Release of Endogenous Catecholamines in the Ischemic Myocardium of the Rat

Part B: Effect of Sympathetic Nerve Stimulation

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SUMMARY. The contribution of centrally originating sympathetic activity to the myocardial extracellular accumulation of noradrenaline during the early phase of ischemia has been assessed in a perfused (Langendorff) rat heart preparation isolated except for its sympathetic innervation. A 10-minute electrical stimulation (4 Hz, 5 V) of the left cervicothoracic ganglion during normal perfusion causes the overflow of 177.5 ± 13.7 pmol noradrenaline/g heart, whereas such stimulation during ischemia liberates only 21.5 ± 3.6 pmol/g (collected during reperfusion). When neuronal reuptake is blocked by desipramine, corresponding values are 321.5 ± 22.5 pmol/g (normal flow) and 151.8 ± 22.4 pmol/g (ischemia). After combined blockade of neuronal uptake, extraneuronal uptake, and α2-receptors, nerve stimulation liberates 674 ± 22 pmol/g during normal flow and 206 ± 24.3 pmol/g during ischemia. These results suggest that, in vivo, centrally originating neural activity would not lead to substantial accumulation of noradrenaline within the extracellular space of the ischemic myocardium. This failure of accumulation is due to both a functioning neuronal uptake of noradrenaline and a failure of neurotransmission.


MYOCARDIAL ischemia in the isolated rat heart produces overflow of noradrenaline even in the absence of a functioning sympathetic innervation (Schomig et al., 1984). However, such noradrenaline overflow is only minor during the first 10 minutes of ischemia. A disadvantage of the isolated rat heart as a model of ischemia is that it cannot reflect a neural contribution to intramyocardial noradrenaline release. Stimulation of sympathetic discharge has been demonstrated to be very potent in facilitating arrhythmias (Verrier et al., 1974; Kliks et al., 1975) in both the normal and the ischemic myocardium, and this may be particularly important during the early phase of ischemia (Schomig et al., 1984) when nonneural mechanisms alone do not produce significant noradrenaline overflow. Thus, in vivo, an enhanced cardiac sympathetic activity (Schwartz and Stone, 1980) might be expected to play a role in modulating locally mediated noradrenaline release. The contribution of sympathetic activation has been studied in further experiments in which sympathetic nerve stimulation has been performed during the first 20 minutes of ischemia.

Methods

Rats (150–200 g) were anesthetized with thiobutabarbital (50 mg/kg, ip). The thorax was opened and a cannula inserted and tied into the ascending aorta for retrograde coronary perfusion (Langendorff technique). Hearts were perfused at 4 ml/min per g with a modified Krebs-Henseleit solution (Dart et al., 1984) gassed with oxygen and with pH adjusted to 7.4 with CO2. Temperature at the point of entry into the aorta was 37.5°C. A polyethylene cannula was introduced into the heart through the inferior vena cava for collection of coronary venous effluent. The completeness of perfusate recovery then was assessed, and experiments in which this failed to exceed 85% were rejected. The left cervicothoracic ganglion and exiting cardiac nerves then were dissected free for subsequent electrical stimulation. Stimulation was performed with bipolar platinum electrodes with a stimulation pulse width of 2 msec and frequency of 4 Hz. Stimulation voltage was 5 V. When not being stimulated, the ganglion and nerve were superfused with warmed Krebs-Henseleit solution. During periods of myocardial ischemia, the hearts were superfused with warmed paraffin and the intramyocardial temperature was monitored with a fine temperature-sensitive probe. Intramyocardial temperature was maintained in the range 36–38°C. Hearts were weighed at the end of each experiment. Samples for subsequent noradrenaline estimation were put on ice, immediately mixed, and stabilized by 1:1 addition of perchloric acid. Samples were stored at -80°C until subsequently assayed. All samples in these experiments were assayed by a radioenzymatic method (Da Prada and Zurcher, 1976). The pharmacological agents used in this study (desipramine, yohimbine, corticosterone) were all dissolved in ethanol, and final concentrations of ethanol were in all cases less than 0.05%.

Statistical evaluation for nonpaired data was done by analysis of variance and for paired data by the paired t-test. Results are expressed as means ± SEM.

Results

The effect of a 10-minute stimulation of the left cervicothoracic ganglion on noradrenaline overflow during normal flow is shown in Figure 1. In the
overflow of noradrenaline into the coronary venous effluent during a continuous 10-minute period of electrical stimulation (4 Hz, 5 V) of the left cervicothoracic ganglion. During all periods of stimulation, the noradrenaline concentration in the effluent was significantly higher (P < 0.05) than during the pre- and poststimulation period. Mean values ± SEM; n = 6.

In the next series of experiments, a 10-minute stimulation was performed during complete stop flow ischemia, and the coronary venous effluent was collected, in aliquots, up to 10 minutes after the end of the ischemic period. Under these circumstances, only 21.5 ± 3.6 pmol/g heart was recovered (Fig. 2). When such stimulation was performed in further experiments in the presence of desipramine (100 nm), the total noradrenaline output was 321.5 ± 22.5 pmol/g heart (Fig. 2). In further experiments, stimulation was performed in the presence of desipramine (100 nm), yohimbine (1 µM), and corticosterone (30 µM) in order to block neuronal uptake (Titus and Spiegel, 1962; Koe, 1976), α2-receptor-mediated presynaptic inhibition (Starke et al., 1975), and extraneuronal uptake (Iversen and Salt, 1970), respectively. Under these conditions, the noradrenaline overflow was 674 ± 22 pmol/g heart (Fig. 2).

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Stimulation for 10 minutes during ischemia in the presence of desipramine (100 nm), yohimbine (1 µM), and corticosterone (30 µM) yielded 206 ± 24.3 pmol/g heart noradrenaline (Fig. 2). This was significantly less (P < 0.05) than that obtained with such stimulation under conditions of normal flow, and not significantly different from the results obtained with desipramine alone.

The results of 1-minute stimulation periods are shown in Figures 3 and 4. The reproducibility of repeated 1-minute stimulations has been previously documented for postganglionic nerve stimulation (Dart et al., 1983), and similar reproducibility was found with ganglion stimulation. Figure 3 shows the overflow of noradrenaline produced by 1-minute stimulation during normal flow, compared, in the same experiments, with that produced by stimulation during ischemia. In these experiments, samples were collected during stimulation (for normal flow) and during the first 4 minutes poststimulation. Stimulation during ischemia yielded significantly less (P < 0.05) noradrenaline than identical stimulation during normal flow, both during stimulation at 0 to 1 minute of ischemia and during stimulation at 9 to 10 minutes of ischemia. In additional experiments, desipramine (100 nm) abolished the differences between ischemic and normal flow stimulation for
ischemic stimulation between 0 and 1 minute. For stimulation between 9 and 10 minutes of ischemia, however, the addition of desipramine was only partly effective in restoring the difference between ischemic and nonischemic stimulation. Under these circumstances, the difference between normal flow and ischemic stimulation remained statistically significant (P < 0.05).

The efficacy of sympathetic stimulation between 19 and 20 minutes of ischemia was assessed with and without neuronal uptake blockade with 100 nM desipramine. In the absence of uptake blockade, the spontaneous overflow of noradrenaline and its variability was too great to allow detection of an additional stimulation induced release. As shown in Figure 4, even with uptake blockade, nerve stimulation did not result in a statistically significant further increase in noradrenaline overflow in comparison to 20 minutes of ischemia without nerve stimulation (26.9 ± 3.4 vs. 20.6 ± 1.8 nmol/g).

**Discussion**

The results presented in these experiments show that sympathetic nerve stimulation during a 10-minute period of total ischemia does not lead to a substantial trapping of noradrenaline within the extracellular space of the myocardium, since any such noradrenaline would be expected to be washed out during reperfusion. The failure of sympathetic nerve stimulation to produce noradrenaline trapping within the extracellular space of the ischemic myocardium could be explained in two ways: a relative failure of release from the nerve terminals (due to impaired neurotransmission or autoinhibition), or an enhanced removal (by uptake and metabolism) of released noradrenaline. Previous experiments with this preparation have shown the effects of blockade of neuronal uptake and autoinhibition during normal flow. Under these conditions, uptake blockade alone increased the noradrenaline overflow by 60% (Dart et al., 1983) and yohimbine (α2-blockade) alone resulted in a 2-fold increase in noradrenaline overflow (Dart et al., 1984). A combina-
tion of yohimbine and desipramine increased the overflow of noradrenaline by more than 3-fold (Dart et al., 1984).

In these experiments, a combined blockade of neuronal uptake, extraneuronal uptake and presynaptic $\alpha_2$-receptors more than tripled the noradrenaline overflow produced by nerve stimulation during normal flow. However, even with this combined blockade nerve stimulation during ischemia yielded only one-third as much noradrenaline as during normal flow conditions, indicating the importance of an additional factor. A possible explanation for the difference between ischemic and nonischemic stimulation under these circumstances is that, during ischemia, there is a partial failure of neural transmission through the ischemic myocardium or a failure of transmitter exocytosis which is ATP dependent (Baker and Knight, 1978). Such neurotransmission failure may increase with time, since a 1-minute stimulation, in the presence of neuronal uptake blockade, at the end of a 10-minute ischemic period still produced a substantially smaller overflow than identical stimulation during normal flow, whereas, during a 1-minute ischemic period, the difference between ischemic and normal flow stimulation was abolished in the presence of neuronal uptake blockade. In addition, stimulation between 19 and 20 minutes of ischemia failed to produce an additional release of noradrenaline from the ischemic myocardium.

Evidence that neuronal uptake is partly responsible for the failure of stimulation during ischemia to lead to extracellular accumulation of noradrenaline is provided by experiments in which neuronal uptake is blocked with desipramine. As already discussed, this factor was more important in preventing noradrenaline accumulation during very early ischemia. The difference between a nonischemic and an ischemic stimulation was abolished by desipramine, suggesting that at this time neuronal uptake was of major importance. Although, even during neuronal uptake blockade, the noradrenaline overflow produced by 10 minutes of stimulation during ischemia was significantly less than during normal flow, it was nonetheless considerably greater (7x) than that obtained by stimulation during ischemia in the absence of neuronal uptake blockade. Stimulation of 10 minutes during ischemia in the presence of a combined blockade of neuronal and extraneuronal uptake and autoinhibition yielded more noradrenaline than with neuronal uptake blockade alone. However, the difference was small, and not significant, suggesting that extraneuronal (i.e., corticosterone blockable) uptake and autoinhibition are not of major importance in explaining the difference between ischemic and nonischemic stimulation. However, in a recent study in dogs (Forfar et al., 1983), yohimbine produced a greater reduction in blood flow to ischemic territory, compared to normal myocardium. The effect of yohimbine in modulating the noradrenaline released by stellate ganglion stimulation was also more marked in the ischemic as compared to the normal myocardium.

Sympathetic stimulation during the early phase of myocardial ischemia does not, therefore, give rise to an accumulation of noradrenaline within the extracellular space of the myocardium. To a large extent, this failure of noradrenaline trapping is explained by the activity of the neuronal reuptake process. Information about the continuing activity of neuronal reuptake at later stages of ischemia is not available from these experiments, but since at later stages neurotransmission within the myocardium is impaired, subsequent neural stimulation (i.e., after 20 minutes of ischemia) does not lead to an additional extracellular noradrenaline accumulation. In contrast to neurally induced release, locally mediated release of noradrenaline plays a progressively important role, and after 30 minutes of ischemia, an extracellular noradrenaline concentration in excess of 1 $\mu$g is likely (Schomig et al., 1984). Local factors can therefore induce the release of toxic amounts of noradrenaline from the sympathetic nerve terminals (Waldenström et al., 1978).

Studies involving the collection and determination of noradrenaline overflow from the heart do not, of course, give direct information concerning the moment-to-moment changes in concentration of transmitter at myocardial receptor sites. In the studies presented here, measurements of postsynaptic activity were not made, so that it is not possible to compare the results directly with those of in vivo experiments in which stimulation of the sympathetic nervous system has been shown to be detrimental with respect to arrhythmias (Verrier et al., 1974; Kliks et al., 1975). Chronic cardiac denervation has been shown to be protective against the development of arrhythmias during subsequent experimental coronary occlusion (Schaal et al., 1969; Ebert et al., 1970). Acute denervation is, however, less effective in this regard (Ebert et al., 1970), suggesting that locally mediated release of noradrenaline from nerve terminals may be of more importance than centrally originating sympathetic impulses, even though the presence of early cardiac sympathetic activity has been demonstrated during ischemia (Malliani et al., 1969).

In conclusion, these studies provide no evidence to support an important role of sympathetic activation in causing extracellular accumulation of noradrenaline within the ischemic myocardium. This potential accumulation of noradrenaline is limited both by the neuronal uptake process and by failure of neurotransmission. In contrast, local factors play an increasingly important role in releasing noradrenaline during the course of ischemia.

This work was supported by the Deutsche Forschungsgemeinschaft (SFB 90 - Cardiovacular System).
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Received March 5, 1984; accepted for publication August 14, 1984.

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INDEX TERMS: Sympathetic stimulation • Catecholamine release • Noradrenaline uptake • Myocardial ischemia • Isolated rat heart
Release of endogenous catecholamines in the ischemic myocardium of the rat. Part B: Effect of sympathetic nerve stimulation.
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doi: 10.1161/01.RES.55.5.702

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