Effect of Myocardial Ischemia on Stimulation-Evoked Noradrenaline Release
Modulated Neurotransmission in Rat, Guinea Pig, and Human Cardiac Tissue

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The effect of myocardial ischemia and its major metabolic changes, such as anoxia, acidosis, and hyperkalemia, on exocytotic noradrenaline release was investigated in rat, guinea pig, and human cardiac tissue. Noradrenaline release was evoked by electrical field stimulation, and the effect of each experimental intervention on stimulation-evoked noradrenaline release (S2) was intradividually compared with the release induced by a control stimulation (S1). In perfused hearts, 10 minutes of global ischemia caused a reduction of noradrenaline overflow in rat hearts (mean S2/S1, 0.31), whereas the overflow was increased in guinea pig hearts (S2/S1, 1.89). This species-dependent effect may be caused by quantitatively different responses to facilitating and suppressing factors of noradrenaline release in both species. Anoxia and substrate-free perfusion increased noradrenaline overflow in guinea pig hearts (S2/S1, 2.40) but had no significant effect in rat hearts (S2/S1, 0.75). Acidosis (pH 6.0) resulted in a suppression of noradrenaline release in rat hearts (S2/S1, 0.16), whereas it had only a minor inhibiting effect in guinea pig hearts (S2/S1, 0.67). Hyperkalemia had a comparable effect in both species (S2/S1 at 15 mmol/L K⁺, 1.17 in rat and 1.14 in guinea pig; and S2/S1 at 20 mmol/L K⁺, 0.64 in rat and 0.41 in guinea pig). To obtain results regarding the modulation of noradrenaline release in human myocardium, human atrial tissue was incubated, and the effect of anoxia, acidosis, and hyperkalemia on stimulation-evoked noradrenaline release was investigated. Anoxia had a moderate facilitating effect on stimulation-evoked noradrenaline release (S2/S1, 1.20), whereas acidosis (S2/S1, 0.35) and hyperkalemia resulted in a suppression (S2/S1 at 15 mmol/L K⁺, 0.63; and S2/S1 at 20 mmol/L K⁺, 0.03). When the same studies were performed in incubated rat and guinea pig atrial tissue, stimulation-evoked noradrenaline release was modulated by the same metabolic factors as in perfused hearts. In conclusion, stimulation-evoked noradrenaline release in ischemic tissue is determined by facilitating and suppressing factors in guinea pig, rat, and human cardiac tissue. In human hearts, the suppressing factors dominate even more than in rat hearts, whereas in guinea pig hearts, the facilitating factors outweigh the suppressing factors during early myocardial ischemia. (Circulation Research 1993;73:496-502)

KEY WORDS • acidosis • energy metabolism • myocardial ischemia • K⁺ • noradrenaline release

Sympathetic activity in acute myocardial ischemia is thought to be closely associated with the progression of myocardial injury1,2 and the pathogenesis of malignant arrhythmias.3,4 Although increased systemic sympathetic activity is well established during acute myocardial infarction,5 local effects of ischemia on the activity of efferent cardiосympathetic neurons and the release of neurotransmitters remain controversial.6,8 Local accumulation of noradrenaline in the interstitial space of ischemic myocardium may be due to either an exocytotic or nonexocytotic release from nerve terminals. Nonexocytotic noradrenaline release is known to occur after more than 10 minutes of myocardial ischemia, independent of central sympathetic activity.7,8 Exocytotic release evoked by central sympathetic activation, on the other hand, is thought to determine the extracellular noradrenaline concentration within the ischemic myocardium during the first 10 minutes. Previous studies about modulation of this local release, however, showed contradictory results. Noradrenaline release evoked by electrical stimulation of sympathetic nerves has been found to be suppressed after 10 minutes of global ischemia in rat hearts,9 whereas a marked overflow of noradrenaline was reported after 4 minutes of anoxia in rabbit10 and dog11 hearts after coronary artery occlusion even without neural stimulation. In pig hearts, noradrenaline release was found to be slightly increased only during the first 10 minutes of regional ischemia.12 Dry et al13 recently suggested an increased exocytotic release of noradrenaline from bovine adrenal chromaffin cells by anoxia. When noradrenaline release was evoked by potassium

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depolarization, anoxia markedly increased noradrenaline release from guinea pig hearts\textsuperscript{14} and rat atrial tissue.\textsuperscript{15} Investigations on sympathetic neurotransmission in human hearts are conflicting: Increased cardiac noradrenaline overflow during short-term myocardial ischemia induced by coronary angioplasty was proposed by one study,\textsuperscript{16} whereas another study\textsuperscript{17} found a net uptake of noradrenaline under comparable experimental conditions. So far, no direct information exists regarding modulation of stimulation-evoked noradrenaline release by ischemia in human cardiac tissue.

The overall decreased or increased noradrenaline release during ischemia or anoxia demonstrated in the various studies mentioned may depend on differences in species and experimental conditions. In different species, variable results regarding stimulation-evoked noradrenaline release within ischemic myocardium may be caused by quantitatively different effects of single metabolic changes occurring during ischemia. In the present study, therefore, exocytotic noradrenaline release evoked by electrical field stimulation was investigated in perfused hearts and incubated atrial tissue, and the effect of each experimental intervention was compared between three different species (the guinea pig, rat, and human) to address the following questions: (1) Is there a species difference regarding the effect of ischemia on stimulation-evoked noradrenaline release? (2) How do single factors of ischemia such as anoxia, acidosis, and hyperkalemia affect exocytotic noradrenaline release? (3) How is stimulation-evoked noradrenaline release modulated in ischemic myocardium of human hearts?

**Materials and Methods**

**Isolated Perfused Hearts**

Male Wistar rats (200 to 250 g) and guinea pigs (250 to 300 g) (Thomae, Biberach, Germany) were anesthetized with thiopental sodium (75 to 100 mg/kg IP). After injection of 500 U heparin intravenously, the thorax was opened. Hearts were removed and weighed (rat hearts, 1.02±0.13 g; guinea pig hearts, 1.30±0.15 g). The aorta was cannulated for isolated perfusion (Langendorff technique), as has been described before.\textsuperscript{7} Two 10×7-mm concavely shaped metal paddles were placed in opposite positions on each heart, touching the hearts in a manner such that the interventricular septum was located between both paddles. Exocytotic noradrenaline release was induced by electrical field stimulation at 5 V (effective voltage), 6 Hz, and a pulse length of 2 milliseconds for 1 minute. All hearts were perfused at a constant flow of 5 mL/min per gram heart weight with a modified Krebs-Henseleit solution gassed with 95% O\textsubscript{2}–5% CO\textsubscript{2} and composed of (mmol/L) NaCl, 125; NaHCO\textsubscript{3}, 16.9; Na\textsubscript{2}HPO\textsubscript{4}, 0.2; KCl, 4.0; CaCl\textsubscript{2}, 1.85; MgCl\textsubscript{2}, 1.0; glucose, 11.0; and sodium EDTA, 0.027. pH was adjusted at 7.4. The temperature of the perfusate was kept constant at 37°C. To inhibit neuronal uptake of noradrenaline and prevent nonexocytotic noradrenaline release, all experiments were done in the presence of desipramine hydrochloride (100 mmol/L, CIBA-GEIGY, Basel, Switzerland).\textsuperscript{7,8,18} After 20 minutes of equilibration, two electrical stimulations 15 minutes apart were performed to induce noradrenaline release, and the effect of each experimental intervention on stimulation-evoked noradrenaline release (S2) was intraindividually compared with a stimulation-evoked release during a control condition (S1). The second stimulation was performed during the last minute of the experimental intervention. The effect of each intervention was analyzed by calculating the individual S2/S1 ratio.

Ischemia was induced by global stop flow over various periods of time; after ischemia, the hearts were perfused at the same flow rate as before stop flow. Anoxia was induced by perfusing the hearts with a glucose-free solution gassed with 95% N\textsubscript{2}–5% CO\textsubscript{2} at an unchanged flow rate. A PO\textsubscript{2} of less than 5 mm Hg was achieved by addition of 0.5 mmol/L sodium dithionite.\textsuperscript{18} In the series of experiments done to investigate the effect of acidosis, pH was reduced by changing PO\textsubscript{2}. To investigate the effect of hyperkalemia, [K\textsuperscript{+}], was increased by exchanging Na\textsuperscript{+} and K\textsuperscript{+} in the perfusate.

One-minute samples for the determination of noradrenaline in the venous overflow were collected into prechilled tubes containing sodium EDTA immediately before, during, and for 3 minutes after the electrical stimulation.

**Incubated Atrial Tissue**

Male Wistar rats and guinea pigs were treated as described above. Hearts were removed, and atria were cut out and weighed (70 to 100 mg). After excision, specimens were placed in Krebs-Henseleit solution. Human atrial tissue was acquired from patients undergoing coronary artery surgery, after obtaining their informed consent. The atrial appendage was cut out during the routine cannulation of the right atrium. Immediately after excision, specimens were placed in ice-cold Krebs-Henseleit solution. Each tissue specimen was divided into two to four parts, each weighing 70 to 100 mg. In one series of experiments, noradrenaline content was determined in rat, guinea pig, and human atrial tissue as well as in rat and guinea pig ventricular tissue.

For electrical stimulation, tissue specimens were placed between two paddle electrodes made of wire netting (10×5 mm; distance, 2 to 3 mm), which touched specimens and allowed diffusion and medium exchange from all sides. Throughout all experiments, tissues were transferred every 5 minutes to tubes containing 3 mL Krebs-Henseleit solution (37.5°C) gassed with 95% O\textsubscript{2}–5% CO\textsubscript{2}. pH was adjusted to 7.4 by changing CO\textsubscript{2} concentration, and pH in the incubation medium was repeatedly measured by a blood gas analyzer (ABL 30, Willich, Germany). Immediately after transferring the tissues to the next tube, the previous medium was cooled on ice and stabilized for determination of noradrenaline. After an equilibration period of 20 minutes, two electrical stimulations of 5 V, 4 Hz, and a pulse length of 2 milliseconds were performed over a period of 5 minutes each with an interval of 20 minutes between them. Noradrenaline was determined in the medium during the 5-minute period before, during, and after stimulation. Desipramine (100 nmol/L) was present throughout the experiments but not during the equilibration period.

Experimental series with energy depletion were performed by gassing a glucose-free solution with 95% NO\textsubscript{2}–5% CO\textsubscript{2} for 10 minutes. Anoxia (PO\textsubscript{2}, <5 mm Hg) was achieved by adding sodium dithionite (1 mmol/L) to the incubation medium. For the experiments to investigate the effect of acidosis, PCO\textsubscript{2} was increased, adjusting pH at 6.5 or 6.0 for 15 minutes. Hyperkalemia was
induced by exchanging Na⁺ and K⁺ in the incubation medium for 10 minutes.

The second electrical stimulation was always performed during the last 5 minutes of each experimental intervention.

**Determination of Noradrenaline**

Noradrenaline samples were stabilized by addition of Na₂-EDTA (10 mmol/L) and stored at −60°C until assayed. Endogenous noradrenaline was measured by using a high-performance liquid chromatography (HPLC) method as has been described in detail before. 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%, the limit of detection was 0.1 nmol/L, and the coefficient of variation was 5.9%.

**Statistical Methods**

Stimulation-evoked noradrenaline release in both perfused hearts and incubated tissue was calculated as cumulative noradrenaline outflow in the two periods during and following electrical stimulation. The results in human atrial tissue specimens of the same individual were averaged and taken as n=1. All results are expressed as the arithmetic mean for noradrenaline release and noradrenaline content of tissue and as the geometric mean for S2/S1 ratios. Statistical differences were tested with Student’s t test for paired data of noradrenaline outflow (S1 and S2). A value of P<.05 was considered significant. All values are given as the arithmetic mean±SEM or geometric mean for S2/S1 ratios.

**Results**

**Effect of Ischemia, Anoxia, Acidosis, and Hyperkalemia on Stimulation-Evoked Noradrenaline Release in Rat and Guinea Pig Perfused Hearts**

In control experiments, two consecutive electrical field stimulations in the same preparation without intervention resulted in comparable noradrenaline outflows from isolated perfused rat hearts (S1, 194±27 pmol/g; S2, 209±26 pmol/g; n=10) and guinea pig hearts (S1, 195±28 pmol/g; S2, 200±47 pmol/g; n=10) with mean S2/S1 ratios of 1.10 and 0.94, respectively. To document exocytotic release of noradrenaline, a series of experiments was performed in the presence of the Na⁺ channel blocker tetrodotoxin (1 μmol/L). When tetrodotoxin was added 10 minutes before the second stimulation, stimulation-evoked noradrenaline release was completely suppressed in both rat (S1, 71±5 pmol/g; S2, 1±1 pmol/g; n=4; P<.01) and guinea pig (S1, 158±18 pmol/g; S2, 1±1 pmol/g; n=8; P<.01) hearts. In another series of experiments, the contribution of extraneuronal uptake (uptake₁) was determined by use of the uptake₂ blocker corticosterone. Corticosterone, added 10 minutes before the second stimulation, caused a minor increase of noradrenaline release at 1 μmol/L (S2/S1, 1.23 in rat [n=4] and 1.05 in guinea pig [n=4]) and at 30 μmol/L (S2/S1, 1.22 in rat [n=4] and 1.20 in guinea pig [n=4]). Thus, regarding the response to corticosterone, no species-dependent effect was detected. Atropine sulfate was added in a third series of experiments to block muscarinic acetylcholine effects. Stimulation-evoked noradrenaline release was not changed by an administration of atropine (10 μmol/L) 10 minutes before the second stimulation (S2/S1, 1.09 in rat hearts [n=4] and 1.07 in guinea pig hearts [n=4]). Noradrenaline release induced by electrical field stimulation, therefore, was not significantly influenced by a potential concomitant release of endogenous acetylcholine in this preparation.

The effect of ischemia on exocytotic noradrenaline release was investigated by stopping perfusion flow over various periods of time. Ischemia for a period of 10 minutes markedly decreased noradrenaline overflow in rat hearts (S1, 136±15 pmol/g; S2, 44±6 pmol/g; n=10; P<.01), whereas noradrenaline release in guinea pig hearts was increased (S1, 250±24 pmol/g; S2, 457±34 pmol/g; n=14; P<.01) (Fig 1). This release of noradrenaline was characterized as calcium dependent, since Ca²⁺-free perfusion 5 minutes before ischemia abolished any noradrenaline overflow induced by electrical stimulation in both species (S2/S1, 0.06 in rat [n=5, P<.05] and 0.11 in guinea pig [n=5, P<.05]). The time course of the effect of ischemia on exocytotic noradrenaline release is demonstrated in Fig 2. The differential effect of ischemia in rat and guinea pig hearts was most pronounced after 10 minutes of ischemia. At 20 minutes of ischemia, stimulation-evoked noradrenaline release was no longer increased in guinea pig hearts (S1, 226±33 pmol/g; S2, 210±36 pmol/g; n=8).

In further experiments, the effects of anoxia, acidosis, and hyperkalemia on exocytotic noradrenaline release were studied (Figs 3 and 4, upper panels). Anoxia and substrate-free perfusion at an unchanged flow rate resulted in a marked increase of noradrenaline release in guinea pig hearts (S1, 283±51 pmol/g; S2, 680±122 pmol/g; n=6; P<.05). This increase was even more pronounced than during ischemia. Noradrenaline release in rat hearts remained unchanged (S1, 141±15 pmol/g; S2, 120±33 pmol/g; n=6). No facilitating effect of anoxia was detected when glucose was present throughout anoxia in guinea pig hearts (S1, 179±17 pmol/g; S2, 189±14 pmol/g; n=6) and rat hearts (S1, 89±16 pmol/g; S2, 67±10 pmol/g; n=6).

Acidosis (pH 6.0) resulted in a suppression of noradrenaline release in rat hearts (S1, 134±21 pmol/g; S2, 25±6 pmol/g; n=6; P<.01) and had minor inhibiting
effects in guinea pig hearts (S1, 302±30 pmol/g; S2, 205±26 pmol/g; n=6). Acidosis had no effect in rat hearts when pH was changed to 6.5 (S1, 171±41 pmol/g; S2, 162±37 pmol/g; n=6) but a slight increasing effect in guinea pig hearts (S1, 224±34 pmol/g; S2, 289±22 pmol/g; n=6; *P<.05).

Increased [K+] in the perfusate reduced the exocytotic noradrenaline release when [K+] exceeded 15 mmol/L (Fig 4, upper panel). In both species, hyperkalemia (20 mmol/L) had a similar inhibiting effect (in rat hearts: S1, 121±12 pmol/g; S2, 78±9 pmol/g; n=6; *P<.05; and in guinea pig hearts: S1, 143±28 pmol/g; S2, 53±3 pmol/g; n=6; *P<.05).

**Effect of Anoxia, Acidosis, and Hyperkalemia on Stimulation-Evoked Noradrenaline Release From Incubated Atrial Tissue**

To investigate the effects of major components of ischemia in human cardiac tissue, an experimental model for investigation of stimulation-evoked noradrenaline release in atrial tissues was used. Studies in this model allow direct comparisons between human, rat, and guinea pig cardiac tissue.

First, tissue noradrenaline content was determined without intervention or stimulation (Table). Tissue concentration of noradrenaline both in atrial and ventricular myocardium was twice as high in guinea pig hearts as in rat hearts, whereas human atrial tissue took a mean position between rat and guinea pig atrial tissue. The ratio between atrial and ventricular tissue noradrenaline concentration was similar in guinea pig hearts (atrial concentration/ventricular concentration, 2.5) and in rat hearts (atrial concentration/ventricular concentration, 2.2). In control experiments without experimental intervention, electrical field stimulation elicited comparable noradrenaline release during two consecutive stimulations in guinea pig, rat, and human cardiac tissue (human: S1, 89±6 pmol/g; S2, 89±6 pmol/g; n=30; rat: S1, 167±21 pmol/g; S2, 163±19 pmol/g; n=8; and guinea pig: S1, 166±34 pmol/g; S2, 155±23 pmol/g; n=8). To demonstrate exocytotic neuronal release in this methodological approach, tetrodotoxin (1 μmol/L) was added before the second stimulation. Tetrodotoxin completely suppressed stimulation-induced noradrenaline release (S2, 0 pmol/g in all three species [n=4 each]; *P<.05). To exclude significant interaction of endogenous acetylcholine with noradrenaline concomitantly released by electrical field stimulation, atropine sulfate (10 μmol/L) was added 10 minutes before the second stimulation. Although a trend was observed in guinea pig atrial tissue (S2/S1, 1.41; n=4), noradrenaline release was not changed in human (S2/S1, 1.10;
Data were comparable in guinea pig atrial tissue (S1, 227±31 pmol/g; S2, 162±30 pmol/g; n=8; P<.05). The strongest suppressing effect of acidosis was observed in human atrial tissue (S1, 67±12 pmol/g; S2, 33±11 pmol/g; n=6; P<.01). No effect was detected in any of the species when pH was changed to 6.5.

Hyperkalemia resulted in a concentration-dependent reduction of exocytotic noradrenaline release. At the highest [K⁺] (20 mmol/L), noradrenaline release in human atrial tissue was almost completely suppressed (S1, 81±12 pmol/g; S2, 8±7 pmol/g; n=6; P<.01) but was not so markedly changed in rat atrial tissue (S1, 152±27 pmol/g; S2, 116±18 pmol/g; n=8; P<.05) or guinea pig atrial tissue (S1, 205±34 pmol/g; S2, 109±12 pmol/g; n=7; P<.05). Hyperkalemia of 15 mmol/L had no effect on noradrenaline release in guinea pig atrial tissue (S1, 240±44 pmol/g; S2, 292±44 pmol/g; n=6) and rat atrial tissue (S1, 153±39 pmol/g; S2, 141±36 pmol/g; n=6), whereas noradrenaline release was already reduced in human atrial tissue (S1, 104±28 pmol/g; S2, 60±13 pmol/g; n=6; P<.05) (Fig 4, bottom).

Discussion

During myocardial ischemia, central sympathetic activity is enhanced, resulting in an increased activation of efferent sympathetic cardiac neurons up to a maximum of 40% to 50%. In addition, the release of noradrenaline itself may be affected and modulated by metabolic changes within the ischemic myocardium, potentially leading to an overall increased or decreased release of noradrenaline. This modulation may influence the occurrence of malignant arrhythmias during early myocardial ischemia. In the present study, stimulation-evoked noradrenaline release was affected by stop-flow ischemia in guinea pig and rat hearts in a qualitatively different way during the first 10 minutes. Noradrenaline overflow underwent a time-dependent increase in guinea pig hearts and decrease in rat hearts. Release of noradrenaline due to electrical field stimulation used in these experiments could be characterized as calcium dependent and exocytotic. Without any stimulation, no overflow of noradrenaline was observed after 10 minutes of stop-flow ischemia in either rat or guinea pig hearts, confirming that nonexocytotic release starts after more than 10 minutes of stop-flow ischemia. In a previous study, stop-flow ischemia suppressed noradrenaline release due to electrical stimulation of the left cardiac sympathetic nerves in perfused rat hearts. In guinea pigs, an increased stimulation-evoked noradrenaline release due to ischemia has not been described before, although increased exocytotic noradrenaline release due to anoxia, was previously proposed in bovine chromaffin cells. The different effects of brief ischemia on noradrenaline release in guinea pig and rat hearts in the present study may help us understand the conflicting results in former studies, which demonstrated an increased noradrenaline overflow in rabbit and canine hearts after 3 to 4 minutes of anoxia and in pig hearts during the first 10 minutes of regional ischemia even without any stimulation, suggesting a differential modulation of noradrenaline release by myocardial ischemia in these species. In addition, the interpretation of conflicting results must reflect the methodological differences between the involved studies, eg, anoxia compared with ischemia. The hy-
Evidence for Differing Effects of Metabolic Changes During Ischemia on Sympathetic Neurotransmission

Within the ischemic myocardium, energy stores are depleted; potassium ions, protons, and adenosine accumulate; and Ca\(^{2+}\) transport is affected.\(^{26}\) Each of these major changes might affect the local release of noradrenaline.\(^{20,24}\) The importance of local energy metabolism of nerve endings for modulation of noradrenaline release has been demonstrated in \([H]noradrenaline-loaded\) rat vasa deferentia with an enhanced sensitivity of noradrenaline release to K\(^+\) depolarization during anoxia.\(^{15}\) Comparable results were obtained in perfused guinea pig hearts when K\(^+\)-evoked noradrenaline release was increased during energy depletion.\(^{14}\) In the present study, anoxia and glucose-free perfusion increased noradrenaline release, both in perfused hearts and in incubated atrial tissue, but this effect was pronounced only in guinea pig hearts. Anoxia without glucose deprivation had no effect on stimulation-evoked noradrenaline release. This is in line with former investigations on metabolic requirements of exocytotic\(^{14,21}\) and nonexocytotic\(^{18}\) noradrenaline release during energy depletion, demonstrating an effect only after interruption of both oxidative phosphorylation and anaerobic glycolysis. The facilitating effect of anoxia on noradrenaline release seems to be independent of the stimulus of noradrenaline release, as long as the release is due to exocytosis. All studies with anoxia were performed with an unchanged flow, therefore avoiding accumulation of metabolites and preventing acidosis but limiting direct comparison to an ischemic condition. Whereas the facilitating effect of anoxia was most pronounced in guinea pig hearts, the suppressed overflow during acidosis was predominant in rat and human cardiac tissue. This suppressing effect was only observed when pH was below 6.5. In previous studies, comparable results were reported with no effect of acidosis on stimulation-evoked noradrenaline release in rat hearts when pH was not below 6.5.\(^{23}\) but with a marked effect on sympathetic neurotransmission in the canine heart at a pH below 6.5.\(^{24}\) The relevance of studies investigating the effect of a pH below 6.5 may be illustrated by findings that demonstrate a fall in extracellular\(^{27}\) and intracellular\(^{28}\) pH during myocardial ischemia to 6.2 after 10 minutes of stop-flow ischemia in rat hearts. A rapid intracellular equilibration of pH was demonstrated for changes of Pco\(_2\).\(^{29}\) Increasing [K\(^+\)] up to 20 mmol/L had a concentration-dependent inhibiting effect in all three species when [K\(^+\)] exceeded 10 mmol/L. Neurotransmission in human cardiac tissue was most susceptible to hyperkalemia and was almost completely inhibited by 20 mmol/L [K\(^+\)]. Most investigations demonstrated an increase of [K\(^+\)] to 10 to 20 mmol/L within an ischemic period of 20 minutes.\(^{27,20}\) The present experiments covered this range of increased [K\(^+\)]. Inhibiting effects were already reported for hyperkalemia (12 mmol/L [K\(^+\)]) in the canine heart,\(^{24}\) in perfused rat hearts when [K\(^+\)] exceeded 13 mmol/L,\(^{31}\) and in isolated dog veins.\(^{32}\) High [K\(^+\)] and low pH may alter action potential characteristics of sympathetic neurons and indirectly decrease Ca\(^{2+}\) entry by voltage-dependent Ca\(^{2+}\) channels, an essential process for noradrenaline release from nerve terminals.\(^{33}\) This was previously suggested as a possible explanation for suppressed neurotransmission due to hyperkalemia and acidosis.\(^{24,34}\) Based on this hypothesis of modulated neurotransmission, the quantitatively different responses of guinea pig, rat, and human cardiac tissue to anoxia, acidosis, and hyperkalemia could be explained by species-dependent sensitivities of the action potential characteristics of nerve terminals to these metabolic changes, eg, by a potentially different susceptibility of Ca\(^{2+}\) channels. This concept cannot be proved by the present study. Another possible explanation for the species-dependent effects of metabolic changes on neurotransmission considers a potentially concomitant release of other neurotransmitters by electrical field stimulation, eg, acetylcholine from parasympathetic nerve endings.\(^{35,37}\) Acetylcholine interacts with noradrenaline release by presynaptic inhibition,\(^{35,36}\) and a different release of acetylcholine in rat and guinea pig cardiac tissue could produce an autonomic imbalance. However, a significant interaction of endogenous acetylcholine with exocytotic noradrenaline release was not observed in the present study, as indicated by the lacking effect of atropine on noradrenaline release due to field stimulation. These data confirm the study of Du et al,\(^{37}\) who demonstrated a failure of cholinergic modulation of noradrenaline release during acute myocardial ischemia in rat hearts.

Modulation of Neurotransmission in Human Cardiac Tissue

The demonstrated species differences emphasize the need for studies on human hearts. A direct approach to investigating the effect of ischemia on neurotransmission in isolated perfused human hearts is hampered by ethical considerations and methodological difficulties. Therefore, two indirect approaches have been used to determine how ischemia affects neurotransmission in human hearts.

First, the effect of ischemia can be investigated in vivo during coronary angioplasty, when coronary arteries are occluded and noradrenaline release is assessed from the coronary arteriogenous concentration differences of noradrenaline. Two previous studies reported no consistent data. The first study demonstrated a decreased difference of coronary arteriogenous noradrenaline concentration after 2 minutes of coronary artery occlusion.\(^{17}\) and the second study suggested an increased cardiac spillover of noradrenaline after 1 minute of coronary artery occlusion.\(^{16}\) This approach is limited by the short periods of ischemia and by the difficulty in distinguishing the release of noradrenaline from ischemic and nonischemic myocardium. Ischemia may also be induced in vivo by pacing the hearts of patients with coronary artery stenosis. In a study of this kind, myocardial ischemia did not induce a significant release of noradrenaline into the coronary sinus.\(^{38}\)

In a second indirect approach, experiments are performed with incubated human cardiac tissue. Experimental studies with this model allow multiple experimental interventions; however, they are restricted to changes that can be induced in the incubation medium, therefore excluding ischemia as an intervention. The validity of this model for investigations of noradrenaline
release has found approval, and both an exocytotic and a nonexocytotic release have been demonstrated in human atrial tissue as it has been described in perfused rat heart.\(^\text{29}\) In the present study, acidosis and increased \([K^+]\) suppressed noradrenaline release in human atrial tissue, and energy depletion increased the release slightly. The data show a dominance of suppressing factors over facilitating factors. Direct comparison between human cardiac tissue and cardiac tissue of rat and guinea pig within the same methodological approach suggests a similarity between rat and human cardiac tissue regarding the modulation of noradrenaline release. This similarity and the decrease of stimulation-evoked noradrenaline release by ischemia in perfused rat hearts may therefore support the hypothesis that in human hearts, even in the presence of a stimulated central sympathetic nervous system, the myocardium is protected from excess noradrenaline release during the first 10 minutes of myocardial ischemia because of metabolic changes such as acidosis and hyperkalemia.

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