Presynaptic modulation of evoked NE release contributes to sympathetic activation after pressure overload

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Submitted 13 September 2003; accepted in final form 26 January 2004

Akers, Wendell S., and Lisa A. Cassis. Presynaptic modulation of evoked NE release contributes to sympathetic activation after pressure overload. Am J Physiol Heart Circ Physiol 286: H2151–H2158, 2004. — Activation of the sympathetic nervous system is well documented in heart failure. Our previous studies demonstrated an increase in evoked norepinephrine (NE) release from left ventricle (LV) slices at 10 days of pressure overload. The purpose of this study was to test the hypothesis that presynaptic modulation of NE release contributes to sympathetic activation after pressure overload. We examined the functional status of the presynaptic α2- and β2-receptors and ANG II subtype 1 (AT1) receptors in LV slices from 10-day aortic constricted (AC) and sham-operated (SO) rats. Evoked 3H overflow from LV slices preloaded with [3H]NE was increased in AC rats. The α2-agonist UK-14,304 decreased evoked 3H overflow with no differences between groups. The β2-agonist salbutamol increased evoked 3H overflow with greater sensitivity in slices from AC rats. The β2-antagonist propranolol decreased evoked 3H overflow from LV slices of AC rats but not controls. ANG II increased evoked 3H overflow with greater sensitivity in slices from AC rats. These data support the hypothesis that aberrant presynaptic modulation of catecholamine release contributes to sympathetic activation after pressure overload.

Congestive heart failure activates a variety of neurohumoral systems including the pituitary vasopressin, renin-angiotensin, and sympathetic nervous systems (13, 33, 49). Sympathetic nervous system activation has been well documented in patients with heart failure as demonstrated by increases in plasma catecholamine levels (49), cardiac norepinephrine (NE) spill-over rates (13, 26, 33), and skeletal muscle sympathetic nerve firing rates (26, 33). Direct demonstration of elevated cardiac interstitial NE has also been documented in failing myocardium (12). Increases in interstitial NE in the heart are most likely the result of an increase in cardiac NE release and a decrease in neuronal NE uptake (3, 37). Understanding the mechanisms that contribute to activation of the sympathetic nervous system is of significance in the pathophysiology of heart failure because results from clinical studies demonstrate that sympathetic activation is associated with increased mortality in heart failure (13, 26, 49).

A variety of experimental animal models exist for the study of cardiac hypertrophy and dysfunction (28). A common experimental model of cardiac hypertrophy and subsequent cardiac dysfunction is surgical aortic constriction in rats, which produces sustained pressure overload to the heart. Previous studies in our laboratory demonstrated neurohumoral activation of the sympathetic nervous and renin-angiotensin systems early (within 3–10 days) in response to pressure overload (4). Moreover, using left ventricle (LV) slices to examine neuronal NE uptake, previous studies in our laboratory (3) demonstrated a reduction in the maximal capacity for [3H]NE uptake at 10 days of pressure overload. Coupled with reductions in [3H]NE uptake, the evoked release of [3H]NE was increased in LV slices from rats after 10 days of pressure overload thereby demonstrating alterations localized to cardiac sympathetic nerve terminals (4). Other evidence that demonstrates sympathetic activation in response to pressure overload is a reduction in left ventricular NE content associated with an increase in NE turnover in the heart (20–22, 45). Collectively, previous results demonstrate elevations in sympathetic drive in response to pressure overload; however, the mechanisms [i.e., increase in efferent central nervous system (CNS) sympathetic outflow and alterations at peripheral sympathetic nerve terminals] responsible for sympathetic activation have not been clearly defined.

There is compelling evidence indicating that the release of NE from sympathetic nerve terminals is modulated by endogenous or exogenous substances acting at receptor sites associated with cardiac nerve terminals. Previous studies in our laboratory (3) demonstrated that the sympathetic nerve terminals that innervate the rat LV possess functional α2- and β2-adrenergic receptors that are capable of negative and positive modulation of NE release, respectively. The purpose of this study was to test the hypothesis that aberrant presynaptic modulation of catecholamine release contributes to sympathetic activation early in the development of cardiac hypertrophy and failure. Using the LV tissue-slice system previously established in our laboratory, we examined the functional status of α2-adrenergic receptors because this is a well-documented autoregulatory mechanism for negative-feedback control of catecholamine release. In addition, we examined the functional status of presynaptic β2-adrenergic receptors and ANG II subtype 1 (AT1) receptors as facilitatory modulators of catecholamine release. Our rationale for examination of these facilitatory receptors is based on previous studies that demonstrated alterations in epinephrine (30) and ANG II (4, 29) levels in the pressure-overload model and in the pathophysiology of congestive heart failure.

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MATERIALS AND METHODS

Animals. Male Sprague-Dawley rats (275–325 g, 7–9 wk of age; Harlan Sprague Dawley; Indianapolis, IN) were used in all experiments. Rats were housed two per cage with free access to food and water. All studies were approved by the Institutional Animal Care and Use Committee of the University of Kentucky.

Surgical induction of pressure overload. Rats were randomly assigned to one of two surgical treatment groups: aortic constricted (AC) or sham operated (SO). Rats were anesthetized with ketamine hydrochloride plus acepromazine maleate (90 and 0.02 mg/kg ip, respectively; Fort Dodge Laboratories; Fort Dodge, IA) and prepared for surgery under aseptic conditions. After a midline abdominal laparotomy was performed, pressure overload was induced by supra-renal abdominal aortic constriction using a tantalum Weck hemoclip (Pilling Weck; Research Triangle Park, NC) tightened to the diameter of a 22-gauge needle (4). Control rats underwent sham surgery consisting of midline laparotomy and isolation of the suprarenal abdominal aorta without constriction. The muscle was sutured and the skin was closed using surgical wound clips.

LV slices. Preparation of LV slices was based on previously established methods (3). The heart was quickly removed and placed in ice-cold Krebs buffer that contained (in mM) 108 NaCl, 14.9 NaHCO3, 5.5 dextrose, 4.7 KCl, 1.2 MgSO4, 1.2 KH2PO4, 0.11 ascorbic acid, and 0.004 EDTA; pH 7.4. The LV was dissected from the atra and right ventricle, and LV slices of 500 μm thickness (30–50 mg) were obtained from the septal wall region using a McVilwain tissue chopper. Methods for examining evoked [3H]NE overflow from superfused slices are based on those previously described (3). Slices were transferred to a metabolic shaker and preincubated for 30 min in 25 ml of oxygenated Krebs buffer at 37°C. Slices were incubated for 30 min in fresh Krebs buffer that contained [3H]NE (0.01 μM) to allow radiolabeled NE uptake into sympathetic nerve terminals via the neuronal uptake-1 transporter. Slices (14 slices/rat) were transferred to Plexiglas superfusion chambers (total volume, 1 ml) that contained two platinum wire electrodes. Desipramine (10 nM) was included in the buffer to inhibit NE reuptake from the synapse into the sympathetic nerve terminal by the neuronal uptake-1 transporter during electrical field stimulation (3). Pargyline (1 μM) was included in the buffer to inhibit metabolism of NE by monoamine oxidase (3, 50, S1). Slices were superfused (1 ml/min) for 90 min with Krebs buffer to allow basal [3H]NE outflow to stabilize before initiation of experimental protocols to examine the specific effect of presynaptic modulators on evoked [3H]NE overflow. Basal [3H]NE outflow represents nonspecific tissue binding or superficially bound [3H]NE removed during the 90-min superfusion period. Each slice was electrically stimulated with a priming stimulus provided by a Grass stimulator (60 V, 2 ms duration; model S44, Grass Instrument; Quincy, MA) during the equilibration period to increase calcium loading into sympathetic nerve terminals and thereby allow for equivalent release during two identical electrical stimulations (S1 and S2) in the absence and presence of specific presynaptic modulators.

Experimental protocols for presynaptic modulators. During each experimental protocol, 5-ml samples of superfusate (total, 20 samples/slice) were collected for 90 min during which each slice was electrically stimulated with a Grass stimulator to determine the effects of each presynaptic modulator on evoked [3H]NE overflow. All presynaptic modulators were included in the superfusion buffer 15 min before the stimulus. At the end of the experiment, each slice was weighed and solubilized in 500 μl of tissue solubilizer (TS-2; Research Products International; Mt. Prospect, IL) overnight at 50°C. Scintillation cocktail (10 ml, 3a70B; Research Products International) was added to determine the [3H] content via liquid scintillation spectrometry using a Packard TriCarb liquid scintillation analyzer, which has a 63% counting efficiency for [3H].

The experimental design for determining the effects of presynaptic receptor agonists on evoked [3H] overflow in AC and SO rats was an across-slice comparison such that two slices served as controls and five slices were exposed to different concentrations of drugs. For studies to examine the presynaptic effects of ANG II (1–1000 nM), propranolol (10 μM), yohimbine (1 μM), and losartan (1 μM), slices were electrically stimulated at 5 Hz for 2 min (total, 600 pulses). For studies to examine the presynaptic effects of UK-14,304 (10–1,000 nM), slices were electrically stimulated at 50 Hz for 1 s (total, 50 pulses). The stimulation conditions (50 Hz for 1 s; total, 50 pulses) were chosen based on previous results, which demonstrated that agonist stimulation of the α2-autoreceptor is best characterized at stimulations of higher frequency and low numbers of electrical pulses to minimize autoregulation by endogenously released NE (17, 18, 45). For studies to examine the presynaptic effects of salbutamol (1–1,000 nM), slices were electrically stimulated at 0.5 Hz for 2 min (total, 60 pulses). The stimulation conditions (0.5 Hz for 2 min) were chosen based on previous results that demonstrated optimal detection of β2-receptor facilitation of NE release at stimulations of lower frequency (6, 31). Moreover, the α2-receptor antagonist yohimbine was included in the superfusion buffer of all slices based on previous studies, which demonstrated that autoregulation through the α2-adrenergic receptor effectively masked β2-adrenergic regulation of evoked NE release (6, 31). For studies to examine presynaptic ANG II receptors in LV slices from control rats, slices were electrically stimulated (5 Hz for 2 min; total, 600 pulses) in the absence (S1) and presence (S2) of either ANG II (10 nM), losartan (1 μM, AT1-receptor antagonist), or ANG II (10 nM) plus losartan (1 μM).

Data and statistical analysis. The LV-to-body weight ratio (LV/BW) was calculated by dividing the LV weight (in g) by the BW for each rat and multiplying by 1,000. Calculations of fractional release, basal [3H] outflow, and evoked [3H] overflow have been previously described (3). Fractional release is the amount of [3H] in each sample expressed as the percentage of tissue [3H]; it is calculated by dividing the amount of [3H] in each superfusate sample by the total amount of [3H] present in the tissue at the time of sample collection. Basal [3H] overflow is the amount of [3H] in the 5-min sample before electrical stimulation. Evoked [3H] overflow is the summation of increases in the amount of [3H] in superfusate samples collected during and after an electrical stimulation minus the basal [3H] outflow. For studies to examine the effects of an AT1 receptor antagonist on increased evoked [3H] overflow by ANG II in LV slices from control rats, a ratio (S2/S1) of evoked [3H] overflow was calculated to determine the effects of the drug. In studies using LV slices from AC and SO rats, we did not use conventional calculation of S2/S1 ratios to determine the effects of drugs due to differences in baseline evoked [3H] overflow between groups (i.e., a greater denominator for S1 in slices from AC rats compared with SO controls). Thus absolute values of fractional release were used to examine the effects of each presynaptic modulator.

Data are presented as means ± SE. For each study to examine the effects of drug on evoked [3H] overflow, a two-way ANOVA was performed with aortic constriction as a between-group factor and drug concentration as a within-group repeated measure. Duncan’s multiple-range test was used for post hoc comparisons. A P value <0.05 was considered statistically significant.

RESULTS

Abdominal aortic constriction resulted in a significant increase in LV weight (SO, 0.904 ± 0.023 g; AC, 1.070 ± 0.021 g; P < 0.05) after 10 days of pressure overload, which remained evident when normalized to BW (LV/BW: SO, 2.50 ± 0.04; AC, 3.08 ± 0.12; P < 0.05). No significant difference was observed in the BWs (SO, 362 ± 8 g; AC, 353 ± 10 g) of the two groups. The effects of electrical stimulation on evoked [3H] overflow were examined using LV slices from SO and AC rats. LV slices from SO and AC rats were an
were similar in weight and tissue 3H content (data not shown). Statistical analysis demonstrated a significant increase in evoked 3H overflow from LV slices of AC rats compared with SO rats (SO, 2.97 ± 1.13; AC, 4.06 ± 1.35; P < 0.05).

Presynaptic α2-adrenergic receptors. The concentration-dependent effects of the α2-agonist UK-14,304 on evoked 3H overflow were examined to determine the functional status of cardiac presynaptic α2-adrenergic receptors after 10 days of pressure overload (n = 6/group). After 10 days of pressure overload, LV hypertrophy had developed in AC rats (LV/BW: SO, 2.58 ± 0.05; AC, 3.01 ± 0.33; P < 0.05). In the absence of UK-14,304, evoked 3H overflow from LV slices of AC rats was significantly increased compared with SO controls at a stimulation of 50 Hz and 50 electrical pulses (Fig. 1). Statistical analysis demonstrated a significant effect of UK-14,304 concentration [F(5,50) = 8; P < 0.05] to decrease evoked 3H overflow from LV slices of SO and AC rats with no between-group differences. To confirm that the stimulation conditions did not evoke sufficient levels of endogenous NE to compete with UK-14,304 at the presynaptic α2-receptor, the α2-agonist yohimbine (1 μM) was included in the superfusion buffer of two slices. Yohimbine did not significantly influence evoked 3H overflow in slices from SO and AC rats (data not shown).

In separate studies, the effects of the α2-adrenergic receptor antagonist yohimbine on evoked 3H overflow from LV slices were examined after 10 days of pressure overload (n = 6/group). After 10 days of pressure overload, LV hypertrophy had developed in AC rats (LV/BW: SO, 2.42 ± 0.06; AC, 2.85 ± 0.06; P < 0.05). In the absence of yohimbine, evoked 3H overflow from LV slices of AC rats was significantly increased compared with SO controls at a stimulation of 5 Hz and 600 electrical pulses (Fig. 2). Statistical analysis demonstrated a significant effect of yohimbine (P < 0.05) on evoked 3H overflow from LV slices of SO and AC rats with no between-group differences. Inclusion of yohimbine (1 μM) in the superfusion buffer resulted in a significant increase in evoked 3H overflow from LV slices of AC and SO rats.

Presynaptic β2-receptors. To determine the functional status of cardiac presynaptic β2-adrenergic receptors after 10 days of pressure overload, the concentration-dependent effects of the β2-agonist salbutamol on evoked 3H overflow from LV slices were examined (n = 6/group). After 10 days of pressure overload, LV hypertrophy had developed in AC rats (LV/BW: SO, 2.58 ± 0.05; AC, 3.01 ± 0.33; P < 0.05). At a stimulation of 0.5 Hz and 60 electrical pulses, evoked 3H overflow from LV slices was not significantly different between SO and AC rats (Fig. 3). Statistical analysis demonstrated a significant [F(1,10) = 13; P < 0.05] between-group difference in response to salbutamol and a significant [F(5,50) = 8; P < 0.05] within-group effect of salbutamol concentration on evoked 3H overflow. Salbutamol increased evoked 3H overflow from LV slices.
slices from AC rats, ANG II increased evoked $^3$H overflow in a concentration-dependent manner with a threshold effect for significance at 3 nM. Moreover, in the presence of ANG II (3, 100, and 300 nM), evoked $^3$H overflow was significantly greater in LV slices from AC rats compared with the same ANG II concentration in LV slices from SO control rats.

To identify the ANG II receptor subtype responsible for facilitation of evoked $^3$H overflow in LV slices, we determined the effects of the AT$_1$ receptor antagonist losartan on evoked $^3$H overflow from LV slices of control rats ($n = 3$ rats; Fig. 6). Inclusion of losartan (1 $\mu$M) in the superfusion buffer did not influence evoked $^3$H overflow (5 Hz and 2 min). ANG II (10 nM) resulted in a significant increase in evoked $^3$H overflow that was totally abolished in the presence of losartan. Additional studies determined the effects of losartan (1 $\mu$M) on evoked $^3$H overflow from LV slices after 10 days of pressure overload ($n = 4$/group). After 10 days of pressure overload, LV hypertrophy had developed in AC rats (LV/BW: SO, 2.68 ± 0.09; AC, 3.43 ± 0.49; $P < 0.05$). Evoked $^3$H overflow was significantly increased in LV slices from AC rats compared with SO controls at a stimulation of 5 Hz and 600 electrical pulses (Fig. 7). Inclusion of losartan in the superfusion buffer did not influence evoked $^3$H overflow from LV slices of AC or SO rats.

**DISCUSSION**

The present study yielded three principal findings related to the status of the cardiac sympathetic nervous system in the aortic constriction model of pressure overload. First, catecholamine release was increased at cardiac sympathetic nerve terminals devoid of CNS input. Second, the presynaptic $\alpha_2$-autoreceptor functioned normally to negatively modulate NE release. Third, the facilitatory presynaptic ANG II and $\beta_2$-adrenergic receptors exhibited increased sensitivity and may contribute to increased NE release. All of these findings occurred early (i.e., 10 days) in response to pressure overload coincident with the reported time course for the development of cardiac hypertrophy but preceding reported declines in

![Fig. 3. Effects of salbutamol on evoked $^3$H overflow from LV slices of SO and AC rats. Experimental design was the same as described for Fig. 1 with the exception of the electrical stimulus (0.5 Hz for 2 min). Salbutamol resulted in a significant increase in evoked $^3$H overflow from LV slices of AC and SO rats; however, the lowest effective concentration of salbutamol to increase evoked $^3$H overflow in LV slices from AC rats was lower compared with SO controls. $^*P < 0.05$, significantly different from absence of drug; $\dagger P < 0.05$, significantly different from SO control at same concentration.](http://ajpheart.physiology.org/)

![Fig. 4. Effects of propranolol on evoked $^3$H overflow from LV slices of SO and AC rats. Experimental design was the same as described for Fig. 1 with the exception of the electrical stimulus (5 Hz for 2 min). In the absence of drug, evoked $^3$H overflow was significantly different from absence of drug; $P < 0.05$, significantly different from SO control at same concentration.](http://ajpheart.physiology.org/)
baseline cardiac function (4). These data support the hypothesis that aberrant presynaptic modulation of catecholamine release may be an initial contributing factor to cardiac sympathetic activation and ultimately to subsequent defects in postsynaptic myocyte β-adrenergic receptors and cardiac dysfunction.

We used the LV tissue slice system for measurement of evoked NE release, a method previously established in our laboratory (3). Our previous results demonstrate that electrically evoked [3H]overflow from LV slices is calcium dependent, depends on the neuronal uptake transporter for recapture of evoked [3H] from the synapse, and is modulated by functional presynaptic α2- and β2-adrenergic receptors. The observation that LV slices from pressure-overloaded rats released greater amounts of [3H]NE with an electrical stimulus is consistent with our previous results (3). Moreover, these results demonstrate that elevations in sympathetic activity (i.e., increased [3H]overflow) are present at the level of the cardiac sympathetic nerve terminal, as the tissue slice model is devoid of direct CNS activity. Results from this study confirm and extend our previous findings by demonstrating the presence of functional presynaptic α2- and β2-adrenergic receptors in LV slices capable of modulating evoked [3H]overflow in LV slices after pressure overload. The existence of AT1 receptors for facilitation of NE release from rat atria slices has been previously documented (44). Results from this study extend previous

Fig. 5. Effects of ANG II on evoked [3H] overflow from LV slices of SO and AC rats. Experimental design was the same as described for Fig. 1 with the exception of the electrical stimulus (5 Hz for 2 min). Administration of ANG II resulted in an increase in evoked [3H] overflow from LV slices of SO and AC rats; however, the lowest effective concentration of ANG II to increase evoked [3H] overflow in LV slices from AC rats was lower compared with SO controls. Moreover, in the presence of ANG II, evoked [3H] overflow was greater from LV slices of AC rats compared with SO controls. *P < 0.05, significantly different from absence of drug; **P < 0.05, significantly different from SO control at same concentration.

Fig. 6. Characterization of the ANG II receptor responsible for facilitation of evoked [3H] overflow from LV slices of control rats. LV slices were prepared from nonoperated control rats. Slices received two stimuli (5 Hz for 2 min) in the absence (S1) and presence (S2) of either ANG II (All, 10 nM), losartan (Los, 1 μM), or ANG II plus losartan (All/Los). A ratio of evoked [3H] overflow (S2/S1) was used to determine the effects of drugs. Administration of ANG II resulted in an increase in evoked [3H] overflow that was totally eliminated in the presence of losartan. Losartan did not influence evoked [3H] overflow from LV slices. *P < 0.05, significantly different from slices not exposed to ANG II (control); n = 3 rats.

Fig. 7. Effects of losartan on evoked [3H] overflow from LV slices of SO and AC rats. Experimental design was the same as described for Fig. 1 with the exception of the electrical stimulus (5 Hz for 2 min). In the absence of drug, evoked [3H] overflow was significantly greater in LV slices of SO or AC rats. *P < 0.05, significantly different from SO control at the same concentration; n = 4 rats.
findings by demonstrating functional AT1 receptors for facilitation of evoked NE release in the rat LV. Because desipramine was included in the superfusion buffer for all of our studies, the observed increase in evoked NE release is not the result of previously observed reductions in cardiac NE neuronal uptake after pressure overload (3, 45). Rather, mechanisms governing NE release such as presynaptic receptors are suggested as the primary contributors to enhanced NE release.

Our approach to studying presynaptic receptor function encompassed the use of exogenous agonists and antagonists. Using agonist stimulation, we defined the functional responsiveness of inhibitory and facilitatory presynaptic receptors. We used receptor antagonists to determine whether tonic stimulation by an endogenous agonist accounted for the observed increase in evoked 3H overflow after pressure overload. We hypothesized that an increase in evoked 3H overflow after pressure overload resulted from the loss of an inhibitory presynaptic modulator and/or an increase in a facilitatory presynaptic modulator.

The most extensively examined inhibitory presynaptic receptor is the α2-adrenergic autoreceptor, which has been demonstrated to operate in cardiac tissue from a number of species (34, 47). Previous results demonstrated the presence of functional α2-autoreceptors in rat atrial slices (17, 18), LV slices (3), the isolated rat heart (10), and human atrial appendages (42). In agreement, results from this study demonstrate functional α2-adrenergic autoreceptors in LV slices after 10 days of pressure overload. Moreover, because we used exogenous agonist and electrical stimulation conditions that precluded autoreceptor activation by an endogenously released agonist, pressure overload did not alter the functional responsiveness of the α2-autoreceptor. We also examined the effects of the α2-receptor antagonist yohimbine on evoked NE release from LV slices after 10 days of pressure overload. Given that evoked NE release was greater in LV slices from AC rats, the observed effect of yohimbine in slices from AC rats most likely resulted from autoreceptor antagonism, of an endogenous agonist (i.e., NE). Collectively, results from this study demonstrate that the presynaptic α2-autoreceptor in LV remains functional despite an already-evident increase in sympathetic activity and may act to limit further heightened cardiac sympathetic drive during the acute period after pressure overload by exerting a tonic inhibitory effect on NE release from sympathetic nerve terminals.

To our knowledge, this is the first study to demonstrate an increase in the functional activity of the facilitatory presynaptic β2-receptor in the LV in response to pressure overload. Presynaptic β2-receptors facilitating NE release have been previously demonstrated in the rat heart (3, 6, 31) and human atria (42). Results from this study demonstrate an enhanced functional responsiveness of the presynaptic β2-receptor to exogenous agonist stimulation after pressure overload. NE has low affinity for β2-adrenergic receptors, and thus presynaptic β2-receptors are generally not considered autoreceptors (19). In this study, the ability of propranolol to decrease evoked NE release in LV slices from AC rats may have resulted from antagonism of an endogenously released substance from cardiac sympathetic nerve terminals with high affinity (i.e., epinephrine) for β2-adrenergic receptors. In support, previous results have demonstrated elevations in plasma (45) and cardiac (4) epinephrine levels in response to pressure overload. Moreover, an increase in circulating epinephrine was previously demonstrated in human heart failure (30). Alternatively, antagonism by propranolol in LV slices obtained from pressure-overloaded rats may have resulted from antagonism of high concentrations of NE at presynaptic β2-adrenergic receptors. Future studies will determine whether the effect of propranolol to decrease evoked 3HNE release results from the release of epinephrine from cardiac sympathetic nerve terminals to act at facilitatory presynaptic β2-receptors.

Considerable evidence demonstrates cardiac-specific activation of the components of the renin-angiotensin system in conditions of myocardial hypertrophy, ischemia, and failure (4, 16, 29). Moreover, we previously reported a marked (fivefold) increase in AT1 receptor density in the LV at 10 days of pressure overload (4). In this study, the facilitatory presynaptic AT1 receptor responded with enhanced functional responsiveness to exogenous ANG II to increase evoked NE release at 10 days of pressure overload. The observed increase in functional responsiveness to exogenous ANG II after pressure overload may have resulted from an increase in the density of AT1 receptor sites at presynaptic sympathetic nerve terminals. However, in this study, losartan did not influence evoked 3H overflow, which suggests that endogenous ANG II does not tonically regulate NE release in rat LV slices.

Results from this study demonstrate that the presynaptic α2-autoreceptor may serve as a compensatory mechanism to limit cardiac NE spillover as a result of enhanced functional responsiveness of the presynaptic AT1 and β2-receptor after pressure overload. However, our results with losartan demonstrate that endogenous ANG II is not the mediator of the observed increase in NE release after 10 days of pressure overload. This may be in part due to the fact that circulating ANG II levels are not elevated in this model during this early phase of compensatory cardiac hypertrophy (4). In contrast, in the presence of propranolol, the increase in NE release after

Fig. 8. Schematic diagram of processes that contribute to presynaptic modulation of norepinephrine (NE) spillover from cardiac sympathetic nerve terminals. Role of the examined presynaptic receptors in the modulation of NE release is depicted. Alterations in the function of specific presynaptic modulators in response to pressure overload in this study are depicted by shading. MAO, monoamine oxidase; COMT, catechol-O-methyltransferase; U1, neuronal uptake-1 transporter; U2, extraneuronal uptake-2 transporter; α and β, α- and β-adrenergic receptors; AT1, ANG II subtype 1 receptor.
pressure overload was effectively eliminated. We suggest that the potential evoked release of epinephrine (with NE) from LV slices of AC rats, with subsequent effects at presynaptic β2-receptors with enhanced sensitivity, may contribute to the observed increase in evoked NE release with short-term pressure overload. Results from this study suggest that blockade of facilitatory presynaptic β2-receptors may be a potential mechanism contributing to improved mortality and reductions in sympathetic drive with the nonselective β-blocker carvedilol in the treatment of heart failure (7, 40).

The use of α2-adrenergic agonists in combination with standard drug therapies for patients with congestive heart failure has recently received renewed interest for the potential of these agents to suppress cardiac sympathetic activity and NE spillover (25, 35). The functional significance of presynaptic α2-adrenergic receptors in regulating cardiac and peripheral NE spillover has been demonstrated in patients with heart failure (2, 39, 46). Moreover, clonidine administration has been demonstrated to reduce cardiac and systemic NE spillover in animal models (24, 52) and in patients with heart failure (1, 8, 32). However, the use of these drugs in patients with heart failure have so far been limited because of initial concerns for a negative inotropic effect due to abrupt sympathetic withdrawal and the recently reported results of a randomized, placebo-controlled trial demonstrating the adverse events of moxonidine administered to patients with heart failure (11, 23, 27). Future studies are warranted in this patient population using lower doses of drugs that reduce NE spillover by targeting specific peripheral presynaptic receptors.

Isolated cardiac tissues, primarily atrial appendages or strips, have been used extensively in studies to examine alterations in [3H]NE spillover from cardiac sympathetic nerve terminals (3, 6, 10, 17, 31, 36–38, 41–44). However, certain assumptions and limitations must be acknowledged in the interpretation of results obtained using these methods particularly when the results are used to examine alterations in NE spillover during pathophysiological conditions of cardiac hypertrophy or failure. First, electrical field stimulation may result in the release of acetylcholine from parasympathetic cardiac nerve fibers or cotransmitters (i.e., neuropeptide Y and epinephrine) from sympathetic nerve terminals (14, 36). Stimulation of presynaptic muscarinic receptors by acetylcholine can inhibit the release of NE (5, 9, 43). Thus reciprocal antagonism between the local parasympathetic and sympathetic nervous systems or alterations in release of cotransmitters under pathological conditions may also contribute to changes in NE spillover from cardiac sympathetic nerve fibers. Finally, it has become increasingly apparent that information exchange at synapses is bidirectional. For example, recent evidence suggests that postsynaptic cells can also release a variety of retrograde signals that can modulate presynaptic neurons (15, 36, 48).

In conclusion, our results demonstrate that pressure overload results in specific alterations in presynaptic receptors located on cardiac nerve terminals that collectively result in enhanced NE spillover in response to electrical field stimulation (Fig. 8). Specifically, although presynaptic α2-autoreceptors function to limit heightened sympathetic drive, presynaptic AT1 and β2-receptors exhibit enhanced functional responsiveness that may contribute to early sympathetic activation after pressure overload. The ability of propranolol to inhibit evoked NE release only in LV slices from rats with pressure overload suggests the release of an endogenous substance with affinity for presynaptic β2-adrenergic receptors. The significance of these results relates to mechanisms contributing to sympathetic activation in cardiac hypertrophy before development of heart failure. Drug therapies directed against presynaptic AT1 and β2-receptors as well as α2-agonists may prove effective in limiting sympathetic activation to the heart in hypertension and heart failure.

ACKNOWLEDGMENTS

The authors thank Vicki English for providing excellent technical skill in performing the catecholamine release studies and DuPont-Merck for the gift of losartan.

GRANTS

This work was supported by National Heart, Lung, and Blood Institute Grant HL-52987 (to L. A. Cassis) and by an internal pilot research grant on the biology of aging (to W. S. Akers; Dr. P. Wise).

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