Nonexocytotic Release of Endogenous Noradrenaline in the Ischemic and Anoxic Rat Heart: Mechanism and Metabolic Requirements

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The release of endogenous noradrenaline and its deaminated metabolite dihydroxyphenylglycol in the myocardium have been studied in the isolated perfused heart of the rat subjected to three models of energy depletions: ischemia, anoxia, and cyanide intoxication. Anoxia and cyanide intoxication were combined with substrate deficiency at constant perfusion flow. All three energy-depleting procedures caused a similar noradrenaline overflow, which followed a constant delay of 10 minutes without increased release, amounted to more than 25% of total heart content within 40 minutes. This noradrenaline overflow was not diminished in the absence of extracellular calcium and was inhibited by the uptake, blocker desipramine in all three experimental models, indicating a common and nonexocytotic release mechanism. In the presence of glucose, neither anoxia nor cyanide intoxication resulted in a measurable noradrenaline overflow. Conversely, blockade of glycolysis or glucose depletion prior to ischemia or cyanide poisoning accelerated the noradrenaline overflow, demonstrating a key role of the sympathetic nerve cells' energy status in causing nonexocytotic catecholamine release. Blockade of energy metabolism in the presence of oxygen (cyanide model) resulted in the overflow of high amounts of dihydroxyphenylglycol that was not inhibited by uptake blockade. The release of the lipophilic dihydroxyphenylglycol by diffusion reflects deamination of axoplasmic noradrenaline by monoamine oxidase. Since saturation of the enzyme could be excluded in this model, dihydroxyphenylglycol release can be taken as a mirror of cytoplasmic noradrenaline concentration. The results obtained by these studies indicate that nonexocytotic catecholamine release is a two-step process induced by energy deficiency in the sympathetic varicosity. In a first step, noradrenaline is lost from storage vesicles, resulting in increasing axoplasmic concentrations. The second step is the rate-limiting transport of intracellular noradrenaline across the cell membrane by the uptake, carrier that has reversed its normal net transport direction. (Circulation Research 1987;60:194-205)

In myocardial ischemia, stimulation of the heart by catecholamines is thought to have deleterious consequences. An acceleration of cell damage may be caused in the border zone of an infarction by increasing energy demand and calcium influx in myocytes of critically underperfused areas. The arrhythmogenic effects of stimulated sympathetic neural activity or increased catecholamine concentrations in the heart are well documented, and substantial information has accumulated during recent years concerning the protection provided by β-adrenergic blockade in myocardial ischemia.

During acute infarction, the myocardium at risk may be affected by increasing concentrations of circulating catecholamines and/or local noradrenaline release induced by reflex stimulation of cardiac sympathetic nerves. In severely ischemic myocardium, however, both processes may be limited in increasing local catecholamine concentrations, since plasma catecholamines have reduced access to underperfused myocardium and exocytotic release rapidly fails in the ischemic area. In acute infarction, another source of catecholamines has to be considered that is independent from central stimulation or external access. Local metabolic release induced by ischemia may cause extremely high noradrenaline concentrations (1 μM) to accumulate within the ischemic myocardium, and may be a main cause of the detrimental catecholamine effects mentioned above.

Therefore, the aim of the following study was to describe the metabolic requirements for this local release to occur, and to elucidate further the mechanism of this release that is not influenced by the usual means of modulating stimulation-induced catecholamine liberation.

All experiments were performed in the isolated heart of the rat, using a modified Langendorff preparation. An ischemia model does not permit sufficient manipulation of individual components contributing to the ischemic reaction; therefore, anoxia and cyanide intoxication were used as additional experimental models of energy deficiency in the presence of unaltered perfusion flow to achieve more detailed information about noradrenaline release mechanisms.

Materials and Methods
Male Wistar rats (180–250 g) were anesthetized with thiobutabarbital (50 mg/kg, i.p.). After injection

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of 500 U heparin, the thorax was opened, the hearts were rapidly removed, and the ascending aorta cannulated for retrograde coronary perfusion. The hearts were perfused at a constant flow of 5 ml/min/g heart weight using a multichannel peristaltic pump. In all experiments, the initial perfusion was done with a modified Krebs-Henseleit solution (KHS; composition in mmol/l: NaCl 125, NaHCO3 16.9, Na2HPO4 0.2, KCl 4.0, CaCl2 1.85, MgCl2 1.0, glucose 11, EDTA 0.027). If not stated otherwise, the buffer was gassed with oxygen and the pH adjusted to 7.4 with CO2. The temperature of the chamber and of the perfusate at the point of entry into the aorta were adjusted to 37.5°C. After 20 minutes initial preperfusion in the presence of 1.8 mM pyruvate, the hearts were subjected to the experimental protocol.

Samples for the estimation of endogenous noradrenaline and 3,4-dihydroxyphenylglycol (DOPEG) were taken from the coronary venous effluent, cooled on ice, stabilized either by the addition of sodium EDTA to a final concentration of 10 mM (HPLC method) or by the addition of perchloric acid to a final concentration of 300 mM for radioenzymatic assay. The samples were stored at −80°C until subsequently assayed either by a radioenzymatic assay or by a high-pressure liquid chromatography (HPLC) method. In a few series, the hearts were rapidly frozen at the end of the perfusion experiment for determination of noradrenaline content by an HPLC method.

Agents used in this study were corticosterone (Fluka, Buchs, Switzerland), desipramine (Ciba-Geigy, Basel, Switzerland), iodoacetate (Merck, Darmstadt, Federal Republic of Germany), pargyline hydrochloride (Sigma, St. Louis, Mo.), potassium cyanide (Merck), Ro 4-1284 (2-hydroxy-2-ethyl-3-isobutyl-11b-H-benzo(a)-quinolizine; Hoffmann-La Roche, Basel, Switzerland), and sodium dithionite (Merck). The agents not soluble in water were dissolved in ethanol, with final concentrations less than 0.05%. For control experiments, identical solvent and ion concentrations were used.

**Experimental Series**

**ISCHEMIA EXPERIMENTS.** The hearts were subjected to total and global ischemia by stopping perfusion flow and were subsequently reperfused at the initial flow rate. In independent experiments, various periods of ischemia were investigated. If drugs were used, they were added 20 minutes (desipramine) or 5 minutes (iodoacetate) prior to ischemia and continued to the end of the experiment. Samples for noradrenaline estimation were taken throughout the last minute before ischemia and during the first 5 minutes of the reperfusion period.

**EXPERIMENTAL SERIES WITH NORMOXIA, HYPOXIA, ANOXIA, AND BLOCKADE OF ENERGY METABOLISM.** The experiments were done at constant flow (5 ml/min/g heart weight). Hypoxia was induced by gassing the perfusion buffer with a N2/C02 mixture. Anoxia was produced by the addition of the reducing agent, sodium dithionite, with a final concentration of 0.5 mM. Hypoxia and anoxia were confirmed by measuring the pO2 with a pH/blood gas analyzer. Oxidative phosphorylation was inhibited by 1 mM cyanide. Samples for HPLC determination of noradrenaline and DOPEG were taken cumulatively for 2 minutes. If not indicated otherwise, the sampling periods were 12–10 and 2–0 minutes prior to, and 0–2, 3–5, 8–10, 13–15, 18–20, 28–30, 38–40, and 58–60 minutes after, the start of metabolic blockade. The results are given in the text and figures for the individual sampling periods, or values of cumulative overflow, are calculated by linear interpolation and integration.

**Biochemical Methods**

**RADIOENZYMATIC ASSAY.** Radioenzymatic determination of noradrenaline concentrations in perfusate was performed with the catechol-O-methyltransferase (COMT) method as described by Da Prada and Zürcher. The coefficient of variation of the method used was less than 5% intra-assay and less than 10% interassay. Desipramine (100 nM) and iodoacetate (0.5 mM) were without effect on blanks and on the estimate of standard concentrations of noradrenaline.

**HPLC method**

**Preparation of tissue samples for noradrenaline determination.** The hearts were homogenized in 2 ml of ice-cold 0.1 M HCl containing 10 mM sodium EDTA. The homogenate was maintained at 0°C and centrifuged at 3000g for 10 minutes. The supernatant (0.1 ml) was diluted with 2.9 ml KHS containing 10 mM Na-EDTA. From this sample, catecholamines were extracted in the same way as described for perfusate samples.

**Solvent extraction.** The pH of the 3-ml sample (perfusate or diluted supernatant) was adjusted to 8.5 by the addition of 1 ml NH4Cl-NH4OH buffer (2 M, pH 8.5) containing 0.5% Na-EDTA and 0.2% diphenylborate for ion-pair formation with catecholamines. According to the method of Smedes et al., catecholamines and DOPEG were extracted into 5 ml organic phase consisting of 99% n-heptane and 1% octanol in the presence of 0.25% tetraoctylammonium bromide. Back extraction of catecholamines into the aqueous phase was performed by shaking the organic phase with 0.15 ml of 0.2 M phosphoric acid. To increase the recovery of DOPEG, the same step was repeated after the addition of 1 ml octanol. Because of the high and constant recovery of noradrenaline (98%) and DOPEG (92%) achieved by this extraction method, the addition of an internal standard was unnecessary.

**HPLC separation and electrochemical detection.** The aqueous phase (0.1 ml) was injected into the HPLC system. Separation was performed using a 5-μm C18 reversed phase column (Latek) with a flow of 0.8 ml/min, with the solvent 0.2 M phosphate buffer (pH 3.0) containing sodium EDTA (40 μM) and octylsulfate (25 μM). Quantitative analysis was performed by electrochemical detection (BAŚ LC 4B) at 0.6 V.
The retention time was 4.5 minutes for noradrenaline and 6.2 minutes for DOPEG. The limits of detection of the method used were 0.1 pmol/l for noradrenaline and 0.2 pmol/l for DOPEG, and the coefficients of variation were 5.9% and 5.8%, respectively. None of the drugs used in the experiments interfered with extraction, with HPLC separation, or with detection of noradrenaline and DOPEG. The parallel estimation of samples with the HPLC method and the radioenzymatic assay revealed a correlation coefficient of 0.953 (n = 80).

Statistical Methods

In the text and figures, results are expressed as means ± SEM. The significance of differences was assessed by Student's t test or analysis of variance.

Results

The overflow of endogenous noradrenaline from the isolated perfused heart of the rat was investigated in 3 experimental models of energy depletion.

Effect of Total Ischemia on Overflow of Endogenous Noradrenaline

Increasing amounts of endogenous noradrenaline were found in the perfusate during reperfusion when ischemic periods were prolonged for more than 10 minutes (Figure 1). This release was markedly temperature dependent, with an increasing overflow after a shorter delay at 42°C and with an impeded and reduced overflow at 30°C (p < 0.01; Figure 2). Calcium-free perfusion led to a small increase of noradrenaline overflow after ischemic periods of 20 minutes (252 ± 23 vs. 202 ± 16, p < 0.1) and 40 minutes (1061 ± 27 vs. 838 ± 84, p < 0.05; Figure 1). Substrate-free preperfusion (Figure 1) or blockade of glycolysis (Figure 1) prior to ischemia resulted in a shift of the release curves to shorter ischemic periods. Under all experimental conditions, noradrenaline overflow was reduced significantly (p < 0.01) by addition of the uptake, blocker desipramine.

Effect of Hypoxia and Anoxia on Overflow of Endogenous Noradrenaline and DOPEG

Hypoxic or anoxic perfusion combined with the lack of glucose in the perfusate induced an overflow of noradrenaline that can be detected after a lag period of between 5 and 10 minutes. After reaching peak overflow values at 30 or 40 minutes of anoxia and glucose deficiency, the release decreased because of extensive noradrenaline depletion of the hearts (Figure 3). For better comparison with the ischemia-induced release, the cumulative overflow is given in Figure 4. The data

![Figure 1](http://circres.ahajournals.org/.../images/1.jpg)
demonstrate that during the first 30 minutes of anoxia the noradrenaline overflow exceeded that found with ischemia by about 100%. This fact may be explained at least in part by the continuous washout of released noradrenaline in the anoxia model. In contrast, in ischemia high concentrations of catecholamines accumulated in the interstitial space, reducing the noradrenaline concentration gradient from intracellular to extracellular space and being available for neuronal and extraneuronal uptake. The cumulative overflow observed during hypoxic (Po2 = 40 mm Hg) substrate depletion is still significantly higher than that found under ischemic conditions (cumulative 20 minutes: 294 ± 22 vs. 202 ± 16 pmol/g; 40 minutes: 1173 ± 54 vs. 838 ± 84 pmol/g; p < 0.01).

Calcium-free perfusion from the onset of anoxia did not significantly change the enhanced noradrenaline overflow (Figure 4). The addition of desipramine reduced the cumulative release by more than 80% during a 30-minute period (p < 0.01). Thus, the effects of desipramine and of calcium-free perfusion were not different between anoxia and ischemia-induced noradrenaline overflow, indicating some common underlying mechanism.

The presence of glucose in the perfusate completely prevents noradrenaline release, irrespective of oxygen pressure and of the presence of the reducing agent dithionite, which is used in all anoxia experiments to achieve oxygen pressures of less than 1 mm Hg. A direct effect of this substance influencing noradrenaline release, besides its metabolic effect through reduced oxygen concentration, is unlikely for two reasons: 1) Any release observed in the presence of dithionite is completely abolished by the addition of glucose. 2) Hypoxia (Po2 = 40 mm Hg, without dithionite) produces a qualitatively similar but less pronounced effect when compared with anoxia (Po2 < 1 mm Hg, in the presence of 0.5 nM dithionite). It is impossible to determine dithionite effects in the presence of oxygen, since the substance rapidly oxidizes to sulfate under these circumstances.

In the anoxia experiments, DOPEG decreased within 10 minutes after lowering the oxygen pressure from basal levels (1.4 ± 0.3 pmol/g/min) to undetectable values.

**Effect of Cyanide Intoxication on Overflow of Endogenous Noradrenaline and DOPEG**

The time course of noradrenaline and DOPEG overflow induced by metabolic blockade and the effects of uptake blockade or calcium deficiency are described in the Figures 5, 6, 7, and 8. Starting 10 minutes after withdrawal of glucose and addition of cyanide, noradrenaline was progressively released. After having reached peak values after 30 minutes, the overflow continuously decreased (Figure 5). The cumulative noradrenaline release during 40 minutes was 874 ± 49 pmol/g. This value is similar to the release found with 40 minutes stop flow (838 ± 84 pmol/g; Figure 1).
Corresponding to ischemic and anoxic conditions, the addition of the uptake blocker desipramine attenuated the noradrenaline overflow (100 nM: 259 ± 35 pmol/g cumulative at 40 minutes; p < 0.01; Figure 5).

The concentration dependence was comparable to that described previously for ischemic conditions. In contrast, blockade of extraneuronal uptake (uptake) by 0.1 mM corticosterone resulted in a nonsignificant tendency to increased noradrenaline overflow (1010 ± 66 vs. 901 ± 31 pmol/g cumulative during 40 minutes; Figure 6). A similar tendency also was shown for ischemia-induced overflow.

Calcium-free perfusion (starting 20 minutes prior to metabolic blockade) accelerated the noradrenaline release after an unchanged lag period (Figure 7). The cumulative release amounts to 1284 ± 76 pmol/g during 40 minutes, which was more than 40% higher when compared to calcium-containing perfusion (p < 0.01). The blockade of release by desipramine, 100 nM, however, was more pronounced with calcium-free perfusion (126 ± 10 pmol/g cumulative at 40 minutes p < 0.01; Figure 7).

Glucose-free normoxic perfusion for 60 minutes moderately increased the cumulative noradrenaline overflow to 271 ± 54 pmol/g within 60 minutes (Figure 8). The subsequent addition of cyanide resulted in a shortened lag period and an accelerated noradrenaline release (1307 ± 160 pmol/g cumulative 40 minutes, p < 0.05). In contrast, the addition of glucose to the perfusate prevented any noradrenaline release in the presence of cyanide (Figure 8). This observation excluded any direct noradrenaline-releasing effect of
overflow of noradrenaline

A 0 10 20 30 40 60 min

B

dOPEQ

pmol/g

0 10 20 30 40 60 min

FIGURE 7. Overflow of endogenous noradrenaline (Panel A) and DOPEG (Panel B) from perfused rat hearts induced by blockade of energy metabolism (1 mM cyanide, glucose-free perfusion 0–60 minutes). The time course of overflow was studied in the presence of 1.85 mM calcium (n = 7), with calcium-free buffer starting 20 minutes prior to metabolic blockade (n = 14), and with calcium-free buffer and uptake, blockade by 100 nM desipramine (DMI), both starting 20 minutes prior to metabolic blockade (n = 7).

cyanide, besides its indirect effect through energy metabolism.

The overflow of the most important deaminated metabolite of noradrenaline (DOPEG) was determined in these experiments because it can serve as an indicator of axoplasmic noradrenaline concentrations in the cyanide model. DOPEG was released increasingly from the onset of metabolic blockade by the withdrawal of glucose and addition of 1 mM cyanide to the perfusion buffer (Figure 5, 6, 7, and 8). At the time when the noradrenaline concentrations were beginning to rise, DOPEG concentrations were already high and leveled off in a plateau phase (10–30 minutes) before becoming parallel to noradrenaline as catecholamine depletion of the hearts progressed.

Neither blockade of uptake, by desipramine (Figure 5) nor blockade of uptake, by corticosterone (Figure 6) nor calcium-free perfusion (Figure 7) had any effect on the initial rise of DOPEG concentrations and the height of the plateau. The plateau phase, however, was prolonged after uptake, blockade by desipramine, which preserved the noradrenaline content of the heart. Energy depletion by glucose-free preperfusion resulted in an accelerated initial DOPEG release and in a shortened plateau phase (Figure 8). Depression of glycolysis (glucose-free perfusion) alone or isolated blockade of oxidative phosphorylation (cyanide in the presence of glucose) had minor effects on DOPEG overflow (Figure 8).

Effect of Metabolic Blockade on Noradrenaline Content of Hearts

After 60 minutes of anoxic and glucose-free perfusion, 2622 ± 278 pmol noradrenaline/g heart was found in the coronary venous effluent (n = 6). Virtually no DOPEG was present. The noradrenaline content of the hearts was reduced after this time to 538 ± 54 pmol/g (n = 6), in comparison to 3303 ± 116 pmol/g after normoxic perfusion in the presence of glucose (n = 7). A similar noradrenaline depletion was observed after 60 minutes of substrate-free perfusion combined with cyanide intoxication (content of the hearts: 472 ± 51 pmol/g; n = 7). In this experiment, the cumulative overflow of noradrenaline and DOPEG amounted to 1886 ± 107 pmol/g and 761 ± 72 pmol/g, respectively.

FIGURE 8. Overflow of endogenous noradrenaline (Panel A) and DOPEG (Panel B) from isolated perfused rat hearts induced by interventions in the aerobic and/or anaerobic energy metabolism. The time course of overflow was studied in 4 experimental groups (each group n = 7): 1) glucose-free perfusion with 1 mM cyanide (0–60 minutes); 2) perfusion with 11 mM glucose and 1 mM cyanide (0–60 minutes); 3) perfusion without glucose (0–60 minutes); and, 4) glucose-free perfusion with 1 mM cyanide (0–60 minutes) following a 60-minute period of glucose-free preperfusion.
Oxygen Dependence of Noradrenaline and DOPEG Release

In one series of experiments with aerobic (cyanide) and anaerobic (glucose-free) energy metabolism blockade, the effect of oxygen pressure on the overflow of endogenous noradrenaline and DOPEG was studied. In Figure 9, the cumulative overflow of noradrenaline and DOPEG is given for 10, 20, and 30 minutes of metabolic blockade. At all 3 periods, the DOPEG overflow clearly depended on the presence of oxygen with maximal release at oxygen pressures of more than 150 mm Hg and half-maximal release at 50 mm Hg. At 20 and 30 minutes, the noradrenaline overflow was in reverse proportion to DOPEG, with identical half-maximal effect. The combined overflow of noradrenaline and DOPEG was unaffected by oxygen pressure. These results demonstrated the oxygen dependence of deamination of noradrenaline by monoamine oxidase (MAO), and show that a high percentage of noradrenaline was a potential substrate of MAO. This means that during the process of release, noradrenaline must cross the axoplasm to be available...
for MAO, located at the outer membrane of mitochondria. At 10 minutes of metabolic blockade, the noradrenaline overflow was independent of oxygen, indicating that the small quantities released were not a result of oxidative deamination, but were released by a different mechanism. Blockade of MAO by pargyline prevented any DOPEG release, even at high oxygen pressures, and ensured that all noradrenaline was released unchanged.

**Capacity of Oxidative Deamination**

In another series of experiments, the overflow of DOPEG was induced by the reserpine-like drug Ro 4-1284 (Figure 10) without interfering with energy metabolism. This overflow was shown to be far greater than the release induced by blockade of energy metabolism in the presence of oxygen (Figures 5, 6, 7, and 8). The addition of cyanide in the presence of glucose had no significant influence on the increased DOPEG formation and release (Figure 10). In both experiments, no noradrenaline release was observed (Figure 10). These results indicated that in the cyanide experiments, DOPEG formation by MAO was neither saturated nor blocked.

**Discussion**

**Nonexocytotic Release of Noradrenaline Under Various Conditions of Energy Deficiency**

Nonexocytotic release of endogenous noradrenaline occurred in 3 different experimental models of energy depletion in the isolated heart of the rat: 1) total and global ischemia, 2) hypoxia or anoxia, and 3) blockade of energy metabolism in the presence of oxygen. All 3 models showed identical characteristics of noradrenaline release, indicating a common mechanism that cannot be reconciled with the concept of exocytotic liberation of noradrenaline. In the 3 models studied, the overflow of noradrenaline was not reduced when the experiments were performed under calcium-free conditions as would be expected for exocytotic release, but, on the contrary, showed a tendency to increased release. Blockade of neuronal uptake did not result in an increased overflow of noradrenaline — a constant finding in connection with exocytotic release — but markedly depressed the catecholamine overflow from the hearts. These characteristics of release found under various conditions of energy depletion may be explained by a nonexocytotic release of noradrenaline from the sympathetic varicosities. The mechanism first postulated by Schomig can be summarized as a two-step release, with the first step loss of noradrenaline from the storage vesicles and consequent increased axoplasmic noradrenaline concentrations. The second step is assumed to be a carrier-mediated outward transport of noradrenaline, using the uptake, carrier in reverse of its normal transport direction.

The experiments performed in this study were planned to test this hypothesis and to provide more detailed information about partial processes involved in the overall noradrenaline release, such as the role of oxidative and anaerobic energy metabolism and the significance of oxygen and MAO activity for the cytoplasmic accumulation of noradrenaline. Since stop-flow ischemia permits little variation of the experimental conditions, it was necessary to perform most studies in models with depleted energy and unchanged perfusion flow. This approach provided less complex experimental situations and made possible independent changes in parameters of interest.

**The Role of Energy Metabolism for Nonexocytotic Noradrenaline Release**

The energy status of the sympathetic nerve terminal appears to be the main determinant of nonexocytotic noradrenaline release. Both the interruption of oxidative phosphorylation (either by oxygen deficiency or by cyanide poisoning) and the inhibition or exhaustion of anaerobic glycolysis are necessary for a significant release of noradrenaline from the nerve terminals. Either of both energy-providing processes was sufficient to reduce strongly the release observed. Thus, ongoing glycolysis completely prevented noradrenaline release, even during complete anoxia or cyanide intoxication. This observation may be reason to accept that neither cyanide (used to block oxidative phosphorylation) nor dithionite (used to achieve complete anoxia) has direct toxic effects on noradrenaline release other than those due to energy metabolism.

In all three models, the quantities of released noradrenaline were comparable and the time courses of release were parallel. In all models, a lag of 10 minutes was found between the start of energy depletion and noradrenaline release, which may be due to ongoing glycolysis. Reduction of glycogen stores by glucose-
free preperfusion or blockade of glycolysis reduced this lag and accelerated noradrenaline release.

Carlson et al.20 studied the overflow of [3H]-noradrenaline from the perfused rat heart submitted to incomplete (low flow) or regional ischemia (ligature of the left coronary artery) and described a protective effect of glucose in comparison to perfusion with lactate. With low-flow ischemia, Dart and Riemersma21 found the residual substrate supply adequate to prevent a major overflow of endogenous noradrenaline during ischemia.

Energy depletion of the nerve terminals is both a necessary and sufficient cause for nonexocytotic noradrenaline release to occur during ischemia. Other factors implicated in ischemia, such as acidosis, increased interstitial potassium concentrations, and accumulation of metabolites, are not likely to play a major role in causing this release because in both models with ongoing flow they are present to a much lower degree. These models showed even higher cumulative releases than the stop-flow model. This is in agreement with earlier studies,22 which showed no effect of modest acidosis (pH 6.6) or hyperkalemia (less than 20 mM) on the overflow of endogenous noradrenaline from normoxic perfused rat hearts.

The local elimination of released noradrenaline by extraneuronal uptake (uptake,) has been shown to be of minor importance during ischemia12 and cyanide intoxication.

Therefore, no clear evidence is available to demonstrate that the metabolic status of the cardiac muscle cell determines directly or indirectly ischemia- or anoxia-induced catecholamine release from the sympathetic neuron. Under most conditions, changes of energy status may be assumed to run parallel in nerve terminals and muscle cells. However, discrepancies in metabolic status may develop between both cell types because of quantitative differences in energy stores and energy consuming processes (e.g., contractions). Energy deficiency in the absence of extracellular calcium may expose such a discrepancy. Under these conditions, the loss of high energy phosphates is delayed in the cardiac muscle cell and contracture is retarded.23 The nonexocytotic release of catecholamines, however, is accelerated and increased, if changed at all. It may be erroneous, therefore, to extrapolate directly from the easily measured effects of energy deficiency in the muscle cell to the energy status of the sympathetic nerve terminals in the heart.

**DOPEG Release Reflects Cytoplasmic Noradrenaline Concentrations**

The cyanide model provides some additional insight into the intraneuronal processes leading to noradrenaline release, because in this model information was available concerning noradrenaline concentrations in the cytoplasm of the nerve terminal. In the presence of oxygen, axoplasmic noradrenaline is deaminated by monoaminoxidase and DOPEG is formed.24 In contrast to noradrenaline, the more lipophilic DOPEG25 diffuses passively from the intracellular to the extracellular compartment, where it can be measured. As in the cyanide model, the DOPEG-forming process was neither inhibited nor saturated; the DOPEG release was a mirror of the axoplasmic noradrenaline concentration.26

The measurement of DOPEG release facilitates an understanding of intraneuronal events modulating noradrenaline release at various levels (Figure 11), including substrate supply or energy metabolism, storage vesicles, oxidative deamination, and catecholamine carrier in the axolem. A reduced noradrenaline overflow, for instance, may be the result of an improved energy status preventing noradrenaline release from the storage vesicles. In this case, a low DOPEG release will be found. On the other hand, inhibition of noradrenaline transport at the level of the plasmalemma will result in a similarly decreased noradrenaline overflow, in conjunction with high DOPEG formation and overflow (effect of desipramine).

The observation of low noradrenaline and high DOPEG levels after uptake, blockade can be taken as an additional reason to exclude exocytosis as a relevant mechanism of metabolically induced noradrenaline release. Furthermore, models of release proposed by Uvnás and Aborg27 and by Bogdanski28 cannot adequately describe the underlying mechanism in anoxia or ischemia. Both authors postulate a carrier-mediated transport of catecholamines directly from vesicles attached tightly to the axolem across an intact membrane into the extracellular space. Following their hypothesis, noradrenaline is not released via the axoplasm and therefore cannot be a substrate of MAO during the release process. In their model, catecholamines from the extracellular space have to be the main source of DOPEG formation, and DOPEG release would have to decrease to almost zero when uptake, was blocked. This is in contrast to the DOPEG overflow in the cyanide experiments, which was not reduced by uptake, blockade.

**A Model of Nonexocytotic Noradrenaline Release in Ischemia**

The initial event of noradrenaline release can be assumed to be a disturbed energy metabolism that compromises the capability of storage vesicles to maintain catecholamines.29 The mechanism of noradrenaline loss from the vesicles is not completely understood. It can, however, be derived from DOPEG data in this study that a rise in cytoplasmic noradrenaline preceded the release of the catecholamine from the neuron. In contrast to vesicles of chromaffine cells, which show a low spontaneous loss of catecholamines, storage vesicles obtained from sympathetic neurons are less stable.30 In the absence of adenosine triphosphate (ATP), the half-loss period of noradrenaline from these vesicles was about 10 minutes at a temperature of 37°C in vitro.31-33 This time course corresponds with the fast release of DOPEG when the noradrenaline transport back into the vesicles was blocked by reserpine-like substances such as Ro 4-1284 under normoxic conditions. Thus, our results are compatible
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**FIGURE 11.** Scheme of the noradrenaline release mechanism activated by energy depletion. The various interventions used in this paper and their specific sites of action are indicated. Four different methods [(1), (2), (3), and (4)] have been used to reduce the production of high-energy phosphates and to induce a release of noradrenaline from the storage vesicles into the cytosol. An ineffective noradrenaline transport into the storage vesicles induced by reserpine-like drugs (5) or by energy depletion results in increasing cytoplasmic noradrenaline concentrations. From the cytoplasm of the neuron, noradrenaline may be eliminated by oxidative deamination (A) or carrier-mediated release (B). A) The oxidative deamination of noradrenaline by monoamine oxidase (MAO) forms DOPEG, which diffuses across the plasma membrane into the extracellular space. DOPEG release reflects the cytoplasmic noradrenaline concentration as long as the deamination process is not impaired by oxygen deficiency or blockade of monoamine oxidase (6). B) The outward transport of noradrenaline via the uptake, carrier can be blocked specifically by desipramine and other antidepressants (7). This carrier-mediated release seems to depend on an increased intracellular sodium concentration and gains increasing significance with progressing energy depletion.

With the concept that a high leakage of noradrenaline from the neuronal vesicles is counterbalanced by vesicular inward transport as long as the energy status is undisturbed. Under conditions of energy depletion, however, this transport, which indirectly depends on high energy phosphates, and is severely hampered, resulting in a situation similar to that of transport blockade by reserpine-like substances. The net loss of noradrenaline from the vesicles leads to increased cytoplasmic noradrenaline concentrations reflected by the DOPEG release in the presence of oxygen. It cannot be excluded from current study data that factors other than a compromised vesicular inward transport such as destabilization of the intravesicular storage complex, an increased leakage, or a carrier mediated efflux from the vesicles may play a role in the loss of vesicular catecholamines into the cytoplasm.

Noradrenaline loss from the vesicles does not necessarily result in a noradrenaline release from the neuron when the uptake, carrier is not available for the outward transport from cytoplasm to the extracellular space, and the catecholamines accumulating in the cytosol are metabolized by MAO. Oxidative deamination of noradrenaline by MAO and subsequent formation of DOPEG is critically dependent on the presence of oxygen with a Km value of 50 mm Hg oxygen pressure. This value, derived from the present experiments with neurons in situ, is in accordance with those published for in vitro MAO preparations. At high oxygen pressures and intact energy supply, these mechanisms prevent any major release of noradrenaline into the interstitial space. Under anoxic or ischemic conditions, however, the protective effect of MAO is lost, and all noradrenaline leaking from storage vesicles is available for outward transport by uptake. The uptake, carrier normally provides the inward transport of released noradrenaline and is driven energetically by a sodium and chloride ion cotransport. With intact glycolysis, even high cytoplasmic noradrenaline concentrations — reflected by a high DOPEG release — did not result in a net noradrenaline release (Figure 10). However, under conditions of energy depletion, increasing amounts of noradrenaline were transported outward across the membrane using the uptake, carrier in reverse of its normal transport direction. A more detailed discussion of the concept and literature concerning the outward noradrenaline transport by the uptake, carrier in ischemia is given by Schönig et al. In all experiments, the release of noradrenaline showed a latency of about 5 minutes when compared to the time course of DOPEG release. This may be due to a rather high tolerance to energy depletion of this transport system, which, above all, determines the time course of flooding the myocardium with noradrenaline during ischemia. The importance of intracellular and extracellular ion concentrations and of Na⁺, K⁺-ATPase activity for the phenomenon of reversed transport by uptake, carrier has been described in several pharmacological models. The role of these factors in catechol-
amine release in the ischemic or anoxic heart is under investigation.

In conclusion, the results of the present study demonstrate a mechanism for the release of noradrenaline in myocardial ischemia and anoxia that is different from release mechanisms known so far. Depletion of high-energy phosphates from the sympathetic nerve terminal is a necessary and sufficient cause for initiating a chain of events leading to high concentrations of noradrenaline in the extracellular space of the myocardium, where the neurotransmitter is available for receptor stimulation. The supply of glucose to the nerve cell can completely suppress this process even in the total absence of oxygen. The release of noradrenaline occurs in two steps. First, catecholamines are lost from storage vesicles and accumulate in the axoplasm, where, in the presence of oxygen, it is a substrate for monoamine oxidase. In the second, rate-limiting step, noradrenaline is transported across the axolem from cytoplasm to intersitial space, using the uptake, carrier that has reversed its normal transport direction. This mechanism for local metabolic catecholamine release may provide a basis for the development of specific measures to prevent release and accumulation of noradrenaline in the ischemic myocardium.

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