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Comparison between *Alpha*-1 Adrenoceptor-Mediated and *Beta* Adrenoceptor-Mediated Inotropic Components Elicited by Norepinephrine in Failing Human Ventricular Muscle[1]

TOR SKOMEDAL, KJELL BORTHNE, HALFDAN AASS, ODD GEIRAN and JAN-BJØRN OSNES

Department of Pharmacology, University of Oslo, Medical Department B (H.A.) and Surgical Department A (O.G.), Rikshospitalet, Oslo, Norway

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ABSTRACT

The purpose of our study was to investigate the inotropic response to the endogenous agonist norepinephrine mediated through *alpha*-1 adrenoceptors and to compare this response to that mediated through *beta*-adrenoceptors in failing human ventricular myocardium. We studied *ex vivo* the inotropic effect of norepinephrine in isometrically contracting trabecular myocardium from both ventricles of explanted hearts. By studying influence of appropriate adrenoceptor blockers, qualitative characteristics of the inotropic response and sensitivity of the inotropic response to cholinergic stimulation, it was revealed that norepinephrine evoked both *alpha*-1 and *beta* adrenoceptor-mediated inotropic effects in failing human ventricle myocardium. Quantitatively the inotropic responses to norepinephrine varied markedly between preparations, but the mean responses elicited through the respective adrenoceptor systems were of comparable magnitude. Concomitant stimulation of *alpha*-1 and *beta* adrenoceptors by norepinephrine alone revealed a contribution of an *alpha*-1 adrenoceptor-mediated component to the final and unopposed inotropic response. Differential sensitivity of the two adrenoceptor systems to norepinephrine depending on etiology of heart failure and possibly also thyroid status was observed. It is concluded that norepinephrine evokes an *alpha*-1 adrenoceptor-mediated inotropic effect comparable to that evoked through the *beta* adrenoceptors in failing human ventricular myocardium, and that this *alpha*-1 adrenoceptor-mediated inotropic effect may be of functional importance.

The catecholamine-induced increase in contractile force in mammalian myocardium is mainly due to activation of *beta* adrenoceptors. The existence of myocardial *alpha*-1 adrenoceptors mediating positive inotropic effects is, however, now well established (Osnes *et al.*, 1985; Fedida 1993; Terzic *et al.*, 1993). In rat and rabbit heart the *alpha*-1 adrenoceptor-mediated effect is influencing and contributing to the final inotropic effect of combined receptor activation by the endogenous agonist norepinephrine (Skomedal *et al.*, 1988, 1990a). As *alpha*-1 adrenoceptor-mediated and *beta* adrenoceptor-mediated inotropic effects can be differentially influenced (Endoh and Motomura, 1979; Christiansen *et al.*, 1987), it has been speculated that *alpha*-1 adrenoceptor mediated effects may have increased importance during conditions where *beta*-adrenoceptor mediated effects are attenuated (Christiansen *et al.*, 1987, Skomedal *et al.*, 1988, 1990a).

In the failing human heart where the *beta*-1 adrenoceptor mediated inotropic response is attenuated, it has been difficult to demonstrate an *alpha*-1 adrenoceptor-mediated inotropic effect of functional significance (*e.g.*, Böhm *et al.*, 1988, Brodde *et al.*, 1992; Steinfath *et al.*, 1992; Bristow 1993). Mügge *et al.* (1983) and Brückner *et al.* (1984) reported *alpha*-1 adrenoceptor-mediated inotropic effects in human ventricular myocardium, but these authors did not compare the *alpha*-1 adrenoceptor-mediated inotropic effect and the *beta* adrenoceptor-mediated inotropic effect. The study by Böhm *et al.* (1988) indicated, however, an increasing importance of the *alpha*-1 adrenoceptor-mediated inotropic effect during severe heart failure due to the increasing attenuation of the *beta* adrenoceptor-mediated effect. In all these studies phenylephrine (in the presence of a *beta* receptor antagonist) has been used as the *alpha* adrenoceptor agonist. In addition, Landzberg *et al.* (1991) also observed an *alpha* adrenoceptor-mediated inotropic effect of phenylephrine in humans *in vivo*. In the presence of a *beta* receptor antagonist, Aass *et al.* (1986) found an *alpha*-1 adrenoceptor-mediated inotropic effect of norepinephrine in human ventricular myocardium obtained from patients undergoing mitral valve replacement. Although not compared directly to the *beta* adrenoceptor-mediated inotropic effect, the *alpha*-1 adrenoceptor-mediated

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ABBREVIATIONS: T_{max}, maximal developed tension; TPT, time to peak tension; TR20, time to relaxation to 20% level; RT, relaxation time; pD_2, -log EC_{50}; EC_{50}, concentration giving half maximal effect; TSH, thyroid stimulating hormone.

inotropic effect observed in this study was of such a magnitude that a functional role might be expected. Neumann *et al.* (1993) and Scholz *et al.* (1995, 1996) reported data that indicated that naturally occurring norepinephrine was more effective as agonist compared to phenylephrine at the myocardial *alpha*-1 adrenoceptors mediating increase in contractility in failing human hearts. The functional importance of the *alpha*-1 adrenoceptors in failing human myocardium is thus still unsettled.

The purpose of our studies was to further elucidate the functional role of the myocardial *alpha* adrenoceptors in regulation of contractility also in failing human heart by investigating the relative magnitude of *alpha*-1 adrenoceptor-mediated and *beta* adrenoceptor-mediated inotropic responses in explanted human hearts. In our work we report that despite great variation in individual maximal responses to adrenergic stimulation by norepinephrine, the magnitude of the *alpha*-1 adrenoceptor-mediated inotropic component was comparable to that of the *beta* adrenoceptor-mediated inotropic component. In addition, the contribution from the two adrenergic receptor systems to the inotropic response may differ due to different etiology of heart failure.

Methods

Myocardial preparations. Ventricular myocardium was obtained from heart transplant recipients immediately after explantation of the failing heart. During preparation of the tissue, the surface was kept wet with physiological saline. Thin trabeculae were localized in the ventricular cavities, cut free and placed in a relaxing oxygenated (95% O_2/5% CO_2) physiological salt solution at room temperature. To prevent contracture during transportation and further preparation, we used a Ca^{++}/Mg^{++} concentration ratio of 1:8 comparable to that of St. Thomas' Hospital cardioplegic solution. Experimentally, magnesium cardioplegia has also been shown to effectively protect the myocardium from calcium overload (Ataka *et al.*, 1993). The solution contained (mmol/liter): NaCl 118.3; KCl 3.0; $CaCl_2$ 0.5; $MgSO_4$ 4.0; KH_2PO_4 2.4; $NaHCO_3$ 24.9; glucose 10.0; mannitol 2.2. The tissue was transported to the laboratory and while kept immersed in this solution, muscle strips (about 1 mm in diameter, 8–10 mm long, endocardium as intact as possible) were prepared. The muscle strips were mounted in four organ baths containing the same oxygenated solution at 37°C except for Ca^{++} being 2.5 mmol/liter and Mg^{++} 1.2 mmol/liter. The muscles were driven electrically (field stimulation) at a frequency of 1 Hz with impulses of 5-msec duration and current about 20% above individual threshold (10–15 mA, determined in each experiment). The isometrically contracting muscles were stretched to the maximum of their length-tension curve. The developed tension was recorded by a Grass FT03C force-displacement transducer connected to a Grass RPS7C8B polygraph recorder equipped with 7DAG driver amplifiers, with 7P1F bridge amplifiers and with 7P20C derivators. The direct current signals were analog-to-digital converted by a National Instruments NB-MIO-16X board mounted in an Apple Macintosh Quadra 700 computer. The data acquisition was externally triggered synchronously to the contraction-relaxation cycles by the square wave pulses that triggered the muscles to contract. Each channel was scanned at 1 kHz yielding a time resolution of 1 msec. For the human heart the time window to be scanned was set to 500 msec, *i.e.*, 500 posttrigger scans were sampled for each contraction-relaxation cycle. The logged data were stored in files as time stamped and event marked unfiltred binary clusters by software developed for the purpose in the visual programming language LabVIEW. The software could later open the files for analysis and compute appropriate low-pass filtered trend curves for developed tension *vs.* time for each contraction-relaxation cycle. Areas representative for the different experimental periods (control, stimulated) could be selected to calculate averaged contraction-relaxation cycles for these periods. These cycles were then used to determine values for typical descriptive parameters including T_{max}, TPT and TR20.

Experimental design. The muscles were allowed to equilibrate for 60 to 90 min before addition of agonist. The salt solution was changed in the middle of the equilibration period. Adrenoceptor agonists (phenylephrine, norepinephrine) were added directly to the organ bath in volumes of 25 to 75 μl to give the appropriate final concentrations and were completely mixed in the bath within 2 to 3 sec. Dose-response experiments were performed by cumulatively increasing the concentration of norepinephrine in the organ baths.

The adrenergic receptor antagonists prazosin and timolol were used to block *alpha*-1 adrenoceptors and *beta* adrenoceptors, respectively. Both prazosin and timolol are nonsubtype selective with respect to *alpha*-1 and *beta* adrenoceptors, respectively. The final concentration in the organ bath was 6×10^{-6} mol/liter of both prazosin and timolol as 1.2×10^{-5} mol/liter of both antagonists caused a slowly developing decline in basal contractility. Six $\times 10^{-6}$ mol/liter is about $1000 \times K_d$ for timolol at the *beta* adrenoceptors in human heart (Golf and Hansson, 1986) and thus corresponding to a theoretical occupancy of 99.90% of the receptors. Six $\times 10^{-6}$ mol/liter is about $10{,}000 \times K_d$ for prazosin at the *alpha*-1 adrenoceptors in human heart (Böhm *et al.*, 1988) and thus corresponding to a theoretical occupancy of 99.99% of the receptors.

When used, adrenoceptor antagonists were either given before the agonists to eliminate the contractile response to activation of the respective receptor population, or after development of the contractile response to norepinephrine to reverse and thus verify the contribution of the respective receptor system. When added before agonist, the antagonists were diluted in the incubation solution and were thus allowed to act for 30 to 45 min before addition of agonist. When used, 1.2×10^{-4} mol/liter carbachol was added at steady state inotropic response to norepinephrine. In dose-response experiments the incubation solution also contained ascorbic acid (10^{-4} mol/liter) and atropine (10^{-6} mol/liter).

Explanted hearts. In our report data obtained from 20 explanted hearts are presented. The explanted hearts were failing either due to ischemic cardiomyopathy (postinfarction failure, $n = 10$) or to nonischemic cardiomyopathy (dilated cardiomyopathy ($n = 4$), congenital malformations ($n = 4$), myocarditits ($n = 1$) or toxic (adriamycin) cardiomyopathy ($n = 1$). In the group of patients undergoing heart transplantation due to ischemic cardiomyopathy, nine were men (44–61 yr, mean 54 yr) and one was a woman aged 58 yr. In the group of patients undergoing heart transplantation due to nonischemic cardiomyopathy, six were men (aged 14–57 yr, mean 36 yr) and four were women (aged 24–55 yr, mean 39 yr). All patients were severely symptomatic from their heart failure (New York Heart Association functional class III-IV). Three of the patients in the nonischemic group had relatively normal left ventricular function. Two of these had severe pulmonary hypertension secondary to congenital heart disease (Eisenmenger's syndrome) and the third had Uhl's syndrome with failing of the right ventricle. The other 17 patients all had severely depressed left ventricular function with ejection fraction of 24 ± 6% (mean ± S.E.) and pulmonary capillary wedge pressure of 21 ± 7 mm Hg (mean ± S.E.) with no difference between failure due to ischemic heart disease or not. The nonischemic patients had more severe right ventricular failure revealed as a higher right atrial pressure than the ischemic group, 15 ± 5 *vs.* 4 ± 2 mm Hg (mean ± S.E., P = .001), respectively. The nonischemic group also had a lower resting cardiac index of 1.5 ± 0.3 liter/min/m^2 compared to 2.2 ± 0.4 liter/min/m^2 (mean ± S.E., P = .006) in the ischemic failure group. All patients were receiving supportive medical treatment before transplantation by at least two of the following drugs: digitalis (mostly digitoxin), loop diuretics (furosemide, bumetanide), ACE-inhibitors (captopril, enalapril, lisinopril), spironolactone and nitrates (isosorbidmono/dinitrate). Some patients also received one or more of the following drugs: aspirin, warfarin, lovastatin, dipyri-

damol and potassium-chloride. In addition some patients used sedatives (oxazepam, levomepromazine, haloperidol), antiasthmatics (inhalation of budesonide, ipratropium, salbutamol), paroxetine, chlormezanone/acetaminophen, omeprazole or glipizide. Due to serious arrhythmias, three patients with ischemic and two patients with nonischemic cardiomyopathy also received amiodarone. All the patients, except one, receiving amiodarone were euthyroid as judged by TSH values. One patient received thyroxine due to hypothyroidism appearing before amiodarone treatment was started. None of the patients received adrenergic blocking drugs.

General anesthesia during the transplant procedure consisted basically of nitrous oxide, isoflurane and pancuronium. All patients received a benzodiazepine (diazepam, midazolam, oxazepam) and in some patients fentanyl and ketamine were also used. Some patients received nitrates (glyceryl trinitrate, nitroprusside) and a short time infusion (few minutes) of adrenergic agonists (dopamine, epinephrine, isoproterenol) before extracorporal circulation. The hearts were rapidly excised after aortic cross-clamp.

The experiments were performed according to the institutional rules.

Definitions. T_{max} = maximal developed tension; TPT = time to peak tension; TR20 = time to relaxation to 20% level; RT = TR20 − TPT = relaxation time; $pD_2 = -\log EC_{50}$ (EC_{50} = concentration giving half maximal effect). Changes in contractile force was expressed as changes in T_{max}. Horizontal positioning of the dose-response curves were expressed by pD_2 values.

Calculation. The values after responses to agonist were generally calculated as percent of control values (100%). Response curves were constructed according to Ariëns and Simonis (1964), by estimating centiles, *i.e.*, t_{10} to t_{100} for each single time course experiment and EC_{10} to EC_{100} for each single dose-response experiment, and calculating the corresponding means for each experimental group. This was done by a computer program based on linear interpolation between actual observed values. The response values were either expressed as fractional responses in per cent of maximum or were recalculated with control values as 100% and the maxima in percent thereof to also express differences in efficacy. The significance levels of differences were expressed by calculating P according to Wilcoxon one-sample or two-sample tests as appropriate. $P \leq 0.05$ was considered to reflect significant differences.

Drugs. (-)-Norepinephrine bitartrate and timolol maleate were purchased through Norwegian Medical Depot. Ascorbic acid, atropine sulfate, carbamylcholine chloride (carbachol), (-)-phenylephrine hydrochloride and prazosin hydrochloride were purchased from Sigma Chemical Co. (St. Louis, MO). Stock solutions were prepared in purified water and kept at −20°C to avoid oxidation. Further dilutions of the drugs were made fresh daily and kept cool (0–4°C) and dark. Repetitive experiments showed that drug solutions treated in these ways, are stable.

Results

Characterization of the *alpha*-1 adrenergic inotropic effect of phenylephrine. In the presence of 6×10^{-6} mol/liter timolol, activation of *alpha*-1 adrenoceptors by 1.2×10^{-4} mol/liter phenylephrine evoked an inotropic response with the characteristics expected for an *alpha*-1 adrenoceptor-mediated inotropic response in mammalian myocardium. After a lag phase of about 30 sec, a monophasic inotropic response developed with a time to 50% relative response of 96 ± 9 sec (mean ± S.E., $n = 6$) (fig. 1, table 1). TPT, TR20 and RT were not significantly changed compared to control (table 1).

Phenylephrine increased the contractile force by 33.1 ± 5.3% (mean ± S.E., $n = 6$) compared to control level. Under comparable conditions and in preparations from the corresponding hearts and chambers, norepinephrine increased the contractile force by 46.6 ± 7.4% (mean ± S.E., $n = 6$) compared to control level. Thus, based on comparison of responses in preparations from the same heart and chamber, phenylephrine apparently displayed an intrinsic activity of 70.9 ± 10.1% (mean ± S.E., $n = 6$, P = .035) compared to norepinephrine at the human cardiac *alpha*-1 adrenoceptors.

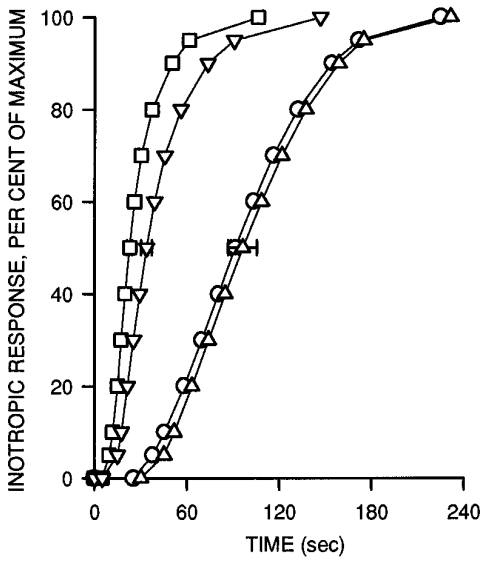

Fig. 1. Time course curves showing the development of the inotropic responses in failing human ventricular myocardium exposed to 1.2×10^{-4} mol/liter norepinephrine in the presence of 6×10^{-6} mol/liter prazosin (*beta* adrenoceptor stimulation, $n = 8$, □); in the presence of 6×10^{-6} mol/liter timolol (*alpha*-1 adrenoceptor stimulation, $n = 14$, ○); alone (concomitant *alpha*-1 and *beta*-adrenoceptor stimulation, $n = 14$, ▽) and to 1.2×10^{-4} mol/liter phenylephrine in the presence of 6×10^{-6} mol/l timolol (*alpha*-1 adrenoceptor stimulation, $n = 6$, Δ). Ordinate: Inotropic response expressed as T_{max} in percent of individual maximal responses. Abscissa, Time in seconds after addition of agonist. The receptor blockers were added 30 to 45 min before the agonists. Horizontal bars (given where they exceed the width of the symbol) represent ± S.E. of time to 50% relative response (see also table 1).

The influence of adrenergic blocking agents on the time course of the inotropic response to norepinephrine. In the presence of 6×10^{-6} mol/liter of the *alpha*-1 adrenoceptor blocker prazosin, 1.2×10^{-4} mol/liter norepinephrine elicited a monophasic positive inotropic response after a lag phase of about 5 sec. Time from addition of norepinephrine to 50% relative response was 22 ± 2 sec (mean ± S.E., $n = 8$) (fig. 1, table 1). This response was sensitive to *beta* adrenoceptor blockade as it was reversed by addition of 6×10^{-6} mol/liter timolol (fig. 2a).

In the presence of 6×10^{-6} mol/liter of the *beta* adrenoceptor blocker timolol, 1.2×10^{-4} mol/liter norepinephrine elicited a monophasic positive inotropic response after a lag phase of about 25 sec. In this situation, time from addition of norepinephrine to 50% relative response was 91 ± 3 sec (mean ± S.E., $n = 14$) (fig.1, table 1). This response was sensitive to *alpha*-1 adrenoceptor blockade as it was reversed by addition of 6×10^{-6} mol/l prazosin (fig. 2b).

Time course of the inotropic response to norepinephrine alone and the reversal of this response by sequential addition of adrenergic blockers. In the absence of adrenoceptor blocking agents, 1.2×10^{-4} mol/liter norepinephrine evoked a monophasic positive inotropic response that developed after a lag phase of about 5 sec. In this

TABLE 1

Characterization of the inotropic responses to phenylephrine and norepinephrine

	Time to 50% Relative Response, Sec after Addition of Agonist	Time to Peak Tension (TPT), % of Control Period	Time to Relaxation to 20% Level (TR20), % of Control Period	Relaxation Time (RT = TR20-TPT), % of Control Period
Phenylephrine (n = 6) (in the presence of timolol)	96 ± 9	102 ± 2	102 ± 1	102 ± 2
Norepinephrine (n = 14) (in the presence of timolol)	91 ± 3	101 ± 1	100 ± 1[a]	100 ± 2[a]
Norepinephrine (n = 8) (in the presence of prazosin)	22 ± 2[b]	78 ± 3[b]	76 ± 2	72 ± 3
Norepinephrine (n = 14) (absence of blockers)	34 ± 4	86 ± 2	79 ± 2	72 ± 4

[a] Significantly different from the corresponding values for norepinephrine in the presence of prazosin and norepinephrine alone ($P < .001$).
[b] Significantly different from the corresponding values for norepinephrine in the presence of timolol ($P < .001$) and norepinephrine alone ($P = .03$).

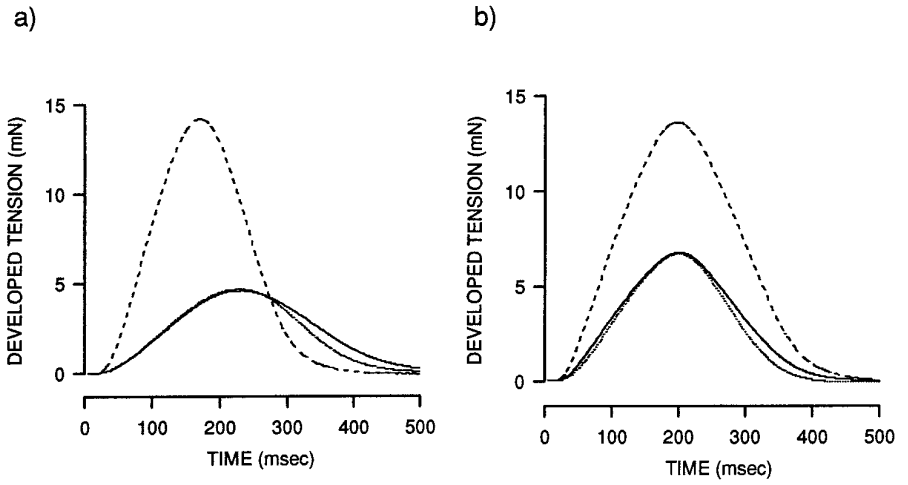

Fig. 2. a, Recording of contraction-relaxation cycles in failing human ventricular myocardium exposed to 1.2×10^{-4} mol/liter norepinephrine in the presence of 6×10^{-6} mol/liter prazosin (*beta* adrenoceptor stimulation). Control contraction before addition of agonist (—); maximal inotropic steady state response (---); reversal of the inotropic response by 6×10^{-6} mol/liter timolol (·····). b, Recording of contraction-relaxation cycles in failing human ventricular myocardium exposed to 1.2×10^{-4} mol/liter norepinephrine in the presence of 6×10^{-6} mol/l timolol (*alpha*-1 adrenoceptor stimulation). Control contraction before addition of agonist (—); maximal inotropic steady state response (---); reversal of the inotropic response by 6×10^{-6} mol/liter prazosin (·····). Ordinate, Developed tension in mN. Abscissa, Time in msec after the initiating stimulus.

situation, time from addition of norepinephrine to 50% relative response was 34 ± 4 sec (mean ± S.E., n = 14) (table 1, fig. 1). The inotropic response thus elicited demonstrated sensitivity both to the *alpha*-1 adrenoceptor blocker prazosin and to the *beta* adrenoceptor blocker timolol. This was demonstrated as the reversal responses to sequential addition of the respective adrenoceptor blockers at the steady state phase of the inotropic response (fig. 3) (see also Skomedal *et al.*, 1988). The inotropic response to norepinephrine thus showed two components: one sensitive to the *alpha*-1 adrenoceptor blocker prazosin, amounting to 26.7 ± 3.5% of the total response (mean ± S.E., n = 12) and one sensitive to the *beta*-adrenoceptor blocker timolol, amounting to 73.3 ± 3.5% of total response (mean ± S.E., n = 12).

Qualitative characteristics of the inotropic responses to norepinephrine in the presence and absence of adrenergic blocking agents. Time to peak tension in the control period was 183 ± 5 msec (mean ± S.E., n = 16). The inotropic responses elicited by 1.2×10^{-4} mol/liter norepinephrine in the presence of prazosin and timolol, respectively, were accompanied by different qualitative changes in the contraction-relaxation cycles. In the presence of 6×10^{-6} mol/liter prazosin, there was a shortening of the duration of the contraction-relaxation cycles (fig. 2a) with a

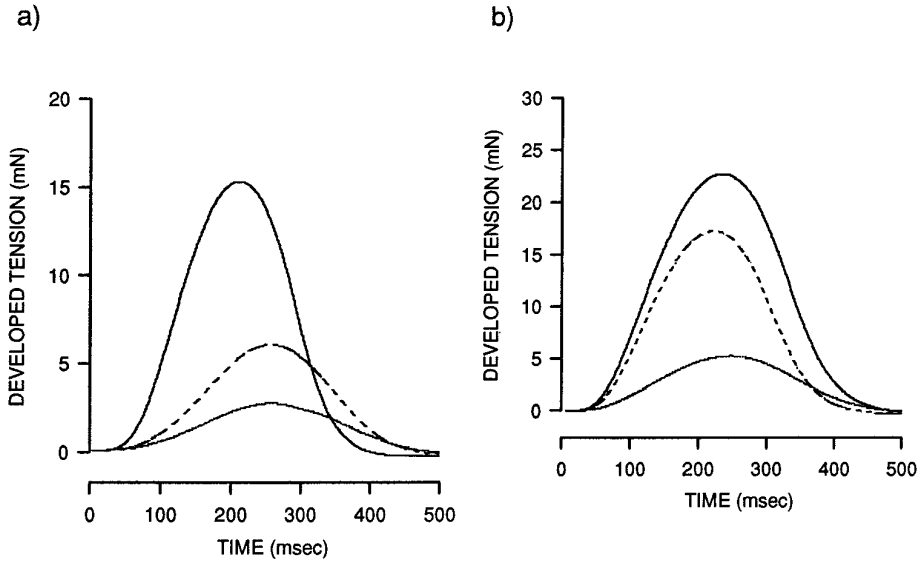

Fig. 3. a) Reversal of the inotropic response to concomitant *alpha*-1 and *beta* adrenoceptor stimulation by norepinephrine: steady state response to 1.2×10^{-4} mol/liter norepinephrine (—), reversal response to 6×10^{-6} mol/liter timolol (---) and reversal response to 6×10^{-6} mol/liter prazosin (·····). b, Reversal of the inotropic response to concomitant *alpha*-1 and *beta* adrenoceptor stimulation by norepinephrine: steady state response to 1.2×10^{-4} mol/liter norepinephrine (—), reversal response to 6×10^{-6} mol/liter prazosin (---) and reversal response to 6×10^{-6} mol/liter timolol (·····). Ordinate, Developed tension in mN. Abscissa, Time in msec after the initiating stimulus.

significant reduction in TPT, TR20 and RT (table 1) reflecting a selective increase in relaxation compared to contraction. The shortening of TPT, TR20 and RT was sensitive to the *beta* adrenoceptor blocker timolol (fig. 2a).

In the presence of 6×10^{-6} mol/liter timolol, the changes in the contraction-relaxation cycles caused by 1.2×10^{-4} mol/liter norepinephrine were markedly different from those described for the presence of prazosin. In this situation there was no shortening of the duration of the contraction-relaxation cycles (fig. 2b). The values for TPT, TR20 and RT were unchanged compared to control (table 1).

The response to norepinephrine in the absence of adrenergic blockers had characteristics similar to those observed in the presence of prazosin with a significant reduction in TPT, TR20 and RT (table 1). This shortening of TPT and TR20 was sensitive to timolol, but not to prazosin as evident from figure 3, a and b.

Influence of cholinergic stimulation on the mechanical responses to norepinephrine in the presence of adrenergic blockers. Activation of muscarinic receptors by 1.2×10^{-4} mol/liter carbachol influenced differently the steady state response to norepinephrine in the presence of prazosin and timolol, respectively. In the presence of 6×10^{-6} mol/liter prazosin, both the inotropic response and the shortening of time to peak tension induced by 1.2×10^{-4} mol/liter norepinephrine were attenuated by carbachol (table 2). In the presence of 6×10^{-6} mol/liter timolol, however, carbachol increased the inotropic response to norepinephrine although time to peak tension was almost unchanged (table 2). The carbachol-induced changes were sensitive to the muscarinic receptor blocker atropine (data not shown).

Dose-response relationships of the inotropic responses to norepinephrine in the presence of adrenergic blockers. Norepinephrine evoked a concentration-dependent increase in contractile force both in the presence of prazosin and of timolol, respectively (fig. 4, table 3). In muscle tissue obtained from hearts failing due to ischemic cardiomyopathy, norepinephrine appeared to be equipotent in the presence of prazosin and timolol, respectively (fig. 4a, table 3). In muscle tissue obtained from hearts failing due to nonischemic cardiomyopathy, however, norepinephrine was apparently about 10 times more potent in the presence of prazosin compared to the presence of timolol (fig. 4b, table 3).

The qualitative changes of the contraction-relaxation cycles were similar to those found in the corresponding time course experiments (cf. fig. 2).

TABLE 2

Effect of carbachol on the response to norepinephrine in the presence of adrenergic blockers

	Inotropic Response, % of Norepinephrine ($n = 4$)	Change in Time to Peak Tension (TPT), % of Norepinephrine ($n = 4$)
Norepinephrine in the presence of timolol		
Control response	100	100
+ Carbachol	107 ± 2[a]	100 ± 0
Norepinephrine in the presence of prazosin		
Control response	100	100
+ Carbachol	44 ± 5[a]	45 ± 7[a]

[a] Significantly different from corresponding control response (P = .014).

Maximal inotropic responses to norepinephrine in the presence of adrenergic blockers. The responses to maximal stimulation by norepinephrine varied considerably in preparations from different individuals. There was, however, an apparent covariation between the inotropic response in the presence of prazosin and in the presence of timolol in preparations obtained from the same heart and chamber (fig. 5) with a rank order correlation of $r^2 = 0.4793$ (P = .013). Thus, the mean values for the maximal effect of the two inotropic response components of norepinephrine were comparable both in time course (single concentration) experiments (fig. 5) and in dose-response (multiple concentrations) experiments (table 3).

Influence of thyroid status on adrenergic inotropic responses to norepinephrine. In one heart failing due to ischemic cardiomyopathy from a patient judged to be hypothyroid as indicated by an elevated TSH value (TSH = 20 nmol/liter), the dose-reponse relationships to norepinephrine deviated from those found in other hearts failing both due to ischemic and due to nonischemic cardiomyopathy (fig. 6). In this situation norepinephrine was about 30 times more potent at the *alpha*-1 compared to the *beta* adrenoceptors. The maximal inotropic responses mediated by the two adrenoceptor systems were, however, still comparable (table 3).

Discussion

Our observations revealed the existence of an *alpha*-1 adrenoceptor-mediated inotropic response that can be elicited by norepinephrine also in the terminally failing human heart ventricle. This is supported by 1) the appearance of a slowly developing inotropic effect of norepinephrine in the presence of 6×10^{-6} mol/liter timolol; 2) the sensitivity of this inotropic response to the *alpha*-1 adrenergic receptor blocker prazosin; 3) the qualitative characteristics of the response and 4) the insensitivity of this inotropic response to be attenuated by concomitant activation of muscarinic cholinergic receptors.

Furthermore, quantitatively the mean inotropic response evoked by activation of *alpha*-1 adrenergic receptors was found to be comparable to that evoked through activation of *beta* adrenergic receptors when expressed as maximal developed tension. This is in contrast to several *ex vivo* studies on failing human myocardium where the *alpha*-1 adrenoceptor-mediated inotropic response to phenylephrine has been found to be small compared to the *beta* adrenoceptor-mediated inotropic response to isoprenaline (*e.g.*, Böhm *et al.* 1988, Brodde *et al.*, 1992; Steinfath *et al.*, 1992; Bristow 1993). Although a great variation in inotropic responses between different hearts was observed in our study, this apparently applied to the *alpha*-1 adrenoceptor-mediated and the *beta*-adrenoceptor-mediated inotropic responses in parallel (fig. 5).

In the presence of 6×10^{-6} mol/liter prazosin, there was a fast developing inotropic response to norepinephrine. Qualitively this response was characterized by a shortening of the duration of the contraction-relaxation cycle. In addition the response was attenuated by concomitant activation of muscarinic receptors. These characteristics are typical for a *beta* adrenoceptor/cyclic AMP-dependent mediated inotropic response in mammalian heart muscle, *e.g.*, of isoprenaline or of norepinephrine in the presence of an *alpha*-adrenoceptor blocker (Ledda *et al.*, 1975, Brückner *et al.*, 1978, Skomedal *et*

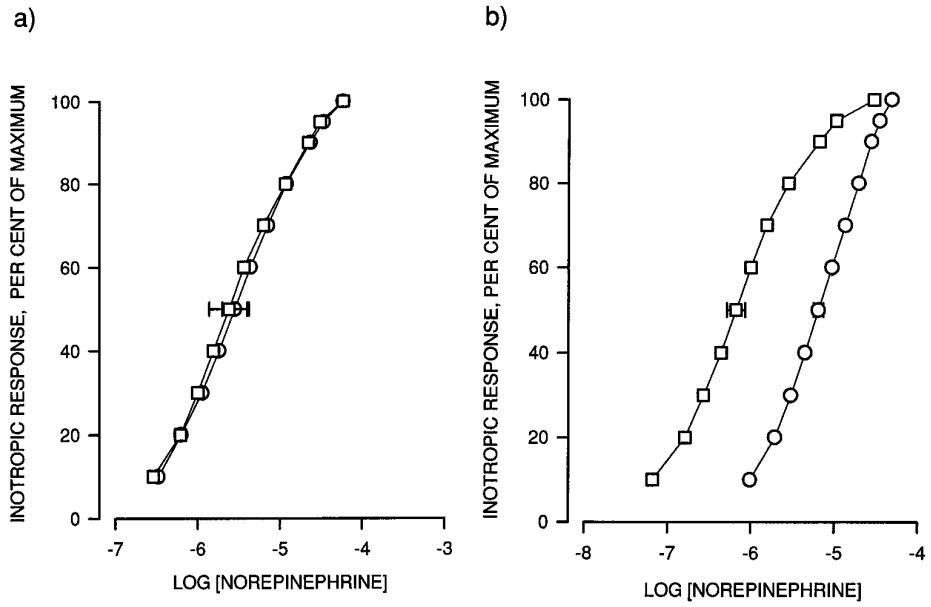

Fig. 4. Inotropic responses expressed as developed tension (T_{max}) to increasing concentrations of norepinephrine in human ventricular myocardium failing a) due to ischemic cardiomyopathy and b) due to nonischemic cardiomyopathy: *alpha*-1 adrenoceptor-mediated response in the presence of 6×10^{-6} mol/liter timolol (○, $n = 6$); *beta* adrenoceptor mediated response in the presence of 6×10^{-6} mol/liter prazosin (□, a, $n = 6$; b, $n = 5$). Ordinate, Inotropic response in percent of individual maxima. Abscissa, Log concentration of norepinephrine (mol/liter). Horizontal bars represent ± S.E. of pD_2 values.

TABLE 3
Potency and efficacy of norepinephrine in human myocardium failing due to different etiology

Etiology of Heart Failure	Potency, pD_2 Value	Efficacy, Inotropic Response, % of Control Period
Nonischemic cardiomyopathy		
Norepinephrine + prazosin ($n = 5$)	6.18 ± 0.11	153 ± 14
Norepinephrine + timolol ($n = 6$)	5.19 ± 0.06[a]	148 ± 10
Ischemic cardiomyopathy		
Norepinephrine + prazosin ($n = 6$)	5.62 ± 0.24	179 ± 10
Norepinephrine + timolol ($n = 6$)	5.55 ± 0.15	171 ± 17
Ischemic cardiomyopathy and hypothyroidism		
Norepinephrine + prazosin ($n = 2$)	4.23 ± 0.35	144 ± 13
Norepinephrine + timolol ($n = 2$)	5.72 ± 0.19	133 ± 7

[a] Significantly different from corresponding value obtained in the presence of prazosin ($P = .008$).

al., 1982, Skomedal and Osnes, 1983, Aass *et al.*, 1983, Skomedal *et al.*, 1985; Aass *et al.*, 1986; reviews: Scholz 1980; Osnes *et al.*, 1985). This response was sensitive to the *beta*-adrenoceptor blocker timolol (fig. 2a) and was taken as the typical *beta* adrenoceptor-mediated component of norepinephrine in failing human ventricle.

In contrast to the situation described above, the inotropic response evoked by norepinephrine in the presence of 6×10^{-6} mol/liter timolol developed slowly. Qualitively this response was characterized by a "symmetrical" change of the contraction-relaxation cycle with unchanged values for TPT, TR20 and RT (=TR20-TPT). In addition, the response was reinforced and not attenuated by concomitant activation of muscarinic receptors. These characteristics are typical for an *alpha*-1 adrenoceptor/cyclic AMP-independent mediated inotropic response in mammalian heart muscle, *e.g.*, of phenylephrine or norepinephrine in the presence of a *beta* adrenoceptor blocker (Ledda *et al.*, 1975, Brückner *et al.*, 1978, Skomedal *et al.*, 1982, Skomedal and Osnes, 1983, Aass *et al.*, 1983, Skomedal *et al.*, 1985; Aass *et al.*, 1986; reviews: Scholz 1980; Osnes *et al.*, 1985). This response was sensitive to the *alpha*-1 adrenoceptor blocker prazosin (fig. 2b) and was thus taken as the typical *alpha*-1 adrenoceptor-mediated component of norepinephrine in failing human ventricle.

Activation of muscarinic receptors by carbachol differentially influenced the *alpha*-1 adrenoceptor and the *beta* adrenoceptor-mediated inotropic responses. This phenomenon is well known from other mammalian species (Endoh and Motomura, 1979) and also described in human ventriclar muscle (Neumann *et al.*, 1993; Scholz *et al.*, 1996). The explanations for these different effects are mainly related to 1) the bidirectional regulation of adenylyl cyclase by *beta* adrenoceptors and muscarinic cholinergic receptors and accordingly to cyclic AMP mediated effects on *e.g.*, sarcoplasmic reticulum and myofilaments (review: Sulakhe and Vo, 1995) and to 2) a possible reinforcement of breakdown of phosphoinositides by stimulation of *alpha*-1 adrenoceptors and of muscarinic cholinergic receptors (*e.g.*, Brown *et al.*, 1985; Poggioli *et al.*, 1986; Ransnäs *et al.*, 1986). The response to cholinergic agonists in this situation can thus be used as a functional test whether the cyclic AMP system is activated.

In experiments with norepinephrine in the absence of adrenoceptor blockers (combined *alpha*-1 and *beta* adrenoceptor stimulation), the time course deviated both from the time course to *alpha*-1 and from the time course to *beta* adrenoceptor stimulation (fig. 1, table 1). Thus, the development of the inotropic response in human failing heart to unopposed stimulation by norepinephrine was influenced by both recep-

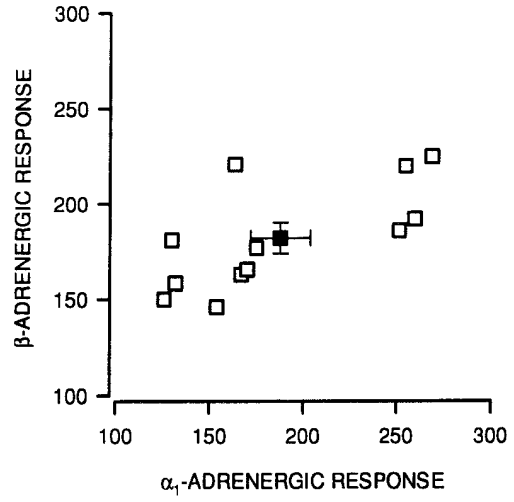

Fig. 5. Maximal inotropic responses in failing human ventricular myocardium to separate *alpha*-1 and *beta* adrenoceptor stimulation by 1.2×10^{-4} mol/liter norepinephrine in the presence of appropriate receptor blockers obtained in corresponding hearts and chambers (□, $n = 12$). ■ and bars represent mean ± S.E. of the separate values. Ordinate, *beta* adrenoceptor-mediated inotropic response in percent of control period. Abscissa, *alpha*-1 adrenoceptor-mediated inotropic response in percent of control period. Rank order correlation, $r^2 = 0.4793$, P = .013.

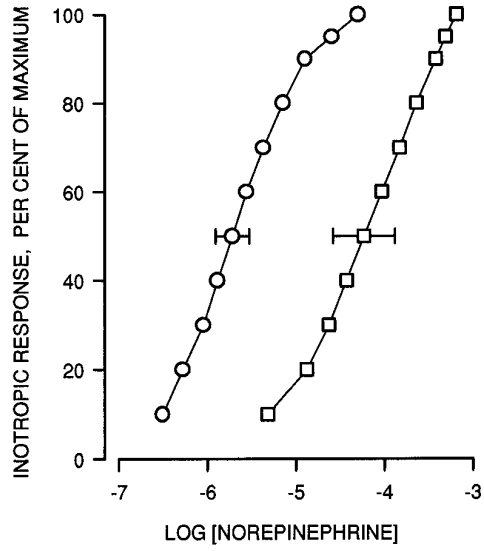

Fig. 6. Inotropic responses expressed as developed tension (T_{max}) to increasing concentrations of norepinephrine in ventricular myocardium from a patient with hypothyroidism failing due to ischemic cardiomyopathy: *alpha*-1 adrenoceptor-mediated response in the presence of 6×10^{-6} mol/liter timolol (○, $n = 2$); *beta* adrenoceptor-mediated response in the presence of 6×10^{-6} mol/liter prazosin (□, $n = 2$). Ordinate, Inotropic response in per cent of individual maxima. Abscissa, Log concentration of norepinephrine (mol/liter). Horizontal bars represent ± S.E. of pD_2 values.

tor populations demonstrating an interaction between the two adrenergic receptor systems. This is in accordance with earlier findings in normal rabbit myocardium (Skomedal *et al.*, 1990b).

Reversal responses to adrenergic receptor blockers revealed a functional contribution of the *alpha*-1-adrenoceptor-mediated component to the final and unopposed response to norepinephrine also in failing human myocardium. The relative contribution of the *alpha*-1 adrenoceptor-mediated (prazosin sensitive) component and of the *beta* adrenoceptor-mediated (timolol sensitive) component to the unopposed inotropic response to norepinephrine found in our work is in general agreement with findings in normal myocardium from rat and rabbit (Skomedal *et al.*, 1988; Osnes *et al.*, 1989, Skomedal *et al.*, 1990a) and in atria from children with congenital heart defects (Borthne *et al.*, 1995). Quantitatively, there is thus a discrepancy between the expression of the *alpha*-1 adrenoceptor-mediated inotropic response during selective activation and activation of the *alpha*-1 adrenoceptor concomitantly with the *beta* adrenoceptors. An inhibitory interaction between the *alpha*-1 and the *beta* adrenoceptors is thus revealed in failing human myocardium and this observation is also in general agreement with findings in normal myocardium from rat and rabbit (Skomedal *et al.*, 1988, 1990a). These response patterns are thus apparently general ones for the dual adrenoceptor regulation of the mammalian myocardium whether normal or failing.

The concentration of norepinephrine used in the time course experiments was chosen to give close to maximal inotropic responses. In dose-response experiments also graded responses were demonstrated and these experiments provided classical dose-response relationships for norepinephrine in the presence of the respective adrenergic blockers. The typical qualitative characteristics of the *alpha*-1 adrenoceptor-mediated and the *beta* adrenoceptor-mediated inotropic responses were observed also in these experiments. Especially, at maximal concentrations of norepinephrine in the presence of 6×10^{-6} mol/liter timolol there was no shortening of the duration of the contraction-relaxation cycle demonstrating the sufficiency of the *beta* adrenoceptor blockade (table 1).

Dose-response experiments indicated differences that may be interesting with respect to etiology of the heart failure. In failure due to ischemic cardiomyopathy, the *alpha*-1 and *beta* adrenoceptor systems were apparently equally sensitive to activation by norepinephrine (fig. 4a). In failure due to nonischemic cardiomyopathy, the *beta* adrenoceptor system was the more sensitive one by about one log unit compared to the *alpha*-1 adrenoceptor system (fig. 4b). This difference in sensitivity at the *beta* adrenoceptors did not parallel the difference in cardiac index observed between the ischemic and nonischemic heart failure groups, respectively. There were no obvious differences in responsiveness (expressed as maximal developed tension) between the two adrenergic systems to activation by norepinephrine although the mean responses were nominally less in nonischemic compared to ischemic cardiomyopathy (table 3). This nominal difference in responsiveness to adrenergic stimulation paralleled, however, the difference in cardiac index between the ischemic and the nonischemic failing hearts.

It has been reported that *beta* adrenergic responsiveness decreases with age in human heart (White *et al.*, 1994). The observed difference in sensitivity to *alpha*-1 and *beta* adrenoceptor stimulation in preparations used for dose-reponse experiments may at least partly be due to age differences as the patients transplanted due to ischemic cardiomyopathy were about 20 yr older (51–61 yr, mean 56 yr) than the patients transplanted due to nonischemic cardiomyopathy (18–53 yr, mean 37 yr). Because our study was not designed to investigate more specifically mechanisms related to *beta*-adrenergic effects, other possible explanations for the ob-

served differences will be speculative only. However, differences in the *beta* adrenergic effector mechanisms both with respect to downregulation and to uncoupling of receptors in ischemic *vs*. nonischemic cardiomyopathy have been reported (Bristow *et al.*, 1991, review: Bristow, 1993).

In several reports the *alpha*-1 adrenoceptor-mediated inotropic effect of phenylephrine (in the presence of *beta* adrenoceptor blocker) has repeatedly been reported to be small compared to the *beta* adrenoceptor-mediated inotropic effect of isoprenaline (*e.g.*, Böhm *et al.*, 1988, Brodde *et al.*, 1992; Steinfath *et al.*, 1992; Bristow 1993). Although Böhm *et al.* (1988) suggested an increased importance of the *alpha*-1 adrenoceptor-mediated inotropic effect in severe heart failure, the *alpha*-1 adrenoceptor-mediated effect of phenylephrine in failing human heart has been considered to be of little interest, *e.g.*, as a compensating mechanism for the attenuated *beta* adrenoceptor-mediated effect that is regularly observed in failing heart muscle (*e.g.*, Brodde *et al.*, 1992). Aass *et al.* (1986) were the first to report an *alpha*-1 adrenoceptor-mediated inotropic effect of norepinephrine in human ventricular myocardium. During the collection period for our data, Neumann *et al.* (1993) and Scholz *et al.* (1995, 1996) presented data in accordance with ours: norepinephrine is apparently able to elicit an *alpha*-1 adrenoceptor-mediated inotropic response in failing human ventricular myocardium that is of a magnitude that makes this component interesting compared to the *beta* adrenoceptor-mediated component. In our work, the *alpha*-1 adrenoceptor-mediated inotropic component of norepinephrine is of a magnitude comparable to the *beta* adrenoceptor-mediated inotropic component and will make it appropriate to reconsider the functional importance of this regulatory mechanism during terminal heart failure.

Norepinephrine and phenylephrine have apparently different intrinsic activities at the *alpha*-1 adrenoceptors in failing human heart. This has not been evident from *ex vivo* experiments in animal myocardium (*e.g.*, Aass *et al.*, 1983) and has, to our knowledge, not been addressed with respect to heart muscle before Neumann *et al.* (1993) and Scholz *et al.* (1995, 1996) presented their data where phenylephrine was found to be a partial agonist with very low intrinsic activity at the human cardiac *alpha*-1 adrenoceptors compared to norepinephrine. In our work phenylephrine exerted an inotropic response of about 70% compared to norepinephrine in preparations obtained from the same hearts. The time course curves were, however, almost superimposed (fig. 1), indicating no major difference between norepinephrine and phenylephrine in changing the kinetics of the involved processes. At present we have no explanation for the apparent difference between norepinephrine and phenylephrine with respect to intrinsic activity at the *alpha*-1 adrenoceptors in failing human heart muscle. To our knowledge, there is for example, no data indicating different intrinsic activity of norepinephrine and phenylephrine at different subtypes of *alpha*-1 adrenoceptors. However, the newly discovered *alpha*-1L subtype (Muramatsu *et al.*, 1995; Noguchi *et al.*, 1995) might be of interest because prazosin apparently is less potent in blocking *alpha*-1 adrenoceptor-mediated inotropic effects in human heart (Mügge *et al.*, 1983, Skomedal *et al.*, 1985) compared to rat and rabbit heart (Skomedal *et al.* 1980, Davey 1986, Hiramoto *et al.* 1988).

One patient was judged to be hypothyroid at the time of transplantation as indicated by an elevated level of TSH. This patient had a known hypothyroidism that was substituted with thyroxine. Due to serious arrhythmia the patient also received amiodarone which may cause hypothyroidism (review: Figge and Figge, 1990). Amiodarone will thus probably reinforce a hypothyroid effect upon the sensitivity of the cardiac *beta* adrenoceptors (*e.g.*, Bjørnerheim *et al.*, 1991). The sensitivity of the *beta* adrenoceptors to norepinephrine in this situation appeared to be markedly reduced compared to the *alpha*-1 adrenoceptors as expressed by a striking preference in stimulation of the *alpha*-1 adrenoceptors. Although these observations are from one heart only, the data are in general agreement with experimental findings in normal animal heart: hypothyroidism is known to increase the sensitivity to *alpha*-1 adrenocepor stimulation and to reduce the sensitivity to *beta* adrenoceptor stimulation (*e.g.*, Nakashima *et al.*, 1971, Kunos *et al.*, 1974, Osnes 1976, Williams and Lefkowitz, 1979, Fox *et al.*, 1985). Thus the thyroid status should apparently be kept in mind when interfering with the adrenoceptors of a failing heart.

In failing heart the *beta* adrenergic response is blunted (*e.g.*, Brodde *et al.*, 1992, Steinfath *et al.*, 1992, Bristow, 1993). Our observations indicated that the *alpha*-1 adrenoceptor-mediated response thus may be of functional importance in comparison with the *beta* adrenoceptor-mediated response. In situations where the *beta* adrenoceptor-mediated inotropic component is further attenuated (cholinergic activity, use of *beta* adrenoceptor blockers, hypothyroidism), the *alpha*-1 adrenoceptor-mediated inotropic component may be even more important also during heart failure. Although speculative, an intriguing possibility is that the *alpha*-1 adrenoceptor-mediated effects may at least support the contractile reserve and may even contribute to the "recovery" of heart function seen in terminal failing hearts that are treated with *beta*-adrenoceptor blockers (see *e.g.*, Eichhorn and Hjalmarson, 1994).

In conclusion, in contrast to findings with phenylephrine as *alpha*-1 adrenergic agonist, the endogenous agonist norepinephrine apparently exerts an *alpha*-1 adrenoceptor-mediated inotropic effect in failing human myocardium that is comparable to the *beta*-adrenoceptor-mediated one when expressed as developed tension. During concomitant activation of adrenergic receptors the final inotropic response to norepinephrine is mediated through both receptor systems. The *alpha*-1 adrenoceptor-mediated inotropic component is thus, although varying, apparently of potential functional importance in failing human ventricular myocardium.

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Send reprint requests to: Dr. Tor Skomedal, University of Oslo, Department of Pharmacology, P.O. Box 1057 Blindern, N-0316 Oslo, Norway.