Age-dependent differential crosstalk between alpha(1)-adrenergic and angiotensin receptors

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BACKGROUND: Previous reports of crosstalk between alpha(1)-adrenergic receptors (α_1 -AR) and angiotensin receptors (ATR) have pointed to the existence of physiological regulation between the sympathetic nervous system and the renin-angiotensin system at the receptor level. This regulation may have an important role in the control of blood pressure and may be modified in different cardiovascular pathologies. Aging is considered to be an independent cardiovascular risk factor. Nevertheless, neither the variation in physiological action or interaction of signal transduction between these two receptors as a result of aging has been established. To clarify these aspects, the interaction between α_1 -AR and ATR was evaluated.

METHODS: The inotropic response of α_1 -AR to agonists was assessed in the presence and absence of angiotensin II using the left atria of 3.5-, 12-, 18- and 24-month-old (young adult, middle aged, elderly and aged, respectively) male Wistar rats. In the four age groups of rat hearts, the activities of tyrosine kinase were measured when just the AT₁R subtype was activated, or when both α_1 -AR and AT₁R were activated. The activities of cytosolic phospholipase A₂ and the levels of cyclic GMP were investigated when just the AT₂R subtype was activated, or when both α_1 -AR and AT₂R were activated.

RESULTS: No effect was found on the cumulative concentration-response curve for phenylephrine when AT₁R was activated in 3.5- or 12-month-old rats. However, in 18- and 24-month-old rats, the maximum positive inotropic response and the negative logarithm of the effective 50% concentration increased markedly. No effect was found on the cumulative concentration-response curve induced by phenylephrine when AT₂R was activated. The activities of tyrosine kinase increased significantly in 3.5- and 12-month-old rats, but there was no difference in 18- and 24-month-old rats when α_1 -AR and AT₁R were both activated compared with when just AT₁R was activated. Cytosolic phospholipase A₂ activity and cyclic GMP levels decreased significantly when both α_1 -AR and AT₂R were activated compared with when just AT₂R was activated.

CONCLUSIONS: In the isolated left atria of elderly and aged rats, the activation of AT₁R enhanced the positive inotropic response induced by the activation of α_1 -AR. The activation of AT₂R had no effect on the positive inotropic response induced by the activation of α_1 -AR. The action of α_1 -AR increased the signal transduction of AT₁R in young-adult and middle-aged rat hearts but had no effect in elderly and aged hearts. The action of α_1 -AR had no effect on AT₂R signal transduction.

Key Words: Age factors; Alpha(1)-adrenergic receptor; Angiotensin type 1 receptor; Angiotensin type 2 receptor; Crosstalk

Interférence différentielle liée à l'âge entre les récepteurs alpha(1)-adrénergiques et les récepteurs de l'angiotensine

HISTORIQUE : Des rapports antérieurs sur l'interférence entre les récepteurs alpha(1)-adrénergiques (RA- α_1) et les récepteurs de l'angiotensine (RAT) ont évoqué l'existence d'une régulation physiologique entre le système nerveux sympathique et le système rénine-angiotensine au niveau des récepteurs. Cette régulation pourrait jouer un rôle important dans le contrôle de la tension artérielle et subir l'influence de diverses pathologies cardiovasculaires. Le vieillissement est considéré comme un facteur de risque cardiovasculaire indépendant. Néanmoins, on n'a pas déterminé l'impact du vieillissement sur les variations des effets physiologiques des deux récepteurs et l'interaction de leurs signaux. Pour clarifier ces questions, les auteurs ont analysé l'interaction entre les RA- α_1 et les RAT.

MÉTHODE : Les auteurs ont étudié la réponse inotrope des RA- α_1 aux agonistes en présence et en l'absence d'angiotensine II, dans des oreillettes gauches de rats Wistar mâles de 3,5, de 12, de 18 et de 24 mois (spécimens respectivement jeunes adultes, d'âge moyen, âgés et très âgés). On a mesuré l'activité de la tyrosine-kinase dans les cœurs de rats des quatre catégories d'âge, lorsque seul le sous-type de RAT₁ était activé ou lorsque les deux types (RA- α_1 et RAT₁) étaient activés. Les auteurs ont aussi analysé l'activité de la phospholipase cytosolique A₂ et les taux de GMP cyclique lorsque seul le sous-type RAT₂ était activé ou lorsque le RA- α_1 et le RAT₂ étaient activés.

RÉSULTATS : Les auteurs n'ont observé aucun effet sur la courbe cumulative concentration-réponse à la phényléphrine lorsque le RAT₁ était activé chez les rats de 3,5 ou de 12 mois. Toutefois, chez les rats de 18 et de 24 mois, la réponse inotrope positive maximum et le logarithme négatif de la dose entraînant 50 % de l'effet maximum ont nettement augmenté. On n'a observé aucun effet sur la courbe cumulative concentration-réponse induite par la phényléphrine lorsque le RAT₂ était activé. Les effets de la tyrosine-kinase ont significativement augmenté chez les rats de 3,5 et de 12 mois, mais on n'a noté aucune différence chez les rats de 18 et de 24 mois lorsque le RA- α_1 et le RAT₁ étaient tous deux activés, comparativement à l'activation du RAT₁ seulement. Les taux d'activité de la phospholipase cytosolique A₂ et du GMP cyclique ont diminué significativement lorsque le RA- α_1 et le RAT₂ étaient activés, comparativement à l'activation du RAT₂ seulement.

CONCLUSION : Dans l'oreillette gauche isolée de rats âgés et très âgés, l'activation du RAT₁ a rehaussé la réponse inotrope positive induite par l'activation du RA- α_1 . L'activation du RAT₂ n'a exercé aucun effet sur la réponse inotrope positive induite par l'activation du RA- α_1 . L'action du RA- α_1 a amplifié la transduction du signal du RAT₁ dans les cœurs de rats jeunes adultes et d'âge moyen, mais n'a exercé aucun effet sur les cœurs des rats âgés et très âgés. L'action du RA- α_1 n'a pas influé sur la transduction du signal du RAT₂.

Angiotensin II (Ang II) has a principal role in mediating the physiological actions of the renin-angiotensin system, which is critical for the development and progression of hypertension and atherosclerosis, cardiac hypertrophy, heart failure and diabetic renal

disease (1,2). There are two main categories of Ang II receptors (ATRs): AT₁R and AT₂R. The expression of AT₁R does not vary significantly with age up to adulthood. AT₂R is expressed at low levels in adult rat cardiac myocytes, while AT₁R and AT₂R expression are

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upregulated in senescent hearts (3). In addition, AT_1R stimulation is known to promote cardiovascular hypertrophy and fibrosis (4), whereas AT_2R activation is believed to oppose those AT_1R -mediated effects (5). Our previous studies also showed that the activation of AT_1R inhibited the signal transduction of AT_2R during the aging process and the activation of AT_2R inhibited signal transduction of AT_1R in every age group (6). Furthermore, experimental evidence suggests that AT_1R blockade by AT_1R antagonists or reduction of Ang II synthesis by angiotensin-converting enzyme inhibitors lowers blood pressure and prevents target-organ disease (7-9).

Alpha(1)-adrenergic receptors (α_1 -ARs) are $G_{q/11}$ -coupled receptors that respond to the neurotransmitters and hormones noradrenaline and adrenaline to mediate physiological effects (10). Acute stimulation of cardiac α_1 -ARs induces positive inotropic effects and electrophysiological alterations, whereas chronic stimulation of cardiac α_1 -ARs leads to cardiac hypertrophy (11).

Experimental evidence suggests that the renin-angiotensin system and the sympathetic nervous system have an effect on each other at the level of the central nervous system and the peripheral vascular system (12-15). Ang II facilitates neurotransmitter release from the presynaptic nerve terminals (16), which can cause vasoconstriction and myocardial damage. Furthermore, Ang II directly stimulates muscle sympathetic nerve activity and facilitates adrenergic sympathetic transmission (17). In addition, stimulation of the sympathetic nervous system promotes renin secretion and Ang II formation (17). Experimental evidence suggests that these two systems also interact at the receptor level and beyond. In vascular tissue neurons, chronic activation of α_1 -AR results in downregulation of AT_1R (18,19). It has been shown that Ang II selectively downregulates α_{1A} -AR subtype messenger RNA and its corresponding receptors in neonatal rat cardiomyocytes (11). Furthermore, evidence suggests that crosstalk between α_1 -AR and ATR in the smooth muscle of rabbit aortas is dependent on the endothelium (20). Furthermore, ATR or α_1 -AR antagonists effectively block downstream signalling and trafficking of the two receptors simultaneously (21). However, the influence of age on the interaction between α_1 -AR and ATR has not been studied. The objectives of the present study were to determine whether the effect of ATR on the α_1 -AR-mediated inotropic response varied and whether the effect of α_1 -AR on ATR signal transduction varied in the hearts of rats in different age groups.

METHODS

Animals

Male Wistar rats (purchased from Vital River Lab Animal Technology Co Ltd, Beijing, China) were used for the experiments. The rats were 3.5 months (young adult), 12 months (middle aged), 18 months (elderly) and 24 months (aged) of age. All experimental procedures and protocols used in the present study conformed to the National Institutes of Health's *Guide for the Care and Use of Laboratory Animals* (22).

Drugs

Ang II, PD123319 (a selective antagonist of AT_2R), prazosin, isoprenaline, phenylephrine (PE) and a tyrosine kinase assay kit system were all obtained from Sigma-Aldrich (USA). Losartan (a selective antagonist of AT_1R) was obtained from Merck and Co Inc (USA). A cytosolic phospholipase A_2 (cPLA₂) assay kit system was obtained from Cayman Chemical Co (USA). An iodine-125-cyclic GMP (cGMP) enzymatic radioimmunoassay kit was obtained from the Shanghai College of Traditional Chinese Medicine (Shanghai, China). All other chemicals were reagent or molecular biology grade and obtained from standard commercial sources.

Inotropic response

Rats were anesthetized with intraperitoneal sodium pentobarbitone (45 mg/kg) and decapitated. The hearts were carefully excised and washed with modified Krebs solution (118.3 mmol/L of sodium chloride, 4.7 mmol/L of potassium chloride, 2.5 mmol/L of calcium

chloride, 1.2 mmol/L of magnesium sulfate, 1.2 mmol/L of potassium dihydrogen phosphate, 0.026 mmol/L of EDTA, 25.0 mmol/L of sodium bicarbonate and 11.1 mmol/L of glucose at a pH of 7.40) and the left atria were rapidly removed. After removing the connective tissue, blood vessels and adherent fat, the ventricles were weighed, minced and incubated in modified Krebs solution for the next step of the experiment. A 10 mL volume of Krebs solution containing 30 μ mol/L propranolol was aerated with 95% O_2 and 5% CO_2 , and maintained at 37°C. The inotropic response of the left atria was measured as described previously (23). The tissues were attached to a force-displacement transducer for measurement of isometric tension and stimulated by electrical pacing (1 Hz for 5 ms at two times the threshold voltage). A resting tension of 0.5 g was applied to all of the preparations. In the experiments examining α_1 -AR- or ATR-mediated positive inotropic response, cumulative concentration-response curves for PE (1 nmol/L to 30 μ mol/L) and Ang II (1 nmol/L to 30 μ mol/L) in the presence of losartan and/or PD123319 or prazosin were generated. The potency of the drugs was expressed using the negative logarithm of the molar concentration of the drugs inducing 50% of the maximal contraction (pD_2) and the maximal contractile response was calculated using a curve-fitting program.

In the experiments examining the effect of AT_1R stimulation on the α_1 -AR-mediated positive inotropic response, cumulative concentration-response curves (CCRCs) for PE were generated first (CCRC I). After washing and a 30 min equilibration period, preparations were incubated with Ang II (100 nmol/L) and PD123319 (1 μ mol/L) for 30 min. After adjusting this tension to baseline, CCRCs for PE were repeated (CCRC II). In the experiments examining the effect of AT_2R stimulation on the α_1 -AR-mediated positive inotropic response, CCRCs for PE were generated first (CCRC I). After washing and a 30 min equilibration period, preparations were incubated with Ang II (100 nmol/L) and losartan (100 nmol/L) for 30 min. After adjusting this tension to baseline, CCRCs for PE were repeated (CCRC III).

Measurement of tyrosine kinase activities

To investigate whether the activation of α_1 -AR had any effect on tyrosine kinase activity that was stimulated by the activation of AT_1R , 100 mg heart fragments were subjected to Ang II (1×10^{-8} mol/L) and PD123319 (5×10^{-7} mol/L) plus prazosin (1×10^{-7} mol/L), and to Ang II and PD123319 plus PE (1×10^{-6} mol/L). Tyrosine kinase activities were determined using the tyrosine kinase assay kit system. Tyrosine kinase activity in the sample was extrapolated from the epidermal growth factor receptor activity graph (absorbance at 492 nm versus units of epidermal growth factor receptor activity). Protein concentration was determined using the Bradford protein assay.

Measurement of cPLA₂ activities

To investigate whether the activation of α_1 -AR had any effect on cPLA₂ activities that were stimulated by the activation of AT_2R , additional 100 mg heart fragments were perfused with Ang II and losartan (1×10^{-7} mol/L) plus prazosin (1×10^{-7} mol/L), and with Ang II and losartan plus PE (1×10^{-6} mol/L). The dose of losartan was chosen based on previous studies that demonstrated a selective AT_1R blockade in response to Ang II (9). cPLA₂ was detected using the cPLA₂ assay kit system. cPLA₂ activity in the sample was extrapolated from the bee venom PLA₂ activity graph (absorbance at 405 nm versus units of bee venom PLA₂).

Measurement of cGMP content

To investigate whether the activation of α_1 -AR had any effect on cGMP content that was stimulated by the activation of AT_2R , additional 100 mg heart fragments were perfused with Ang II and losartan (1×10^{-7} mol/L) plus prazosin (1×10^{-7} mol/L), and with Ang II and losartan plus PE (1×10^{-6} mol/L). cGMP levels were determined by iodine-125-cGMP enzymatic radioimmunoassay kits and expressed as mol/mg protein.

TABLE 1

Effect of angiotensin II on the negative logarithm of the effective 50% concentration (pD_2) to phenylephrine in isolated left atria of rats in different age groups

Age, months	n	pD_2		
		CCRC I	CCRC II	CCRC III
3.5	8	5.47±0.14	5.58±0.13	5.38±0.16
12	8	5.45±0.15	5.59±0.18	5.39±0.15
18	7	5.20±0.25	5.53±0.23*	5.29±0.33
24	6	5.19±0.27	5.54±0.23*	5.30±0.24

* $P<0.05$ compared with control by paired t test. CCRC Cumulative concentration-response curves

TABLE 2

Effect of angiotensin II on the maximum response (R_{max}) to phenylephrine in isolated left atria of rats in different age groups

Age, months	n	R_{max}		
		CCRC I	CCRC II	CCRC III
3.5	8	144.1±13.9	149.6±12.0	139.3±15.5
12	8	137.9±14.7	146.7±17.9	132.1±14.4
18	7	94.5±18.6	118.4±18.1*	81.7±19.0
24	6	55.6±12.0	82.6±12.3**	46.1±8.4

* $P<0.05$; ** $P<0.01$ compared with control by paired t test. CCRC Cumulative concentration-response curves

Statistical analysis

All data are expressed as mean $\pm$ SD. The Student's paired t test was used to determine the statistical significance of differences. All data were evaluated statistically using SPSS 11.5 (SPSS Inc, USA) software. Statistical significance was accepted at $P<0.05$.

RESULTS

Influence of ATR stimulation on α_1 -AR-mediated positive inotropic response in the atria of rats in different age groups

The contractile response to PE (1 nmol/L to 30 μ mol/L) was dose-dependent in the four age groups of rats. The pD_2 values of CCRC I were similar to CCRC II in the left atria of 3.5- (5.47±0.14 versus 5.58±0.13; $P>0.05$) and 12-month-old (5.45±0.15 versus 5.59±0.18; $P>0.05$) rats (Table 1). However, a significant shift to the left of CCRC II was observed in the atria of 18- and 24-month old rats. The pD_2 values were increased in the atria of 18- (5.20±0.25 versus 5.53±0.23; $P<0.05$) and 24-month-old (5.19±0.27 versus 5.54±0.23; $P<0.05$) rats. No significant differences were found in the maximal response to PE between CCRC I and II from the atria of either 3.5- or 12-month-old rats. However, the maximal response of CCRC II was significantly greater than that of CCRC I in the atria of both 18- (118.4±18.1 versus 94.5±18.6; $P<0.05$) and 24-month-old (82.6±12.3 versus 55.6±12.0; $P<0.01$) rats (Table 2).

Ang II and losartan treatment to inhibit the AT_1 R activation and to activate AT_2 R between CCRC I and CCRC III had no influence on the pD_2 in the atria from any of the four age groups (5.47±0.14 versus 5.38±0.16 in 3.5-month-old rats, $P>0.05$; 5.45±0.15 versus 5.39±0.15 in 12-month-old rats, $P>0.05$; 5.20±0.25 versus 5.29±0.33 in 18-month-old rats, $P>0.05$; and 5.19±0.27 versus 5.30±0.24 in 24-month-old rats, $P>0.05$) (Table 1). As well, Ang II and losartan treatment did not modify the maximal contractile response to PE between the CCRC I and CCRC III in the left atria from any of the four age groups of rats (144.1±13.9 versus 139.3±15.5 in 3.5-month-old rats, $P>0.05$; 137.9±14.7 versus 132.1±14.4 in 12-month-old rats, $P>0.05$; 94.5±18.6 versus 81.7±19.0 in 18-month-old rats, $P>0.05$; and 55.6±12.0 versus 46.1±8.4 in 24-month-old rats, $P>0.05$) (Table 2).

Figure 1 Effects of phenylephrine (PE) on angiotensin II (Ang II)-induced tyrosine kinase activities in the left atria of rats in different age groups. * $P<0.05$; ** $P<0.01$ compared with Ang II+PD123319 by paired t test. Pro Protein

Figure 2 Effects of phenylephrine (PE) on angiotensin II (Ang II)-induced cytosolic phospholipase A_2 (cPLA $_2$) activities in the left atria of rats in different age groups. * $P<0.05$; ** $P<0.01$ compared with Ang II+losartan by paired t test. Pro Protein

Effects of PE on Ang II-induced tyrosine kinase activities in rats of different ages

The tyrosine kinase activities induced by Ang II were increased in 3.5- and 12-month old rats when α_1 -AR was activated by PE. In 18- and 24-month-old rats, tyrosine kinase activities induced by Ang II did not significantly increase when PE was present (Figure 1).

Effects of PE on Ang II-induced cPLA $_2$ activity and cGMP content in the hearts of rats in different age groups

When the α_1 -AR was activated by PE, both cPLA $_2$ activity and cGMP content were significantly decreased in all four age groups of rat hearts compared with when only AT_2 R was activated (Figure 2 and Table 3).

DISCUSSION

There are more than 1000 receptors that couple with G proteins; α_1 -AR and ATR are two of them (24). Acute stimulation of cardiac α_1 -AR by neurotransmitters such as noradrenaline and adrenaline induces positive inotropic effects and electrophysiological alterations, whereas chronic stimulation of cardiac α_1 -AR leads to cardiac hypertrophy (11). With respect to the effect of stimulating cardiac receptors on the contractile effects of Ang II, the data from animal experiments are controversial:

TABLE 3
Effects of phenylephrine (PE) on angiotensin II (Ang II)-induced cyclic GMP (cGMP) content in the left atria of rats in different age groups

Age, months	cGMP (fmol/mg)	
	Ang II+losartan	Ang II+losartan+PE
3.5	102.7±12.7	78.3±12.0**
12	81.0±9.4	58.5±10.0**
18	69.8±5.6	53.3±9.7**
24	57.7±8.0	40.5±6.0*

* $P < 0.05$; ** $P < 0.01$ compared with Ang II+losartan by paired *t* test

positive inotropic effects (25), negative inotropic effects (26) and no effects on contractility (27) have been described. Activation of both α_1 -AR and ATR involves G proteins, and their respective signalling cascades are important for mediating cardiovascular homeostasis. Physiologically important interactions between the adrenergic and ATR systems have been described. Furthermore, important interactions between α_1 -AR and ATR also exist. Evidence suggests that Ang II induces transcription and expression of α_1 -AR in rat vascular smooth muscle cells (28). However, it is unknown whether Ang II has any direct effect on α_1 -AR-mediated positive inotropic response and signalling in cardiac myocytes from rats of different ages; this potential interaction has not been investigated previously in any tissue.

In left atrial myocytes from rats in four different age groups, we found a concentration-dependent positive inotropic effect of PE. This finding is consistent with previous work on muscle strip preparations (29,30). The effect is clearly α_1 -AR-mediated because it can be blocked by prazosin. In the present study, it was confirmed that activation of AT₁R has no effect on the positive inotropic effect induced by PE in 3.5- or 12-month-old rats. However, in 18- and 24-month-old rats, the maximum positive inotropic response induced by PE increased markedly when AT₁R was activated and the pD₂ also increased significantly.

Cardiac muscle contractile function is largely related to the intracellular Ca^{2+} concentration of cardiac muscle cells and the sensitivity of the myofilaments to Ca^{2+} (31). Ang II had no positive inotropic effect in rats during normoxia, but increased contractility during low-flow ischemia (32). These results are consistent with a relative intracellular alkalinization that occurs secondary to Ang II action to stimulate Na^+/H^+ exchange. In addition, Ang II contributes to cardiac muscle contractile augmentation by increasing myofilament calcium sensitivity (33). In the present study, our outcome data indicated that the activation of AT₁R augmented the positive inotropic effect of PE in the aging rat, likely due to myocardial blood flow decreasing in the aging heart and activating Na^+/H^+ exchange. Although the integrating capability of AT₁R and Ang II in the aged heart is not very clear, AT_{1A} and AT_{1B} messenger RNA levels were markedly upregulated (5.6-fold) in the left ventricle of 24-month-old rats compared with three-month-old rats (34,35). AT₁R can integrate more Ang II when there is a sufficient extrinsic source of Ang II. Experimental evidence suggests that cardiovascular function depending on the renin-angiotensin system was augmented in the aging heart and our experimental result indicated that cardiac muscle contraction was related to the crosstalk between ATR and α_1 -AR.

In the present study, it was confirmed that activation of AT₂R had no effect on the positive inotropic effect induced by PE in any of the four age groups. This finding is in good agreement with the previous work of Marano and Argiolas (36).

Our experimental results indicated that activation of AT₁R or AT₂R had no positive inotropic effect in any of the four age groups, which is consistent with previous work (32). Thus, signal transduction variation was designed to study the effects of α_1 -AR on AT₁R and AT₂R in the atria of rats of different age groups. Several mechanisms have been shown to mediate the activation of nonreceptor tyrosine kinases and receptor tyrosine kinases by the G-protein

coupled receptors (37-39). Ang II-induced activation of AT₁R stimulates extracellular signal-regulated kinase 1/2 phosphorylation via transactivation of receptor tyrosine kinases or nonreceptor tyrosine kinases. This leads to phosphorylation of tyrosine kinase as well as its subsequent internalization (40), which can promote cell growth, migration and inflammation. Our data show that in the presence of PE, the activities of tyrosine kinases were increased in 3.5- and 12-month-old rats but not in 18- and 24-month-old rats. This result refers to the activation of α_1 -AR augmenting the AT₁R activation, but this effect vanished with age. Experimental evidence (41) suggests that α_1 -AR gene expression was high in adult rats and decreased in both ventricles of senescent rats. Furthermore, α_1 -AR-specific binding was decreased in the aged left ventricular myocardium.

Although the signal mechanisms pertaining to AT₂R are not very clear, three major transduction mechanisms are responsible for AT₂R signalling: the activation of various protein phosphatases causing protein dephosphorylation, the activation of the nitric oxide/cGMP system and the stimulation of phospholipase A₂ with subsequent release of arachidonic acid (42,43). Our studies demonstrated that PE causes a decrease of cPLA₂ activities and cGMP content stimulated by Ang II in all four age groups, which suggests that α_1 -AR activation can inhibit the activation of AT₂R in all rat hearts. The traditional viewpoint considered that the major effects of ATR were induced by AT₁R, but recently, increasing experimental evidence suggests that in senescent hearts and some pathological conditions such as hypertension and myocardial hypertrophy, AT₂R actually has a more important role (44,45). The results of the present study suggest that α_1 -AR inhibits the signal mechanism of AT₂R. Ang II acting at AT₁R has well-documented effects on cardiovascular structure such as the promotion of cardiovascular hypertrophy and fibrosis (46), which are believed to be opposed by AT₂R stimulation (47). The present study shows that the activation of α_1 -AR mediates the contribution of AT₁R and AT₂R.

CONCLUSION

The present study demonstrates the crosstalk between α_1 -AR and ATR in the left atria of rats in different age groups. The beneficial or detrimental effects of the crosstalk remain to be investigated. The present study has several limitations. First, the isolated-perfused heart model does not allow investigation of the later effects of crosstalk, such as the effect on cellular proliferation. Second, it does not distinguish whether changes in enzyme activities or cGMP content are localized predominantly in cardiac myocytes or also in matrix cells that express receptors. However, this is the first time that the presence of interaction has been reported in the hearts of differently aged rats. Nevertheless, the onset and role of this alteration in the cause of cardiac senescence needs further evaluation. In the future, studies should provide a better understanding of cross-regulation between the two receptors and potential mechanisms for these crosstalks.

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