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Modification of aortic contractility in the cardiomyopathic hamster

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1 The functional arterial response in the cardiomyopathic hamster compared with inbred control, was investigated in thoracic aortae. For this purpose, vessels were cut into 6-mm rings and mounted in 20-ml organ baths.

2 In a first experimental series, the function of the endothelium was evaluated. Dose-response curves to acetylcholine (0.1 nM-10 μM) on phenylephrine (0.3 μM)-preconstricted rings of cardiomyopathic hamsters and inbred age-matched controls were comparable (log[EC_{50}] of -7.08 ± 0.12 and -7.18 ± 0.12, respectively; $n=4$).

3 Changes in contractility of cardiomyopathic hamster endothelium-denuded aortae were investigated. Dose-response curves to phenylephrine (1 nM-0.1 mM), angiotensin II (10 pM-0.3 μM), 5-hydroxytryptamine (5-HT) (1 nM-0.1 mM) and KCl (1 mM-0.1 M) were performed. Increased sensitivity in cardiomyopathic hamster aortae, compared to controls, was observed with phenylephrine (log[EC_{50}] of -7.25 ± 0.05 and -6.83 ± 0.05, respectively, $n=6$, $P<0.001$) and angiotensin II (log[EC_{50}] of -8.67 ± 0.07 and -8.26 ± 0.06, respectively, $n=6$, $P=0.001$) but not with 5-HT or KCl. A decreased maximum response in cardiomyopathic, compared to control, was observed with 5-HT (1.28 ± 0.06 g vs 1.56 ± 0.07 g, respectively, $n=6$, $P=0.03$). Comparable results were found in aortae with an intact endothelium.

4 No difference in the maximum contractile response to the G-protein activator, NaF (3, 10 and 30 mM) was observed in either group of animals.

5 Phorbol 12-myristate 13-acetate (PMA, 1-10 μM) was used to assess changes in the activity of protein kinase C (PKC). Contractility to PMA was increased in cardiomyopathic hamster aortae compared to controls (0.22 ± 0.02 g vs 0.07 ± 0.03 g at 3 μM, respectively, $n=6$, $P=0.003$).

6 Finally, cardiomyopathic hamsters aortae were found to be less sensitive when exposed to increasing concentrations of Ca^{2+} (10 μM-1 mM) in KCl-depolarized rings (0.58 ± 0.04 g in cardiomyopathic vs 0.79 ± 0.06 g in control aortae at 0.3 mM, $n=8$, $P=0.03$).

7 In conclusion, aortae from cardiomyopathic hamsters are more sensitive to phenylephrine and angiotensin II, but not to 5-HT, than those of controls. The increase in sensitivity does not implicate Ca^{2+} channels or Ca^{2+} itself since cardiomyopathic hamsters aortae are not more sensitive to KCl- and Ca^{2+}-induced contraction. The greater effect of PMA on cardiomyopathic hamster aortae suggests that the increase in sensitivity to phenylephrine and angiotensin II involves an enhanced activity of PKC.

Keywords: Cardiomyopathic hamsters; contractility; aortic rings; phenylephrine; angiotensin II; 5-hydroxytryptamine; G-proteins; PKC; Ca^{2+}

Introduction

The cardiomyopathic hamster is a widely used experimental model for studying congestive heart failure (Bajusz, 1969; Liu & Tilley, 1980; Sonnenblick *et al.*, 1985; Sole & Liew, 1988; Schlenker & Burbach, 1990; Weisman, 1993; Carbone *et al.*, 1995; Lambert *et al.*, 1995b). The cardiac disease of this strain is genetically determined and it has been well characterized in terms of myocardial injuries. The evolution of the disease shows generally four steps (Jasmin & Eu, 1979; Jasmin & Proschek, 1982; Hunter *et al.*, 1984; Chemla *et al.*, 1991; Ver Donck *et al.*, 1994). Firstly, a prenecrotic stage takes place in the first months with no pathological manifestations. Secondly, myocardial multifocal necrosis appears between 60 and 90 days. Thirdly, a period of cicatrization and a gradual evolution towards cardiac hypertrophy occurs between 90 and 120 days. Finally a stage of severe cardiac failure is observed from the 120th day until the death of the animals. Classical pathological symptoms associated with cardiac heart failure can be observed such as decreased cardiac output, pulmonary oedema and venous congestion.

Only a few studies have been performed to investigate the status of vascular reactivity in this animal model. For example, decrease in the vasorelaxant response to acetylcholine has been reported in the micro-circulation of cardiomyopathic hamsters using cheek pouch arterioles (Mayhan & Rubinstein, 1992). Microvascular spasms have also been reported and pointed out as a possible cause of myocardial injuries (Factor *et al.*, 1982). Finally, Hunter & Elbrink (1982) have observed an increased contractility in aortic spiral strips of cardiomyopathic hamsters to noradrenaline, phenylephrine, isoprenaline, histamine and 5-hydroxytryptamine (5-HT). The aim of the present study was to investigate further vascular reactivity and to assess the contribution of the different vasoconstrictor signalling pathways in the modifications observed.

Methods

Vessel preparation

The experiments were performed on thoracic aortae taken from cardiomyopathic hamsters (CHF 146, Canadian Hybrid Farms, Halls Harbour, NS, Canada) and age-matched inbred

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control (CHF 148) of either sex (125–150 day old, unless specified otherwise). The animals were killed by decapitation after being narcotized by a short exposure to a 100% CO_2 atmosphere. The vessels were cleaned of all fat and connective tissue and cut into 6-mm rings. The endothelium was removed mechanically in some rings by inserting one arm of a fine forceps into the lumen and then rolling back and forth ten times on a gauze soaked with physiological solution. The rings were then mounted in 20-ml organ baths filled with Krebs-Henseleit buffer of the following millimolar composition: NaCl 118, KCl 4, $CaCl_2$ 2.5, KH_2PO_4 1.2, $MgSO_4$ 1, $NaHCO_3$ 24, D-Glucose 11. The solution was gassed with 95% O_2: 5% CO_2 and warmed at 37°C. The preparations were then connected to a strain gauge (Grass FT03) and isometric tension was recorded on a Grass polygraph (model 79). The preparations were stretched to an optimal 2 g resting tension, as determined with repeated KCl 20 mM contractions. In preparations in which endothelial cells were removed, the addition of 1 μM acetylcholine to KCl (20 mM) preconstricted rings did not induce any relaxation. The preparations were allowed to stabilize for 30 min before experimentation. Each ring was used for a single dose-response curve (Furchgott & Zawadzki, 1980; Dumont & Lamontagne, 1995).

Histological determinations were made to evaluate the thickness of the vessel media. The vessels were cut by cryotomy into 100 μm slices. Staining was with Gill's haematoxylin (3 min) and Gomori's trichromide (3 min) (Hould, 1984). Media thickness of both control and cardiomyopathic aortae were compared by computer image analysis (Moussa & Cartilier, 1996).

Experimental protocols

The function of the endothelium in cardiomyopathic hamsters was compared to that in age-matched controls in a first experimental series, by performing dose-response curves to acetylcholine (0.1 nM–10 μM) after preconstriction with phenylephrine (0.3 μM). In this particular protocol, hamsters of 30, 75, 125, 200 and 300 days of age were used in order to assess age-dependent modification of endothelial cells.

To investigate possible changes in vascular contractility in cardiomyopathic hamsters, cumulative dose-response curves to phenylephrine (1 nM–0.1 mM), angiotensin II (10 pM–0.3 μM) and 5-HT (1 nM–0.1 mM) were performed in intact and endothelium-denuded rings of cardiomyopathic and age-matched controls. The contractile response of smooth muscle cell depolarized with KCl (1 mM–0.1 M) was also tested in endothelium-denuded rings of both cardiomyopathic hamsters and age-matched controls.

The following series of experiments was performed in order to identify the component of the signalling pathways involved in the increased contractility observed with the preceding studies. To assess changes in the activity of G-proteins, dose-response curves to NaF (3–30 mM) were obtained in endothelium-denuded aortic rings of cardiomyopathic hamsters and age-matched controls. Likewise, the activity of protein kinase C (PKC) was assessed with dose-response curves to phorbol 12-myristate 13-acetate (PMA, 1–10 μM).

Finally, sensitivity to Ca^{2+} in cardiomyopathic and control hamsters was tested by use of a modified Krebs-Henseleit buffer in which $CaCl_2$ was omitted, 40 mM KCl was added, and NaCl was reduced to 78 mM in order to maintain the isotonicity. Propranolol (1 μM) and phentolamine (10 μM) were added to the modified solution to eliminate adrenoceptor responses that would have resulted from depolarization-induced catecholamine release (Kähönen *et al.*, 1994). This depolarizing physiological solution was used to keep voltage-gated Ca^{2+} channels open. Cumulative dose-response curves to $CaCl_2$ (10 μM–1 mM) were then obtained in endothelium-denuded aortic rings of cardiomyopathic hamsters and age-matched controls.

Statistical anaylsis

Values represent the mean±s.e.mean. Dose-response curves which exhibited sigmoidal shape were analyzed by a curve-fitting analysis programme (De Léan *et al.*, 1978). This programme allows the estimation of parameters, such as EC_{50}, maximum response and slope factor, and statistical comparison of several dose-response curves. All other dose-response curves were compared by analysis of variance (Systat for Windows). Probability values (P) smaller than 0.05 were considered significant.

Drugs

A stock solution of angiotensin II (Sigma Chemical, St Louis, MO, U.S.A.) 1 mM was prepared in Krebs-Henseleit buffer and kept at −20°C until use. Propranolol (Sigma) and phentolamine (Sigma) were added directly to the buffer. All other drugs were prepared as 10 mM stock solutions: PMA (Sigma) was dissolved in dimethyl sulphoxide (DMSO), phenylephrine (ICN Biochemicals, Cleveland, Ohio), acetylcholine (Sigma) and 5-HT were dissolved in distilled water. The final concentration of DMSO in the bath was 0.14% and did not induce any contraction. All subsequent dilutions of drugs were made in water or buffer.

Results

No difference was observed when comparing wall thickness of vessels from cardiomyopathic and control hamsters (Table 1).

Phenylephrine (0.3 μM) induced a tension of 1.34±0.23 g in aortic rings of 125-day-old cardiomyopathic hamsters and of 1.14±0.63 g in age-matched controls ($n=4$, $P>0.05$). Acetylcholine (0.1 nM–10 μM) added to these preconstricted rings induced a similar relaxation, with comparable log[EC_{50}] (−7.08±0.12 vs −7.18±0.12 for cardiomyopathic and control animals respectively, $n=4$, $P>0.05$) and maximal responses (60.4±13.9 vs 57.1±13.6% relaxation for cardiomyopathic and control animals respectively, $n=4$, $P>0.05$). The same experimental protocol performed with cardiomyopathic and control animals of 30, 75, 200 and 300 days of age yielded comparable results (data not shown).

Vessels of cardiomyopathic hamsters were more sensitive to phenylephrine-induced constriction than were those of control animals, the difference being modest in the presence of an intact endothelium (log[EC_{50}] of −6.55±0.07 vs −6.36±0.07, respectively, $n=6$, $P=0.008$) but more pronounced without endothelium (log[EC_{50}] of −7.25±0.05 vs −6.83±0.05 respectively, $n=6$, $P<0.001$, Figure 1). Comparable maximum responses to phenylephrine in intact and denuded aortic rings were observed between cardiomyopathic and control animals (Figure 1).

Intact and endothelium-denuded rings constricted to an-

Table 1 Aortic media thickness of control and cardiomyopathic hamsters of 125–150 days of age

	n	*Media* (mm²)	*Lumen* (mm²)	*Media/Lumen* (%)
Control	3	0.308±0.006	0.452±0.009	68.3±1.2
Cardiomyopathic	2	0.253±0.004	0.395±0.008	64.3±0.3

Between 5 and 15 aortic slices per animal were analyzed and averaged to obtain a representative value. *n:* number of aortae analyzed.

giotensin II up to a concentration of 30 nM. Above this concentration, rings relaxed despite further addition of the vasoconstrictor. Dose-response curves obtained in such conditions displayed a bell-shaped relation. These curves were fitted as two-step dose-response curves with the final step constrained to zero. Comparable maximum responses to angiotensin II were observed between cardiomyopathic and control animals. However, aortae taken from cardiomyopathic animals were more sensitive to angiotensin II than those of control animals, either with intact ($\log[EC_{50}]$ of -8.41 ± 0.1 vs -8.06 ± 0.1, $n=6$, $P<0.001$) or denuded rings ($\log[EC_{50}]$ of -8.67 ± 0.07 vs -8.26 ± 0.06, $n=6$, $P=0.001$, Figure 2).

In contrast, intact and endothelium-denuded rings of cardiomyopathic hamsters were not more sensitive to 5-HT-induced constriction. Moreover, maximum responses were reduced in cardiomyopathic hamster vessels compared with control (1.36 ± 0.05 g vs 1.68 ± 0.06 g, respectively, $n=6$, $P<0.001$) in the presence of an intact endothelium, and (1.28 ± 0.06 g vs 1.56 ± 0.07 g, respectively, $n=6$, $P=0.03$) without endothelium (Figure 3).

Dose-response curves to KCl (1 mM–0.1 M) performed in endothelium-denuded aortic rings taken from cardiomyopathic and control animals were found to be statistically comparable ($\log[EC_{50}]$ of -1.69 ± 0.02 vs -1.69 ± 0.01, respectively, $n=6$, $P>0.05$).

Endothelium-denuded aortic rings responded to the G-protein activator, NaF (3–30 mM), in a dose-independent fashion, with no measurable contraction at 3 mM and a maximum response at 10 mM. No difference in maximum response to NaF was observed with this concentration (1.90 ± 0.30 g in cardiomyopathic vs 2.18 ± 0.60 g in control aortae at 10 mM, $n=4$, $P>0.05$).

PKC activation with PMA induced stronger contractions in cardiomyopathic than in control animals at 3 μM (0.22 ± 0.02 g vs 0.07 ± 0.03 g, respectively, $n=6$, $P=0.03$) and at 10 μM (0.70 ± 0.04 vs 0.49 ± 0.08 g, respectively, $n=6$, $P=0.048$, Figure 4).

In contrast, aortic rings from cardiomyopathic animals were less sensitive to a cumulative addition of $CaCl_2$ to KCl-depolarized rings compared with those of control animals

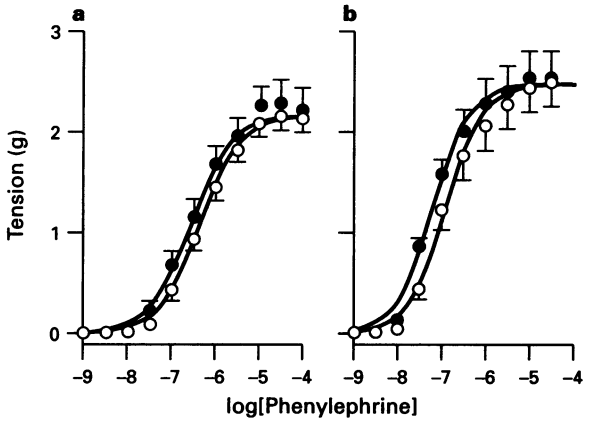

Figure 1 Dose-response curves to phenylephrine using intact (a) and endothelium-denuded (b) aortic rings of cardiomyopathic (●) and control (○) hamsters. Phenylephrine was more potent in cardiomyopathic than in control vessels in both intact ($\log[EC_{50}]$ of -6.55 ± 0.07 and -6.36 ± 0.07, respectively, $n=6$, $P=0.008$) and endothelium-denuded ($\log[EC_{50}]$ of -7.25 ± 0.05 and -6.83 ± 0.05, respectively, $n=6$, $P<0.001$) preparations.

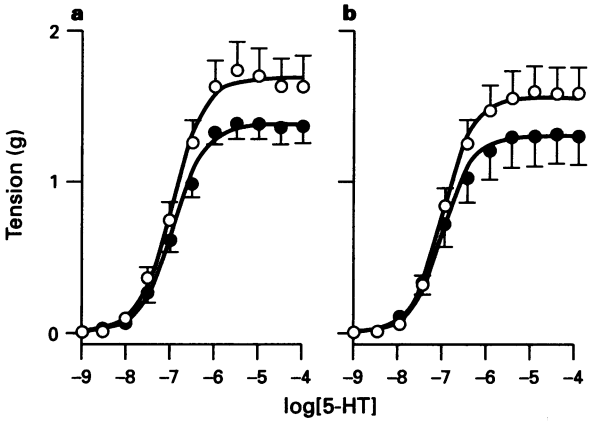

Figure 3 Dose-response curves to 5-hydroxytryptamine (5-HT) using intact (a) and endothelium-denuded (b) aortic rings of cardiomyopathic (●) and control (○) hamsters. Maximum response to 5-HT was decreased in cardiomyopathic vessels compared to control in both intact (a, 1.36 ± 0.05 g vs 1.68 ± 0.06 g, respectively, $n=6$, $P<0.001$) and endothelium-denuded (b, 1.28 ± 0.06 g vs 1.56 ± 0.07 g, respectively, $n=6$, $P=0.03$).

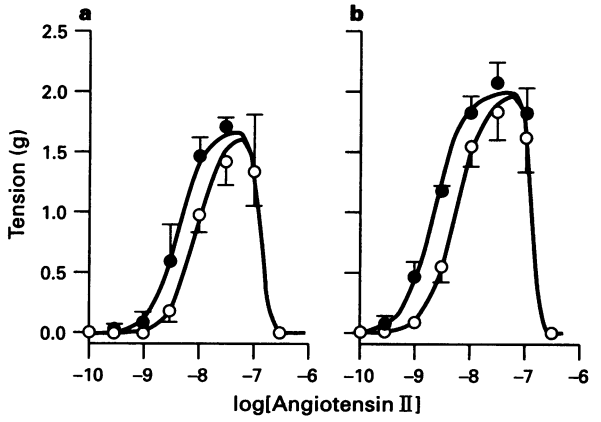

Figure 2 Dose-response curves to angiotensin II on intact (a) and endothelium-denuded (b) aortic rings of cardiomyopathic (●) and control (○) hamsters. Angiotensin II was more potent in cardiomyopathic than in control vessels in both intact ($\log[EC_{50}]$ of -8.41 ± 0.1 and -8.06 ± 0.1, respectively, $n=6$, $P<0.001$) and endothelium-denuded ($\log[EC_{50}]$ of -8.67 ± 0.07 and -8.27 ± 0.07, respectively, $n=6$, $P=0.001$) preparations.

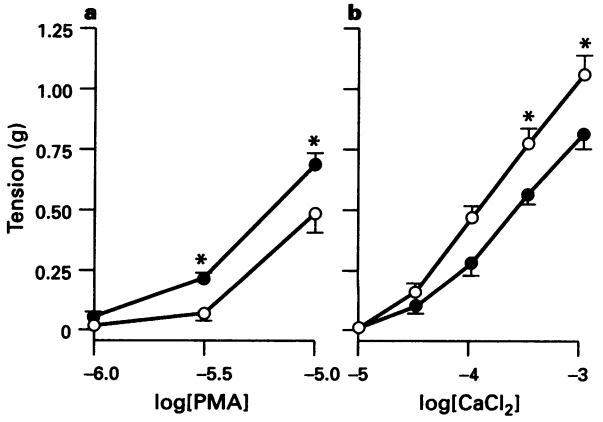

Figure 4 Changes in tension as a function of the logarithm of PMA concentration (log[PMA], a, $n=6$) and $CaCl_2$ concentration ($\log[CaCl_2]$, b, $n=8$) of endothelium-denuded control (○) and cardiomyopathic (●) aortic rings (*$P<0.05$).

(0.58±0.04 g vs 0.79±0.06 g at 0.3 mM, respectively, $n=8$, $P=0.03$ and 0.83±0.06 g vs 1.08±0.08 g at 1 mM, respectively, $n=8$, $P=0.045$, Figure 4).

Discussion

The aim of this study was to evaluate the vascular reactivity in cardiomyopathic (CHF 146) and age-matched inbred control (CHF 148) hamsters. It has been reported by Mayhan & Rubinstein (1992) that the endothelial cell layer in the microcirculation of cardiomyopathic hamsters is damaged. A recent study has demonstrated altered endothelium-dependent vascular responses in femoral arteries of dog with rapid ventricular pacing heart failure (Kaiser *et al.*, 1989). However, this canine model of heart failure differs from the cardiomyopathy of the hamster in many points, and comparison should be made with caution. In the present study, comparable responses to acetylcholine on phenylephrine-preconstricted aortic rings of cardiomyopathic and control hamsters suggest that in these animals, the endothelium is intact and fully functional in large conduit arteries.

It has been observed that aortic strips of cardiomyopathic hamsters are more sensitive to different peptide and non-peptide vasoconstrictors (Hunter & Elbrink, 1982). To verify the latter, dose-response curves to phenylephrine, angiotensin II and 5-HT were performed in aortae from cardiomyopathic and control hamsters of 125–150 days of age. Intact and endothelium-denuded rings were used. Aortae from cardiomyopathic hamsters showed an increased sensitivity to phenylephrine and angiotensin II, without any increase in maximum response, regardless of the presence of an intact endothelium. The comparable maximum response obtained in both groups of animals suggest that there is no modification in either cellular volume or cell number in the vascular wall. This is further supported by the absence of histological evidence of increased media thickness in the cardiomyopathic vessels. On the other hand, the increased sensitivity might suggest modifications in the receptor population or changes in the vasoconstrictor signalling pathways. In contrast, an unchanged sensitivity and a decreased maximal response were observed with 5-HT, which rules out a generalized increased contractility to all vasoconstrictors in cardiomyopathic hamsters. Interestingly, diabetic rat aortae show an increased contractility to noradrenaline, but not to 5-HT (MacLeod & McNeill, 1985). Thus, the vascular response to 5-HT seems to be affected differently from other vasoconstrictors in pathologies.

Karliner *et al.* (1981) reported an increased population of α_1-adrenoceptors (postsynaptic) in the right ventricular muscle strips of cardiomyopathic hamsters. In agreement with these observations, Böhm *et al.* (1986) demonstrated an increase in the response to α_1-adrenoceptor agonist, phenylephrine in the isolated papillary muscle of cardiomyopathic hamsters. Interestingly, the number of β-adrenoceptors increases at an early stage, but is reduced during the severe hypertrophic stages of the disease (Saito *et al.*, 1994). Cardiac receptors to angiotensin II (AT_1) are also increased in CHF 146 cardiomyopathic hamsters (Lambert *et al.*, 1995a). It is therefore possible that an increased number of both α_1-adrenoceptors and AT_1 receptors in the aortae of cardiomyopathic hamsters could explain the increased sensitivity that we observed. According to this, receptor-independent contraction induced by KCl was not increased in the cardiomyopathic hamster.

The increase in sensitivity of the vessels to phenylephrine and angiotensin II are comparable, with a reduction in the EC_{50} value of approximately half a logarithmic unit. Such a similar difference could perhaps be best explained by a modification in the signalling pathways common to both vasoconstrictors. To characterize this possibility further, different steps of the vasoconstriction signalling pathways were stimulated and evaluated separately. Until now, no studies have clearly reported modifications in the cardiomyopathic vascular smooth muscle cell G-protein activity. In the cardiomyocytes of cardiomyopathic animals, a decoupling between G_s and adenylyl cyclase occurs so that NaF or forskolin are no longer effective during severe stages of the disease (Sethi *et al.*, 1994). F^- is known to form AlF_4^- complex and replace gamma phosphate of GTP increasing stimulation of G-protein coupled to phospholipase C, which in turn induces hydrolysis of PIP_2 in vascular and non-vascular smooth muscle cells (Nishizuka, 1984; Berridge & Irvine, 1984; Bigay *et al.*, 1985). Thus, in our studies, NaF was used to stimulate directly G-proteins of cardiomyopathic and control hamster vessels. Since under our conditions, NaF did not induce a dose-dependent response, it is impossible to assess any change in sensitivity to this vasoconstrictor. On the other hand, maximum responses to NaF were comparable between cardiomyopathic and control aortae. This observation is in agreement with our results obtained with phenylephrine and angiotensin II.

It has been reported that PIP_2-IP_3 and 1,2 diacylglycerol(DAG)-PKC pathways are enhanced in the cardiomyopathic heart, leading to hypertrophy (Kawaguchi *et al.*, 1994). On the other hand, recent studies suggest that DAG pathways increase in the early stages of the disease (30 days) and decrease in the later stages (Okumura *et al.*, 1994). However, little is known about IP_3 and DAG formation in the vascular smooth muscles of cardiomyopathic hamsters. It is well recognized that PKC phosphorylates both myosin light chain (MLC) and MLC-kinase and decreases MLC-phosphatase activity leading to contraction of the vascular smooth muscle cells (Ikebe *et al.*, 1987). Phosphorylation of two regulatory proteins, calponin and caldesmon, may also be involved in PKC-induced contraction of smooth muscles (Walsh *et al.*, 1994). Since phorbol esters mimic DAG and activate directly several isoenzymes of PKC (Walsh, 1994), PMA was used in our study to assess a possible increase in the activity of these enzymes in the aortae of cardiomyopathic hamsters. Our results showed that the response to phorbol ester stimulation is enhanced in aortae of cardiomyopathic hamsters compared to control animals and this is certainly an interesting possible explanation of the higher sensitivity of cardiomyopathic aortae to phenylephrine and angiotensin II. However, even though IP_3 is probably not implicated in PMA-induced contraction, we cannot rule out a contribution of IP_3 in the increased contractility to phenylephrine and angiotensin II.

Finally, an increased sensitivity to Ca^{2+} or enhanced Ca^{2+} influx might also explain the higher sensitivity of cardiomyopathic vessels. An intracellular Ca^{2+} overload is generally observed in the cardiomyocytes as well as skeletal muscles of cardiomyopathic hamsters (Strobeck *et al.*, 1979; Ward & Cameron, 1984; Jasmin & Proschek, 1984; Olbrich *et al.*, 1988; Ver Donck *et al.*, 1994). Decreased genetic expression and activity of Na^+-K^+ ATPase have been characterized and seem to lead to the higher concentration of intracellular Ca^{2+} (Makino *et al.*, 1985; Tsuruya *et al.*, 1994). This excess in Ca^{2+} is observed at about 30 days of age and seems to be one of the major causes of the lesions in the cardiac cells (Ver Donck *et al.*, 1994). The mitochondria are also overloaded in Ca^{2+}. This in turn makes the H^+-ATPase less functional and a lack of ATP is observed (Proschek & Jasmin, 1982). Since in our studies both cardiomyopathic and control aortae contracted similarly with increasing doses of KCl, modification in the functionality of voltage-gated Ca^{2+} channels or Ca^{2+} overload, although possibly present, might not explain the increased contractility observed with phenylephrine and angiotensin II. Moreover, cardiomyopathic hamster aortae were less sensitive to an increasing concentration of Ca^{2+} in depolarized vessels indicating that their contractile apparatus is certainly not more sensitive to Ca^{2+}, at least in the absence of a fully stimulated PKC. These latter results are similar to those observed with 5-HT. It would be tempting to hypothesize that vasoconstriction produced by 5-HT relies less on PKC, compared with phenylephrine and angiotensin II, explaining the different results observed. On the other hand, Ca^{2+} would play a more important role in 5-HT induced constriction. Al-

though not demonstrated in vascular smooth muscle, Chopra *et al.* (1994) have reported that constriction of large bronchioles with 5-HT was less affected by PKC inhibition, compared with small bronchioles.

In conclusion, our data showed that the endothelium is fully functional in aortae of cardiomyopathic hamsters, whereas the vessels are more sensitive to phenylephrine and angiotensin II, but not to 5-HT. The increased contractility observed in cardiomyopathic hamsters with angiotensin II and phenylephrine may be due to an increased activity of PKC. The importance of an enhanced vascular reactivity in the pathophysiology of the cardiomyopathic hamster to these vasconstrictors is unknown and should be of interest in further studies.

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