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C. Wicher, G.-A. Biewald

Chronic treatment with doxorubicin (DXR) or other anthracyclines causes impairments in myocardial function and, eventually, results in dilatative cardiomyopathy (DCM). It is also associated with changes in the action of the renin-angiotensin II (AII) system and AII receptor density. Aim of the present study was to investigate effects of DXR perfusion (up to 60 min) of isolated, vagally innervated rat hearts on vagus effects regarding cardiac activity and possible changes by DXR in the modulation of vagus effects by angiotensin II (AII), for comparison with known chronic anthracycline effects. Electrical (surface ECG) and mechanical (left-ventricular pressure, LVP) cardiac activity were recorded. Vagus stimulation (10 Hz, 10 V) caused prolonged ECG intervals, reduced heart rate (HR) and negative inotropic effects (reduced left-ventricular systolic pressure P_s , decelerated contraction). Perfusion with DXR-containing Tyrode (10–40 $\mu\text{mol/L}$ DXR) caused dose-dependent and progressive functional impairments, similar to those known from long-term anthracycline treatment in humans: prolonged ECG intervals, decreased HR, reduced P_s and a prolonged contraction period. The effects of heart vagus stimulation on ECG and LVP were reduced during DXR perfusion.

However, DXR perfusion did not affect the positive chronotropic and inotropic effects of AII (1 $\mu\text{mol/L}$, applied subsequently to DXR) as well as the modulatory function of AII on cardiac vagus actions: AII blocking effects were comparable to control. These results are at some variance with clinical findings in long-term anthracycline treatment. It is concluded that short-time DXR administration was not enough for establishing changes in the AII system observed during long-term treatment. *J Clin Basic Cardiol* 1999; 2: 259–66.

Key words: doxorubicin, myocardial function, ECG, vagus stimulation, angiotensin II

Doxorubicin (DXR = adriamycin, a glycoside antibiotic originally isolated from the fungus *Streptomyces peucetius* var. *caesius* [1]) was clinically used as an antitumoral antibiotic in the treatment of leukaemias, lymphomas and some solid tumors [2]. However, long-term administration of DXR might cause cardiac functional alterations, such as sinus bradycardia, prolonged atrioventricular conduction and, consequently, reduced heart rate [1]. Chronic treatment of rats with DXR caused ECG alterations, including widening of the QRS complex and elongated P-Q intervals, the appearance of distinct S-wave through and occasionally with an increase or flattening of the T wave [2]. Frequently a development of a myocarditis was observed during DXR therapy with clinical manifestations like a dilated and poorly contracting heart [3, 4].

Three intracellular mechanisms of action are ascribed to anthracyclines: interactions with DNA synthesis, binding to cell membranes (altering the membrane functions and intracellular Na^+ or Ca^{2+} concentrations) and stimulation of lipid peroxidation to form oxygen radicals [1]. Especially the effects of DXR on intracellular calcium homeostasis seemed to be associated with the development of chronic cardiomyopathy [1]. DXR causes alterations in mitochondrial Ca^{2+} transport in the heart ranging from inhibition of Ca^{2+} uptake by mitochondria upon exposure to DXR *in vitro* [3] to stimulation of mitochondrial Ca^{2+} uptake in rabbits chronically intoxicated with DXR [5]. DXR inhibits the $\text{Na}^+/\text{Ca}^{2+}$ exchanger [6], the oxygen consumption and the ATP production of mitochondria in an *in vitro* rat heart preparation [7].

In sarcoplasmic reticulum vesicles of rabbit cardiac muscle, DXR (2.5–10 $\mu\text{mol/L}$) had a biphasic effect on calcium release channels (ryanodine receptor channels, [8]): initially, DXR activated this channel, then, after a few minutes, the channel was irreversibly inhibited. This biphasic effect on channel function may correspond to the clinically observed

adverse effects of DXR, which causes a dilated cardiomyopathy after prolonged usage [9]. Combination of DXR and caffeine therapy enhances DXR antitumor activity in terms of prolonging survival in carcinoma-bearing mice [10] and men [11], possibly *via* inhibition of opening of IP_3 -dependent calcium channel and/or enhancing of opening of ryanodine receptors by caffeine [12].

Also, morphological changes by DXR of cardiomyocytes were described: electron micrographs of the myocardium of DXR-treated mice showed narrowing of myofibrils, oedematous cytoplasm, and mitochondrial swelling. Light microscopic examination revealed that intracellular spaces between myocardial cells were widened indicating shrinking of myocardial cells [13].

Concerning the dramatic effects of DXR on cellular mechanisms it should be expected that DXR might also affect modulatory influences on cardiac functions. One important modulator of cardiac and circulatory functions is angiotensin II (AII). In isolated hearts, AII evokes positive chronotropic and inotropic effects *via* activation of cardiac AII receptors [14] which belong to the AT1 subtype [15–17]. Under *in vivo* conditions, AII increases cardiac contractility by activation of the sympathetic nervous subsystem [18]. AII also affects the antagonistic interaction of the sympathetic and parasympathetic nervous subsystems: AII dose-dependently inhibits the effects of vagus stimulation on heart rate and cardiac contractility [19].

Chronic dilated cardiomyopathy (DCM), which can be induced by long-term DXR treatment, causes an up-regulation of the α - and β -adrenergic system as well as of the renin-angiotensin system [20, 21] whereas the angiotensin receptor density in peripheral organs becomes reduced [22]. Consequently, in patients with heart failure [23] and dilated cardiomyopathy [24], sympathetic tone was increased while the parasympathetic tone was reduced, eventually resulting in a

shift of the sympatho-vagal balance towards the sympathetic side. On the other hand, a generally reduced autonomic nervous activity is reported for DCM patients that could contribute to the observed cardiac desensitization [25].

This study investigates time- and concentration-dependent effects of short-time DXR perfusion on electrical (surface ECG) and mechanical (left-ventricular pressure) cardiac activity as well as on its modification by vagus stimulation and angiotensin II in isolated rat hearts (modified Langendorff preparation). Observed alterations in cardiac activity during DXR perfusion are discussed and compared with those observed during the pathogenesis of the DCM (chronic anthracycline treatment).

Materials and Methods

The experiments were performed on adult female HanWistar rats (200–250 g body weight, Institute for Pharmacology and Toxicology, Friedrich Schiller University Jena). The rats were anaesthetized by combinatory injection containing ketamine and rompun: 1 mg ketamine hydrochloride/kg body weight (Mallinckrodt Vet, Burgwedel, Germany) and 0.2 mg rompun/kg body weight (rompun = 2-(2,6-Xylidino)-5,6-dihydro-4 H-1,3-thiazine-hydrochloride, Bayer Leverkusen, Germany), whereby about 50 per cent of the bolus was i.m. injected into each hindlimb of awake rats. About 5 minutes after injection, the right *N. vagus* was cut in the mid-neck region, and the distal trunk was carefully dissected free using fine forceps. The vagus was kept moist using isotonic saline solution. Surgery had a duration of 10 minutes in maximum. The rats were killed by cervical dislocation. The heart with intact right vagus trunk was quickly removed and immersed in cold (4 °C) Tyrode solution (in mmol/L: 150 NaCl, 5.4 KCl, 2.5 CaCl₂, 0.5 MgSO₄, 11.1 glucose, 5 HEPES, pH = 7.4). The aorta was cannulated and the heart was mounted in a perfusion apparatus (Langendorff preparation). The heart was retrogradely perfused through the aorta at a constant perfusion pressure of 40 cm H₂O. After washing the heart by perfusion with oxygenated Tyrode (37 °C) for 10 min, the heart was perfused by recirculating Tyrode solution. The hearts were allowed to beat spontaneously.

After a 30 min stabilization period, the distal end of the right cardiac *N. vagus* was positioned on a platinum electrode which was connected with an electrical stimulator (Technical and Scientific Equipment, Bad Homburg, Germany). In each stimulation period, a 10 s impulse train of square pulses (5 ms single pulse duration, stimulation voltage: 10 V, stimulation frequency: 10 Hz) was applied on vagus nerve (control recording contains 3 such stimulation periods, with temporal distances each of 1 minute at least). In our experiments, we stimulated only the right vagus trunk, because it is known that the cardiac innervation of the right vagus is generally stronger than that of the left one [26, 27]. Right vagus stimulation effects on cardiac actions were indeed stronger than effects by left vagus stimulation [19]. The vagus nerve was stimulated by a constant frequency of 10 Hz which corresponds to physiological conditions of *N. vagus* [28].

Experimental protocol

In 15 experiments, 30 min after preparation of the heart, a control recording of LVP and ECG (see above) were performed. Then doxorubicin (DXR, CALBIOCHEM NOVA-BIOCHEM, La Jolla, USA, tested dose range 10–40 µmol/L, one dose per experiment, 5 rats per DXR dose) was administered to the Tyrode solution. Recording was repeated up to the individual end of spontaneous heart beating period, once

per 5 min. In dependence on applicated DXR dose the isolated hearts beat after DXR application for up to 60 min (10 µmol/L DXR) and 35 min (40 µmol/L DXR), respectively.

In another 15 experiments, 30 min after the end of heart preparation, 3 control vagus stimulations (right *N. vagus*, stimulation parameters as described above) were performed (once per minute). Subsequently DXR was added (10–40 µmol/L, one dose per rat). After a 4 min perfusion period, vagus stimulations were repeated every 5 min up to the individual end of spontaneous beating of the heart.

In 5 separate experiments, after control stimulation, 10 µmol/L dantrolene (dantrolene sodium, Research Biochemicals International, Natick, USA, an inhibitor of the intracellular ryanodine receptor which causes a blockade of calcium-induced calcium release from sarcoplasmic reticulum [29, 30]), and 5 minutes later 20 µmol/L DXR were administered to Tyrode solution.

In 5 other experiments, 10 mmol/L caffeine (caffeine 1,3,7-trimethylxanthine, Research Biochemicals International, Natick, USA, a stimulator of the ryanodine receptor which causes an opening of ryanodine receptor channels [12, 31, 32]), and 5 min later 20 µmol/L DXR were added to the perfusion solution. Vagus stimulations were repeated every 10 min starting after addition of the drugs up to the individual end of spontaneous beating of the heart.

In 5 separate experiments, 20 min after addition of 20 µmol/L DXR, 1 µmol/L angiotensin II (AII, BACHEM Heidelberg, Germany) was administered to perfusion solution. After 4 min perfusion, next vagus stimulation was performed, stimulations were repeated all 5 min up to the individual end of spontaneous heart beating.

Parameters and data analysis

The time course of left-ventricular pressure (LVP) was recorded *via* pressure transducer (pressure evaluation unit, FMI, Seeheim, Germany), amplified (amplifier, FMI, Seeheim, Germany) and recorded by TIDA software (HEKA Elektronik, Lambrecht, Germany). Systolic pressure (P_S) was calculated from LVP. Time courses of contraction and relaxation periods of LVP were described by single exponential functions (time constants: τ_C for contraction, and τ_R for relaxation). ECG was recorded directly from the cardiac surface *via* surface electrodes which were positioned near the heart base and the apex. From ECG recording, the PQ, QRS, SαT and QT intervals were measured similarly as described in [34]. In our analysis, the isoelectric point was determined by taking the average of 9 voltage values directly before the Q wave. The Q wave was taken as the point where the isoelectric line intercepts an interpolation of the upstroke of the R wave. The S wave was taken as a clear minimum after the R wave. The apex of the T wave (αT) was taken as the clear maximal point after the S wave. The QRS (from the apex of the Q wave to the apex of the S wave) and SαT (from the apex of the S wave to the apex of the T wave) intervals were calculated from these data. The P, Q, R, S and T wave voltages (mV) were measured by determination of the averaged signal from the isoelectric baseline. The heart rate was calculated from R-R interval. All measurements were made on tracings of 10 consecutive cardiac cycles.

Direct doxorubicin-induced changes in the electrocardiographic intervals were determined from individual rats as changes from the pretreatment values. Vagus stimulation effects (taken from the end of each stimulation period) as well as angiotensin II-induced modulation of vagus stimulation effects were determined as changes from pre-stimulation values (control values) and from vagus stimulation-induced values before angiotensin II addition, respectively.

Pre- and posttreatment figures were compared to each other by ANOVA (for one factor, combined with Dunnett's post test, PRISM, GraphPad Software, San Diego, USA). All data are means $\pm$ standard errors of the mean (SEM) of independent experiments.

Results

Control recording of LVP and ECG and vagus stimulation

In isolated hearts, a spontaneous heart rate of $184 \pm 47/\text{min}$ was recorded (Table 1). The systolic pressure P_S amounted to 67.5 ± 24.2 mmHg. Fitting single exponential functions to the time courses of contraction and relaxation revealed a time constant of contraction τ_C of 11.9 ± 5.1 ms and a time constant of relaxation τ_R of 31.4 ± 6.8 ms. The time parameters of the ECG were: PQ time: 51.7 ± 5.0 ms, QRS time: 11.9 ± 3.6 ms, S α T time: 15.6 ± 3.2 ms (QT interval: 27.5 ± 3.4 ms). As a characteristic ECG amplitude the R-S amplitude was recorded after 10-fold magnification: 3.18 ± 0.79 V. Because of the strong dependency of ECG amplitudes on the momentary position of ECG electrodes on the cardiac surface, the other ECG peak amplitudes had great deviations and were therefore not considered. For comparison control figures of ECG and LVP parameters recorded on isolated, vagally innervated rat hearts and in anaesthetized rats [33] are given in Table 1.

Stimulation of the right vagus (square pulse trains, 10 Hz, 10 V, 10 s stimulation period) caused reductions in heart rate by about 52 ± 14 % and in left-ventricular pressure amplitude (P_S) by about 48 ± 18 %. The time constant of contraction (τ_C) was prolonged by about 34 ± 11 %. In the ECG, there were significant prolongations of the S α T interval (83 ± 18 %) during vagus stimulation, but only slight prolongations in the PQ and QRS intervals (by about 5 % each, ECG changes not shown). Vagus stimulation effects during Tyrode perfusion were set to 100 % in each experiment.

AII modulation (under physiological conditions)

In Figure 1 original registrations of LVP in isolated heart and effects of vagus stimulation on LVP before and after addition of $2 \mu\text{mol/L}$ AII to Tyrode (without DXR) are shown (physiological AII effect).

In this experiment, AII caused an increase in heart rate by about 32 % (from 217/min to 287/min, positive chronotropic effect of AII), an increase in the left-ventricular systolic pres-

sure (from 66 to 84 mm Hg, increase of 40 %) and a decrease in τ_C (from 13.7 to 8.6 ms, decrease by about 37 %, positive inotropic effects of AII). The S α T interval of ECG was reduced by about 20 % (not shown).

AII also caused a nearly complete inhibition of vagus stimulation effects (Figure 1): vagus stimulation after AII addition caused only a slight reduction in heart rate and a slight increase in τ_C (by about 5 % each), and it had nearly no effects on systolic pressure (Figure 1) and on S α T interval of ECG (ECG effects not shown).

DXR effects on ECG and LVP

Depending on the DXR concentration in the Tyrode solution, the isolated hearts were still beating spontaneously for up to 60 min after addition of $10 \mu\text{mol/L}$ and for up to 35 min after addition of $40 \mu\text{mol/L}$ DXR. Within this time, progressive impairments of ventricular function were observed, such as reductions in HR, prolonged ECG times (QRS time and S α T time), decreased systolic pressure P_S and increased τ_C . Nevertheless, τ_R remained nearly unchanged during DXR perfusion. An example of single cycles of original two-chan-

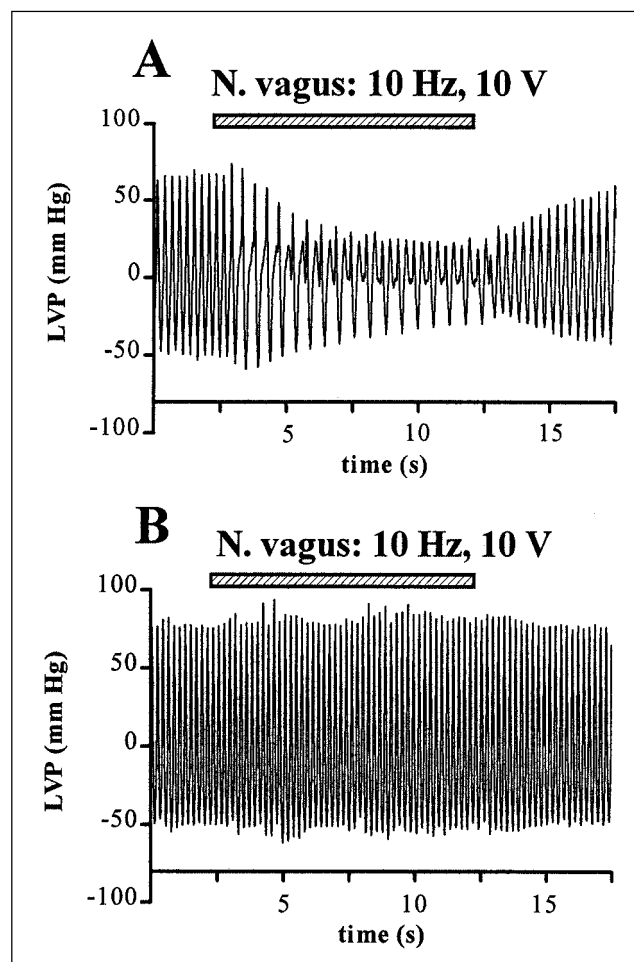


Figure 1. Effects of angiotensin II on time course of left-ventricular pressure (LVP) and on vagus stimulation effect, recorded in isolated rat hearts (modified Langendorff preparation). Time periods of vagus stimulation (right N. vagus, 10 Hz, 10 V, 10 s stimulation period) are marked by horizontal bars. **A:** control recording before AII application, **B:** recording 5 min after addition of $2 \mu\text{mol/L}$ AII to perfusion solution. Note clear reductions in heart rate and systolic pressure caused by vagus stimulation during Tyrode perfusion (**A**). AII had direct positive chronotropic and inotropic effects and inhibited the vagus stimulation effects (**B**).

Table 1. Control figures of ECG and LVP parameters recorded on isolated, vagally innervated rat hearts. Means and SD from 15 experiments. For comparison, control figures of ECG parameters and heart rate recorded in anaesthetized rats [33] are given. Note, that no considerable differences occur between ECG parameters recorded in isolated heart and anaesthetized rat. Heart rate is significantly smaller in isolated hearts ($P < 0.05$).

	Isolated rat heart	Anaesthetized rat (literature)
ECG		
QRS time	11.9 ± 3.6 ms	11.7 ± 6.2 ms
S α T time	15.6 ± 3.2 ms	16.5 ± 6.0 ms
QT interval	27.5 ± 3.4 ms	28.2 ± 4.0 ms
PQ time	51.7 ± 5.0 ms	46.4 ± 6.2 ms
RS amplitude	3.18 ± 0.79 V	
LVP		
Systolic pressure P_S	67.5 ± 24.2 mmHg	
Time constant of contraction τ_C (exponential fit)	11.9 ± 5.1 ms	
Heart Rate	$184 \pm 47/\text{min}$	$305 \pm 16/\text{min}$

nel recordings of ECG and LVP from isolated rat heart before and 30 min after addition of 20 $\mu\text{mol/L}$ DXR to Tyrode is given in Figure 2. Reduced left-ventricular pressure amplitude and slower increase in pressure during contraction phase are evident.

These changes were stronger after addition of 40 $\mu\text{mol/L}$ DXR. The dose-dependence of changes in HR, $S_{\alpha T}$ and τ_C are shown in Figure 3. In this figure changes in $S_{\alpha T}$ and τ_C are given without a correction for frequency-dependent changes. Values of all parameters recorded in control stimulation (Tyrode perfusion) were set to 100 %. Significant changes ($P < 0.05$) in the above parameters occurred after perfusion times of 10 min or more with at least 20 $\mu\text{mol/L}$ DXR in Tyrode. As an example, after 30 min perfusion with 20 $\mu\text{mol/L}$ DXR the heart rate was reduced to $63 \pm 9\%$ of control, the $S_{\alpha T}$ interval of ECG was increased by $81 \pm 30\%$ of control and the time constant τ_C was increased by $65 \pm 20\%$ of control.

DXR effects on vagus stimulation and AII modulation

An example of original time course of left-ventricular pressure (left column: without vagus stimulation) as well as of effects of vagus stimulation on left-ventricular pressure (right column: vagus stimulation effect) in isolated rat heart (modified Langendorff preparation) is given in Figure 4.

During DXR perfusion, P_S and HR as well as the effects of vagus stimulation on ECG and LVP parameters were strongly

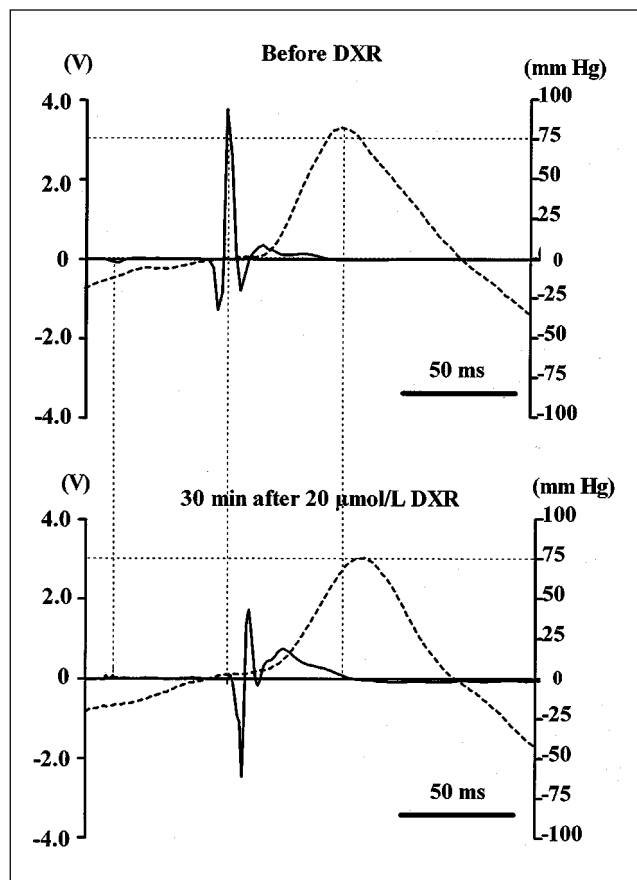


Figure 2. Superimposed single time courses of left-ventricular pressure (LVP, dashed line) and electrocardiogram (ECG, solid line), recorded in isolated rat hearts before (above) and 30 min after addition of 20 $\mu\text{mol/L}$ doxorubicin (DXR, below) to Tyrode solution. P wave was chosen as common origin of the curves. Note that DXR induces a slowing in contraction phase and increases in ECG intervals (PQ and $S_{\alpha T}$).

decreased (for example see the second line of curves in Figure 4, LVP was recorded after 24 min perfusion with 20 $\mu\text{mol/L}$ DXR-containing Tyrode: "24 min DXR").

AII was administered 25 min after heart perfusion with DXR-containing Tyrode. Also in this perfusion solution, AII had positive chronotropic and inotropic effects (third line of figures: "31 min DXR, 5 min AII", left part) and the effects of vagus stimulation on heart rate and LVP were further reduced after addition of 1 $\mu\text{mol/L}$ AII to the DXR-containing perfusion solution (third line of figures, right part). Therefore, modulatory action of AII regarding vagus stimulation effects was not affected by DXR in isolated rat heart. At the end of experiment (last line in Figure 4), the heart beat irregularly and feebly, and the vagus stimulation effects had disappeared.

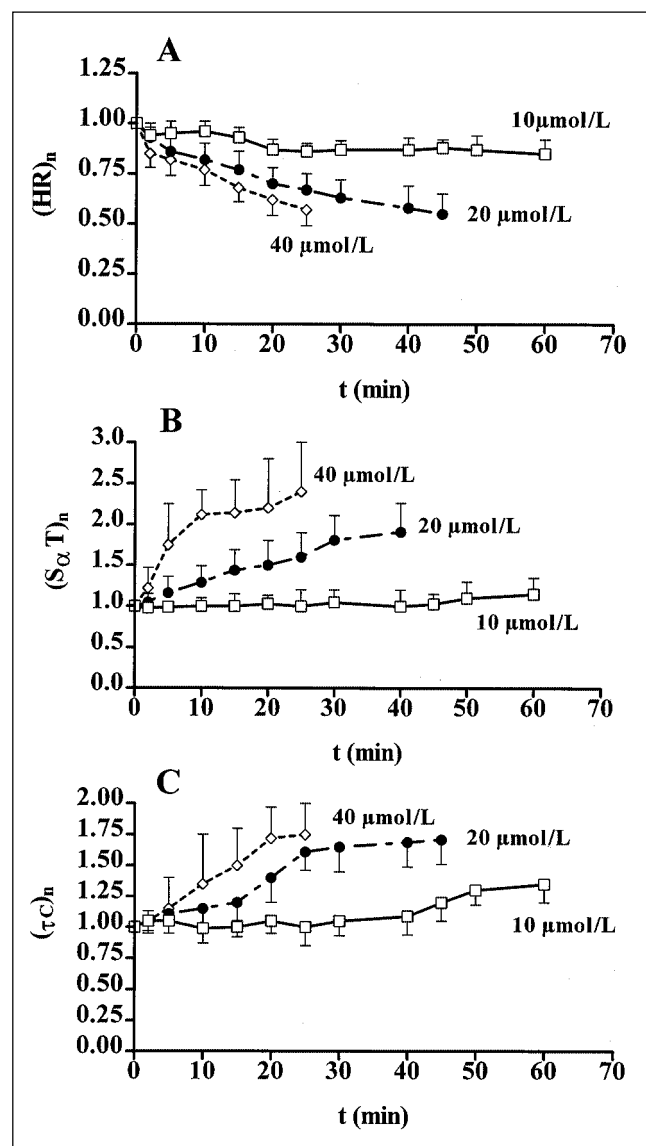


Figure 3. Normalized changes in heart rate (HR, A), $S_{\alpha T}$ (B) time of the ECG and time constant of left-ventricular contraction τ_C (C) during perfusion of isolated heart with doxorubicin (DXR, 10–40 $\mu\text{mol/L}$)-containing Tyrode (parameters before DXR addition were set to 1). 10 $\mu\text{mol/L}$ DXR: solid line and open squares, 20 $\mu\text{mol/L}$ DXR: dash-dotted line and filled circles, 40 $\mu\text{mol/L}$ DXR: dashed line and open rhombs. Means and SD of 5 experiments. Note that DXR perfusion caused reductions in heart rate by up to 50 %, and increases in $S_{\alpha T}$ by up to 150 %, and increase in τ_C by up to 75 % (after 25 minutes perfusion with 40 $\mu\text{mol/L}$ DXR-containing Tyrode).

The time dependence of DXR perfusion on vagus stimulation effects on HR, S α T and τ_C are shown in Figure 5: Higher DXR concentrations as well as longer perfusion duration caused smaller vagus stimulation effects. As an example, after 30 min perfusion with 20 μ mol/L DXR in Tyrode (see "end of experiment" in Figure 5), chronotropic vagus effect was reduced to 58 ± 20 %, vagus stimulation effect on S α T was reduced to about 44 ± 14 % of each control vagus stimulation effect.

In Figure 6 the time courses of the parameters HR, S α T, P $_s$ and τ_C during one characteristic DXR-experiment are given. Vertical arrows give the effects of vagus stimulation at several time points of the experiment. We found reductions

in HR and P $_s$ (by about 37 % and 55 %, respectively) and increases in S α T and τ_C (by about 59 % and 29 %, respectively) during DXR perfusion (direct DXR effects). Furthermore, vagus stimulation effects on all parameters were progressively reduced (by up to 70 % regarding HR; by up to 57 % regarding S α T, by up to 70 % regarding P $_s$ and by up to 54 % regarding τ_C) during 20 minutes of DXR perfusion. After addition of AII (1 μ mol/L), significant improvements (though only for a few minutes) were obtained including an increase in HR and P $_s$, and shortenings in S α T and τ_C (positive chronotropic and inotropic

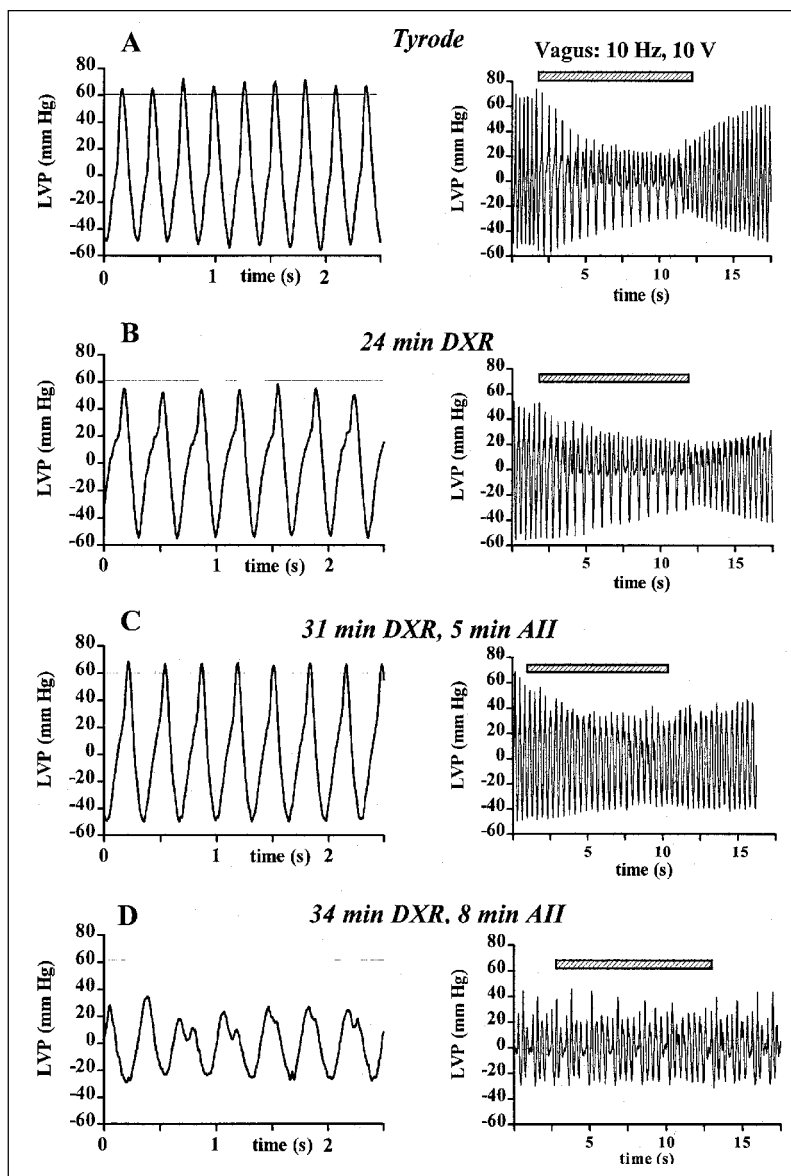


Figure 4. Time courses of left-ventricular pressure (LVP) in isolated rat heart before (left figures) and during vagus stimulation (10 Hz, 10 V, 10 s stimulation period, right figures), recorded during Tyrode perfusion, Tyrode + doxorubicin (DXR) perfusion and Tyrode + DXR + angiotensin II (AII) perfusion. 20 μ mol/L DXR and 1 μ mol/L AII were applied in this experiment. AII was added 26 minutes after DXR administration. **A** Tyrode perfusion, **B** after 24 min perfusion with Tyrode + DXR, **C** after 5 min perfusion with Tyrode + DXR + AII (31 min after DXR application) and **D** 3 minutes later than **C** (after 8 min perfusion with Tyrode + DXR + AII, 34 min after DXR application = end of the experiment). Horizontal bars (right figures) mark the time period of vagus stimulation.

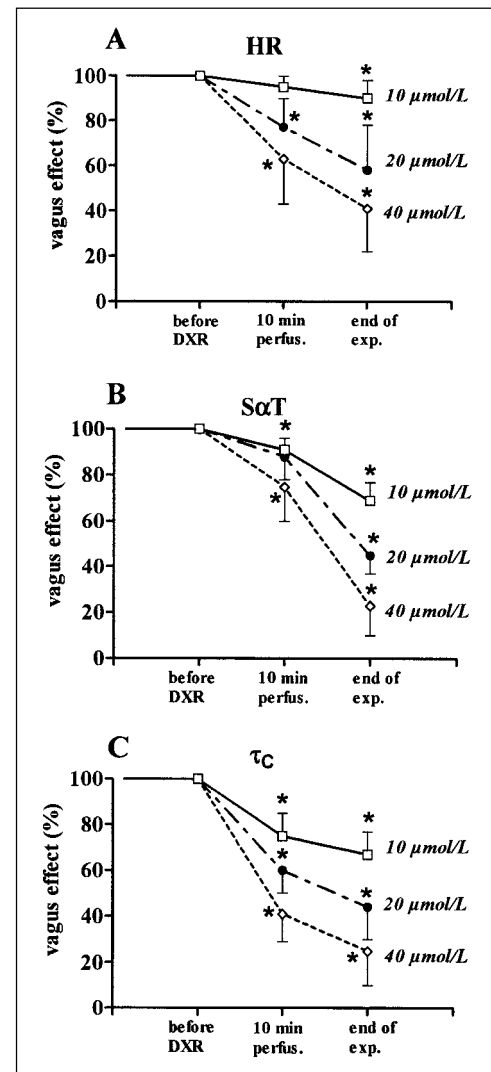


Figure 5. Dose-dependence of the changes in vagus stimulation (right *N. vagus*, 10 Hz, 10 V) effects on heart rate (HR, **A**), S α T time (**B**) and time constant of contraction τ_C (**C**) during perfusion of isolated rat hearts with doxorubicin (DXR)-containing Tyrode. 10 μ mol/L DXR: solid line and open squares, 20 μ mol/L DXR: dash-dotted line and filled circles, 40 μ mol/L DXR: dashed line and open rhombs. Vagus stimulation effects before DXR addition to Tyrode were set to 100 %. Vagus stimulation effects were recorded 10 min after start of DXR perfusion and shortly before the end of each experiment (duration of experiments was the shorter the higher the DXR doses: hearts beated about 60 min after addition of 10 μ mol/L DXR, 40 min after addition of 20 μ mol/L DXR and 25 min after addition of 40 μ mol/L DXR). Means and SD of 5 experiments. Asterisks, significant changes compared with control values before DXR perfusion ($P < 0.05$).

AII effects). Consequently, after a period of 5 to 10 minutes, all parameters, except P_S approached control values (recorded before DXR application). Also the modulatory effect of AII regarding vagus stimulation effects was preserved during DXR perfusion: Vagus stimulation caused progressively smaller effects, especially on HR and P_S . At the end of the experiment, AII had nearly completely suppressed the vagus effects regarding cardiac action (Figure 6).

Dantrolene and caffeine modulate the DXR effects on cardiac action and vagus stimulation

The effects of dantrolene and caffeine regarding DXR-induced changes in heart rate and chronotropic vagus stimulation effect are shown in Figure 7. Dantrolene (10 $\mu\text{mol/L}$) increased the DXR effect regarding its influence on heart rate (Figure 7A) and cardiac contractility (τ_C , Figure 7C). After a 40 min perfusion period with dantrolene and DXR (20 $\mu\text{mol/L}$), heart rate was significantly ($P < 0.05$) more strongly reduced (by about 15 %) than after perfusion with DXR-containing Tyrode solution. The DXR effects on systolic pressure and time constant of contraction τ_C were also increased after perfusion with dantrolene and DXR (both by about 15 %). Additionally, reductions in heart rate and in cardiac contractility caused by vagus stimulation (right *N. vagus*, 10 Hz, 10 V) were smaller after perfusion with dantrolene and DXR, compared with stimulation effects after perfusion with DXR-containing Tyrode.

In contrast to this, caffeine (10 mmol/L) reduced the DXR-induced effects: after a 40 min perfusion period with caffeine and DXR (20 $\mu\text{mol/L}$), the decreases in HR (Figure 7B) and P_S (not shown) were significantly ($P < 0.05$) smaller (by about 15 %) than after perfusion with DXR-containing Tyrode solution. Vagus stimulation effects after perfusion with caffeine and DXR were stronger than those after DXR application alone.

Discussion

The aim of this study was to investigate changes in cardiac vagus function and its modulation by AII during short-time perfusion of DXR in isolated hearts. There was a good parallelity between ECG parameters recorded in the isolated rat heart and previously reported values for anaesthetized [33] or conscious DXR-treated rats [34]. Heart rate *in vitro* was significantly lower than in anaesthetized rats or even conscious animals where the heart rate is regulated by the vegetative innervation (*in vivo*).

The DXR dose in clinical anticancer therapy amounted to maximal 550 mg per square meter of body-surface area [1]. This clinically applied dose approximately corresponds to about 20 $\mu\text{mol/L}$ (estimated for an adult man with about 1.5 square meters of body-surface area and about 80 kg body weight). Therefore, DXR concentrations in our experiments (10–40 $\mu\text{mol/L}$) were in a comparable range as reported from clinical studies [1], but were also comparable with *in vivo* experiments on rats (4 mg DXR/kg body weight, applied up to 5 times per week [34]), experiments on calcium release channels from cardiac sarcoplasmic reticulum of dog cardiac muscles (2.5–10 $\mu\text{mol/L}$, [9]) and experiments on isolated sar-

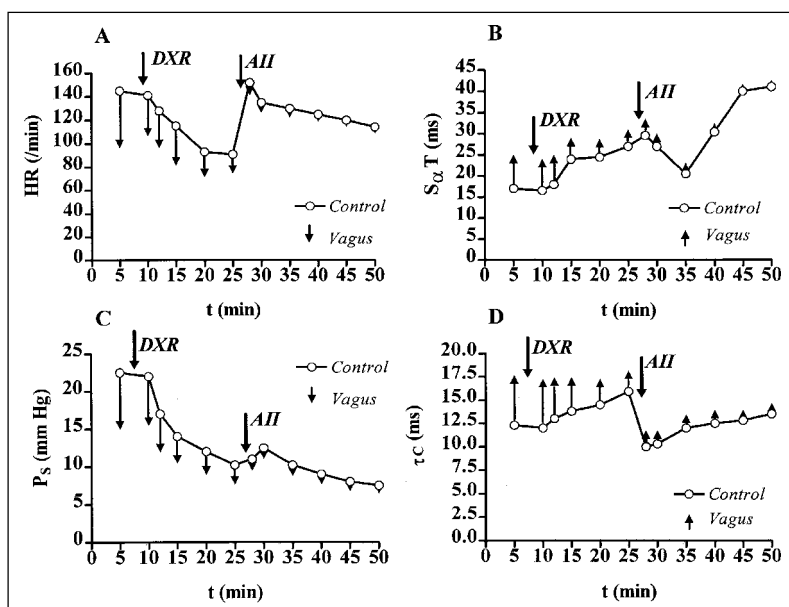


Figure 6. Time courses of heart rate (HR, **A**), $S\alpha T$ time (**B**), systolic pressure P_S (**C**) and time constant of contraction τ_C (**D**) and changes in above parameters during vagus stimulation (right *N. vagus*, 10 Hz, 10 V), recorded in an isolated rat heart (modified Langendorff preparation) before and during doxorubicin (DXR) perfusion (20 $\mu\text{mol/L}$) and after addition of angiotensin II (AII, 1 $\mu\text{mol/L}$). AII was added 20 min after DXR application to Tyrode solution. Small vertical arrows which originate from the curves, show changes in the parameters mentioned above, caused by vagus stimulation. Note, that vagus effects were progressively reduced during DXR perfusion. Addition of AII caused positive chronotropic and inotropic effects for a short time. Additionally, AII addition reduced vagus stimulation effects regarding above parameters (especially HR and P_S). At the end of experiment, all vagus effects were nearly completely disappeared.

colemmal vesicles of dog hearts (100 $\mu\text{mol/L}$, [6]). We found significant changes in various ECG parameters including heart rate as well as an impaired left-ventricular contractility, when the isolated heart was perfused with Tyrode which contained 20 $\mu\text{mol/L}$ DXR or more. Our findings regarding changes in the ECG qualitatively correspond to those previously reported for rats where DXR was administered long-term (over 4–6 weeks, [2]), as well as for patients receiving chronic treatment with anthracyclines [1]. Therefore, DXR had strong effects on cardiac actions not only in chronic administration, but also under the condition of short-time application in the isolated heart.

In our experiments, the survival time of beating hearts was inversely correlated to the DXR concentration. This agrees with similar findings in rats where DXR was chronically administered: the number of surviving rats was generally smaller after longer-lasting application of higher DXR doses [34].

Furthermore, in the presence of DXR, we found a concentration-dependent reduction in the efficacy of vagus stimulation on heart action: Vagus stimulation effects recorded at the end of the period of perfusion with DXR-containing Tyrode solution, amounted only to about 20–40 % of the effects of control stimulation. These findings are in good accordance with clinical observations in DCM patients where the sensitivity of cardiac actions on vagal stimulation was also reduced [25].

It could be shown that dantrolene (an inhibitor of the intracellular ryanodine receptor) increased the DXR effects: reductions in heart rate and cardiac contractility caused by DXR were potentiated by dantrolene, and the smaller vagus stimulation effects observed in the presence of DXR were further reduced. On the other hand, caffeine (an opener of ryanodine receptor, [12, 31, 32]) reduced (but not completely) the DXR

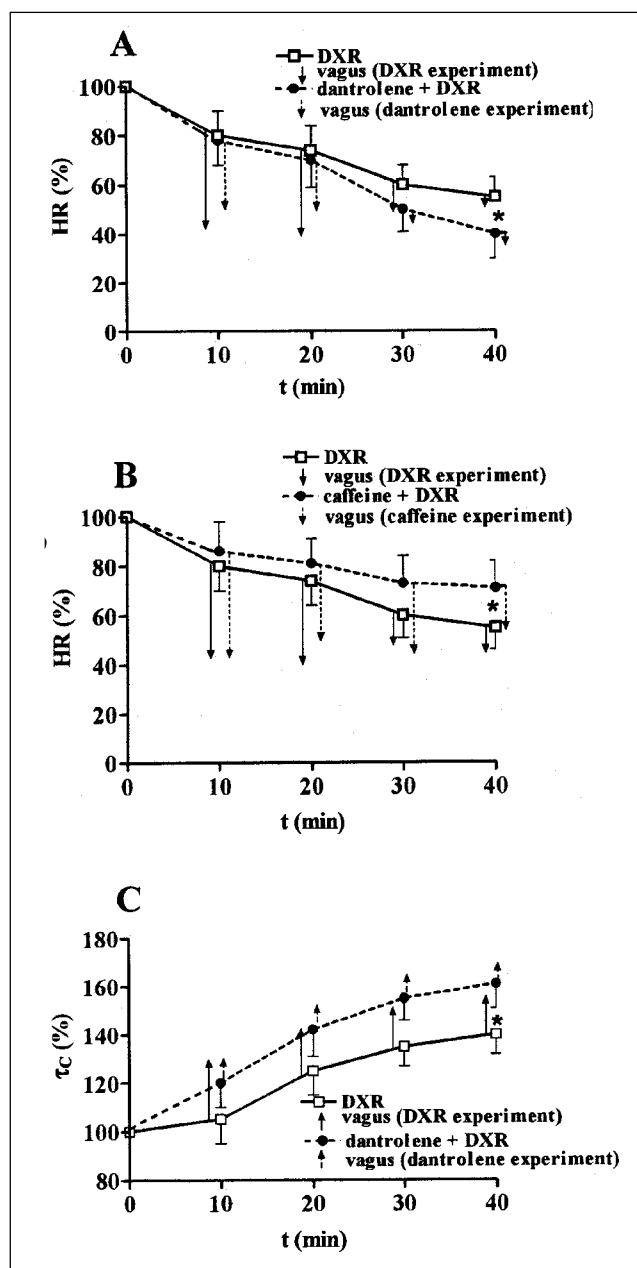


Figure 7. Heart rate (HR, A, B) and time constant of contraction τ_c (C) during perfusion (for 40 min) of isolated rat hearts with doxorubicin (DXR, 20 $\mu\text{mol/L}$)-containing Tyrode solution, and after addition of dantrolene (10 $\mu\text{mol/L}$, A, C) and caffeine (10 mmol/L, B) to DXR-containing Tyrode solution, respectively. Values were normalized on HR or τ_c of control recording (during Tyrode perfusion). Drugs were applied at the time point "0 min". Means and SD of 5 experiments with DXR perfusion (solid line and open squares in A–C) and of 5 experiments each where a perfusion with dantrolene (A,C) or caffeine (B) + DXR was performed (dashed lines and filled circles). Vagus stimulation (right *N. vagus*, 10 Hz, 10 V, 10 s stimulation period) effects were recorded in intervals of 10 min starting after drugs administration (the arrows represent HR reductions / τ_c increases caused each by vagus stimulation). Full line arrows, vagus stimulation effects during DXR perfusion; dashed line arrows, vagus stimulation effects during perfusion with dantrolene/caffeine+DXR containing solution). Note that HR was significantly ($P < 0.05$, asterisks) reduced (A) / increased (B) after 40 min perfusion with dantrolene+DXR / caffeine+DXR, compared with HR in DXR perfusion experiments. τ_c was significant increased after 40 min perfusion with dantrolene+DXR, compared with HR in DXR perfusion experiments. Caffeine reduces, dantrolene increases the observed DXR actions regarding HR, τ_c and vagus stimulation effects.

effects on heart action and vagus effects. These observations could be a sign that intracellular ryanodine receptors seem to be involved in mediation of DXR effects regarding the reduction of intracellular calcium concentration [8].

In contrast, the angiotensin II (AII)-induced modulation of vagus effects were not affected by DXR perfusion: After addition of 1 $\mu\text{mol/L}$ AII subsequently to DXR, we observed clear effects of AII on heart rate and cardiac contractility (positive chronotropic and inotropic effects). Also the modulatory role of AII regarding vagus control in the DXR-perfused heart was comparable with AII modulation effects under control conditions. These findings could base on the different intracellular mechanisms underlying DXR effects on the one hand and AII effects on the other hand which do not seem to interact: AII binds on myocardial AT1 receptors and increases the intracellular calcium level *via* calcium release from intracellular stores (activation of IP₃ receptors) as well as *via* phosphorylation of L-type Ca²⁺-channels (PKC activation) and calcium inward current [15–17, 35]. DXR mediates its action *via* intracellular myocardial ryanodine receptors (at least partially) which were initially activated and subsequently irreversibly inhibited by DXR [9]. Thus, it should not be surprising that the modulatory role of AII was preserved also during short-time DXR perfusion, and was comparable with AII effects under physiological conditions [19].

Clinical data indicate that chronic anthracycline treatment causes functional changes in the renin-angiotensin system: the available concentration of angiotensin II or its precursors in the plasma as well as the activation of α - and β -adrenergic system were increased [20, 21], whereas the density of angiotensin II receptors in the peripheral effector organs was markedly reduced [22]. Concluding from our results, the short DXR-contamination period applied in our experiments (maximal 60 min perfusion) seemed not to be sufficient to cause comparable changes in the AII system *in vivo*.

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