



Review Article

On inotropic effects of UTP in the human heart

J. Neumann^{a,*}, B. Hofmann^b, U. Gergs^a^a Institute for Pharmacology and Toxicology, Germany^b Cardiac Surgery, Medical Faculty, Martin-Luther University Halle-Wittenberg, 06097, Halle (Saale), Germany

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ABSTRACT

Uridine 5'-triphosphate (UTP) exerts a positive inotropic effect (PIE) in isolated electrically driven isolated right atrial trabeculae carneae from patients undergoing heart surgery. This review discusses some aspects of the current knowledge on the putative receptor(s) involved and the potential biochemical transduction steps leading to the PIE.

All mammalian cells contain uridine 5'-triphosphate (UTP), a pyrimidine derivivate. UTP is essential to form RNA and is therefore crucial for cellular function [1]. UTP can, at least under some conditions, stimulate the degradation of poly(A)⁺RNAs, UTP can stimulate enzymes directly involved in poly(A)⁺RNA, or formation of uridine cyclic phosphate at the 3' end of the RNAs [2]. In several mammalian species (Table 1, Fig. 1), UTP like ATP exerts substantial concentration- and time-dependent positive inotropic effects (PIE), notably in the human atrium where uridine 5'-diphosphate UDP is nearly ineffective (Fig. 1). While ATP has a biphasic effect (suggesting the activation of two different, but presently unknown receptors) on force of contraction in human atrium, an initial decline in force followed by a pronounced PIE, UTP exerts hardly any initial negative inotropic effect in human atrium but only a sustained positive inotropic effect (Fig. 1). The time course of the PIE of UTP is much slower than the time course of the PIE of β -adrenoceptor agonists in the human atrium (Fig. 1): this is one piece of evidence that different mechanisms are operative for the PIE of UTP compared to β -adrenergic stimulation in cardiomyocytes. In isolated right atrial preparations or living animals, UTP did not increase the rate of the heart beat (mouse: [3]). The PIE of UTP could be of clinical interest: the content of UTP in the extracellular space can be altered and thus might actively modulate contractility. UTP can be degraded by enzymatic (using for instance the enzyme CD39) break down on the surface of cardiovascular cells (Fig. 2) or by enzymes present in the

plasma [4] to UDP, UMP, uridine, pyrophosphate or phosphate. For instance, the concentrations of ATP (UTP levels were not reported and thus would be a valuable goal of a further study), were increased after application of the CD39 inhibitor polyoxotungstate (Na₆[H₂W₁₂O₄₀]), (see Fig. 2) [5]. Interestingly, under normal conditions CD39 was mainly expressed on endothelial cells, but after myocardial infarction CD39 was also detected on the surface of cardiomyocytes using immunohistochemistry [5]. The extracellular concentration of UTP is in the range of 10–100 nM. Conceivably, drugs might be given to patients that inhibit the degradation of UTP in the plasma or on cell membranes in the heart and this would be expected to lead indirectly to a PIE in the heart by increasing UTP levels in the circulation and hence in the vicinity of sarcolemmal receptors [5]. Vascular effects of UTP have to be considered: depending upon the species tested, the anatomic location of the vessel (coronary versus peripheral vessel) and even the prevalent disease state of the vessel investigated, both vasoconstrictory and vasodilatory effects of UTP have been reported [6, 7, 8]. This is an important issue for any drug with a PIE. If the drug induced a vasoconstriction of coronary arteries, cardiac ischemia would be induced and the supply of oxygen for the heart would be diminished and heart failure would be worsened. Likewise, vasoconstriction of peripheral resistance vessels would induce hypertension which would also be detrimental for cardiac function because more cardiac output would be required and hence the oxygen demand of the heart would increase and hence heart failure would be

* Corresponding author.

E-mail address: joachim.neumann@medizin.uni-halle.de (J. Neumann).

Table 1
Species dependent cardiac effects of UTP, ATP, Adenosine.

Species/tissue	UTP	ATP	Adenosine
Human atrium	PIE, antiadrenergic: [37]	Biphasic: NIE, PIE: [38]	Biphasic: NIE, PIE [39, 40, 41]
Human ventricle	n.d.	n.d.	No effect alone Antiadrenergic: [29, 30] NIE, NCE [45, 46]
Rat atrium	PIE: [42]	NIE, PIE: [42] NIE, PIE: biphasic [43] PIE: [44]	NIE, NCE [45, 46]
Rat ventricle		PIE: [47] NIE: [43]	NIE: [45]
Neonatal rat cardiomyocytes	PIE, PCE: [22]		
Guinea pig atrium		PIE: [48]	NIE, NCE [49, 50] [50]
Guinea pig ventricle			
Mouse atrium	PIE, no NIE	PIE, NCE	
Mouse ventricle	PIE: [37]	PIE: [47]	
Chicken ventricle	PIE: [51]	PIE: [51]	

PIE = positive inotropic effect; NIE = negative inotropic effect; NCE: negative chronotropic effect, PCE = positive chronotropic effect. Overview of the contractile effects of UTP, ATP and adenosine in several mammalian cardiac preparations. Species and regional differences are apparent.

expected to occur. For instance, infusion of UTP in patients in arterial vessels (forearm, nine healthy volunteers, mean age 27.2 years) led to vasodilatation (6). In vitro in porcine (six months old, mechanically removed endothelium) and human coronary arteries (heart transplant recipients, endothelium removed with Trion-X-100) UTP and moreover a metabolically stable derivate of UTP (UTP_γS) induced vasoconstriction with a EC₅₀-value of 30 μM [7, 8]. There seems, however, to be a role for the endothelium in human coronary arteries. If the endothelium is functionally intact, then UTP induces vasodilation in isolated human coronary arteries [9] and presumably in patients. Hence, it is probably not feasible to treat patients with heart failure as a consequence of coronary heart disease with UTP as a PIE, because in these patients UTP would induce vasoconstriction of the coronary arteries. However, if the endothelium were intact (patients with idiopathic cardiomyopathy that is without involvement of coronary arteries) UTP might be a useful

inotrope. The advantage to current inotropes like β-adrenoceptor agonists (e.g. dobutamine) or phosphodiesterase inhibitors (e.g. milrinone) come from the fact that UTP increase force of contraction without elevating cyclic AMP levels which is quite likely to induce cardiac arrhythmias.

In the intact outer cell membrane of cardiomyocytes and other cells types present in the human heart, there are proteins like P2X₇-receptors, pannexins, connexins or anion-maxi-channels [10, 11]. These proteins might extrude UTP (like ATP which is much better investigated) out of cells but pannexins and connexins transport UTP possibly bidirectionally and thus might also transport UTP from the outside into the inside of cells: hence addition of 100 μM UTP to atrial preparations to the organ bath would generate a gradient of UTP from the outside to the inside of cardiomyocytes: however this hypothesis has to be proven or disproven experimentally by using transport inhibitory drugs (Fig. 2). The concentration of UTP in the cell is about a tenth of the concentration of ATP (around 10 mM) (rat heart: [12], rat cardiomyocytes: [13]): so physiologically there is a gradient of UTP from the inside to the outside of the cell. Whatever the mechanism(s) of release (conceivably P2X₇-receptors, pannexins et cetera) might be, at least releases of UTP from endothelial cells and from cardiomyocytes have been reported: the released UTP may lead to autocrine or paracrine effects of UTP mediated by P2Y₂- (or P2X₂-) receptors located in the sarcolemma. Furthermore, it might be interesting to investigate whether P2-receptors exist in the intracellular space of cardiomyocytes. At least in rat liver, UTP can elevate Ca²⁺-transport into mitochondria by stimulation of functional P2-receptors (which have been identified also by immunological methods: [14]) and in addition, intracellular P2-receptors on lysosomes (in HEK-239 cells) and in nuclei (mouse liver cells) have been reported (review: [15]). If that were also the case, which needs to be elucidated, in cardiomyocytes, an interesting role for UTP acting on an intracellular signal transduction system might exist.

At least in the extracellular space a physiological regulation of the levels of UTP in the heart has been shown: upon cardiac ischemia, UTP levels in the plasma increase in pigs and humans [16]. Moreover, upon thrombin stimulation, UTP is released from erythrocytes [17]: such a release of UTP is expected to occur during myocardial infarction and might be another source of increased UTP in the vicinity of cardiomyocytes. In any case, released UTP might lead to a PIE, to cardiac protection against ischemia, tachycardia and other kinds of arrhythmia

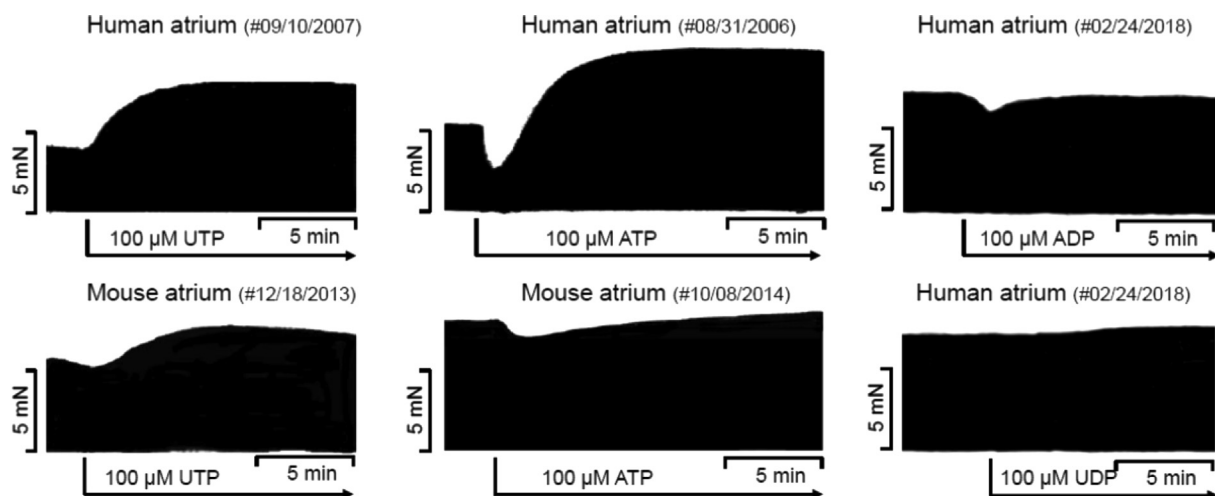


Fig. 1. Typical effects (original tracings) of UTP, UDP, ATP and ADP (100 μM, each) on force of contraction (ordinates) in isolated electrically driven (1 Hz) trabeculae carneae from patients undergoing bypass surgery (human atrium) or wild type mouse left atrium (mouse atrium). Numbers indicate date of experiment. Time scale indicated in the abscissae. Ordinates give force of contraction. ATP elicited biphasic inotropic effects; ADP solely transient negative inotropic effect, UTP a pronounced monophasic positive inotropic effect (PIE) and UDP induced a minor PIE (unpublished original observations). This study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki and has been approved by the ethics committee of the medical faculty of the Martin Luther University Halle-Wittenberg (hmbü 04.08.2005) and patients gave informed consent.

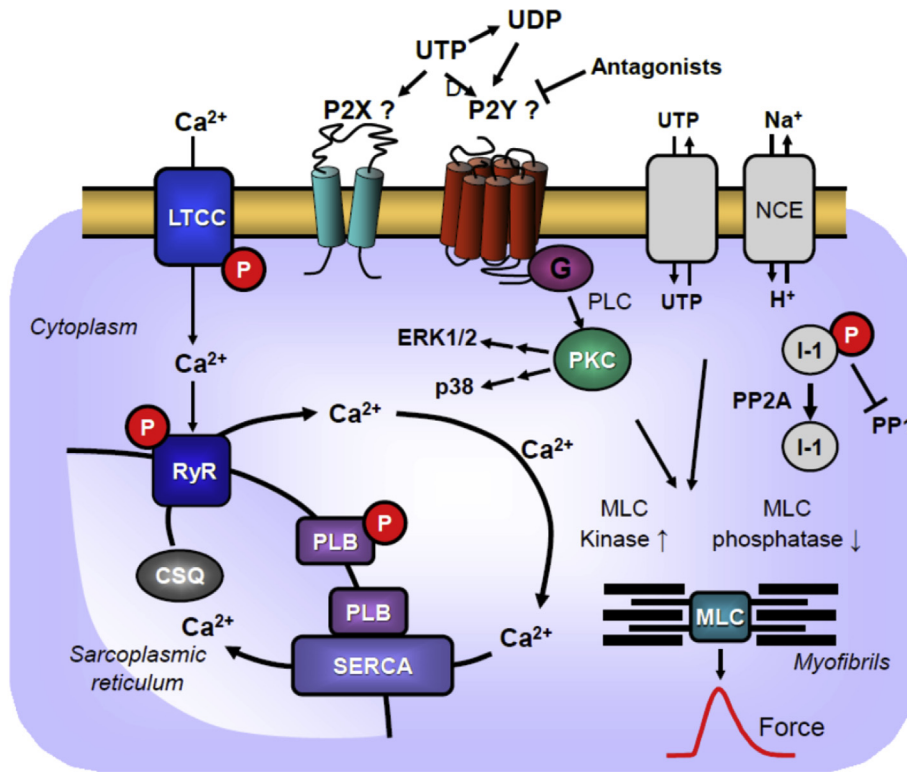


Fig. 2. Scheme: Putative mechanism(s) of the positive inotropic effect of UTP. UTP might act on P2X- or P2Y-receptors in human cardiomyocytes. UTP can be converted by ecto-nucleoside triphosphate diphosphohydrolases (CD39) to less active UDP. Antagonists might be able to differentiate between actions on these P-receptors. In addition, UTP might be transported in both directions through proteins in the sarcolemma. UTP possibly via P2Y- or P2X-receptor stimulation can transiently enhance the phosphorylation state and activity of ERK1/2 [17] and p38, which might explain the initial PIE of UTP. These phosphorylations might also increase the current through the L-type Ca²⁺ channel (LTCC) and/or release of Ca²⁺ from the sarcoplasmic reticulum (SR) via RYR2; both processes would increase force of contraction by increasing the Ca²⁺ acting on myofilaments. In diastole, Ca²⁺ is pumped via SERCA from the cytosol into the SR. Activity of SERCA is increased by phosphorylation of phospholamban. Moreover, UTP might act via altering phosphorylation state of NCE (Na⁺/Ca²⁺ exchanger [55, 56]) to increase the Ca²⁺ influx into the cytosol or lower the pH of the cell. Moreover, sustained PIE of UTP might follow from an increase in the phosphorylation state of the myosin light chains (MLC) by activation of MLC kinase (following MAP kinase pathways) and/or inhibition of the activity of MLC phosphatase [57, 58]. The latter effect might follow from inhibition of PP2A (a serine/threonine phosphatase: PP) activity by MAP kinases and subsequent increased phosphorylation state and thus activation of I-1 (a specific inhibitory protein of PP1) which will lead to decreased activity of PP1. Reduced activity of PP2A [25] (and/or PP1) can increase phosphorylation of additional proteins [59] and might thus increase the Ca²⁺ sensitivity of myofilaments which would increase force of contraction.

[18]. Whether UTP really causes arrhythmia or is an innocent bystander, cannot be decided from previous work: only a correlation was observed [18]: if ischemia was induced in pig hearts in vivo by occluding a coronary artery the level of UTP in the blood (in the coronary sinus) increased at times where arrhythmias occurred [18]. More work in this respect is clearly warranted to prove causality.

A role of UTP in cardiac protection is suggested by the observation that UTP pre-treatment (before induction of ischemia) of neonatal rat cardiomyocytes attenuated the ischemia induced increase in mitochondrial Ca²⁺-levels [19]. These effects in vitro were thought to be mediated by a degradation product of UTP namely pyrophosphate (discussed in [20]). However, pyrophosphate was ineffective in vivo, but the protection mediated by UTP was absent in P2Y^{-/-} mice, suggesting, that the protection were mediated by this receptor. The signal transduction system of UTP (Fig. 1, Table 1) in the heart exhibits species differences. UTP, like ATP it is known to increase the phosphorylation states of MAP kinases in cardiomyocytes. For instance, UTP led to concentration- and time-dependent increases in the phosphorylation states of ERK1/2 and p38 in neonatal rat cardiomyocytes [21], adult mouse cardiomyocytes [21] and isolated human atrial preparations [22]. The increases in the phosphorylation states of ERK1/2 were transient (within 10–20 min) whereas the increases in contractile response were permanent for the time studied (at least 30 min: [22], and Fig. 1). Thus MAP kinases could be involved in the initiation of the PIE of UTP but the mechanism for the maintenance of the PIE of UTP is unknown and is suggested to result from other protein phosphorylation events. Previously, it had been shown that inhibition of p38 MAP kinase by the drug SB203580 exerted in vitro an increase in contractility [23], probably by increasing the Ca²⁺ sensitivity of the myofilaments [24]. More recently evidence was provided that p38 might modulate force of contraction by altering the activity of SERCA, a protein that is important for the Ca²⁺ homeostasis of the heart [25].

Others have also shown that the UTP-dependent protection of cardiomyocytes was independent of ERK-phosphorylation as an inhibitor of ERK phosphorylation, called U0126, did not attenuate the UTP-mediated cardiac protection against hypoxia [26]. An attractive hypothesis would be that an increase in the phosphorylation state of myosin light chains (MLC, Fig. 2) might explain the sustained PIE of UTP in the human heart.

It is difficult to say whether the PIE of UTP in human right atrium, is region specific or a general phenomenon: UTP apparently has not yet been studied with respect to a PIE in isolated preparations from the left human atrium, the right human ventricle or the left human ventricle. Such data are expected with interest. Moreover, the receptor(s) mediating the positive inotropic effect of UTP in the human heart have not yet been clearly identified. Basically, one can distinguish P1- and P-2 receptors (originally called purinoceptors, but as UTP is a pyrimidine and acts on these receptors, the abbreviation P-receptor is currently used [27]). Adenosine acts on P1-receptors which are subdivided into A1-, A2A-, A2B- and A3-receptors [28]. ATP (and UTP) act on P2-receptors, that are subdivided into P2X- and P2Y-receptors. P2X-receptors are ion channels. Their conductance are typically increased by stimulation with ATP (or possibly UTP), whereas P2Y-receptors belong to the family of G-protein coupled receptors and are usually thought to act via alteration (activation or inhibition) of adenylyl cyclase (AC) activity or phospholipase C-pathways. As seen in Table 2, there is in general a lack of potent, selective, specific antagonists for some P2-receptors, which is a limitation for pharmacology studies on the effects of UTP. As soon as newer antagonists (more potent, more selective, more specific) become available, more information on the receptor(s) involved in the PIE of UTP in the human heart should emerge. Another avenue to define the receptor(s) which mediate the PIE of UTP in the atrium, would be the study of knock out (KO) mouse models, possibly also KO mice of orphan receptors from the P2-receptor family. Some data in this regard have been produced

Table 2
Putative receptors for UTP in the human heart.

Receptor	Agonist	Antagonist	Human UTP effect antagonist action	UTP in KO mice
P2X1	ATP	PPADS (good), suramin (good)	-	
P2X2	ATP	PPADS (good), suramin (good)	-	
P2X3	ATP	PPADS (good), suramin (good)	-	
P2X4	ATP	PPADS (poor),	?	
P2X5	ATP	PPADS (good), suramin (good)	-	
P2X6	ATP	PPADS (poor)	?	
P2X7	ATP	PPADS (poor)	?	
P2Y1	ATP	PPADS (good), RB2 (good), suramin (good)	-	
P2Y2	UTP, ATP	RB2 (poor), suramin (good) AR-C1118925a	-	-
P2Y4	UTP, ATP	PPADS (poor), PSB-1454 (good)	-	-
P2Y6	UTP, UDP	PPADS (poor), suramin (poor) MRS2578 (good)	-	-
P2Y11	ATP, UTP	RB2 (good), suramin (good)	-	
P2Y12	ADP	RB2 (good), suramin (good)	-	
P2Y13	ADP	PPADS (poor), RB2 (good), suramin (good)	-	
P2Y14	UDP-glucose		-	
CystLT1R	UDP		-	
CystLT2R	UDP		-	

[33, 30, 52, 53, 54]: no or negligible contractile effect in isolated electrically driven trabeculae 21, 3: carneae from human right atrium. It is apparent that presently only negative reports (-), indicating lack of identification of underlying receptor for PIE of UTP in the human heart is available. A question mark indicates that no clearcut published information is to be found in the literature.

(Table 2). However, these data have not yet positively identified which KO mouse would show a lack of UTP effects in the mouse heart. Moreover, there are receptors in the mouse which are not present in humans and vice versa. Hence, it cannot be ruled out that work on KO mouse might fail to predict which receptor is used by UTP in the human heart. Based on work from tissues other than the heart (Table 2), UTP is mainly expected to act via P2Y₂, P2Y₄, P2Y₆, P2Y₁₁ or possibly P2Y₁₄-receptors [27, 28, 29, 30]. However, our present data do not allow one to identify any of these receptors as the receptor that mediates the PIE of UTP in the isolated human right atrial trabeculae (Table 2), because the PIE of UTP were not antagonized by pre-incubation of the samples with typical antagonists of P2Y-receptors, that are currently available. While P2Y-receptors can act in various tissues via AC or phospholipases, using inhibitors of the activity of AC or phospholipases, we could not block the PIE of UTP in the human heart, which also argues against an action of UTP on P2Y receptors on the sarcolemma what would then translate to altered activity of AC and phospholipases in the interior of the cell [3]. However, there are species differences in the inotropic effects of UTP (see Table 1). For instance, in the mouse atrium, UTP or ATP initially exert a negative inotropic effect which is reversible over time. The reversible negative inotropic effect in mouse atrium can be regarded as the mixture of a persistent negative inotropic effect mediated by a certain receptor, the effect of which is overcome in due time by a positive inotropic effect of another receptor (Fig. 1). Some even claim that UTP might act via P2X-receptors [31], but for some P2X-receptors good antagonists are currently also not available (Table 2). Another explanation for the lack of known P-receptors antagonists to block the PIE of UTP in the human atrium is the hypothesis that these effects are mediated via orphan

receptors (no antagonists available) or heteromeric P-receptors (review: [32, 33]) with incompletely understood antagonist sensitivities [34].

From the literature the time course of PIE of UTP action in the human heart is comparable with that of the PIE of stimulation of α_1 -adrenoceptors by noradrenaline in the human heart, that is probably mediated via phosphorylation of (regulatory myosin light chain) MLC-2 [35]. One would test the assumption that UTP acts via the latter mechanism by studying whether the PIE of UTP in human atrium is accompanied by MLC-2 phosphorylation, but that has not yet been reported (Fig. 2). We speculate that UTP in intact form might enter the cardiomyocyte: this might be thermodynamically conceivable if there were local compartments of UTP. In other words, UTP was initially measured in homogenized cardiomyocytes [13]. However, we know now that for instance local concentrations can be high and cytosolic near the sarcolemma can be low: the accepted paradigms for the existence of intracellular compartments include Ca^{2+} and cyclic AMP. Hence, we suggest it might be an interesting avenue to synthesize UTP sensitive fluorescent dyes and stain cardiomyocytes to ascertain whether compartments of UTP in the cell exist (or not). Conceivably, intracellular UTP (if it would enter from the outside of the cell) might be used by MLC kinase to phosphorylate MLC-2 and thus increase its phosphorylation state (and thus force) or pyrophosphate (a degradation product of UTP) might inhibit MLC phosphatase, increase the phosphorylation state of MLC-2 (and thus force: compare Fig. 2). One could alternatively study whether the PIE of UTP were blocked by myosin kinase inhibitors which have been shown to block the PIE of α_1 -adrenergic receptors in the heart [35], or whether the PIE of UTP is enhanced in the presence of inhibitors of the activity of MLC phosphatase, for instance a Rho-associated kinase leads to MLC phosphorylation by inactivating myosin phosphatase. Fittingly, the Rho-kinase inhibitor (+)-(R)-trans-4-(1-aminoethyl)-N-(4-pyridyl) cyclo-hexanecarboxamide (Y-27632, 50 μ M) blocked the effect of PIE of α_1 -adrenergic receptors in the heart [34]. However, this approach has also its limitation because typical MLC kinase inhibitors like 1-(5-chloronaphthalene-1-sulfonyl)1H-hexahydro-1,4-diazepine (ML-9) at the concentrations (10–50 μ M) usually used, will also inhibit cAMP- and cGMP-dependent protein kinases and might thus not lead to conclusive findings [36]. Interestingly, ERK kinase inhibitors like PD-98059 (10 μ M) and SB-203580 (10 μ M) did not reduce the PIE of α_1 -adrenergic receptors in the heart [35] which is opposite to our finding that the PIE of UTP was attenuated by the ERK inhibitor U0126 [21], possibly suggesting that the PIE of α_1 -adrenergic receptors and UTP in the heart do not use identical signal transduction pathways [35].

Other known positive inotropic mechanisms (which are used by ATP in the heart) like an increase in intracellular pH and increased Ca^{2+} -transients in the cytosol [32] could be measured after stimulation of human atrial myocytes with UTP. In summary, there is a PIE of UTP in the human heart. However, the receptor(s) involved and exact signal transduction mechanism(s) for the PIE remain to be elucidated.

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