Effect of Digitalis Glycosides on Norepinephrine Release in the Heart
Dual Mechanism of Action

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The effect of ouabain on exocytotic and nonexocytotic norepinephrine release was investigated in perfused rat and guinea pig hearts. The overflow of endogenous norepinephrine and its neuronal metabolite 3,4-dihydroxyphenylethanolamine N-oxide (DOPEG) was determined by high-pressure liquid chromatography. DOPEG served as the indicator of free axoplasmic norepinephrine concentrations. The overflow of the norepinephrine cotransmitter neuropeptide Y (NPY) was determined by radioimmunoassay and NPY was used as marker for exocytotic release. Electrical stimulation of the left stellate ganglion resulted in exocytotic norepinephrine release in rat and guinea pig hearts. Ouabain caused an increase in stimulation-induced norepinephrine overflow from rat and guinea pig hearts by 40%. However, overflow of NPY was decreased by 40%, indicating a reduced exocytosis rate. Ouabain increased both norepinephrine and NPY overflow, suggesting enhancement of exocytosis, when neuronal catecholamine uptake (uptake) was blocked by desipramine or when presynaptic α2-adrenoceptors were inhibited by yohimbine. The results demonstrate an interaction of ouabain with both calcium-dependent exocytosis and uptake of norepinephrine. Under calcium-free conditions, ouabain or potassium-free perfusate resulted in norepinephrine release from hearts when the axoplasmic norepinephrine concentration was elevated by the reserpine-like agent Ro 4-1284. This release was independent from neural activity, not accompanied by NPY overflow, and suppressed by the uptake blocker desipramine. These findings are in keeping with carrier-mediated nonexocytotic norepinephrine release that is caused by reversal of the transport direction of the uptake carrier. During myocardial ischemia nonexocytotic norepinephrine release was accelerated and enhanced by inhibition of Na⁺,K⁺-ATPase before ischemia. This study demonstrates the potential of digitalis glycosides to interact both with transmitter exocytosis and with the neuronal catecholamine transport system by Na⁺,K⁺-ATPase inhibition. Interaction with the catecholamine transport system involves both inhibition of norepinephrine inward transport and induction of norepinephrine outward transport, resulting in nonexocytotic norepinephrine release. (Circulation Research 1991;68:1628–1637)

Facilitation by cardiac glycosides of norepinephrine release from sympathetic nerve endings is well documented (for review, see Reference 1). In part, the cardiovascular effects of digitalis glycosides have been attributed to their ability to increase both spontaneous and nerve stimulation–induced norepinephrine overflow by inhibition of Na⁺,K⁺-ATPase (for review, see Reference 2). However, contradictory data have been published concerning mechanisms of this facilitation of norepinephrine release. Particularly, conflicting results about the calcium dependency of this effect gave rise to confusion (e.g., see References 1, 3, and 4); therefore, the role of exocytosis as the exclusive cause of increased norepinephrine overflow induced by cardiac glycosides has been challenged. Inhibition of local norepinephrine elimination via neuronal catecholamine uptake (uptake) see Reference 6) and induction of calcium-independent nonexocytotic norepinephrine release have also been discussed as pathways for the enhancing effect of cardiac glycosides on extracellular norepinephrine accumulation and overflow.

In this study the overflow of endogenous norepinephrine, its neuronal metabolite 3,4-dihydroxyphenylethanolamine N-oxide (DOPEG), and neuropeptide Y (NPY) were used to reinvigate the effects of digitalis glycosides on calcium-dependent and calci-
um-independent processes involved in the extracellular accumulation of norepinephrine in the heart. The effect of ouabain on calcium-dependent exocytotic norepinephrine release induced by electrical stimulation of the stellate ganglion was studied in rat and guinea pig hearts that were perfused in situ with intact sympathetic innervation. Calcium-independent nonexocytotic norepinephrine release was examined in isolated perfused hearts of either species. This type of release has been demonstrated to occur independent from central sympathetic activity and to gain relevance during myocardial ischemia.

Endogenous norepinephrine was measured to avoid problems resulting from loading sympathetic nerve endings with labeled exogenous transmitters such as an inhomogeneous distribution of \(^{[3}H\)norepinephrine within the tissue and within the sympathetic nerve terminal.

NPY, which is stored with norepinephrine in sympathetic neurons, has been demonstrated to be coreleased with norepinephrine in guinea pig hearts during exocytic transporter release but not with nonexocytotic, carrier-mediated efflux during anoxia and ischemia. Thus, in this study NPY was used as a marker for norepinephrine exocytosis in guinea pig hearts. In rat hearts neuronal NPY content and release were too low to be used for this purpose.

DOPEG, the main norepinephrine metabolite arising from deamination of axoplasmic norepinephrine by monoamine oxidase, is diffusible across the neuronal cell membrane and served as an indicator for free axoplasmic norepinephrine concentrations. In the present study, overflow of DOPEG from hearts was measured to define conditions for nonexocytotic norepinephrine release induced by cardiac glycosides.

Materials and Methods

Male Wistar rats (180–220 g; Ivanovas, Kislegg, FRG) or male guinea pigs (250–300 g; Thomae, Biberach, FRG) were anesthetized with thiobutabarbital (50 mg/kg i.p.). In all study protocols the hearts were perfused at a constant flow of 4 ml/min (rats) or 7 ml/min (guinea pigs). If not otherwise indicated, the hearts were perfused with a modified Krebs-Henseleit solution containing (mM) NaCl 125, NaHCO\(_3\) 16.9, Na\(_2\)HPO\(_4\) 0.2, KCl 4.0, CaCl\(_2\) 1.85, MgCl\(_2\) 1.0, glucose 11, and EDTA 0.027. The perfusate was gassed with oxygen, and the pH was adjusted to 7.4 with carbon dioxide. The temperature of the perfusate was adjusted to 37.5°C at the point of entry into the ascending aorta. Hearts were weighed at the end of the experiment (mean weight: rats, 0.8 g; guinea pigs, 1.5 g).

Sympathetic Stimulation

For details of sympathetic stimulation, see References 8, 9, and 17. Hearts were left in situ. After cannulation of the aorta a thin tube was placed in the right atrium for collection of the coronary venous effluent. Preparations with a perfusate recovery of less than 85% were rejected. The left cervicothoracic ganglion was dissected free for electrical stimulations. After an equilibration period of 20 minutes, stimulation was performed for two 1-minute periods with a bipolar platinum electrode at a pulse width of 2 ms, a voltage of 5 V, and a stimulation frequency of 4 Hz for rat hearts and 12 Hz for guinea pig hearts. At this voltage the effects on norepinephrine overflow and heart rate were maximal. During stimulation, perfusion pressure increased temporarily by less than 10 mm Hg, whereas the perfusion flow remained constant. The influence of varying stimulation frequencies on norepinephrine and NPY overflow has been described previously. There was an interval of 10 minutes between both stimulations. One-minute samples were taken immediately before, during, and for 1 minute (rats) or 4 minutes (guinea pigs) after electrical stimulation. The first stimulation (S\(_1\)) was used as an individual control, and the drug to be tested was added before the second stimulation (S\(_2\)). The effect of the respective intervention was characterized by calculating the S\(_2\)/S\(_1\) ratio for each heart.

Isolated Heart Perfusion

For details of heart perfusion, see Reference 10. Hearts were rapidly removed, and the ascending aorta from each animal was cannulated for coronary perfusion. For parallel perfusion of control and drug-perfused hearts a multichannel peristaltic pump was used. When calcium-free perfusate was used, hearts stopped beating immediately after the omission of calcium. Samples for determination of norepinephrine, DOPEG, and NPY (in the case of guinea pig hearts) were taken for 2-minute periods. In ischemia experiments perfusion was totally interrupted while the temperature in the surrounding chamber was kept constant at 37.5°C. After reinstallation of perfusion flow, the effluent was collected for 1-minute periods over 5 minutes.

Determination of \(^{[3}H\)Norepinephrine Uptake

Norepinephrine uptake was determined by measuring the clearance of \(^{[3}H\)norepinephrine from the perfusate in isolated perfused guinea pig hearts. A 1-ml bolus of \(^{[3}H\)norepinephrine (New England Nuclear, Dreieich, FRG) with a norepinephrine content of 100 pmol and a total activity of 3 µCi was injected into the perfusion system and proportionally distributed to the heart and blank channels. The coronary effluent was sampled at 2-minute intervals before and after the administration of \(^{[3}H\)norepinephrine, and the samples were counted in a liquid scintillation counter (LKB, Gräfelfing, FRG). The amount of \(^{[3}H\)norepinephrine that was extracted from the hearts is expressed as the percentage of total \(^{[3}H\)norepinephrine applied. Radioactivity decreased nearly to background levels within 5 minutes after the \(^{[3}H\)norepinephrine bolus.
Determination of Endogenous Norepinephrine, DOPEG, and NPY

The catecholamine samples were stabilized by the addition of Na₂EDTA (10 mM) and the NPY samples by adding phosphate buffer containing 3% bovine serum albumin. All samples were stored at −60°C until assayed.

Endogenous norepinephrine and DOPEG were measured by using a high-pressure liquid chromatography (HPLC) method as described by Schömig et al. After a two-step extraction, separation was performed with a reversed-phase counterion HPLC system. Electrochemical detection was used for quantitative analysis. Recovery was 98% for norepinephrine and 92% for DOPEG, the limits of detection were 0.1 and 0.2 nmol/l, and the coefficients of variation were 5.9% and 5.8%, respectively.

A specific radioimmunoassay was used to measure endogenous NPY. The sensitivity of the radioimmunoassay (i.e., the amount of the peptide that displaced 10% of the label) was 0.7 fmol/tube. The 50% intercept was 4.0 fmol/tube. With routine radioimmunoassay there was an intra-assay variation of 15% and an interassay variation of 17%.

Agents Used

The following agents were used: desipramine hydrochloride (CIBA-GEIGY, Basel, Switzerland), ouabain (Merck, Darmstadt, FRG), Ro 4-1284 (2-hydroxy-2-ethyl-3-isobutyl-9,10-dimethoxy-1,2,3,4,6,7-hexahydro-11b-H-benzo[a]-quinolizine; Hoffmann-La Roche, Basel, Switzerland), and yohimbine hydrochloride (Sigma Chemical Co., Munich). None of the drugs used in the experiments had any influence on the perfusion flow or interfered with extraction, separation, and detection of norepinephrine and DOPEG, or with radioimmunoassay of NPY.

Statistical Methods

Results are expressed as the arithmetic mean for data and as the geometric mean for S₀/S₁ ratios. Statistical differences were tested with Student’s t test for paired data. If S₀ and S₁ were different in control experiments, the statistical difference between the control and intervention groups was tested by using Student’s t test for unpaired data. A value of p<0.05 was considered significant.

Results

The influence of ouabain on exocytotic norepinephrine release was examined in hearts perfused in situ with intact sympathetic innervation in the presence of extracellular calcium. In this preparation cardiac sympathetic fibers were stimulated electrically.

Interaction of ouabain with nonexocytotic release was investigated in isolated perfused hearts in the absence of extracellular calcium to avoid exocytotic release. No electrical stimulation of sympathetic nerves was performed.

Effect of Ouabain on Stimulation-Induced Release and Neuronal Uptake of Norepinephrine

In the absence of drugs, two subsequent periods of stimulation (S₀ and S₁) of the left stellate ganglion in the same preparation resulted in comparable norepinephrine overflow from in situ perfused rat hearts. Norepinephrine overflow was evoked by 1-minute electrical stimulation of the left stellate ganglion at 4 Hz. Ouabain was added or potassium was removed 5 minutes before the second stimulation (S₁). In control experiments (n=14) the perfusate remained unchanged. The effect of ouabain in the presence of desipramine (300 nM, n=7) throughout the experiment is shown at the right. Control experiments (n=10) were without the addition of ouabain. Results are shown as the S₀/S₁ ratio of both stimulations and are geometric mean±SEM; *p<0.05.
Ganglion stimulations and perfused second stimulation drug.

Reference a2-adrenoceptors (norepinephrine: NA, upper panel) and neuropeptide Y (NPY, lower panel) from in situ perfused guinea pig hearts. Transmitter release was evoked by 1-minute electrical stimulation of the left stellate ganglion at 12 Hz. Either neuronal catecholamine uptake had been inhibited by 300 nM desipramine (shown at the left, n=9; control, n=7) or presynaptic a2-adrenoceptors had been blocked by 1 µM yohimbine (shown at the right, n=9; control, n=7). Desipramine and yohimbine were administered from 10 minutes before the first stimulation (S1) throughout the experiments. Ouabain application started 5 minutes before the second stimulation (S2). Results are shown as the S2/S1 ratio of both stimulations and are geometric mean±SEM; *p<0.05.

Throughout the experiment resulted in a decrease in norepinephrine (S2/S1, 0.68; n=7; p<0.05) and NPY overflow (S2/S1, 0.56; n=7; p<0.05) during the second period of stimulation (Figure 3). However, additional application of ouabain before the second stimulation led to a relative increase in the overflow of both norepinephrine (S2/S1, 1.18; n=9; p<0.05) and NPY (S2/S1, 1.02; n=9; p<0.05) in comparison to the yohimbine control (Figure 3).

Control experiments with two periods of stimulation after blockade of uptake1 by 300 nM desipramine demonstrated good comparability of norepinephrine overflow in the rat (S2/S1, 0.85; n=10) and of norepinephrine (S2/S1, 0.81; n=7) and NPY overflow (S2/S1, 1.11; n=7) in the guinea pig. Ouabain before S1 still increased stimulation-induced norepinephrine overflow (rat: S2/S1, 1.24; n=7; guinea pig: S2/S1, 1.38; n=9; p<0.05) (Figures 1 and 3). However, in contrast to the experiments without additional uptake blockade, ouabain induced an increase in
NPY overflow from guinea pig hearts (S2/S1, 1.48; \( n=9; p<0.05 \)) (Figure 3). This observation indicates that with inactive uptake, ouabain increases exocytotic transmitter release.

Uptake, in the guinea pig heart was determined by measuring the clearance of \(^{3}H\)norepinephrine during perfusion with Krebs-Henseleit solution. After a bolus injection of \(^{3}H\)norepinephrine 47.8±1.7% (\( n=6 \)) of the radioactivity was extracted by the hearts. Repeated bolus injections demonstrated stability of uptake over time (10 minutes, 48.1±3.5%; 45 minutes, 51.4±2.4%). Perfusion with ouabain (300 \( \mu M \)) markedly decreased uptake after 5 minutes (24.1±1.4% versus 52.3±0.6%; \( n=6, p<0.05 \)) and abolished it completely after 40 minutes. For comparison, desipramine (300 \( nM \)) completely inhibited \(^{3}H\)norepinephrine uptake after 5 and 40 minutes (data not shown).

**Effect of Ouabain on Nonexocytotic Norepinephrine Release During Normoxia and Ischemia**

In this part of the study hearts were perfused with calcium-free Krebs-Henseleit solution to prevent exocytotic norepinephrine release.

Nonexocytotic norepinephrine release requires elevation of free axoplasmic norepinephrine.\(^{15,16}\) Perfusion of rat hearts with the reserpine-like agent Ro 4-1284\(^{21}\) resulted in a marked overflow of DOPEG, indicating a rise of free axoplasmic norepinephrine (Figure 4). No detectable norepinephrine overflow was induced by 1 \( \mu M \) Ro 4-1284.

Ouabain (1 \( mM \)) alone had no effect on either overflow of norepinephrine or DOPEG. An increase in norepinephrine overflow from hearts was found only when Ro 4-1284 was combined with Na\(^+\),K\(+\)-ATPase inhibition by ouabain (cumulative overflow over 10 minutes, 169.8±14.7 pmol/g). DOPEG overflow then was considerably lower compared with experiments without ouabain (Figure 4), demonstrating competition of two elimination processes for axoplasmic norepinephrine: oxidative deamination and transmembrane transport. Blockade of uptake, by desipramine (100 \( nM \)) attenuated norepinephrine overflow induced by ouabain and Ro 4-1284 by about 80%, suggesting carrier-mediated nonexocytotic norepinephrine efflux. In guinea pig hearts ouabain (100

**Figure 4. Effect of Na\(^+\),K\(+\)-ATPase inhibition by 1 \( \mu M \) ouabain (left panels; \( n=3\times7 \)) or omission of potassium (right panels; \( n=3\times7 \)) on the overflow of endogenous norepinephrine (upper panels) and 3,4-dihydroxyphenylethylenglycol (DOPEG, lower panels) from isolated rat hearts perfused with calcium-free Krebs-Henseleit solution. The figure demonstrates the effects of the reserpine-like-acting substance Ro 4-1284 (1 \( \mu M \)) and inhibition of sodium pump activity in the absence and presence of neuronal catecholamine uptake blockade by desipramine (100 \( nM \)). The time course of drug exposure is indicated in the figure. Data are arithmetic mean±SEM.**
μM) and Ro 4-1284 (10 μM) resulted in a comparable overflow of DOPEG and norepinephrine (cumulative norepinephrine overflow over 10 minutes, 131.9±6.9 pmol/g; n=4). No concomitant overflow of NPY was detected, excluding significant exocytotic transmitter release (data not shown).

To verify whether the effect of ouabain was due to Na⁺,K⁺-ATPase inhibition, the sodium pump was inhibited by omission of potassium from the perfusate. Potassium-free perfusion combined with Ro 4-1284 elicited an increased norepinephrine overflow that was accompanied by a decreased DOPEG overflow. Again, norepinephrine overflow was reduced by about 80% when uptake, was blocked by desipramine (Figure 4). These effects on norepinephrine overflow of suppressed Na⁺,K⁺-ATPase were reversible: restitution of Na⁺,K⁺-ATPase activity by increasing potassium from 0 to 4 mM abolished norepinephrine overflow within 5 minutes, whereas DOPEG overflow increased (Figure 5).

To investigate whether carrier-mediated nonexocytotic norepinephrine release occurring during myocardial ischemia is influenced by Na⁺,K⁺-ATPase inhibition, ischemia-induced norepinephrine release from rat hearts was determined in the absence and presence of ouabain. Ischemia lasting 10 minutes did not result in norepinephrine overflow from rat hearts during reperfusion. Norepinephrine overflow induced by 20 minutes of ischemia amounted to 162.3±56.1 pmol/g. Preperfusion with ouabain 1 mM over 30 minutes before the ischemic period led to norepinephrine overflow of 93.6±13.8 pmol/g during 10 minutes of ischemia (p<0.05) and nearly doubled norepinephrine overflow caused by 20 minutes of ischemia (305.4±49.8 pmol/g, p<0.05) (n=7 in each series). In contrast to this finding, blockade of uptake with desipramine (100 nM) before interruption of perfusion prevented most of the norepinephrine overflow caused by 20 minutes of ischemia (19.7±2.8 pmol/g, p<0.05) (Figure 6).

Discussion

In the present study the effects of ouabain on extracellular norepinephrine accumulation have been investigated in perfused rat and guinea pig hearts and have been related to exocytotic and nonexocytotic processes by using the overflow of NPY and DOPEG as tools for discrimination between two different release mechanisms (Figure 7). Norepinephrine overflow caused by exocytosis, the principal way of norepinephrine release under physiological conditions, was enhanced mainly by inhibition of catecholamine elimination via neuronal uptake. The additional potential of cardiac glycosides to enhance exocytosis rate was masked by effective presynaptic autoinhibition. On the other hand, digitals glycosides induced and accelerated nonexocytotic carrier-mediated norepinephrine release, which may gain relevance during myocardial ischemia.

Enhancement of Extracellular Norepinephrine Accumulation Caused by Exocytosis

Ouabain enhanced norepinephrine overflow after left stellate ganglion stimulation both from rat and guinea pig hearts and led to a parallel increase in NPY overflow from guinea pig hearts when either uptake was blocked by desipramine or presynaptic autoinhibition was prevented by yohimbine. A corelease of NPY with norepinephrine has been shown to occur exclusively during exocytotic transmitter release.13 With inactive uptake, or blocked presynaptic autoinhibition, therefore, ouabain enhanced norepinephrine overflow from the heart through an increase in exocytosis rate. Since exocytosis is a calcium-mediated process, this increase is most likely due to improved intracellular calcium availability subsequent to an increase of axoplasmic sodium.24,25 There are various theories about the mechanism of
increased axoplasmic calcium concentrations after Na⁺,K⁺-ATPase inhibition, such as augmented calcium entry, reduced calcium extrusion from the cell, or displacement of calcium from intracellular storage sites.²⁴,²⁶,²⁷

However, an increased exocytosis rate was not the predominant way by which ouabain exerted its enhancing action on stimulation-induced norepinephrine overflow. With active autoinhibition through presynaptic α2-receptors and a primarily intact uptake, system, ouabain increased extracellular norepinephrine accumulation by a mode of action different from augmentation of exocytosis rate. In fact, exocytosis rate was even suppressed by ouabain. This was indicated by the marked reduction of NPY overflow after nerve stimulation in the presence of ouabain.

Suppression of exocytosis rate was mediated by autoinhibition, and blockade of presynaptic autoinhibition by yohimbine resulted in a ouabain-induced increase of both norepinephrine and NPY overflow during electrical stimulation, indicating increased exocytosis. The results suggest ongoing control of exocytosis by autoinhibition even in the presence of enhanced intracellular sodium.

High concentrations of yohimbine (IC₅₀ approximately 10 μM) have been described to inhibit norepinephrine overflow evoked by acetylcholinesterase in the absence of sympathetic stimulation.²⁸ These effects can be neither excluded nor substantiated in our experimental model with lower yohimbine concentrations (1 μM) and electrical stimulation to induce release of the neurotransmitters.

A similar dissociation between norepinephrine and NPY overflow as seen with ouabain was found when desipramine was administered (Figure 2). This effect of desipramine on stimulation-induced norepinephrine overflow. With active autoinhibition through presynaptic α2-receptors and a primarily intact uptake, system, ouabain increased extracellular norepinephrine accumulation by a mode of action different from augmentation of exocytosis rate. In fact, exocytosis rate was even suppressed by ouabain. This was indicated by the marked reduction of NPY overflow after nerve stimulation in the presence of ouabain.

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rine and NPY overflow had been demonstrated to be an indirect consequence of uptake, inhibition and subsequently elevated extracellular norepinephrine concentrations\(^9\) that suppress exocytosis via activation of presynaptic inhibitory \(\alpha_{2}\)-adrenoceptors.\(^{29,30}\) Similarly, ouabain increased stimulation-induced norepinephrine overflow by inhibition of uptake\(_1\). The expected potentiation of exocytosis by ouabain was masked by effective presynaptic autoinhibition secondary to norepinephrine accumulation within the synaptic cleft caused by uptake blockade (Figure 7). In the presence of uptake blockade by desipramine the effect of ouabain on uptake\(_1\) was eliminated and the effect on exocytosis was unmasked. Nerve stimulation resulted in a relative increase of exocytosis rate indicated by the enhanced overflow of both norepinephrine and NPY.

Inhibition by ouabain of uptake\(_1\)^5,31 was confirmed for the experimental model of the perfused guinea pig heart by measuring \(^{3}\)H\)norepinephrine extraction. The results are in accordance with the close relation between Na\(^\pm\),K\(^\pm\)-ATPase inhibition and suppression of uptake\(_1\) by ouabain that had been demonstrated for various species and tissues.\(^32\) There was an important difference between the kinetics of uptake inhibition by ouabain compared with that by desipramine. This finding reflects the different modes of action of both substances. Desipramine binds to the norepinephrine binding site of the uptake\(_1\) carrier\(^33,34\) and inhibits amine transport in both directions. Uptake inhibition by cardiac glycosides is thought to be a consequence of increased axoplasmic sodium concentrations.\(^31\) The inhibitory action of augmented axoplasmic sodium on catecholamine uptake can be derived from the role of the transmembrane sodium gradient for norepinephrine transport\(^35,36\): For energy, the cotransport of norepinephrine and sodium depends on the transmembrane sodium gradient, which is maintained by Na\(^+,\)K\(^+\)-ATPase. The physiological high transmembrane sodium gradient directed from outside to inside facilitates movement of norepinephrine from the extracellular space to the axoplasm. Since pump inhibition by cardiac glycosides involves a rise in axoplasmic sodium, reuptake of extracellular norepinephrine on the one hand is hampered. On the other hand, movement of norepinephrine from the axoplasm to the extracellular space is facilitated by reversal of the transport direction of the carrier.

**Induction of Carrier-Mediated Nonexocytotic Norepinephrine Release**

This reversal of transport direction of the neuronal catecholamine carrier results in a calcium-independent efflux of norepinephrine when axoplasmic norepinephrine concentrations are increased. Such nonexocytotic release has been described for various pharmacological and pathophysiological conditions.\(^7,10,15,16,37,38\) Overflow of endogenous norepinephrine after Na\(^+,\)K\(^+\)-ATPase inhibition by either ouabain or potassium-free perfusion occurred when axoplasmic norepinephrine was elevated by reserpinelike agents such as Ro 4-1284 or trimethylin.\(^37\) The rise in axoplasmic norepinephrine was indicated by a marked overflow of DOPEG after inhibition of vesicular norepinephrine uptake by Ro 4-1284 or blockade of vesicular H\(^+\)-ATPase by trimethylin.\(^39\) This type of cardiac norepinephrine release was found in the absence of extracellular calcium, not accompanied by NPY overflow and suppressed by the uptake, inhibitor desipramine, suggesting norepinephrine efflux across the neuronal plasma membrane with the uptake\(_1\) carrier in reverse of its usual transport direction. Induction by ouabain of carrier-mediated release of endogenous norepinephrine from the axo-plasma is in keeping with the finding of calcium-independent release of \(^{3}\)H\)norepinephrine after Na\(^+,\)K\(^+\)-ATPase inhibition from sympathetic nerve endings loaded with labeled norepinephrine.\(^7,15,38\)

Because the Na\(^+,\)K\(^+\)-ATPase of the rat is particularly insensitive to cardiac glycosides,\(^40\) high concentrations of ouabain were necessary to induce nonexocytotic norepinephrine release from rat hearts. Ancillary properties of cardiac glycosides were excluded by Na\(^+,\)K\(^+\)-ATPase inhibition by potassium-free perfusion, which had effects on norepinephrine overflow comparable to those of ouabain. Moreover, norepinephrine efflux was completely suppressed when Na\(^+,\)K\(^+\)-ATPase was reactivated by the addition of potassium. These data emphasize the functional link between axoplasmic sodium concentration and transmembrane norepinephrine transport.

Nonexocytotic release has been demonstrated to be the predominant mechanism of extracellular norepinephrine accumulation during myocardial energy deficiency.\(^10,41,42\) Ischemia-induced norepinephrine release is assumed to occur in two steps. First, norepinephrine is lost from its storage vesicles as a consequence of impaired energy supply and accumulates in the axoplasm.\(^16\) In a second, rate-limiting step free axoplasmic norepinephrine is transported into the extracellular space by the uptake\(_1\) carrier in reverse of its usual transport direction\(^16\) as a consequence of increased axoplasmic sodium concentrations.\(^37\) In this study, Na\(^+,\)K\(^+\) pump inhibition before myocardial ischemia accelerated the time course of ischemia-induced norepinephrine overflow. On the other hand, desipramine was able to suppress norepinephrine overflow during ischemia almost completely. This differential effect of ouabain and desipramine is due to their different modes of interaction with the neuronal catecholamine carrier. Desipramine acts by direct binding to the uptake\(_1\) carrier and blocks both inward and outward transport, whereas digitalis glycosides affect norepinephrine transport indirectly through an increase of axoplasmic sodium concentrations. Therefore, by induction of nonexocytotic norepinephrine release, cardiac glycosides have the potency to act like indirect sympathomimetic agents during myocardial ischemia.

Lorenz et al\(^4\) found an early calcium-independent increase in norepinephrine overflow after Na\(^+,\)K\(^+\)-ATPase inhibition that might be due to nonexocyt-


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