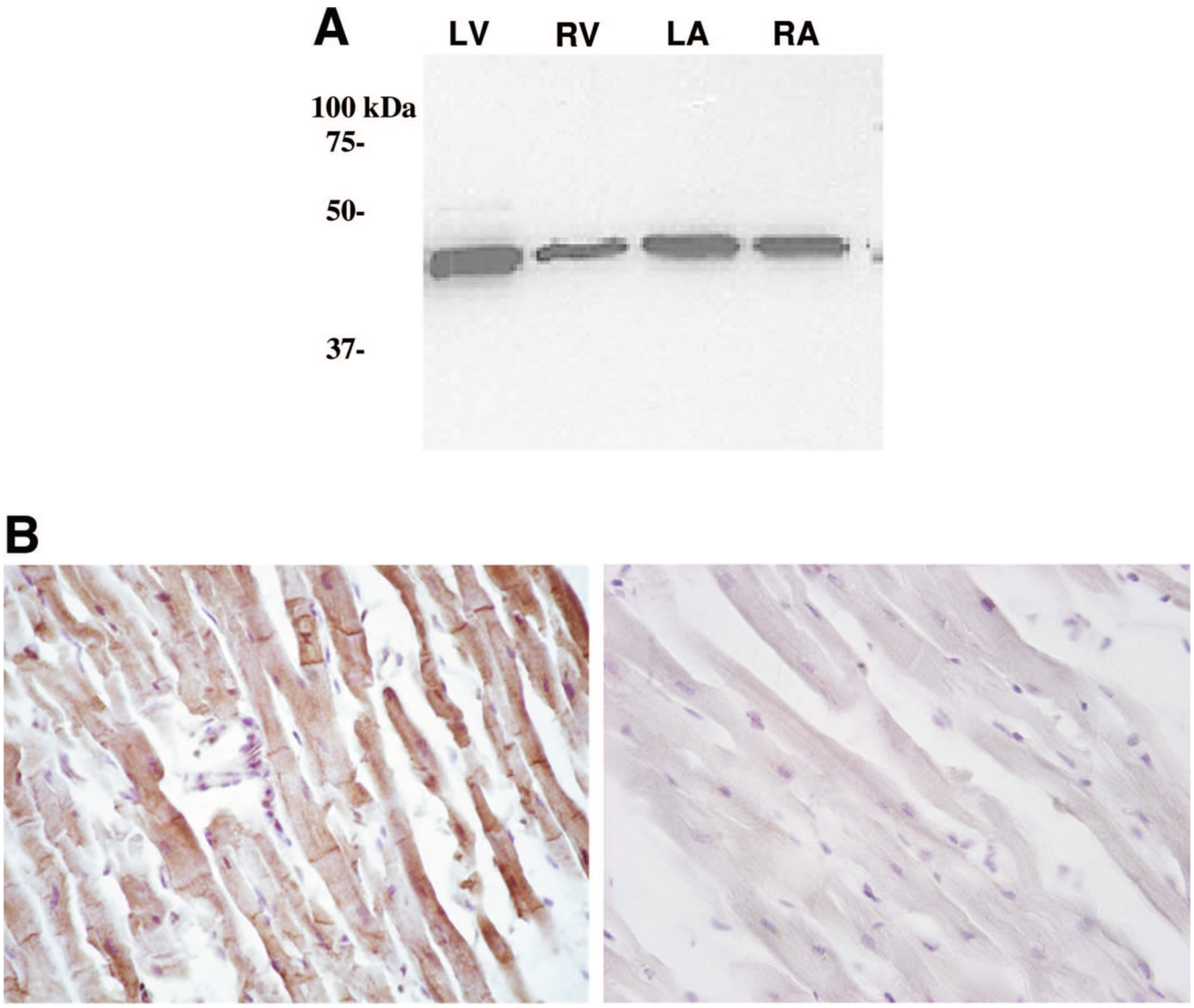

Figure 6.

$P2Y_6$ was detected using specific antibodies in immunohistochemistry and Western blot. a, Western blot on $P2Y_6$ in left and right ventricles (LV and RV) and atria (LA and RA) of human heart. The receptor was detected in both ventricles and atria of the human heart with a band of approximately 45 kD, which is in agreement with the expected size. No band was found in the control membrane, indicating that the band represents the $P2Y_6$ receptor. b, The same antibody was used in immunohistochemistry of human left ventricular heart tissue obtained from the explant heart at transplantation. Positive immunoreactive staining (brown) was seen for $P2Y_6$ (left) that was absent when control peptide was added or in controls without the primary peptide (right).