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ORIGINAL ARTICLE

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Selective sensory denervation by capsaicin aggravates adriamycin-induced cardiomyopathy in rats

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Abstract Capsaicin-sensitive sensory nerves that contain calcitonin gene-related peptide (CGRP) contribute significantly to cardioprotective mechanisms. In this study, the possible role of capsaicin-sensitive afferent nerves in the development of congestive heart failure was examined in an established model of adriamycin-induced experimental cardiomyopathy in rats. Systemic treatment with capsaicin was utilized to deplete sensory neuropeptides from cardiac afferent nerves. Echocardiography was applied to assess the cardiac function in adriamycin-treated rats pretreated with capsaicin or its vehicle. In control rats, adriamycin treatment produced a reduction in the fractional shortening of the left ventricle and an increase in the ratio of the left atrial diameter and the aortic diameter, indicative of a decreased myocardial contractility and heart failure only at 3–4 weeks post-treatment. In contrast, in capsaicin-pretreated rats, a deterioration of the cardiac function was already evident 1 week after the cessation of adriamycin administration, while the clinical signs associated with cardiomyopathy were more severe and displayed a significantly more rapid progression. Immunohistochemistry revealed a complete depletion of calcitonin gene-related peptide from cardiac sensory nerves after systemic capsaicin treatment. This study has demonstrated that elimination of capsaicin-sensitive afferent nerves promotes the development and progression of adriamycin-induced myocardial dysfunction. The results suggest that interfering with capsaicin/vanilloid receptor function and/or perturbation of the myocardial CGRP metabolism may open up new perspectives concerning prevention and/or alleviation of the pathological changes that follow adriamycin treatment.

Keywords Cardiomyopathy · Adriamycin · Sensory nerves · Capsaicin · Rat · Calcitonin gene-related peptide · Cardioprotection · Echocardiography

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Introduction

The introduction of anthracycline-type cytostatic agents provided a powerful new therapeutic tool for the treatment of a variety of malignancies (Di Marco et al. 1969). However, the anthracyclines also exert some serious side effects, the major risk being the development of congestive cardiomyopathy. The overall incidence of adriamycin-induced cardiomyopathy ranges from 1.7 to 6.8% (Olson and Mushlin 1990), and 2% of all patients who are given adriamycin develop symptoms of congestive heart failure (Henderson and Frei 1980). Its incidence increases with increasing total dose and may be as high as 15–30% (Henderson and Frei 1980; Lefrak et al. 1973). Several mechanisms have been shown to contribute to the development of anthracycline-induced cardiomyopathy, including free radical production and lipid peroxidation, impairment of DNA replication (cf. Singal et al. 1997) and interaction with intracellular calcium homeostasis (cf. Olson and Mushlin 1990).

Although peptidergic cardiac nerves have been demonstrated to play a significant role in cardiac function under pathophysiological and possibly physiological conditions, the possible involvement of cardiac sensory nerves in the pathomechanism of anthracycline-induced toxic cardiomyopathy has not yet been considered. Calcitonin gene-related peptide (CGRP), a 37 amino acid peptide, is to be found in approximately 40% of primary sensory neurons, including those innervating the heart (Franco-Cereceda 1988; Gibbins et al. 1987; Mulderry et al. 1985). CGRP

exerts marked coronary vasodilatatory (Holman et al. 1986) and positive inotropic and chronotropic effects in several species, including the guinea-pig (Franco-Cereceda 1988; Franco-Cereceda and Lundberg 1988), rat (Franco-Cereceda 1988; Franco-Cereceda and Lundberg 1988; Gasparetti et al. 2002) and pig (Franco-Cereceda and Lundberg 1989). CGRP is localized in cardiac primary sensory neurons which are sensitive to the potent sensory neurotoxin capsaicin (Ferdinandy et al. 1997; Franco-Cereceda 1988; Gasparetti et al. 2002) and which express the capsaicin/vanilloid receptor (Zahner et al. 2003). It has been demonstrated that the depletion of CGRP by capsaicin results in a marked increase in the incidence of adverse reactions, which commence following the experimental induction of myocardial ischemia (Franco-Cereceda and Lundberg 1989; Kallner 1998; Kallner and Franco-Cereceda 1998). Further, CGRP is essential for the development of myocardial protection: the depletion of CGRP from sensory nerves by prior pre-treatment with capsaicin greatly inhibits or even abolishes the protective effect of ischemic preconditioning (Ferdinandy et al. 1997; Zhou et al. 1999) or heat stress on reperfusion injury (Song et al. 1999) and attenuates the nitroglycerine-induced improvement of preservation with cardioplegic solution (Zhou et al. 2001). CGRP is also involved in human cardiac pathologies: an early increase in plasma CGRP level has been demonstrated in patients with myocardial infarction (Lechleitner et al. 1992) and administration of this peptide has been shown to delay the onset of myocardial ischemia upon physical exercise in patients with stable angina pectoris (Uren et al. 1993).

With regard to the pivotal significance of capsaicin-sensitive CGRP-containing afferent nerves in myocardial protective mechanisms, the present experiments were conducted in an attempt to reveal a possible role of these particular nociceptive sensory nerves in the development of adriamycin-induced experimental cardiomyopathy in the rat.

Materials and methods

This study conformed fully with the "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) and was approved in advance by the Animal Research Ethics Committee of the University of Szeged.

Adult male Wistar rats weighing 220–250 g at the start of the experiments were used in the study. Experimental cardiomyopathy was induced with adriamycin according to the treatment schedule introduced by Tong et al. (1991). Briefly, the animals (n=17) received a cumulative dose of 15 mg/kg of adriamycin (Pharmacia Italia, Milan, Italy) by the injection of 2.5 mg/kg of the drug intraperitoneally three times a week for 2 weeks. The control rats (n=6) received equivalent amounts of the vehicle (saline). Other groups of animals (n=23) were pretreated with capsaicin (Fluka, 10, 20 and 100 mg/kg subcutaneously injected on 3 consecutive days) or its vehicle (6% ethanol, 8% Tween 80 in saline, n=6) under ether anesthesia 2 weeks prior to the induction of adriamycin treatment. This capsaicin treatment paradigm has been shown to result in a practically complete elimination of CGRP-containing cardiac sensory nerves (Ferdinandy et al. 1997).

Cardiac function was assessed by echocardiographic examination before and at regular intervals after capsaicin and/or adriamycin treatment. Echocardiography was performed essentially as described by Schwarz et al. (1998). The rats were anesthetized with ether, the chest was shaved and the animal was placed in the supine position. Two-dimensional and M-mode echocardiographic examinations were performed in accordance with the criteria of the American Society of Echocardiography, with a Desmin F ultrasound scanner (Echo-Son, Puławy, Poland), using 5 and 7.5 MHz phased-array transducers. The investigator who analyzed the echocardiograms was unaware of the modes of treatment that the animals had undergone.

At all time points, three measurements were made in each animal, and the mean values were calculated and used for statistical evaluation of the data. The left ventricle (LV) was examined in the parasternal long-axis view at the level of the mitral valve, or in the parasternal short-axis view at the level of the papillary muscles. The LV diameters were measured by means of M-mode echocardiography between the endocardial borders. The LV end-diastolic diameter (LVDD) was measured at the longest diameter of the LV. The LV end-systolic diameter (LVSD) was measured at the shortest diameter of the LV. The fractional shortening (FS) was calculated by using the LV diameters (LVDD−LVSD)/LVDD, and was expressed as a percentage. The left atrial diameter (LAD) and aortic diameter (AOD) were measured by M-mode echocardiography at the level of the longest LAD. Three cardiac cycles were measured, and the mean values were calculated and utilized for the evaluation of the data. The ratio LAD/AOD was calculated. Values are given as means ± SEM.

Pericardial and pleural effusion was detected by the examination of fluid accumulation between the layers of the epicardium and the pericardium and between the layers of the visceral and parietal pleuras, respectively, with two-dimensional and M-mode echocardiography. Ascites was visualized from the subcostal four-chamber or short-axis view below the diaphragm by means of two-dimensional ultrasonography.

For immunohistochemical studies additional groups of rats treated with adriamycin (n=3) and capsaicin plus adriamycin (n=3) were perfused via the left heart ventricle with Zamboni's fixative 2 weeks after the completion of the administration of adriamycin. The hearts were removed and after a post-fixation period of 3 h they were placed in a buffer solution and stored at 4°C until sectioning. Transverse sections through the ventricles were cut at a thickness of 20 μm and were processed for immunohistochemical staining with the indirect immunofluorescence technique by using rabbit polyclonal antisera raised against protein gene product 9.5 (PGP 9.5, 1:1000; Ultraclone, Cambridge, UK) and CGRP (1:500, Sigma

Chemicals, St Louis, MO, USA). Goat anti-rabbit IgG labeled with Cy3 (carboxymethylindocyanin, 1:500, Jackson ImmunoResearch Laboratories, West Grove, PA, USA) was used as a secondary antibody. The specimens were viewed under a Leitz DMLB fluorescence microscope equipped with an appropriate filter combination and photographed with a digital camera (Nikon Coolpix 950).

Statistical evaluation of the experimental data was performed with ANOVA, followed by the Bonferroni test. A probability level $p<0.05$ was regarded as a statistically significant difference between groups. For statistical analysis of the survival probabilities of rats treated with adriamycin and capsaicin plus adriamycin, respectively, the life table method was applied (Altman 1991). At the start of the experiments the numbers of animals were 15 and 17 in the adriamycin and the capsaicin plus adriamycin-treated groups, respectively. The events of interest (death of animals) were registered at the end of the adriamycin treatment and also at the end of the 2nd, 3rd, and 4th weeks of the post-treatment period. The values of the survival proportions ± SEM were calculated. To compare the survival experience of the animals of the two groups, the non-parametric log rank test was applied. Since more than one event was registered at certain times of observations, we calculated the X^2 value by a formula described by Altman (1991). Pairwise comparisons between the survival proportions observed at the completion of adriamycin treatment and at the end of the 2nd, 3rd, and 4th weeks of the post-treatment period were performed using Fisher's exact test and the p values were corrected by Bonferroni's method.

Results

The echocardiograms in Fig. 1 illustrate the techniques used to measure the echocardiographic parameters involving AOD, LAD, LVSD, and LVDD. The cardiac parameters measured by echocardiographic examination in control, capsaicin-, adriamycin-, and capsaicin plus adriamycin-treated rats are presented in Table 1. Sequential echocardiographic examination of the control animals demonstrated little change in the cardiac parameters during the period of the study. Since there was no significant difference between the parameters of the two control groups, treated with the vehicles for capsaicin and adriamycin, respectively, for subsequent statistical evaluation, they were lumped together as a single control group. None of the rats in this control group died or developed pathologies associated with cardiac failure during the period of the study. Fractional shortening at the beginning of the study was 25.3±1.5% and did not exhibit any significant change throughout the entire study period. Examination of the rats treated with capsaicin revealed that there was no significant change in FS as compared with the initial baseline value or that for the control group. During the period of the study, the follow-up examinations indicated that there was no change in FS of rats treated only with capsaicin.

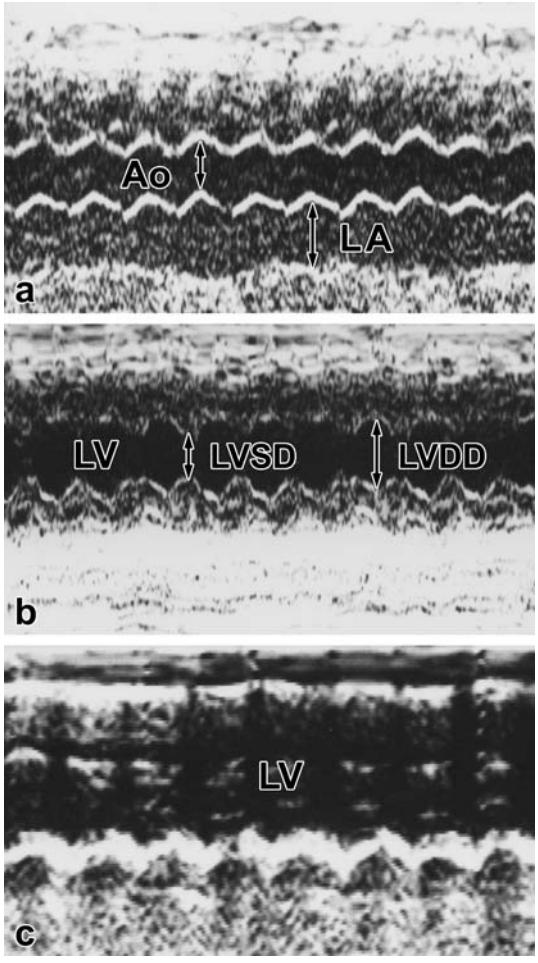

Fig. 1 **a** M-mode echocardiogram of the aorta (*Ao*) and the left atrium (*LA*) illustrating the technique used to measure the aortic (AOD) and left atrial dimensions (LAD). A normal ratio of LAD and AOD is measured in the parasternal long axis view in a control rat. **b** M-mode echocardiogram of the left ventricle (*LV*) in the parasternal long axis view illustrating the method used to measure the ventricular dimensions in a control rat. The left ventricular end-diastolic diameter (*LVDD*) and the left ventricular end-systolic diameter (*LVSD*) measurements are done from the leading edge at the end of the diastole and at the end of the systole. Normal contractility of the left ventricle can be seen. **c** An enlarged LV can be seen by M-mode echocardiography in the parasternal long axis view in a rat treated with capsaicin plus adriamycin. The contractility of the left ventricle is markedly decreased.

In rats treated only with adriamycin, echocardiographic measurements of the LV dimensions 3 and 4 weeks after the completion of drug administration demonstrated significant increases in the LVSD. LVDD did not change significantly in these animals. The ratio LAD/AOD was already significantly increased 1 week after the completion of the treatment (Fig. 2a, Table 1).

In the rats treated with capsaicin plus adriamycin, LVSD was increased significantly already by the end of the 1st week after the completion of adriamycin administration. By the end of the 3rd week after the completion of adriamycin administration, both LVDD and LVSD were increased significantly. The ratio LAD/AOD was already

Table 1 Cardiac parameters measured by echocardiographic examination in control, capsaicin-, adriamycin-, and capsaicin plus adriamycin-treated rats. *AOD* aortic diameter, *LAD* left atrial diameter, *LVVD* left ventricular end diastolic diameter, *LVSD* left ventricular end systolic diameter, *FS* fractional shortening

Treatment period	AOD				LAD				LAD/AOD			
	Control	Capsaicin	Adriamycin	Capsaicin + adriamycin	Control	Capsaicin	Adriamycin	Capsaicin + adriamycin	Control	Capsaicin	Adriamycin	Capsaicin + adriamycin
Start	3.42 ±0.12	3.06 ±0.22	3.39±0.12	3.29±0.09	3.77 ±0.26	3.35 ±0.26	3.59±0.15	3.56±0.12	1.10 ±0.04	1.10 ±0.06	1.06±0.05	1.08±0.04
End	3.37 ±0.09	3.37 ±0.24	3.36±0.13	3.03±0.14	3.63 ±0.12	3.56 ±0.26	4.33±0.16*	3.59±0.17	1.08 ±0.02	1.07 ±0.05	1.27±0.07	1.20±0.05
1 week[a]	3.35 ±0.10	3.45 ±0.15	3.47±0.13	3.00±0.17	3.52 ±0.07	3.80 ±0.13	4.42 ±0.16*[#]	4.10±0.22*[#]	1.05 ±0.01	1.10 ±0.06	1.28 ±0.05*[#]	1.37±0.07*[#]
2 weeks[a]	3.60 ±0.16	3.28 ±0.16	3.28±0.14	3.28±0.12	4.11 ±0.28	3.41 ±0.28	4.44 ±0.17*[#]	5.54±0.25*[#]	1.14 ±0.03	1.25 ±0.07	1.37 ±0.05*[#]	1.69±0.05*[#]
3 weeks[a]	3.52 ±0.16	3.20 ±0.11	3.27±0.16	3.36±0.14	3.97 ±0.26	3.64 ±0.23	5.03 ±0.22*[#]	5.31±0.27*[#]	1.13 ±0.02	1.14 ±0.06	1.53 ±0.06*[#]	1.58±0.05*[#]
4 weeks[a]	3.62 ±0.12	3.48 ±0.19	3.15±0.17	3.24±0.16	3.87 ±0.14	3.79 ±0.36	4.69 ±0.22*[#]	5.41±0.31*[#]	1.07 ±0.02	1.09 ±0.05	1.49 ±0.07*[#]	1.67±0.06*[#]
Treatment period	LVDD				LVSD				FS			
	Control	Capsaicin	Adriamycin	Capsaicin + adriamycin	Control	Capsaicin	Adriamycin	Capsaicin + adriamycin	Control	Capsaicin	Adriamycin	Capsaicin + adriamycin
Start	5.46 ±0.19	5.20 ±0.26	5.33±0.30	5.46±0.17	4.11 ±0.14	3.94 ±0.24	4.06±0.17	4.11±0.14	0.25 ±0.01	0.24 ±0.02	0.24±0.02	0.25±0.01
End	5.70 ±0.11	5.31 ±0.34	5.52±0.21	5.68±0.24	4.31 ±0.23	4.04 ±0.30	4.23±0.19	4.23±0.21	0.24 ±0.05	0.24 ±0.02	0.23±0.02	0.25±0.02
1 week[a]	5.65 ±0.14	5.53 ±0.18	5.40±0.17	6.01±0.30	4.33 ±0.13	4.19 ±0.17	4.00±0.16	4.95±0.27*[#]	0.23 ±0.02	0.24 ±0.02	0.26±0.02	0.17±0.02*
2 weeks[a]	5.76 ±0.11	5.49 ±0.22	5.40±0.32	5.83±0.22	4.41 ±0.12	4.21 ±0.26	4.28±0.27	5.03±0.19*[#]	0.23 ±0.02	0.23 ±0.03	0.22±0.02	0.14±0.02*[#]
3 weeks[a]	5.83 ±0.18	5.58 ±0.57	5.65±0.29	6.45±0.24*[#]	4.33 ±0.07	4.18 ±0.43	4.69±0.18*	5.52±0.21*[#]	0.26 ±0.04	0.25 ±0.03	0.17±0.02	0.14±0.02*[#]
4 weeks[a]	5.80 ±0.14	5.81 ±0.44	5.40±0.51	6.68±0.28*[#]	4.45 ±0.12	4.31 ±0.48	4.64±0.19*	5.75±0.24*[#]	0.23 ±0.03	0.26 ±0.03	0.14 ±0.02*[#]	0.13±0.03*[#]

Values are expressed in mm as mean ± SEM obtained from at least five animals
*Significantly different from the initial baseline value
[#]Significantly different from the values of the corresponding control group
[a]Time after completion of the treatment with adriamycin

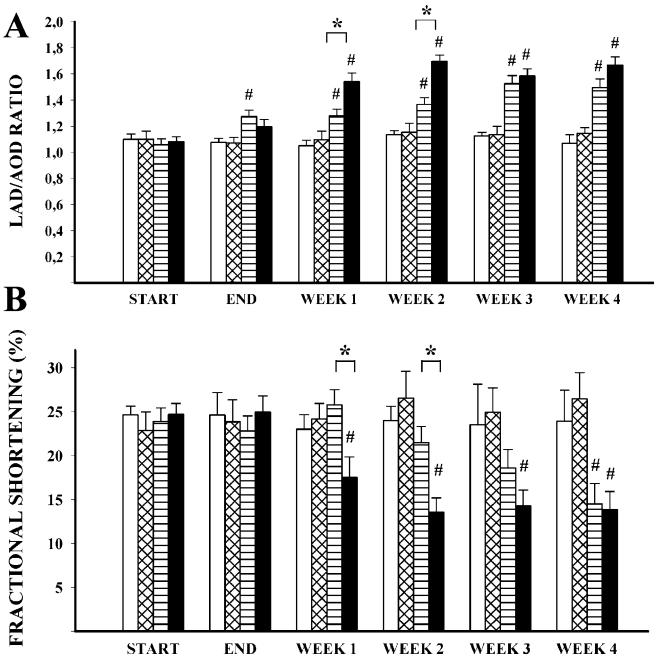

Fig. 2 **a** Ratio of LAD and AOD and **b** fractional shortening of the left ventricle in control (*open bars*), capsaicin-treated (*cross-hatched bars*), adriamycin-treated (*horizontally hatched bars*), and capsaicin plus adriamycin-treated (*filled bars*) groups of rats before (*Start*), and 3 days (*End*) and 1–4 weeks after the completion of the adriamycin treatment. The asterisk and the hash sign denote statistically significant differences ($p<0.05$) between selected groups or in comparison with the initial control value respectively. Each column depicts the mean of values obtained from at least five rats.

significantly increased 1 week after the completion of adriamycin treatment (Fig. 1, Table 1).

In the rats treated with adriamycin, the echocardiographic examinations revealed a progressive reduction in FS, indicating a decrease in cardiac contractility (Fig. 1c), from the 2nd week onwards after the completion of the administration of the drug, but this became statistically significant only in the 4th week post-treatment (Fig. 2b, Table 1). In contrast, a marked and significant reduction in FS was already observed 1 week after the completion of adriamycin treatment in the rats pretreated with capsaicin. Statistical analysis of the data showed that, with the exception of the 4th week post-treatment, the FS in the capsaicin plus adriamycin-treated rats was markedly lower than that in the rats treated only with adriamycin (Fig. 2b, Table 1).

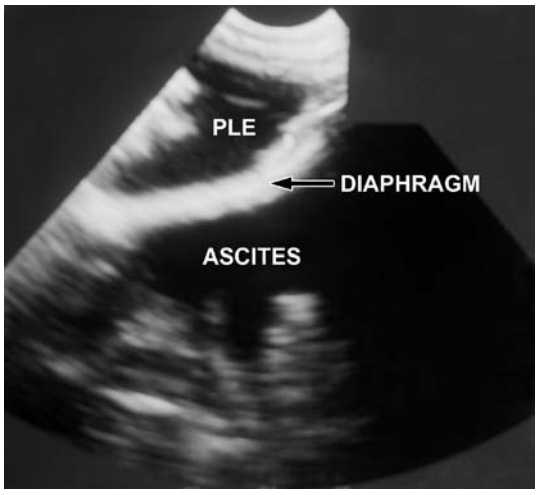

Fig. 3 Demonstration of the large amount of pleural effusion (*PLE*) and ascites in the subcostal cross-sectional view of an adriamycin-treated rat by two-dimensional ultrasound examination.

Echocardiography and ultrasonographic examination of the chest and abdomen revealed the accumulation of fluid in the pericardial, pleural, and abdominal cavities in adriamycin-treated animals (Fig. 3). The cumulative incidences of pericardial effusion, pleural effusion, and ascites by the end of the study period were 8, 3, and 8 of the 15 adriamycin-treated rats, and 11, 3, and 8 of the 17 capsaicin plus adriamycin-treated rats, respectively.

The statistical analysis of the survival probabilities revealed that 33% of the vehicle-treated and 35% of the capsaicin-treated animals have survived the 6-weeks period after the start of adriamycin administration. The data on the survival times, the number of rats at risk, the observed numbers of events, and the survival proportions are shown in Table 2. The non-parametric log rank test resulted an X^2 value of 2.907 corresponding to a $0.2>p>0.1$ probability which is higher than the level of significance chosen for this study ($p<0.05$). The pairwise comparisons using Fisher's exact test have not shown significant differences in the survival ratios between these two groups.

Immunohistochemical studies were performed to reveal possible changes in the population(s) of cardiac nerves which, in turn, may contribute to the aggravation of adriamycin-induced cardiomyopathy in capsaicin-pretreated rats. In control rats, immunohistochemical demonstration of cardiac nerves using an antiserum against protein gene product (PGP) 9.5, a panneuronal marker, revealed a

Table 2 Survival statistics of rats treated with adriamycin and capsaicin plus adriamycin

Survival time[a]	Adriamycin			Capsaicin plus adriamycin		
	Number of rats at risk	Number of events	Survival proportion ± SEM	Number of rats at risk	Number of events	Survival proportion ± SEM
0	15	3	0.80±0.92	17	2	0.88±0.07
2 weeks	12	1	0.73±0.10	15	5	0.58±0.09
3 weeks	11	1	0.66±0.11	10	2	0.47±0.10
4 weeks	10	5	0.33±0.08	8	2	0.35±0.10

[a]Time after the completion of the administration of adriamycin

dense innervation of both the left and right ventricles (Fig. 4a, b). CGRP-containing nerves innervating the ventricles were far less numerous (Fig. 4e). Examination of the distribution of PGP 9.5-positive nerve fibers of the heart ventricles of rats treated with adriamycin or capsaicin plus adriamycin 2 weeks after completion of adriamycin treatment disclosed an innervation pattern similar to that of the controls (Fig. 4c, d). In contrast, in rats treated with capsaicin plus adriamycin, but not in rats treated only with adriamycin, CGRP-containing nerves could not be detected 2 weeks after cessation of adriamycin treatment (Fig. 4f).

Discussion

The present findings confirm previous reports by showing that the administration of adriamycin to rats over a period of 2 weeks was followed by a progressive deterioration of cardiac function resembling congestive cardiomyopathy (Tong et al. 1991). Follow-up measurements of the cardiac parameters revealed that the changes indicative of an impaired cardiac function were most marked from the 3rd week onwards after the cessation of adriamycin administration. The FS displayed a noteworthy reduction by 3 weeks after the cessation of treatment, and by the 4th week the reduction reached the level of statistical significance. This is in agreement with the previous finding that the cardiac function began to deteriorate 3 weeks after the cessation of drug administration when this treatment schedule was used (Tong et al. 1991). Similarly as in previous reports, only minor changes in LVDD were detected in the course of the study, but LVSD exhibited significant increases from the 3rd week onwards (Schwarz et al. 1998). The animals showed other signs of congestive heart failure, as indicated by pericardial and pleural effusions and ascites. These findings corroborate the earlier observations and furnish further evidence that adriamycin causes a progressive deterioration of cardiac function resembling congestive cardiomyopathy, which

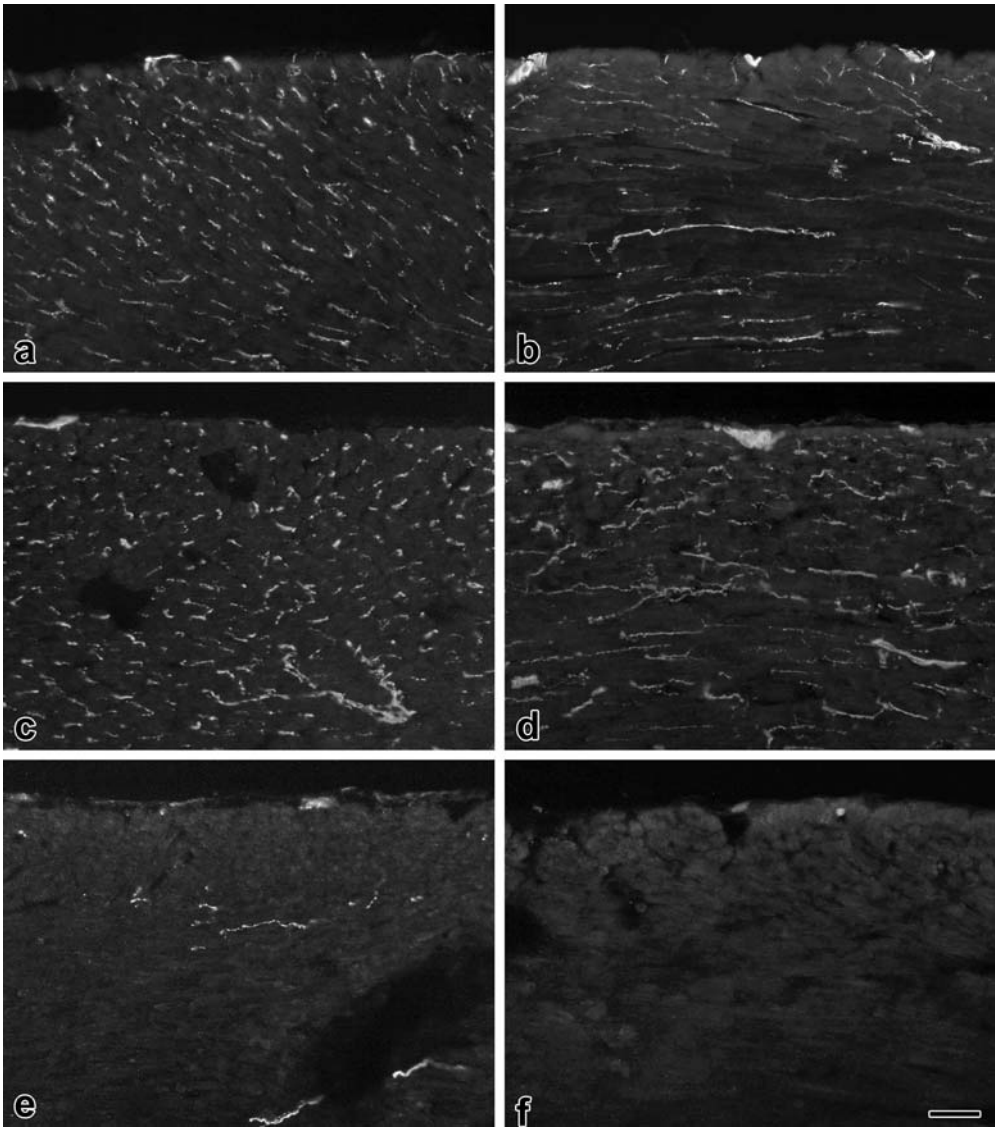

Fig. 4 **a**–**d** Protein gene product (PGP) 9.5-immunoreactive and **e**, **f** calcitonin gene-related peptide (CGRP)-immunoreactive nerve fibers in tissue sections obtained from the left (**a**,**c**, **e**,**f**) and right (**b**,**d**) heart ventricles of control (**a**, **b**) and capsaicin plus adriamycin-treated (**c**–**f**) rats. The distribution pattern of PGP 9.5-positive cardiac nerves is apparently similar in control and treated rats. CGRP-immunopositive nerves cannot be detected in the capsaicin-pretreated rat. The *scale bar* in **f** indicates 50 μm and applies to all microphotographs.

can be reliably monitored by means of follow-up echocardiographic examinations in the rat.

However, the most important observation in the present study was the demonstration of a marked aggravation and, in particular, acceleration of the development of the symptoms of adriamycin-induced congestive heart failure in capsaicin-pretreated rats. Hence, capsaicin pre-treatment resulted in a marked deterioration of the cardiac function even only 1 week after the cessation of adriamycin administration, as indicated by a significant reduction in FS and a significant increase in the ratio LAD/AOD. In particular, 1 and 2 weeks after the completion of adriamycin treatment FS was significantly reduced and the ratio LAD/AOD was significantly increased in the capsaicin-pretreated rats as compared not only with the baseline values, but also with the values obtained in the rats treated only with adriamycin. Similarly as in the rats treated only with adriamycin, pericardial and pleural effusions and ascites were observed in many animals upon ultrasonographic examination. Capsaicin treatment per se produced neither significant changes in the cardiac parameters examined nor clinical signs of an impaired cardiac function. The statistical analysis of the life tables and the pairwise comparisons of the survival ratios observed at different times in the course of the study did not reveal significant differences between the survival parameters of animals treated with adriamycin and capsaicin plus adriamycin, respectively. These findings, therefore, indicate that systemic treatment with capsaicin did not significantly influence adriamycin-induced mortality.

The immunohistochemical findings of the present study confirm and extend previous observations (Wharton et al. 1986; Ferdinandy et al. 1997) by showing a complete depletion of CGRP from cardiac afferent nerves after capsaicin treatment. In addition, PGP 9.5 immunohistochemistry failed to reveal a marked loss of cardiac nerves 2 weeks after the completion of adriamycin or capsaicin plus adriamycin treatments. These observations suggest that capsaicin-insensitive afferent and autonomic nerve fibers are not affected at a post-treatment period when significant impairments in functional parameters have already been detected. Earlier studies disclosed that, in the rat, CGRP is the predominant peptide present in afferent nerves innervating the cardiac ventricles, since in contrast with guinea-pigs (Wharton et al. 1986), substance P cannot be demonstrated in this tissue in the rat (Onuoha et al. 1999).

Calcitonin gene-related peptide plays a cardinal role in the myocardial protection provided by ischemic preconditioning, heat stress, or nitroglycerine which is dependent on the integrity of the capsaicin-sensitive sensory nerves and is abolished after capsaicin treatment (Ferdinandy et al. 1997; Song et al. 1999; Zhou et al. 1999). Accordingly, we suggest that the loss of capsaicin-sensitive CGRP-containing cardiac afferent nerve fibers may be the most likely explanation for the marked acceleration and aggravation of the adriamycin-induced cardiac pathology in the capsaicin-pretreated rats. Elimination of an important cardioprotective mechanism by the depletion of CGRP through capsaicin pre-treatment may result in the earlier onset and rapid progression of the myocardial changes leading to congestive cardiomyopathy. Several lines of available experimental data support this assumption. Capsaicin pre-treatment has been found to result in a significant aggravation of the pathological changes in a pig model of experimentally induced myocardial ischemia (Franco-Cereceda and Lundberg 1989; Kallner 1998; Kallner and Franco-Cereceda 1998). In rats, the intravenous infusion of CGRP markedly reduced the myocardial damage produced in an ischemia–reperfusion injury model (Wu et al. 2001). CGRP has also been shown to reduce ischemia–reperfusion-induced cardiac arrhythmias and mortality in rats (Zhang et al. 1994). The fundamental contribution of CGRP in preconditioning-induced cardioprotection is well documented (Li et al. 1996, 2000; Peng et al. 1996; Song et al. 1999; Zhang et al. 1994; Zhou et al. 1999). Finally, the principal role of capsaicin-sensitive afferent nerves in the preconditioning-induced release of CGRP and cardioprotection has also been clearly established (Csont et al. 2003; Ferdinandy et al. 1997; Hu et al. 2003; Li et al. 1996; Song et al. 1999). Taken together, these observations strongly suggest that local release of sensory neuropeptides, in particular CGRP, from capsaicin-sensitive afferent nerves may be a significant protective mechanism which counteracts the deleterious effects of adriamycin in this experimental model of congestive cardiomyopathy. In addition, other mechanisms may also contribute to cardioprotection afforded by capsaicin-sensitive afferent nerves. Hence, stimulation of capsaicin-sensitive afferents has been suggested to exert a protective effect by triggering cardiogenic sympathoexcitatory reflexes (Schultz 2003; Zahner et al. 2003).

Further, endocrine mechanisms may also contribute to the cardioprotective effect of capsaicin-sensitive afferent nerves. Hence, B-type natriuretic peptide has been shown to limit the size of ischemia-induced infarcts in isolated rat hearts (D'Souza et al. 2003) and the stretch-induced release of atrial natriuretic peptide has been shown to be inhibited in capsaicin-treated rats (Rankin and Scott 1990).

In conclusion, the present study has demonstrated that elimination of capsaicin-sensitive afferent nerves promotes the development and progression of adriamycin-induced myocardial dysfunction. The results suggest that perturbation of the function of capsaicin-sensitive afferent nerves and/or myocardial CGRP metabolism, e.g., by agents interfering with capsaicin/TRPV1 receptors localized on cardiac sensory nerves (Zahner et al. 2003) or with peptide metabolism, may open up new perspectives as concerns prevention and/or alleviation of the pathological changes that follow adriamycin treatment.

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