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Increased influence of endothelium in obese Zucker rat aorta

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Abstract—The ability of endothelium to alter contractile events in phenylephrine (PE)-triggered contraction has been tested on ring segments of the thoracic aorta removed from obese Zucker rats (plasma cholesterol 3·63 mM; n = 8) and from age matched lean rats (plasma cholesterol 2·38 mM; n = 8). In normal medium, PE (1 μM) elicited similar contractions in endothelium-denuded arteries of both strains. However, the presence of endothelium reduced these contractile events and the endothelium-dependent relaxation induced by carbachol (10 μM) was higher in obese rats. In rings incubated in Ca^{2+} free medium containing EGTA (1 mM), PE (1 μM) induced a phasic contraction and a sustained contraction following addition of Ca^{2+} (2·5 mM) to the medium. The phasic contraction was due to intracellular Ca^{2+} release, whereas the sustained response was dependent on extracellular Ca^{2+} influx. In endothelium-free preparations, the size of both the phasic and sustained contraction was similar for the two strains. The Ca^{2+} antagonist gallopamil (1 μM) reduced the sustained contraction of lean (24%) and obese (34%) rats without affecting the phasic contraction. In preparations possessing endothelium, the sustained, but not the phasic contraction, of both strains was inhibited. This inhibitory effect of endothelium on the sustained contraction was significantly higher in obese than in lean rats. Thus, it can be concluded that phenylephrine elicited quantitatively and qualitatively similar contractions in obese and lean rats. In both strains, the endothelium diminished the contraction induced by PE, however, this effect was more pronounced in obese rats than in lean ones. These results may explain, in part, the described absence of atherosclerotic lesions in the obese strain.

Hypercholesterolaemia is one of the most common risk factors associated with atherosclerosis, and the damage of endothelial cells is considered as a possible promoter of the atherosclerotic process (Ross & Glomset 1976; Faggiotto et al 1984). Furchgott & Zawadzki (1980) first reported that the relaxing effect of acetylcholine on rabbit aorta was mediated by a substance released from the endothelial cells, termed 'endothelium derived relaxing factor' (EDRF). Since these original observations, several studies have focused on the implication of endothelial function in pathological states such as vasospasm, hypertension and atherosclerosis (Habib et al 1984; Vanhoutte 1986; Vanhoutte & Lüscher 1987). It has been reported that a decreased endothelium-dependent relaxation is associated with atherosclerotic lesions in aorta from rabbits fed a high cholesterol diet (Habib et al 1984; Jayakody et al 1985; Verbeuren et al 1986). Moreover, the vascular reactivity of these atherosclerotic blood vessels was also affected (Henry & Yokoyama 1980; Verbeuren et al 1986).

The obese Zucker rat exhibits hypercholesterolaemia and hypertriglyceridaemia due to a genetic defect (Bray 1977) and has been proposed as an animal model of atherogenesis (Stoltz et al 1981). Thus the present work was undertaken to study the contractile properties of obese rat aorta in comparison with those of the lean rats. In addition, as a potent basal release of EDRF has been recently demonstrated in rat aorta (Griffith et al 1984; Martin et al 1986) the influence of endothelium was also investigated.

Materials and methods

Male lean (Fa/-) and obese (fa/fa) Zucker rats (14–15 weeks old) were purchased from CSEAL (Orleans, France). One week before the beginning of the experiments the systolic blood pressure was determined by the indirect tail-cuff method (BP recorder, Ugo Basile). The individual values are the average of five consecutive measurements. Following fasting (18 h), blood samples were obtained from the retro-orbital vein under light ether anaesthesia. Plasma cholesterol and plasma triglyceride were determined enzymatically (Abbott Laboratories, ABA 200).

On the day of the experiment, the rats were weighed and then killed by cervical dislocation. The thoracic aorta was removed, cleaned of its surrounding tissue and cut into 2 mm wide rings under a dissecting lens. Two rings from each animal were suspended in organ baths containing 10 mL of physiological solution (see below for composition) under a tension of 2 g at 37°C and gassed with 95% O_2/5% CO_2. Contractile responses were measured using force displacement transducers (Statham UC2) coupled to a Gould 8000 S polygraph. The endothelium of one ring was mechanically disrupted. A 1 h equilibration period was allowed before experimentation. Normal physiological solution was composed of (mM): NaCl, 118; KCl, 5·4; $CaCl_2$, 2·5; KH_2PO_4, 1·2; $MgSO_4$, 0·6; $NaHCO_3$, 25; glucose, 11. Gallopamil, 1 μM was added in some experiments. The Ca^{2+} free-medium, with or without gallopamil, was prepared by substituting $CaCl_2$ with 1 mM EGTA. After equilibration, the preparation was subjected to a dose of phenylephrine (PE, 1 μM) which was determined to elicit a near maximal contraction (> 95%) in the two strains in preliminary experiments. When the contraction was stable, carbachol (10 μM) was tested to verify the integrity of the endothelium (Furchgott & Zawadzki 1980). The tissues were then washed for 50 min in normal physiological solution followed by 20 min in Ca^{2+} free-medium. PE (1 μM) was introduced into the bath and after a 7 min interval 2·5 mM $CaCl_2$ was added. The resultant contractions were recorded for 30 min. At the end of the organ bath experiment the rings were blotted, weighed and their protein content determined by using the Bradford procedure (1976).

Statistics. All parameters are expressed as the mean ± s.e. mean. Statistically significant differences between the various parameters were determined using a one way analysis of variance.

Materials. 1-Phenylephrine hydrochloride, carbachol, ethyleneglycol-bis (β-aminoethyl ether) *N*, *N*, *N'*, *N'*-tetraacetic acid (EGTA) were obtained from Sigma. Gallopamil (D600).

Results

Characteristics of animals and aortas studied. The characteristics of the groups studied are shown in Table 1. Obese rats exhibited significantly higher body weight, plasma cholesterol and plasma triglyceride levels than the age matched lean rats. Conversely, blood pressure, ring aortic weight and absolute amount of total aortic protein were the same in the two strains.

Contractile response to PE in normal and Ca^{2+} free-medium, influence of endothelium. In normal medium, the relaxation induced by carbachol (10 μM) in PE precontracted intact preparations was significantly ($P < 0.05$) higher in obese rats (95 ± 2·8%; n = 8) than in lean rats (85 ± 2·9%; n = 8). No

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relaxation could be elicited in preparations denuded of endothelium (Fig. 1). PE triggered similar contractions in endothelium-free rings of both strains (Fig. 2). The presence of endothelium reduced the PE-response in the obese rats (50%; $P<0.001$) and, to a lesser extent, in the lean rats (36%; $P<0.01$) (Fig. 2).

In Ca^{2+} free-medium, PE (1 μM) induced a phasic contraction in aortic rings incubated for 20 min in a Ca^{2+} free EGTA medium. This phasic contraction was of the same magnitude in obese and lean rings with or without endothelium. After calcium addition ($CaCl_2$, 2·5 mM), a similar sustained contraction was obtained in obese and lean rings without endothelium. The presence of endothelium reduced the sustained contraction of both strains. However, this inhibitory effect of the endothelium was significantly greater in obese rats (Figs 2, 3).

Contractile response to PE in normal and Ca^{2+} free-medium with gallopamil (1 μM), influence of endothelium. In gallopamil containing normal medium, PE induced similar contractions in endothelium-free rings of obese and lean rats. The presence of the endothelium greatly reduced these contractions (Fig. 3). In gallopamil containing calcium free-medium, no difference in the PE-induced phasic contraction was observed in either strain with or without endothelium. The sustained contraction observed after calcium addition was strongly inhibited in intact ring relative to those with a damaged endothelium (Fig. 3).

Effect of gallopamil on the different experimental contractions elicited by PE. Comparison of Figs 2 and 3 shows that in normal physiological medium, gallopamil similarly inhibited the whole-PE induced contraction of endothelium-free rings of obese (36±6·7%) and lean (27±4·6%) rats. However, under these

Table 1. Animals and aortic characteristics.

Parameters (Mean values ± s.e.mean)	Lean Rats (8) (Fa/-)	Obese Rats (8) (fa/fa)
Body weight (g)	327±11·9	468±13·3 ***
Systolic blood pressure (mmHg)	134±6·4	130±2·5
Plasma cholesterol (mmol L^{-1})	2·38±0·101	3·63±0·290 **
Plasma Triglyceride (mmol L^{-1})	0·91±0·008	2·84±0·290 ***
Aortic ring weight (mg mm^{-1})	1·25±0·104	1·12±0·113
Total protein ($\mu g\ mm^{-1}$)	80±2·0	73±5·6

**, ***: $P<0.01$ and $P<0.001$ for the comparison between the two strains.

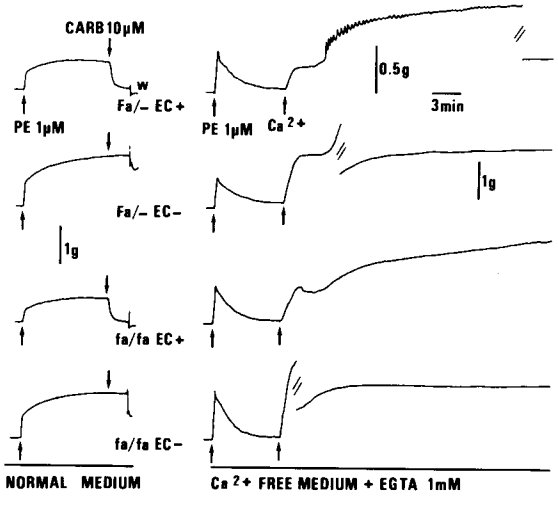

FIG. 1. Representative recordings of contraction of the lean (Fa/-) and obese (fa/fa) rat aortas produced by phenylephrine (PE 1 μM) in normal medium and in Ca^{2+} free EGTA medium in the absence (EC−) or presence (EC+) of endothelial cells. Effect of Ca^{2+} introduced 7 min after PE addition. Note that in normal medium, carbachol (CARB) produces a relaxation only on EC+ preparations. The break in the tension recordings denotes a change in scale. W = wash.

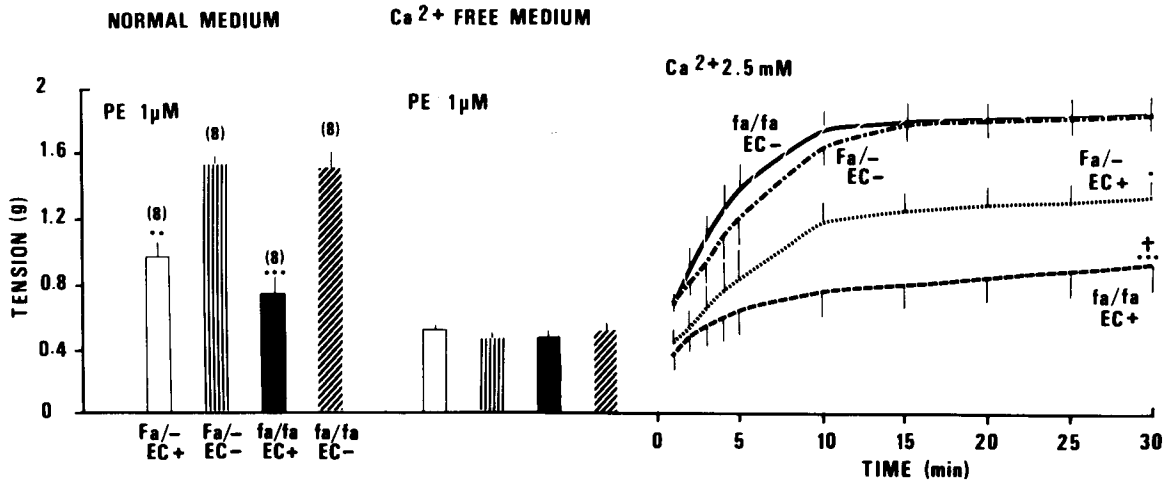

FIG. 2. Effects of phenylephrine (PE 1 μM) on lean (Fa/-) and obese (fa/fa) rats aorta incubated in normal medium (left histogram) or in Ca^{2+} free EGTA medium (four middle histograms). Effect of Ca^{2+} introduced 7 min after PE addition in Ca^{2+}-free medium (right part, see also Fig. 1). Values represent mean ± s.e. mean and number of experiments are in parentheses. **, *** significantly different from corresponding strain (EC−) ($P<0.01$; $P<0.001$) for comparison in normal and Ca^{2+}-free medium + Ca^{2+} 2·5 mM at 30 min only. $P<0.05$ for comparison between Fa/- EC+ and fa/fa EC+ in Ca^{2+}-free medium + Ca^{2+} 2·5 mM at 30 min only.

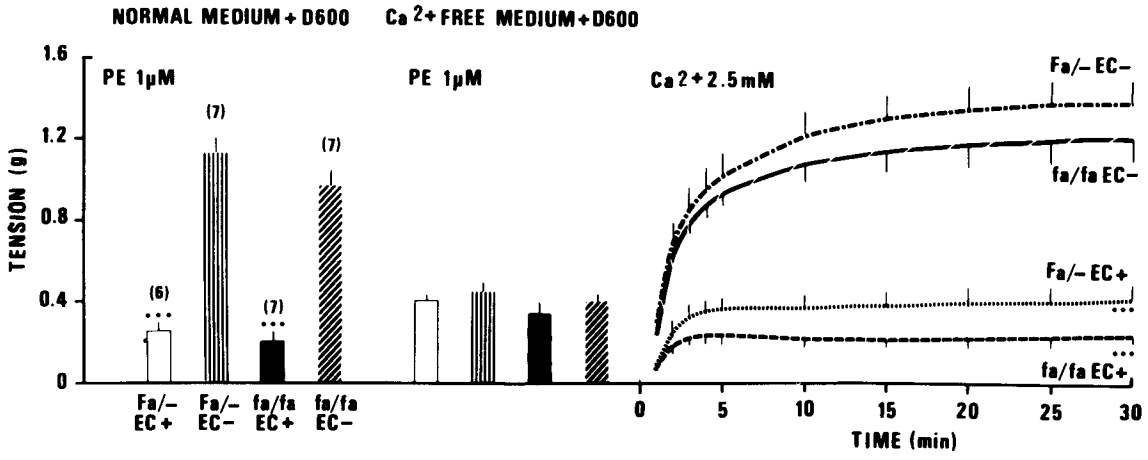

FIG. 3. As Fig. 2, except that the medium contains gallopamil (D600) (1 μM). Note that in these conditions, the contractions of preparations with endothelium (EC+) are higher in Ca^{2+}-free medium (intermediate histograms) than in normal medium (left histograms).

conditions, the inhibitory effect of the endothelium was stronger. In calcium free-medium, gallopamil did not modify the intensity of the phasic contraction elicited by PE in the different groups. Nevertheless, the calcium antagonist decreased the sustained contractions in all preparations. The respective inhibitions were 69 and 24% for lean rats with and without endothelium and 74 and 34% for obese rats with and without endothelium. The inhibiting effect of endothelium on the sustained contraction in the two strains was significantly increased by the presence of gallopamil.

Discussion

The present experiments demonstrate that, despite increased levels of plasma cholesterol and plasma triglyceride, obese rats exhibit similar blood pressure and aortic protein content to lean rats. This leads to comparable contractile properties of the vascular smooth muscle independent of the endothelium influence. In vascular smooth muscle, contractions induced by α-adrenoceptor agonists are mediated by an influx of extracellular Ca^{2+} and by mobilization of intracellular Ca^{2+} stores (Bohr 1963; Sitrin & Bohr 1971; Deth & Van Breemen 1974). In Ca^{2+} free-medium the contractile response induced by α-adrenoceptor agonists can be separated into two phases, a phasic component due to mobilization of intracellular Ca^{2+} stores and a sustained component obtained after readdition of Ca^{2+} to the medium which is dependent on extracellular Ca^{2+} (Deth & Van Breemen 1974; Hester & Carrier 1978; Auguet & Defeudis 1982). Extracellular Ca^{2+} may enter vascular smooth muscle cells through potential-dependent channels (PDC) or through receptor-linked channels (RLC) (Bolton 1979). Calcium entry blockers selectively inhibit potential dependent channels (Fleckenstein 1977; Flaim 1982). The present study shows that in rat isolated aorta incubated in a Ca^{2+} free-medium, the selective α_1-adrenoceptor agonist, PE, induces a phasic contraction which is followed by a sustained tonic contraction after addition of Ca^{2+} to the medium.

In the presence of the calcium entry blocker gallopamil at a level (1 μM) sufficient to nearly abolish the potassium-induced calcium influx due to PDC stimulation (Nghiem et al 1982; Bou et al 1983; Delaflotte et al 1989), the phasic contraction was not modified, whereas the sustained response was similarly reduced in both obese and lean rings without endothelium. It could be argued that gallopamil interferes at the adrenoceptor level but this assumption is ruled out since, unlike prazosin, it is ineffective on the phasic contraction. In addition, higher concentrations are needed to elicit its effect on adrenergic binding sites in rat brain membranes (Fairshurst et al 1980) and smooth muscle (Jim et al 1981).

Thus, the present study shows that PE triggers at least two different types of Ca^{2+} events; intracellular Ca^{2+} release which produces the phasic contraction and extracellular Ca^{2+} influx which produces the sustained contraction. The processes are dependent on α-adrenoceptor activation and thus both the phasic and sustained components of the contraction are dose-dependently inhibited by the α_1-adrenoceptor antagonist prazosin (Delaflotte et al 1989). This has also been previously observed in rabbit aorta (Cauvin et al 1982). Therefore, it may be postulated that, on rat aorta, full adrenoceptor agonists induce extracellular Ca^{2+} influx via two different pathways, only one of them being sensitive to gallopamil. This assumption may be supported by the fact that part of the noradrenaline-dependent influx is resistant to block by other calcium entry blockers, e.g. nifedipine (Godfraind et al 1986). These Ca^{2+} events are comparable in obese and lean rat aortas without endothelium, indicating equal vascular reactivity in the two strains.

Endothelial cells may influence the tone of the underlying smooth muscle cells via basal or stimulated activity (Furchgott & Zawadzki 1980; Martin et al 1986). The present work indicates that both carbachol-induced endothelium-dependent relaxation and the basal influence of endothelium on PE-induced contraction are augmented in obese rats. Since the two strains exhibit similar reactivity, a modification of the vascular smooth muscle sensitivity to endothelium may be ruled out and an increased activity of endothelium may be postulated. Considering the influence of endothelium on the different calcium events in PE-triggered contraction, the results presented here show that in aorta endothelium, spontaneous release of products (e.g. EDRF) decreases the Ca^{2+} influx without modification of intracellular Ca^{2+} release. This difference may be explained by the following assumptions: (i) The basal release of EDRF does not interact with the Ca^{2+} intracellular mobilization. (ii) The basal release of EDRF does not occur in a Ca^{2+} free-medium. This second hypothesis seems the best explanation since the liberation of EDRF by various agonists has been reported to be Ca^{2+} dependent (Long & Stone 1985; Peach et al 1987). Thus in the present study, the influence of the endothelium could only be observed on Ca^{2+} influx when Ca^{2+} was present in the medium

and thus the endothelium was functional. When potential-dependent channels are antagonized by gallopamil, the inhibitory effect of endothelium on Ca^{2+} influx is increased, confirming that, like other calcium entry blockers (e.g. nicardipine, diltiazem), it has no inhibitory effect on the endothelium dependent relaxation (Winquist et al 1985; Jayakody et al 1987). Thus, it may be hypothesized that basally released EDRF is more potent in inhibiting the gallopamil insensitive Ca^{2+} influx through receptor-linked channels in rat aorta than Ca^{2+} influx through potential-dependent channels. In addition, in the presence of gallopamil, the total contraction (phasic and sustained) induced by PE in intact rings was smaller than the PE-induced phasic contraction in Ca^{2+} free-medium. This suggests that in calcium free-medium the phasic contraction is not controlled by endothelial function.

In conclusion, the present results show that, despite high plasma cholesterol levels, there is no impairment of endothelial function in obese rats as previously reported for aorta of rabbits fed a high cholesterol diet (Habib et al 1984; Jayakody et al 1985; Verbeuren et al 1986). On the contrary, an increased influence of endothelium was observed. Although obese rats do not develop atherosclerotic lesions (Amy et al 1988), it is presently unknown whether this augmented endothelial activity reflects a biochemical adaptation to protect the arteries against hypercholesterolaemia.

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