Role of Neuronal K\textsubscript{ATP} Channels and Extraneuronal Monoamine Transporter on Norepinephrine Overflow in a Model of Myocardial Low Flow Ischemia

Christof Burgdorf, Andreas Dendorfer, Thomas Kurz, Edgar Schömig, Ines Stölting, Frank Schütte, and Gert Richard

Institut für experimentelle und klinische Pharmakologie und Toxikologie, Universitätsklinikum Schleswig-Holstein, Campus Lübeck, Germany (C.B., A.D.); Medizinische Klinik II, Universitätsklinikum Schleswig-Holstein, Campus Lübeck, Germany (T.K., I.S., F.S.); Institut für Pharmakologie, Klinikum der Universität zu Köln, Köln, Germany (E.S.); and Herzzentrum Segeberger Kliniken GmbH, Bad Segeberg, Germany (G.R.)

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ABSTRACT

Global myocardial low flow ischemia results in an uniform suppression of norepinephrine (NE) overflow from the heart. We hypothesized that opening of neuronal ATP-sensitive potassium (K\textsubscript{ATP}) channels as well as activation of the extraneuronal monoamine transporter (EMT) mediates attenuation of NE overflow during low flow ischemia. Isolated rat hearts were subjected to low coronary flow of 0.4 ml min\textsuperscript{-1}. Release of endogenous NE was induced by electrical field stimulation. EMT activity was measured as the transport rate of the substrate \(N\)-[methyl-\textsuperscript{3}H]4-phenylpyridinium (\([\text{3}H]\)MPP\textsuperscript{+}). NE overflow decreased by 57\% within 120 min of low flow. Five minutes of reperfusion at normal flow (8 ml min\textsuperscript{-1}) restored NE overflow to baseline. K\textsubscript{ATP} channel blockade with glibenclamide as well as EMT blockade with corticosterone increased NE overflow 1.5- and 2-fold at 120 min of low flow, whereas neither drug affected NE overflow in the absence of flow reduction. At normal flow, K\textsubscript{ATP} channel opening with cromakalim suppressed NE overflow, both in the presence and absence of EMT blockade (14 \pm 4 and 9 \pm 1\%). However, cromakalim had no effect on EMT activity as indicated by an unaffected \([\text{3}H]\)MPP\textsuperscript{+} overflow. In conclusion, activation of both K\textsubscript{ATP} channels and EMT mediate suppression of NE overflow during low flow ischemia. K\textsubscript{ATP} channels impair NE release directly at presynaptic nerve endings, whereas EMT increases NE elimination in a manner independent of K\textsubscript{ATP} channels.

Cardiac sympathetic nerve activity in patients with acute myocardial infarction is critically dependent on the severity of coronary flow reduction and duration of energy deprivation. In myocardial tissue subjected to more than 10 min of stopflow, nonexocytotic norepinephrine (NE) release via the neuronal monoamine transporter (NET) prevails over exocytotic NE release, resulting in progressive interstitial transmitter accumulation (Schömig, 1990; Kurz et al., 1995; Imamura et al., 1996; Hatta et al., 1999; Levi and Smith, 2000; Oka et al., 2002; Sesti et al., 2003). In contrast, during prolonged periods of reduced coronary flow (low flow ischemia), exocytotic NE release decreases, resulting in a uniform suppression of NE overflow from the heart (Du and Riemersma, 1991). Because ischemia in vivo is neither absolute nor homogeneous, heterogeneity of NE release has substantial clinical implication, because it forms the substrate for malignant ventricular arrhythmias (Schömig et al., 1991). So far, the underlying metabolic alterations that affect sympathetic neurotransmission have been studied intensively in the setting of stopflow ischemia; however, less is known concerning the modulation of transmitter release during coronary low flow.

It is well known that adenosine rapidly accumulates in the interstitial space in response to metabolic distress (Fredholm et al., 2001; Mubagwa and Flameng, 2001). Pharmacological blockade of presynaptic adenosine A\textsubscript{1}-receptors attenuates suppression of exocytotic NE release during low flow isch-

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ABBREVIATIONS: NE, norepinephrine; NET, neuronal monoamine transporter; K\textsubscript{ATP} channel, ATP-sensitive potassium channel; EMT, extraneuronal monoamine transporter; DPCPX, 8-cyclopentyl-1,3-dipropylxanthine; CCPA, 2-chloro-\textsuperscript{[\text{3}H]}-cyclopentyladenosine; \([\text{3}H]\)MPP\textsuperscript{+}, \(N\)-[methyl-\textsuperscript{3}H]4-phenylpyridinium; SUR, sulfonylurea receptor.
emia as well as reperfusion, whereas presynaptic α-adrenoceptors lose their autoinhibitory potential (Du and Riemersma, 1991; Burgdorf et al., 2001). In parallel with adenosine accumulation, breakdown of intracellular ATP triggers the opening of ATP-sensitive potassium (K\textsubscript{ATP}) channels in ischemic myocardium (Yokoshiki et al., 1998; Grover and Garlid, 2000). Because K\textsubscript{ATP} channels are present at noradrenergic neurons (Dunn-Meynell et al., 1997, 1998), it is conceivable that axonal hyperpolarization in response to K\textsubscript{ATP} channel opening modulates NE release. Indeed, pharmacological activation of K\textsubscript{ATP} channels has recently been shown to reduce the exocytotic release of NE in nonischemic atrial tissue of guinea pigs (Oe et al., 1999). However, NE overflow from the heart is not only determined by the release itself but also by the elimination of NE from the synaptic cleft. Recently, molecular identification of the extraneuronal monoamine transporter (EMT, uptake\textsubscript{2}) has been reported (Gründemann et al., 1998; Gründemann and Schömig, 2000). The characteristics distinguishing EMT from NET (uptake\textsubscript{1}) include the ability to transport NE in a Na\textsuperscript{+}- and Cl\textsuperscript{−}-independent manner, inhibition by corticosterone, as well as dependence on the membrane potential as the driving force (Kekuda et al., 1998; Wu et al., 1998). In vitro studies using clonal cancer cells of human renal tubules have documented that cell membrane hyperpolarization in response to K\textsubscript{ATP} channel opening enhances cellular catecholamine uptake via EMT (Schömig et al., 1992). From the latter findings, it is tempting to conclude that in addition to neuronal K\textsubscript{ATP} channels, extraneuronal K\textsubscript{ATP} channels may modulate sympathetic neurotransmission during ischemia via EMT. However, previous findings from isolated myocytes and intact rat hearts suggested that the EMT, at least in myocardial tissue, operates independently from K\textsubscript{ATP} channels, because neither K\textsubscript{ATP} channel opening nor blockade influenced cellular catecholamine uptake by EMT (Bryan-Llua and Vuocolo, 1993; Obst et al., 1996).

We therefore sought to evaluate the role of neuronal K\textsubscript{ATP} channels and of EMT on NE overflow during coronary low flow ischemia.

**Materials and Methods**

The present study has been carried out in accordance with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the U.S. National Institutes of Health.

**Heart Preparation.** Seven-week-old male Wistar rats (180–250 g body weight; Charles River, Sulzfeld, Germany) were anesthetized with 150 mg kg\textsuperscript{−1} thiopental sodium intraperitoneally. After medial laparotomy and injection of 500 IU kg\textsuperscript{−1} heparin sodium into the inferior vena cava, the thorax was opened and the heart rapidly removed and weighed. Isolated hearts were mounted in a Langendorff apparatus and were perfused via the ascending aorta with 95% O\textsubscript{2}, 5% CO\textsubscript{2} saturated Krebs-Henseleit solution (37°C, pH 7.4).

**NE Release.** Two 13 × 8 mm concavely shaped metal paddles were attached to the epicardium in opposite positions for electrical field stimulation (effective voltage 5 V, stimulation frequency 6 Hz, pulse width 2 ms). During the experiment, hearts were stimulated twice (1 min each). For determination of endogenous NE release, effluent was collected immediately before, during, and for 2 min after electrical stimulation. The exocytotic nature of stimulation-induced NE release has been documented previously (Seyfarth et al., 1993).

Each heart was equilibrated for 25 min at a constant flow of 8 ml min\textsuperscript{−1}. Unless otherwise stated, desipramine (0.1 μmol l\textsuperscript{−1}) was given to all hearts from the 11th min to prevent nonexocytotic NE release via the neuronal NET (Schömig, 1990). Subsequent to equilibration, hearts were subjected to low coronary flow (0.4 ml min\textsuperscript{−1}, i.e., 95% flow reduction). The first stimulation (baseline NE release) was carried out in the 20th min of equilibration. The second stimulation was performed in four different groups of hearts either in the final minute of equilibration or 5, 30, or 120 min after the onset of low flow (Fig. 1). Additionally, in a separate group with 120 min of low flow ischemia, the second stimulation was induced after 5 min of reperfusion at normal flow of 8 ml min\textsuperscript{−1}. In hearts with continuous perfusion at normal flow, electrical stimulations were evoked at corresponding times to maintain comparability to low flow ischemic perfusion protocols. A, low flow experiments. Baseline NE release (S\textsubscript{1}) was induced in the 20th min of equilibration (i.e., 5 min before low flow); the second stimulation (S\textsubscript{2}) was performed 5, 30, or 120 min after the onset of low flow. In a separate set with 120 min of low flow, the second stimulation was performed after 5 min of reperfusion. B, normal flow experiments. Electrical stimulations were carried out corresponding to protocol A. C, low flow with pharmacological K\textsubscript{ATP} channel blockade (glibenclamide), EMT blockade (corticosterone), or adenosine A\textsubscript{1}-receptor blockade (DPCPX). Drugs were given 2 min before flow reduction until the end of the experiment. S\textsubscript{1} was performed 5 min before low flow; S\textsubscript{2} was carried out either 5, 30, or 120 min after the onset of low flow. D, normal flow with 30 min of pharmacological intervention [glibenclamide, corticosterone, DPCPX, cromakalim (K\textsubscript{ATP} channel opener), diazoxide (K\textsubscript{ATP} channel opener), or CCPA (adenosine A\textsubscript{1}-receptor agonist)]. S\textsubscript{1} was induced 5 min before the addition of drugs; S\textsubscript{2} was carried out in the final minute of drug infusion. In three additional groups, cromakalim was given during the final 15 min of corticosterone or glibenclamide infusion and CCPA during the final 15 min of glibenclamide infusion (not shown). E, normal flow with 1.5 min [\textsuperscript{3}H]MPP\textsuperscript{+} labeling. Pharmacological interventions on EMT activity (cromakalim and corticosterone) were started 10 min after the beginning of [\textsuperscript{3}H]MPP\textsuperscript{+} labeling and were continued throughout the remaining experiment.
by the second stimulation (intervention) versus stimulation-induced NE overflow at baseline. Normalized data were expressed as NE overflow in percentage of baseline. $[^3H]$MPP$^+$ in each 30 s coronary venous fraction was normalized to the total myocardial $[^3H]$MPP$^+$ content present in the tissue when the sample was collected. The results are expressed as fractional $[^3H]$MPP$^+$ overflow.

All data are given as means ± S.E.M. of n experiments. Inner group comparison (NE overflow induced by the second stimulation versus stimulation-induced NE overflow at baseline) was assayed by two-tailed paired Student’s $t$ test. Comparisons between groups (normalized NE overflow, fractional $[^3H]$MPP$^+$ overflow) were assayed by one-way analysis of variance followed by Bonferroni’s post hoc test when three or more groups were compared. Two-tailed unpaired Student’s $t$ test was performed when two groups were compared. A value of $p < 0.05$ was considered statistically significant.

Results

In all preparations, spontaneous NE overflow before electrical stimulation was below the detection limit of 0.1 pmol g$^{-1}$. Furthermore, washout of NE after 5, 30, and 120 min of low flow (each group $n = 8$) remained below the detection limit.

Effects of Low and Normal Coronary Flow on Stimulation-Induced NE Overflow. Directly before flow reduction, stimulation-induced NE overflow did not differ from baseline (294 ± 42 versus 292 ± 40 pmol g$^{-1}$, $n = 8$). In contrast, NE overflow decreased by 37 ± 4 and 56 ± 4% within 5 and 30 min of low flow (5 min: 218 ± 15 versus 348 ± 28 pmol g$^{-1}$, $p < 0.01$, $n = 6$; 30 min: 117 ± 19 versus 260 ± 30 pmol g$^{-1}$, $p < 0.01$, $n = 8$) (Fig. 2). Inhibition of NE overflow remained constant for up to 120 min (115 ± 15 versus 264 ± 28 pmol g$^{-1}$, $p < 0.01$, $n = 8$). Five minutes of reperfusion at normal flow after 120-min ischemia restored NE overflow (263 ± 26 versus 297 ± 25 pmol g$^{-1}$, $n = 8$), suggesting a functional rather than structural alteration of sympathetic neurotransmission during low flow (Fig. 2). Corresponding periods of 5, 30, 120, and 125 min of normal perfusion did not affect NE overflow compared with baseline (5 min: 330 ± 24 versus 342 ± 30 pmol g$^{-1}$, $n = 4$; 30 min: 235 ± 13 versus 238 ± 12 pmol g$^{-1}$, $n = 8$; 120 min: 249 ± 17 versus 264 ± 24 pmol g$^{-1}$, $n = 8$; 125 min: 364 ± 37 versus 378 ± 44 pmol g$^{-1}$, $n = 4$) (Fig. 2).

In a separate set of experiments, NE overflow was determined in the absence of NET blockade by desipramine.
this setting, the absolute amounts of stimulation-induced NE overflow at baseline were 3-fold lower than those without NET blockade. Within 30 min of low flow, NE overflow decreased to 58 ± 6% of baseline overflow (53 ± 7 versus 91 ± 2 pmol g⁻¹, p < 0.01, n = 4), whereas the respective controls with 30 min of normal flow revealed no effect on NE overflow (87 ± 5 versus 94 ± 6 pmol g⁻¹, 93 ± 4%, n = 8). The inhibition of NE overflow at 30 min low flow did not differ significantly from the inhibition that was observed in the presence of NET blockade.

Role of K<sub>ATP</sub> Channels, EMT, and Adenosine A<sub>1</sub>-Receptors on Stimulation-Induced NE Overflow at Low and Normal Coronary Flow. NE overflow induced by the second stimulation (i.e., during pharmacological intervention) was normalized to the intraindividual NE overflow at baseline. Within the separate experimental groups, baseline NE overflow did not differ significantly and was comparable with that of untreated hearts subjected to either low flow or normal flow. Normalized data of untreated hearts served as control.

None of the drugs induced a detectable NE overflow before stimulation. Blockade of K<sub>ATP</sub> channels with glibenclamide (30 µmol l⁻¹) had no effect on NE overflow at 5 min of low flow (n = 8); however, glibenclamide increased NE overflow 1.9- and 1.5-fold at 30 and 120 min (each group n = 4) (Fig. 3). EMT blockade with corticosterone (30 µmol l⁻¹) elicited a 1.6-, 2.4-, and 2-fold increase of NE overflow after 5 min (n = 8), 30 min (n = 6), and 120 min (n = 4) of low flow. Likewise, adenosine A<sub>1</sub>-receptor blockade with DPCPX (1 µmol l⁻¹) significantly enhanced NE overflow after 30 min of low flow (n = 6) (Fig. 3). To assess whether the effect of EMT blockade on NE overflow was only operative in the presence of NET blockade by desipramine, additional experiments were performed without desipramine. After 30 min of low flow, corticosterone (30 µmol l⁻¹) markedly attenuated the suppression of NE overflow (86 ± 7% of baseline NE overflow, n = 8, p < 0.05 versus 58 ± 6% at 30 min of low flow without desipramine and corticosterone).

At normal coronary flow, neither K<sub>ATP</sub> channel blockade with glibenclamide (30 µmol l⁻¹, n = 6) nor EMT blockade with corticosterone (30 µmol l⁻¹, n = 4) had an effect on NE overflow (Fig. 4). Likewise, adenosine A<sub>1</sub>-receptor blockade with DPCPX (1 µmol l⁻¹, n = 6) did not affect NE overflow (108 ± 8% of baseline NE overflow). In contrast, when K<sub>ATP</sub> channels were opened with cromakalim (100 µmol l⁻¹, n = 4), NE overflow was slightly but significantly suppressed (Fig. 4). Interestingly, diazoxide (100 µmol l⁻¹, n = 6), a structurally different K<sub>ATP</sub> channel opener, did not reduce NE overflow (102 ± 4% of baseline NE overflow). The inhibitory effect of cromakalim remained evident in the presence of EMT blockade by corticosterone (30 µmol l⁻¹, n = 6). However, the suppressive effect of cromakalim was not found when K<sub>ATP</sub> channels were blocked with glibenclamide (30 µmol l⁻¹, n = 6) (Fig. 4). Because it is well known that adenosine A<sub>1</sub>-receptors activate K<sub>ATP</sub> channels (Fredholm et al., 2001; Mubagwa and Flameng, 2001), we also determined whether the inhibitory effect of CCPA (adenosine A<sub>1</sub>-receptor agonist) on NE overflow can be antagonized by K<sub>ATP</sub> channel blockade with glibenclamide (30 µmol l⁻¹). In hearts treated with CCPA (0.1 µmol l⁻¹, n = 4) exclusively, NE overflow decreased by 40 ± 6%; however, the suppressive effect of CCPA was still observed in the presence of glibenclamide (n = 4), suggesting that presynaptic adenosine A<sub>1</sub>-receptors modulate NE release independently of K<sub>ATP</sub> channels (Fig. 5).

Role of K<sub>ATP</sub> Channels and EMT on [³H]MPP⁺ Overflow. The modulatory effects of cromakalim (100 µmol l⁻¹) and corticosterone (30 µmol l⁻¹) on EMT activity was assessed at normal coronary flow by means of [³H]MPP⁺ overflow. The drugs were added to the perfusion buffer during steady-state conditions 8.5 min after termination of [³H]MPP⁺ loading. Control hearts did not receive drugs (each group n = 4).

During the loading period, a major fraction of [³H]MPP⁺ was not retained in the heart causing high fractional overflow (Fig. 6). Within 1.5 min after termination of labeling, [³H]MPP⁺ overflow decreased to ~8.5% in control-, cromakalim-, and corticosterone-treated hearts. In control hearts, fractional [³H]MPP⁺ overflow stabilized at a level of 6%. In contrast, [³H]MPP⁺ overflow decreased 2.5-fold 2 min after addition of corticosterone, an effect that persisted until the end of the experiment. K<sub>ATP</sub> channel opening with cromakalim had no modulatory effect on EMT activity, as indicated by an unaffected [³H]MPP⁺ overflow throughout the experiment (Fig. 6).

Discussion

The findings of the present study are as follows. 1) Prolonged periods of myocardial low flow ischemia resulted in a suppression of NE overflow, which is reversible during reperfusion. 2) K<sub>ATP</sub> channel blockade with glibenclamide as well as EMT blockade with corticosterone attenuated the suppression of NE overflow during low flow, whereas neither drug affected NE overflow during low flow, whereas both drugs affected NE overflow during low flow, whereas neither drug affected NE overflow during low flow, whereas both drugs...
overflow in the absence of flow reduction. 3) \( K_{\text{ATP}} \) channel opening with cromakalim decreased NE overflow at normal flow, both in the presence and absence of EMT blockade by corticosterone. 4) Cromakalim had no effect on EMT activity, as indicated by an unaffected [\(^3\)H]MPP\(^+\) overflow. Together, these findings indicate that endogenous activation of \( K_{\text{ATP}} \) channels and EMT mediate suppression of NE overflow during coronary low flow ischemia. Furthermore, the present findings suggest that \( K_{\text{ATP}} \) channels impair NE release directly at the presynaptic nerve ending, whereas EMT increases NE elimination from the synaptic cleft, independently from modulatory \( K_{\text{ATP}} \) channels. Finally, we confirmed previous data of Du and Riemsma (1991) that activation of presynaptic adenosine A\(_1\)-receptors contributes to the suppression of exocytotic NE release during low flow ischemia.

The results of the current study suggest that flow reduction impairs sympathetic neurotransmission as a result of metabolic alterations (\( K_{\text{ATP}} \) channel opening, adenosine A\(_1\)-receptor activation) that may affect both exocytotic catecholamine secretion and extraneuronal catecholamine elimination.

We could demonstrate that activation of EMT contributes importantly to the suppression of NE overflow. This effect occurs specifically in the condition of low flow ischemia because in isolated rat hearts subjected to either normoxic perfusion, hypoxic perfusion, or stopflow ischemia, pharmacological blockade of EMT had no effect on NE overflow (Schönig et al., 1984; Carlsson et al., 1986). However, our findings indicate that the EMT is selectively activated during moderate ischemia because corticosterone did not influence NE overflow in nonischemic conditions. Notably, previous studies using nonmyocardial cells and incubated segments from guinea pig trachealis muscle have documented that cell membrane hyperpolarization stimulates the EMT (Schönig et al., 1992; Bryan-Lluka and Vuocolo, 1993; Kekuda et al., 1998; Wu et al., 1998). \( K_{\text{ATP}} \) channels couple the membrane potential to the metabolic state of the cells (Yokoshiki et al., 1998; Grover and Garlid, 2000). \( K_{\text{ATP}} \) channels are closed during physiological conditions but open during the course of ischemia and thereby evoke hyperpolarization. Therefore, it was suggested that endogenous \( K_{\text{ATP}} \) channel opening during low flow ischemia activates the EMT, resulting in a suppression of NE overflow. Hence, to assess this hypothesis, we have determined whether cromakalim (\( K_{\text{ATP}} \) channel opener) would influence EMT activity. EMT activity was measured by means of fractional [\(^3\)H]MPP\(^+\) overflow. EMT transports the neurotoxin [\(^3\)H]MPP\(^+\) more efficiently than [\(^3\)H]NE as is evident from higher rate constants for inwardly and outwardly directed specific transport (Russ et al., 1992).
As expected, corticosterone decreased [3H]MPP+ overflow, indicating functional suppression of EMT, whereas cromakalim did not affect [3H]MPP+ overflow. However, the lack of effect of cromakalim on EMT activity is in accordance with previous findings from quiescent myocytes and isolated beating rat hearts, that neither membrane hyperpolarization nor depolarization is effective to modulate EMT in myocardial tissue (Bryan-Lluka and Vuocolo, 1993; Obst et al., 1996). Together with the observation that corticosterone increased NE overflow at 5 min of low flow ischemia before KATP channels are opened because glibenclamide had no effect (Fig. 3), our results suggest that activation of EMT during low flow is not induced by KATP channel opening. This contention is further substantiated by the finding that neither cromakalim nor glibenclamide influenced postsynaptic isoprenaline uptake in intact rat hearts (Bryan-Lluka and Vuocolo, 1993). However, the underlying endogenous stimulus for enhanced EMT activity during myocardial low flow ischemia remains unclear.

Previous investigations have focused primarily on the effects of KATP channel modulation on neurotransmitter release in the brain. Because ATP-sensitive K+ conductance is present in noradrenergic neurons (Dunn-Meynell et al., 1997, 1998), the possibility arises that KATP channel opening in response to ischemia influences NE release from the heart. In fact, we found that glibenclamide substantially increased NE overflow after 30 and 120 min of myocardial low flow ischemia, indicating endogenous activation of KATP channels. The lack of effect of glibenclamide at preserved coronary flow is in accordance with previous findings from quiescent myocytes and isolated transmural flow. Furthermore, heterogeneity of NE release during low flow, suggesting that the EMT was suppressed at ASPET Journals on July 12, 2017 Downloaded from jpet.aspetjournals.org

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heart. In addition to adenosine A<sub>1</sub>-receptor stimulation, endogenous activation of K<sub>ATP</sub> channels and of EMT mediates suppression of NE overflow during low coronary flow. K<sub>ATP</sub> channels modulate sympathetic neurotransmission primarily at the presynaptic nerve ending, most likely through inhibition of NE release. EMT influences sympathetic neurotransmission independently from K<sub>ATP</sub> channels at the postsynaptic membrane by increasing NE elimination from the synaptic cleft.

References


Address correspondence to: Christof Burgdorf, Institut für experimentelle und klinische Pharmakologie und Toxikologie, Universitätsklinikum Schleswig-Holstein, Campus Lübeck, Ratzeburger Allee 160, 23538 Lübeck, Germany. E-mail:chi柏rg@compuserve.de