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Supersensitivity mismatch of adenosine in the transplanted human heart: chrono- and dromotropy versus inotropy

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Abstract Supersensitive negative chronotropic and dromotropic effects have been described for adenosine after human heart transplantation. The present study investigated a potential antiadrenergic negative inotropic effect of adenosine in heart transplant recipients compared to normal subjects. Sinus cycle length, PR interval, blood pressure, and inotropic response in vivo were compared in seven orthotopic heart transplant recipients and seven healthy volunteers (controls). Fractional shortening, velocity of circumferential fiber shortening, and systolic pressure/dimension ratio were calculated using M-mode echocardiography. Baseline ventricular contractility was normal in both groups. Although adenosine induced a significant exaggeration of the negative chronotropic and dromotropic effect in the transplant group, the positive inotropic effect of 20 ng/kg × min isoproterenol (FS 53.2 ± 8.8 vs 51.0 ± 4.6 %, P/D 5.8 ± 1.9 vs 6.0 ± 0.8 mm Hg/mm, V_{cf} 0.21 ± 0.04 vs 0.20 ± 0.02 %/ms for heart recipients vs controls) was not reduced by the additional administration of 150 μg/kg adenosine (FS 52.2 ± 8.6 vs 51.7 ± 5.6 %, P/D 5.5 ± 1.5 vs 5.4 ± 0.8 mm Hg/mm, V_{cf} 0.24 ± 0.07 vs 0.21 ± 0.02 %/ms for transplant recipients vs controls). In contrast to a chronotropic and dromotropic supersensitivity, adenosine does not attenuate the catecholamine-induced increase in contractility in the human ventricle in vivo after heart transplantation.

Key words Heart transplantation, adenosine · Adenosine, heart transplantation

Introduction

Enhanced responsiveness to transmitters in denervated organs is termed denervation supersensitivity [6]. After cardiac transplantation, supersensitivity has been shown for catecholamines in terms of enhanced chronotropic, dromotropic, and inotropic response [18, 20]. After parasympathetic denervation, the denervated canine sinus and atrioventricular node also demonstrate supersensitive responses for acetylcholine [10, 14].

The endogenous nucleoside adenosine exhibits a direct negative chronotropic and dromotropic effect in the normal human heart in vivo. Adenosine is also reported to exhibit an antiadrenergic, indirect negative inotropic response in the ventricular myocardium in vitro [2]. Recently, it has been shown that, in contrast to the in vitro studies, adenosine does not attenuate the catecholamine-induced increase in contractility in the innervated human ventricle in vivo [11]. Adenosine shares the atrial and ventricular transmembrane signalling mechanisms with the m_2-cholinoceptors. Besides comparable electrophysiological effects, m_2-cholinoceptor stimulation also exerts a significant antiadrenergic decrease in ventricular contractility in vivo [19]. In view of a similar in vitro potency, this difference in the in vivo effect could be explained by a significantly lower efficacy for adenosine [11]. Therefore, the difference between the in vivo and in vitro responses of adenosine

would appear to be due to a limitation of the maximal tolerable dosage in vivo by severe negative chronotropic and dromotropic effects.

After cardiac transplantation, the chrono- and dromotropic responses of the denervated sinus and atrioventricular nodes to adenosine have been reported to be of increased magnitude and duration. This indicates a negative chrono-and dromotropic adenosine supersensitivity after cardiac transplantation in humans [1, 8]. The purpose of the present study was to investigate a possible indirect, negative inotropic supersensitivity to adenosine. This could potentially unmask an antiadrenergic effect of adenosine on beta-adrenoceptor-stimulated human ventricular contractility in vivo that is not detectable in the innervated human heart.

Methods

The transplant group consisted of seven orthotopic heart transplant recipients (six men and one woman) aged 50 ± 13 years, 30 ± 23 months post-transplantion. Routine annual cardiac catheterization performed within 24 h of the adenosine study documented the absence of transplant vasculopathy and a normal ejection fraction and cardiac index in all heart transplant recipients. Patients receiving theophylline, dipyridamol, verapamil, beta-blockers, or digitalis were excluded from the study. The control group consisted of seven healthy male volunteers, aged 30 ± 2 years. Continuous assessment of myocardial contractility was performed with two-dimensionally guided M-mode echocardiography in the short-axis view using phased array equipment with a 2.5-MHz transducer (Toshiba SSA-270A). Systolic and diastolic blood pressure were monitored with an automated sphygmomanometer (Accutorr); sinus cycle length and PR interval were obtained by simultaneous electrocardiographic recordings. End-systolic and end-diastolic diameters of the left ventricle and the left ventricular ejection time were measured as the mean of five cardiac cycles according to standard guidelines [17]. As instantaneous parameters of the ventricular contractility the fractional shortening [100 X (end-diastolic diameter – end-systolic diameter)/end-diastolic diameter] and the ejection time-incorporating velocity of circumferential fiber shortening (fractional shortening/left ventricular ejection time) were calculated [13]. To control for effects of potentially different afterload conditions, the systolic pressure/dimension ratio as an afterload-independent parameter of inotropy was calculated as systolic blood pressure/end-systolic diameter [16].

Study protocol

After 15 min in supine position, a continuous infusion of isoproterenol was started at a dosage of 20 ng/kg body weight per minute. Ten minutes after initiation of the isoproterenol infusion, a steady state was reached as assessed by echo- and electrocardiographic recordings. A bolus injection of 150 μg/kg body weight adenosine was administered intravenously. Echo- and electrocardiographic recordings, as well as blood pressure measurements, were taken at rest, during isoproterenol steady-state, and after injection of adenosine. Each subject gave written informed consent. The study protocol was approved by the Ethics Committee of the Ludwig-Maximilians University of Munich.

Statistical analysis

All group data are given as mean value ± standard deviation. Within the two groups analysis of variance was performed using the Scheffé range test for multiple comparisons. For comparisons between both groups, the Mann-Whitney-U test was used. All calculated P values are two-tailed. All P values less than 0.05 were considered significant.

Results

Baseline left ventricular function, heart rate, and blood pressure were normal in all subjects. As indicated by an unchanged end-diastolic diameter and systolic blood pressure (with the exception of a slightly increased systolic blood pressure in the control group during isoproterenol and adenosine), pre- and afterload did not change in either groups during all conditions (Table 1). After administration of adenosine, the maximal PR interval increased significantly compared to the isoproterenol measurement in the control group and the isoproterenol and rest measurement in the transplant group.

Chronotropy

Within the control group, the sinus cycle length did not change significantly during isoproterenol infusion or additional administration of adenosine. Within the transplant group, isoproterenol did not change the sinus cycle length either. Additional administration of adenosine resulted in a third degree sinoatrial (SA) block, defined as a sinus arrest longer than 2000 ms, in two heart transplant recipients and in a significant prolongation of the sinus cycle length in the remaining four (one heart transplant recipient had to be excluded due to a demand pacemaker; Fig. 1, Table 2).

Dromotropy

With an insignificant shortening of the PR interval by isoproterenol in the control group as well as in the transplant group, the additional administration of adenosine resulted in a significant negative dromotropic effect in both groups. Within the control group, two volunteers developed a short-term second degree atrioventricular (AV) block. The remaining five control subjects showed a significant prolongation of the PR interval. Within the transplant group, adenosine resulted in a second or third degree AV block in five heart transplant recipients and in a significant prolongation of the PR interval in the remaining two (Table 3).

Table 1 Measurements at rest, during isoproterenol, and during isoproterenol and adenosine (*SBP* systolic blood pressure, *DBP* diastolic blood pressure, *LVET* left ventricular ejection time, *EDD* end-diastolic diameter, *ESD* end-systolic diameter, RR_{max} maximal RR interval)

	SBP (mm Hg)	DBP (mm Hg)	LVET (ms)	EDD (mm)	ESD (mm)	RR_{max} (ms)
Rest						
Control group	118 ± 9	66 ± 7	277 ± 20	49 ± 3	31 ± 3	941 ± 147
Transplant group	114 ± 16	64 ± 8	254 ± 27	44 ± 6	28 ± 8	654 ± 81*[8]
Isoproterenol (20 ng/kg × min)						
Control group	126 ± 9	56 ± 4	244 ± 10*[2]	50 ± 4	24 ± 5*[1]	717 ± 80
Transplant group	119 ± 16	59 ± 12	211 ± 30*[2]	43 ± 6	22 ± 7	557 ± 84*[8]
Isoproterenol and adenosine (150 µg/kg)						
Control group	138 ± 4*[3]	66 ± 13	247 ± 10*[4]	49 ± 4	24 ± 4*[3]	997 ± 266*[5]
Transplant group	123 ± 19	63 ± 12	239 ± 16	43 ± 6	21 ± 7	2059 ± 1540*[3, *5]

*[1] $P < 0.05$; *[2] $P < 0.01$ isoproterenol vs rest; *[3] $P < 0.05$; *[4] $P < 0.01$ isoproterenol and adenosine vs rest; *[5] $P < 0.05$; *[6] $P < 0.01$ isoproterenol and adenosine vs isoproterenol; *[7] $P < 0.05$; *[8] $P < 0.01$ transplant vs control group

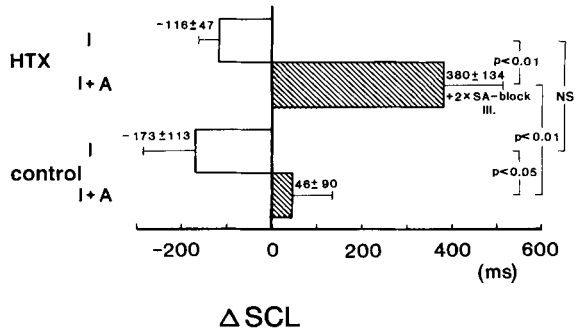

Fig. 1 Increase in sinus cycle length (△SCL) from baseline values in six heart transplant recipients (*HTX*) and seven healthy volunteers (control) during 20 ng/kg × min isoproterenol (*I*), and additional administration of 150 µg/kg adenosine (*I* + A)

Inotropy

Starting from a normal baseline ventricular contractility, the transplant group as well as the control group showed a comparable significant positive inotropic response to isoproterenol as judged from fractional shortening, velocity of circumferential fiber shortening, and systolic pressure/dimension ratio. Additional administration of adenosine was not capable of attenuating this significant increase in contractility in either group. No difference in the inotropic response between heart transplant recipients and controls was present during isoprenaline or isoprenaline and adenosine administration (Fig. 2, Table 4).

Discussion

In the present study, the effect of adenosine on catecholamine-enhanced contractility was investigated in the denervated human heart in vivo. Adenosine did not attenuate the isoproterenol-induced increase in ventricular contractility. In contrast to an exaggerated chronotropic and dromotropic response to adenosine in the denervated heart, this argues against an indirect negative inotropic supersensitivity to adenosine in vivo.

In the myocardium, the actions of adenosine are mediated by A_1-adenosine receptors. In supraventricular tissue (including sinus and atrioventricular node), the A_1-adenosine receptor couples to a distinct subset of membranous potassium channels via a regulatory guanine nucleotide-binding protein. By activating a potassium outward current, adenosine induces a "direct" negative chronotropic and dromotropic response in the normal human heart. In ventricular tissue, A_1-adenosine receptors are coupled via an inhibitory guanine nucleotide-binding protein ($G_{i\alpha}$) to adenylate cyclase, thereby reducing the catecholamine-induced formation of cyclic adenosine monophosphate. In vitro adenosine is reported to exhibit an antiadrenergic or "indirect" negative inotropic response in the beta-adrenergic-stimulated ventricular myocardium [12]. In the normal human heart, in vivo maximal dosages of adenosine do not attenuate the catecholamine-enhanced contractility, most probably due to a low ventricular sensitivity. A further increase in the in vivo dosage of adenosine is limited by a high-degree atrioventricular block and intolerable side effects [2]. In the denervated human heart after orthotopic transplantation, adenosine has been reported to induce an exaggerated prolongation in sinus cycle length and atrioventricular conduction [1, 8]. In the innervated heart, the effects of adenosine may be counterregulated by a complex activation of the sympathetic nervous system. Therefore, it has been suggested that, in the denervated heart, the exaggerated negative chronotropic and dromotropic response may be due to the absence of adenosine-induced sympathetic counterregulation instead of a primary adenosine supersensitivity. This hypothesis was discarded by a recently de-

Table 2 Sinus cycle length (in ms) at rest, during isoproterenol, and during isoproterenol and adenosine [*Iso* isoproterenol (20 ng/kg × min), *Iso + adeno* isoproterenol and adenosine (150 μg/kg), *SAB III* third degree sinoatrial block]

Control group				Transplant group			
Case	Rest	Iso	Iso + adeno	Case	Rest	Iso	Iso + adeno
1	750	770	850	1	730	620	1230
2	1000	910	1200	2	600	500	760
3	1100	840	950	3	640	550	SAB III
4	860	560	940	4	Demand pacemaker		
5	760	640	800	5	760	550	1150
6	940	710	760	6	500	420	SAB III
7	950	720	950	7	640	550	1080
	($n = 7$)	($n = 7$)	($n = 7$)		($n = 6$)	($n = 6$)	($n = 4$)
	909	736	921		645	528	1063
	± 127	± 118	± 145		± 93	± 66	± 192*,**

* $P < 0.01$ isoproterenol and adenosine vs isoproterenol; ** $P < 0.05$ isoproterenol and adenosine vs rest

Table 3 PR interval (in ms) at rest, during isoproterenol, and during isoproterenol and adenosine [*Iso* isoproterenol (20 ng/kg × min), *Iso + adeno* isoproterenol and adenosine (150 μg/kg), *AVB II* second degree atrioventricular block, *AVB III* third degree atrioventricular block]

Control group				Transplant group			
Case	Rest	Iso	Iso + adeno	Case	Rest	Iso	Iso + adeno
1	160	160	AVB II	1	170	140	AVB II
2	170	160	AVB II	2	100	100	AVB III
3	180	160	200	3	150	140	250
4	150	150	200	4	170	130	170
5	190	170	210	5	160	150	AVB II
6	160	140	160	6	160	150	AVB III
7	190	160	200	7	150	140	AVB II
	($n = 7$)	($n = 7$)	($n = 5$)		($n = 7$)	($n = 7$)	($n = 2$)
	171 ± 16	157 ± 9	190 ± 19*		148 ± 25	136 ± 17	210 ± 6**

* $P < 0.05$, ** $P < 0.01$ isoproterenol and adenosine vs isoproterenol

scribed increased shortening of the atrial monophasic action potentials by adenosine in the transplanted human heart [13]. In contrast to the opposing effects of adenosine and the sympathetic nervous system on the sinus and atrioventricular nodes, the effects on atrial repolarization are concordant. Therefore, the increased shortening of the monophasic action potentials, even in the absence of reflex sympathetic activation, is evidence of a primary supersensitivity to adenosine. In addition, due to the extremely short half-time of adenosine, in former in vitro studies in human myocardium [3, 9] as well as in an in vivo animal study [15], adenosine was administered as a continuous, intravenous infusion. In the present study, the inotropic response was assessed online by instantaneous parameters of ventricular contractility, allowing us to evaluate potentially rapid and transient inotropic changes by a bolus injection of adenosine. In contrast to continuos infusions, a biphasic effect in response to a bolus injection offers the advantage of enabling one to differentiate between the early primary effect of adenosine itself and secondary, systemic counterregulation. Until the onset of sympathetic counterregulation in innervated hearts (i.e., reflex tachycardia and vasoconstriction), there is a delay of several seconds after the maximum effect of adenosine [21]. The absence of this delay for the described effects of adenosine argues strongly against an exaggeration due to missing counterregulation.

In view of the supersensitive chrono- and dromotropic response, it was speculated that an exaggeration of the antiadrenergic effect would also lead to a clinically measurable indirect, negative inotropic effect.

Denervation supersensitivity to sympathetic and parasympathetic transmitters is said to be due to either presynaptic (loss of neuronal uptake) or postsynaptic (upregulation of the receptor density or of the second messenger system) changes [5, 18]. In contrast to neurotransmitters, supersensitivity to endogenous metabolite adenosine could only be mediated by changes in the receptor-effector system. The chronotropic and dromotropic, as well as inotropic, effects of adenosine are mediated by the same A_1-adenosine receptor. However, the second messenger systems are different. Thus, one can speculate that the chronotropic and dromotropic super-

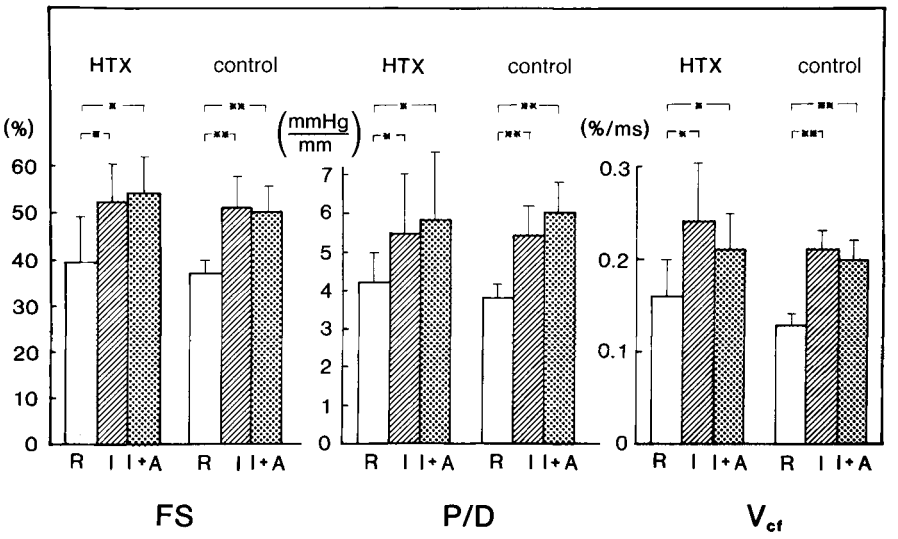

Fig. 2 Fractional shortening (*FS*), systolic pressure/dimension ratio (*P/D*), and velocity of circumferential fiber shortening (V_{cf}) in seven heart transplant recipients (*HTX*) and seven healthy volunteers (control) during rest (*R*), 20 ng/kg × min isoproterenol (*I*), and additional administration of 150 µg/kg adenosine (*I* + A). * $P < 0.05$; ** $P < 0.01$

Table 4 Fractional shortening, velocity of circumferential fiber shortening, and systolic pressure/dimension ratio as indices of ventricular contractility at rest, during isoproterenol, and during isoproterenol and adenosine (*FS* fractional shortening, *P/D* systolic pressure/dimension ratio, V_{cf} velocity of circumferential fiber shortening)

	Control group			Transplant group		
	FS (%)	P/D (mm Hg/mm)	V_{cf} (%/ms)	FS (%)	P/D (mm Hg/mm)	V_{cf} (%/ms)
Rest	37.1 ± 2.7	3.8 ± 0.3	0.13 ± 0.01	39.7 ± 9.9	4.2 ± 0.8	0.16 ± 0.04
Isoproterenol (20 ng/kg × min)	51.7 ± 5.6 *2	5.4 ± 0.8 *2	0.21 ± 0.02 *2	52.5 ± 8.6 *1	5.5 ± 1.5 *1	0.24 ± 0.07 *1
Isoproterenol and adenosine (150 µg/kg)	51.0 ± 4.6 *4	6.0 ± 0.8 *4	0.20 ± 0.02 *4	53.2 ± 8.8 *3	5.8 ± 1.9 *3	0.21 ± 0.04 *3

*1 $P < 0.05$; *2 $P < 0.01$ isoproterenol vs rest; *3 $P < 0.05$; *4 $P < 0.01$ isoproterenol and adenosine vs rest; P = NS for isoproterenol and adenosine vs isoproterenol and for transplant group vs control

sensitivity and the absence of an indirect, negative inotropic response in the denervated human heart may be caused by isolated changes in the atrial second messenger system. Another explanation could be an only subclinical exaggeration of the antiadrenergic negative inotropic effect, not pronounced enough to result in a measurable decrease in ventricular contractility. In contrast to m-cholinoceptors, which exhibit a measurable antiadrenergic effect on ventricular contractility in vivo by using the same transmembrane-signalling mechanisms, A_1-adenosine receptor density in the human ventricular myocardium is reported to be only 8 %–10 % of the m-cholinoceptor density [4]. A_1-receptor stimulation mediates only 40 %–50 % of the maximal effects produced by m-cholinoceptors [4]. Furthermore, the ventricular density of A_1-receptors is reported to be much lower than that of atrial tissue [3]. Therefore, the absence of an indirect negative inotropic effect of adenosine in both innervated and denervated hearts in vivo may be explained by a low sensitivity of ventricular myocardium to adenosine and a limitation of the maximum clinically tolerable dosage by electrophysiological side effects.

These issues should be elucidated by further in vitro studies comparing the efficacy of adenosine in ventricular myocardium and the atrial second messenger system of adenosine in both innervated and denervated human hearts.

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