ELSEVIER

European Journal of Pharmacology 313 (1996) 221–227

ejp

Mesenteric and hepatic vascular reactivity in Donryu rats with and without a cholesterol-supplemented diet

Vera Ralevic *, Gjumrach Aliev, Geoffrey Burnstock

Department of Anatomy and Developmental Biology, University College London, Gower Street, London WC1E 6BT, UK

Received 24 June 1996; accepted 28 June 1996

Abstract

Vascular function of Donryu rats fed on a normal or cholesterol-supplemented diet was examined in the isolated perfused mesenteric arterial bed and portally perfused liver. In mesenteric preparations, frequency-dependent vasoconstriction to electrical field stimulation (4–32 Hz, 1 ms, 90 V, 30 s) and dose-dependent vasoconstriction to noradrenaline (0.15–1500 nmol) was similar in both groups. Dose-dependent vasoconstriction to α,β-methylene ATP (0.05–500 nmol) via P_{2X} purinoceptors was significantly impaired in Donryu rats fed on a cholesterol-supplemented diet. In preparations with raised tone (methoxamine 5–35 μM), there was no significant difference in endothelium-dependent relaxation to acetylcholine and ATP, or endothelium-independent relaxation to sodium nitroprusside. In liver preparations, there was no difference in frequency-dependent vasoconstriction to electrical field stimulation (2–32 Hz, 1 ms, 90 V, 30 s), or dose-dependent vasoconstriction to noradrenaline (0.05–500 nmol) and α,β-methylene ATP (0.05–500 nmol) between the groups. In conclusion, in mesenteric arteries, but not in the hepatic portal vasculature of Donryu rats fed on cholesterol P_{2X} purinoceptor function is impaired, but sympathetic neurotransmission is unaffected. Mesenteric endothelial and smooth muscle function is unimpaired.

Keywords: Cholesterol-feeding; Endothelium; P_{2X} purinoceptor; Mesenteric arterial bed, rat; Sympathetic neurotransmission; Donryu rat

1. Introduction

The Pittsburgh Yoshida rat has recently been presented as a novel model of endogenous hyperlipidemia (Fantappiè et al., 1992; Chinellato et al., 1994a,b). This inbred rat model is characterised by high serum lipids as well as by decreased endothelium-dependent relaxation and morphological alteration of thoracic aortic endothelial cells, particularly after feeding on a cholesterol-supplemented diet (Chinellato et al., 1994a,b). Notably, there is an absence of atheromatous lesions or functional and morphological damage of smooth muscle cells in the aorta (Chinellato et al., 1994a,b). This animal model has been developed by innoculation of the Donryu rat with Yoshida sarcoma cells (Omura et al., 1995). Vascular function of the Donryu rat, in the absence or presence of a high cholesterol diet has not been studied.

In experimental animals and in humans, impaired endothelial function in hyperlipidemia and atherosclerosis has been shown, particularly in the aorta and in coronary arteries (Verbeuren et al., 1986; Cohen et al., 1988; Förstermann et al., 1988; Shimokawa et al., 1988; Osborne et al., 1989; Shimokawa and Vanhoutte, 1989; Kang et al., 1995). Selectively augmented and decreased responses to vasoconstrictor agents in hypercholesterolemia and atherosclerosis have also been described (Al-Jubouri and Al-Bayati, 1981; Verbeuren et al., 1986; Heistad et al., 1984; Ibengwe and Suzuki, 1986; Lopez et al., 1989; Trzeciak et al., 1993; Lamping et al., 1994). Perivascular nerves may also be affected; exposure to a high cholesterol diet throughout development and maturation leads to impairment of sympathetic function of the rat tail artery due to impaired storage and release of transmitter (Panek et al., 1985). In 12-month-old Watanabe heritable hyperlipidemic rabbits, a model of familial hypercholesterolemia, there is a reduction in nerve-mediated constrictor responses of the mesenteric artery (Stewart-Lee et al., 1991, 1992).

The aim of this study was to further characterize vascular function in the Donryu rat before and after dietary supplementation with cholesterol. Specifically, we examined sympathetic constrictor function in the hepatic portal vasculature, and sympathetic constriction and endothelium-dependent and -independent relaxation in the mesenteric arterial vasculature.

* Corresponding author. Tel.: +44 (0)171 387 7050; fax: +44 (0)171 380 7349.

PII S0014-2999(96)00514-6

2. Materials and methods

2.1. Animals

Male Donryu rats (2-month-old) were obtained from Charles River Japan. One group of rats was fed on a standard control diet and water ad libitum. The other group received the same diet enriched with 4% cholesterol, 1% cholic acid, 0.5% thiouracil (CCT diet) for 2 months (Joris et al., 1983; Chinellato et al., 1994a,b).

2.2. Isolated mesenteric arterial bed preparation

Rats were killed by asphyxiation with CO_2. Mesenteric beds were isolated and set up for perfusion as described previously (Ralevic et al., 1993). The abdomen was opened and the superior mesenteric artery exposed and cannulated with a hypodermic needle. The superior mesenteric vein was severed, the gut dissected away and the preparation mounted on a stainless steel grid (7 × 5 cm) in a humid chamber (custom made at University College London, London, UK). The preparation was perfused at a constant flow rate of 5 ml min^{-1} using a peristaltic pump (model 7554-30; Cole-Parmer Instrument, Chicago, IL, USA). The perfusate was Krebs solution of the following composition (mM): NaCl 133, KCl 4.7, NaH_2PO_4 1.35, $NaHCO_3$ 16.3, $MgSO_4$ 0.61, $CaCl_2$ 2.52 and glucose 7.8, gassed with 95% O_2-5% CO_2 and maintained at 37°C. Responses were measured as changes in perfusion pressure (mm Hg) with a pressure transducer (model P23XL; Viggo-Spectramed, Oxnard, CA, USA) on a side arm of the perfusion cannula, and recorded on a polygraph (model 7D; Grass Instrument, Quincy, MA, USA). Preparations were allowed to equilibrate for 30 min prior to experimentation.

Frequency-response curves were produced by passing a current (2–32 Hz, 90 V, 1 ms, 30 s) across each preparation between the metal needle and the wire grid (acting as two electrodes) on which the preparation rested. After a recovery period of 10 min, vasoconstrictor responses of preparations to a range of increasing doses (50-μl bolus injections) of noradrenaline and α,β-methylene ATP were assessed. Preparations from each of a Donryu rat fed a normal and a CCT diet were studied simultaneously, and for each particular dose of a substance preparations were injected one immediately after the other. For noradrenaline, individual doses were applied at intervals of at least 2 min at low doses, but as much as 10 min at high doses, depending on the time it took for the tone to return to baseline. Because of desensitization doses of α,β-methylene ATP were applied at 10 min intervals. Approximately 10 min was allowed between consecutive dose-response curves to agonists.

Tone was raised by the addition of methoxamine (5–35 μM) to the perfusate and vasodilator responses to acetylcholine, ATP and sodium nitroprusside examined.

Table 1
Serum lipid spectrum of Donryu rats with and without a cholesterol-supplemented diet (mg ml^{-1})

	Control diet	High-cholesterol diet
Total serum cholesterol	2.57 ± 0.32	7.05 ± 2.04 [a]
High-density lipoprotein	1.78 ± 0.24	1.35 ± 0.29 [a]
Low-density lipoprotein	0.52 ± 0.15	6.83 ± 2.0 [a]
Triglyceride	0.61 ± 0.14	0.45 ± 0.1

Cholesterol levels were determined from $n = 7$ rats in each group.
[a] Significant difference from control ($P < 0.05$).

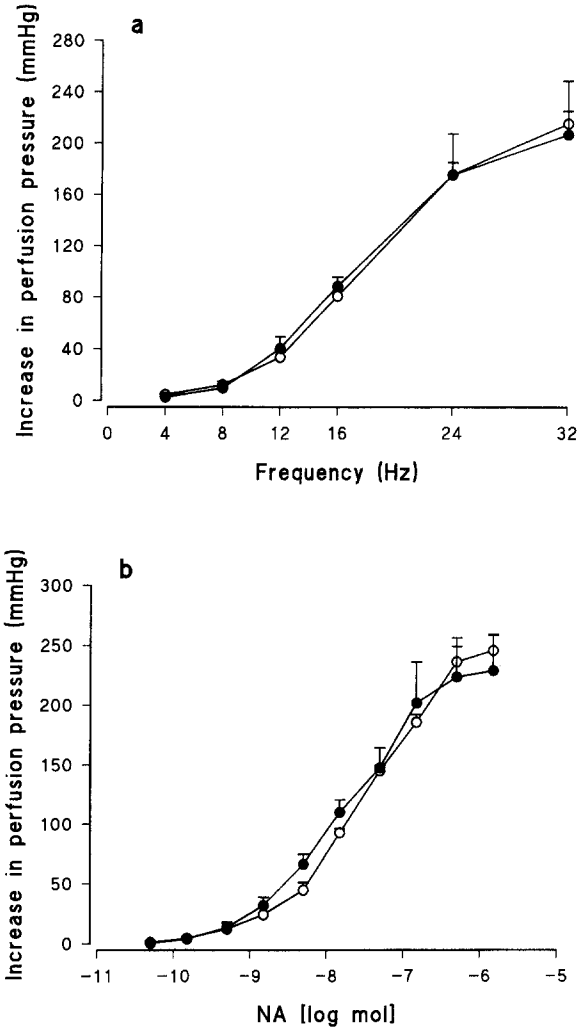


Fig. 1. (a) Frequency-response curves to electrical field stimulation (4–32 Hz, 1 ms, 90 V, 30 s) showing constrictor responses of mesenteric arterial preparations from Donryu rats fed on a control diet (●, $n = 7$) or on a cholesterol-supplemented diet (○, $n = 7$). Data are presented as means with vertical bars indicating S.E.M. (b) Dose-response curves showing constrictor responses to noradrenaline (NA) of mesenteric arterial preparations from Donryu rats fed on a control diet (●, $n = 7$) and Donryu rats fed on a cholesterol-supplemented diet (○, $n = 7$). Data are presented as means with vertical bars indicating S.E.M.

2.3. Isolated liver preparation

Livers were isolated and perfused via the portal vein as described previously (Mathie et al. 1996). The portal vein

was cannulated with a 1-mm bore metal cannula and the liver flushed with approximately 2 ml of Krebs solution. The liver was carefully cut away from surrounding tissues and placed on a metal grid in a humid chamber as described above for the mesenteric arterial bed preparation. The portal vascular bed was perfused at a constant flow rate of 5 ml min^{-1} with Krebs solution at 37°C, gassed with 95% O_2/5% CO_2. Electrical field stimulation (2–32 Hz, 1 ms, 90 V, 30 s) and exogenous application of drugs was as described for the isolated mesenteric arterial bed.

2.4. Cholesterol and triglyceride determinations

Total and high-density lipoprotein (HDL) serum cholesterol levels were determined by the cholesterol oxidase/peroxidase method (Allain et al., 1974) and the low-density lipoprotein (LDL) levels determined by calculation. HDL was determined by this method on serum samples after removal of LDL and very low-density lipoprotein (VLDL) fractions with phosphotungstic acid and $MgCl_2$. Serum triglyceride content was determined enzymatically (Megraw et al., 1979).

2.5. Drugs used

All drugs were applied as 50-μl bolus injections into a rubber septum proximal to the preparations. Drug dilutions were made up daily in distilled water, except for noradrenaline and 5-hydroxytryptamine, which were made up daily as stock solutions in 0.1 mM ascorbic acid and diluted in distilled water. The following drugs were obtained from Sigma: acetylcholine chloride, adenosine 5′-triphosphate (disodium salt), alpha beta methylene ATP (lithium salt), methoxamine hydrochloride, noradrenaline bitartrate and sodium nitroprusside.

2.6. Data analysis

Responses were measured as changes in perfusion pressure (mm Hg) and presented as mean ± S.E.M. Response curves were compared by analysis of variance with repeated measures and differences considered statistically significant when $P < 0.05$. Post-hoc analysis was by Student's t-test.

3. Results

3.1. Animals

Donryu rats fed a normal diet weighed 470 ± 1.3 g ($n = 7$); those on a CCT diet weighed 259.2 ± 1.6 g ($n = 7$). The serum lipid spectrum is summarised in Table 1.

3.2. Mesenteric arterial bed

Basal perfusion pressure of the beds was similar in the two groups: 26.71 ± 4.86 mm Hg ($n = 7$) in controls and 26.17 ± 5.26 mm Hg ($n = 7$) in Donryu rats.

3.2.1. Vasoconstrictor responses to electrical field stimulation

Electrical field stimulation (4–32 Hz, 1 ms, 90 V, 30 s) elicited frequency-dependent vasoconstriction which was

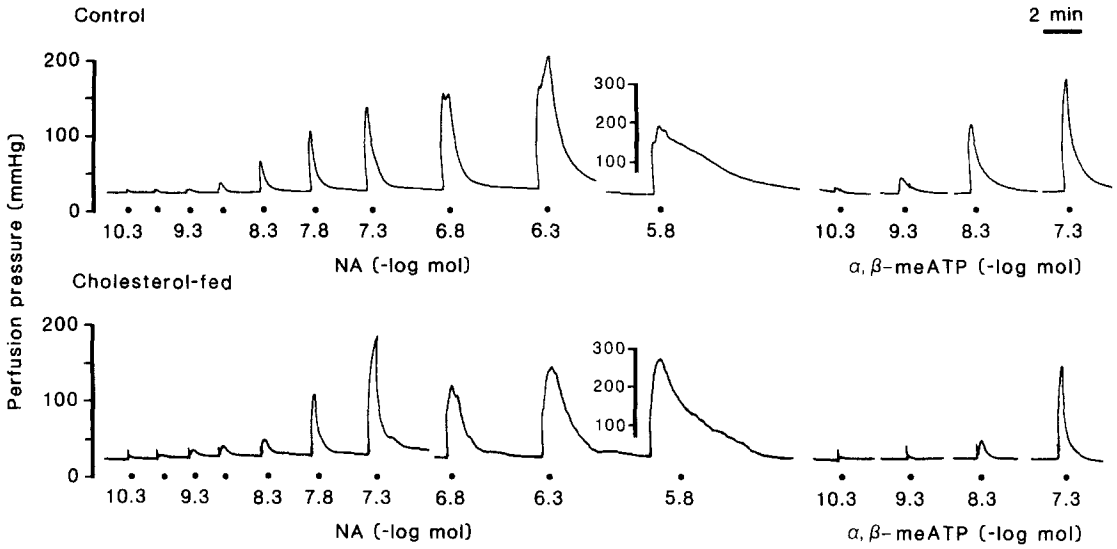


Fig. 2. Representative traces showing vasoconstrictor responses of mesenteric arterial beds from a Donryu rat fed on a control diet (upper trace) and a Donryu rat fed on a cholesterol-supplemented diet (lower trace) to doses (−log mol) of noradrenaline (NA; 0.15–1500 nmol) and α,β-methylene ATP (α,β-meATP; 0.05–500 nmol). For clarity, only alternate doses of NA are labelled at the far left of the trace. Perfusion pressure scale change applies to responses to noradrenaline only for the top dose in the control diet and the top three doses in the high-cholesterol diet. The scale for α,β-methylene ATP is as for the lower doses of noradrenaline.

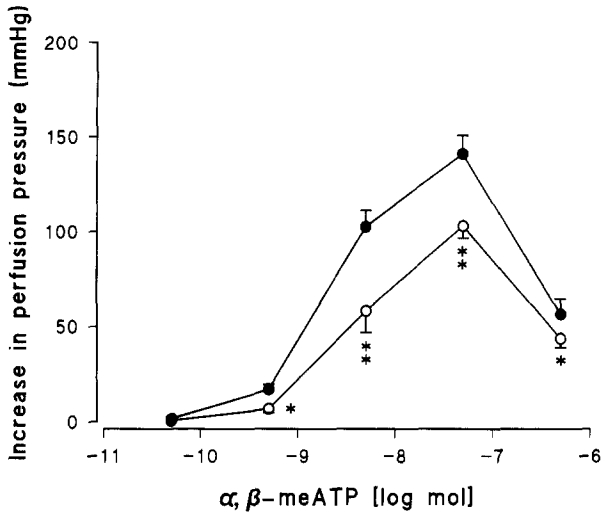


Fig. 3. Dose-response curves showing constrictor responses to α,β-methylene ATP (α,β-meATP) of mesenteric arterial preparations from Donryu rats fed on a control diet (●, $n = 7$) and Donryu rats fed on a cholesterol-supplemented diet (○, $n = 7$) rats. Data are presented as means with vertical bars indicating S.E.M. * $P < 0.05$; ** $P < 0.01$.

similar in Donryu rats fed on a control and CCT diet (Fig. 1a). These responses are due to activation of perivascular sympathetic nerves since they are abolished by guanethidine (Ralevic et al., 1993).

3.2.2. Vasoconstrictor responses to exogenous noradrenaline

Dose-dependent vasoconstrictor responses to noradrenaline (0.05–1500 nmol) were similar in both groups of Donryu rats (Fig. 1b and Fig. 2).

3.2.3. Vasoconstrictor responses to exogenous α,β-methylene ATP

Dose-dependent vasoconstrictor responses to α,β-methylene ATP (0.05–500 nmol) were significantly attenuated in Donryu rats fed on a CCT diet compared to those fed on a control diet ($P = 0.01$) (Figs. 2 and 3).

3.2.4. Vasodilator responses to acetylcholine, ATP and sodium nitroprusside

Methoxamine (5–35 μM) was used to raise the tone of the preparations. There was no difference between the preparations in the degree of tone achieved or in the concentration of methoxamine used to elicit the increase in tone: control diet, 77.84 ± 8.47 mm Hg with 12.5 ± 3.9 μM methoxamine ($n = 7$); CCT diet, 69.09 ± 6.41 mm Hg with 15.0 ± 3.9 μM methoxamine ($n = 7$).

There was no significant difference in dose-dependent relaxation to acetylcholine (0.0005–50 nmol), ATP (0.005–50 nmol) or sodium nitroprusside (0.005–50 nmol) between the groups (Fig. 4).

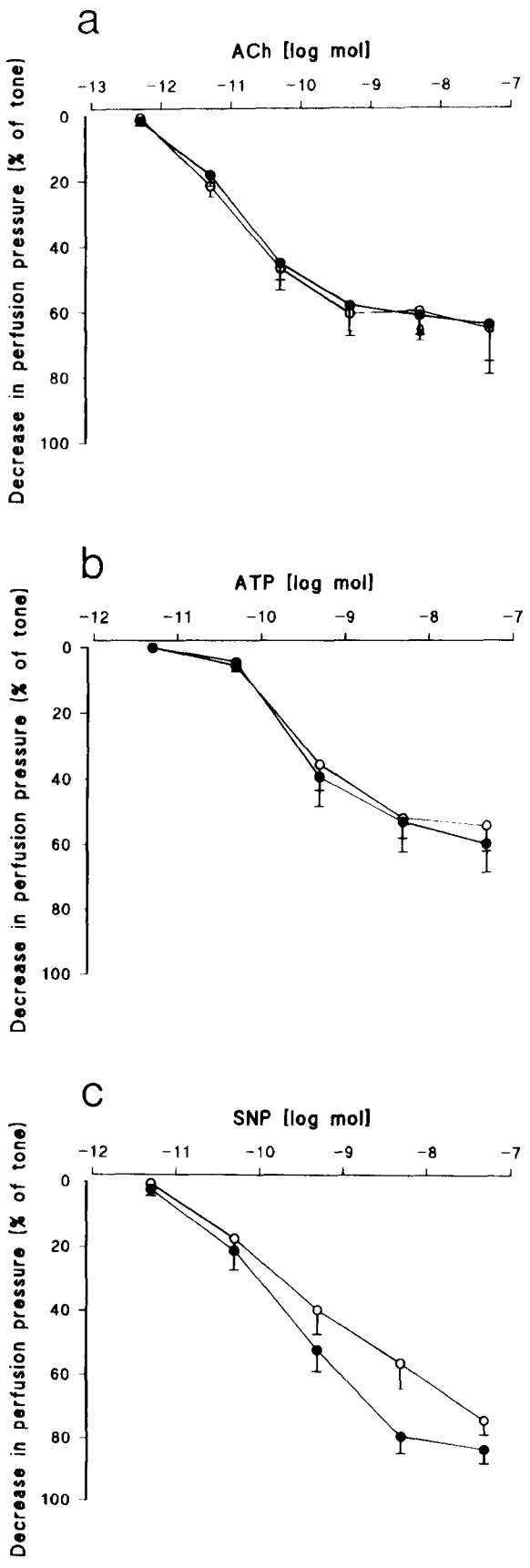
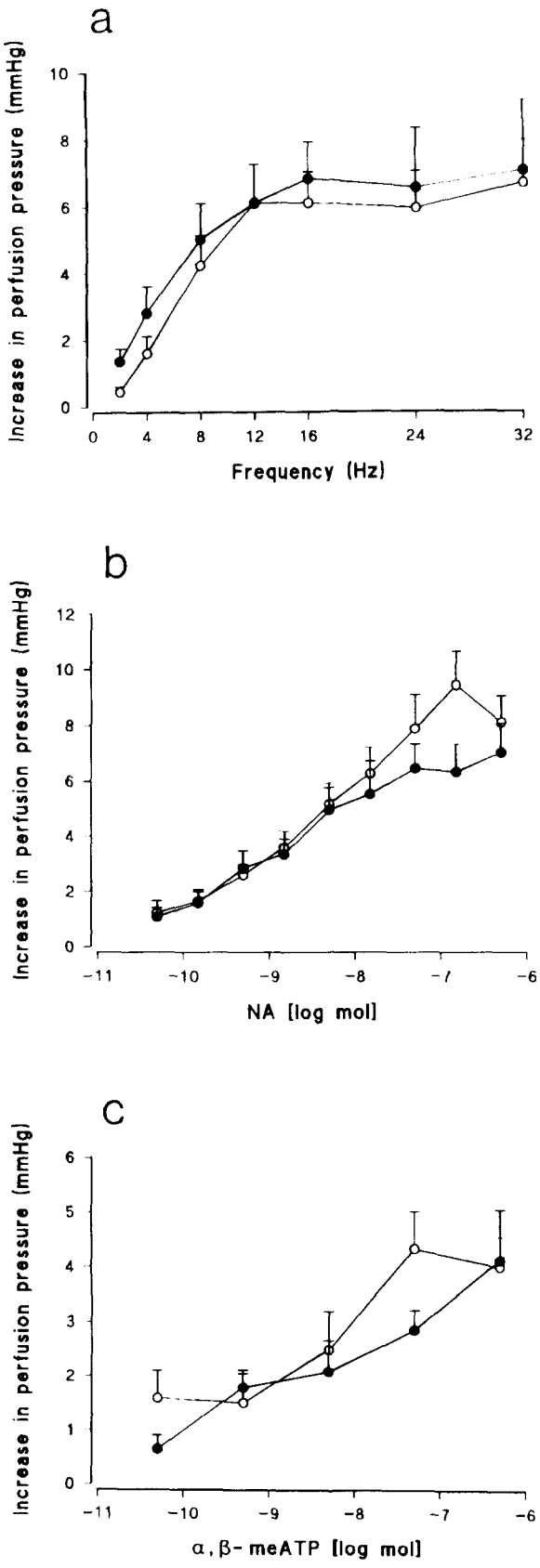


Fig. 4. Dose-response curves showing vasodilatation of mesenteric arterial preparations from Donryu rats fed on a control diet (●, $n = 7$) and Donryu rats fed on a cholesterol-supplemented diet (○, $n = 7$) to: (a) acetylcholine (ACh), (b) ATP and (c) sodium nitroprusside (SNP). Data are presented as means with vertical bars indicating S.E.M.

3.3. Hepatic portal vascular bed

Basal perfusion pressure was similar in livers from Donryu rats fed on a control diet (2.80 ± 0.89 mm Hg, $n = 6$) and CCT diet (2.33 ± 0.92, $n = 7$).



3.3.1. Vasoconstrictor responses to electrical field stimulation

Electrical field stimulation (2–32 Hz, 1 ms, 90 V, 30 s) elicited frequency-dependent vasoconstriction which was similar in both groups (Fig. 5a).

3.3.2. Vasoconstrictor responses to exogenous noradrenaline and α,β-methylene ATP

Exogenous noradrenaline (0.05–1500 nmol) and α,β-methylene ATP (0.05–500 nmol) elicited similar dose-dependent vasoconstriction in both groups (Fig. 5b).

4. Discussion

The rat is not generally favoured as a model to study atherosclerosis because of its resistance to develop atherosclerotic lesions. However, the characterization of elevated serum lipid levels in the Yoshida heritable hyperlipidemic rat, together with reports of impaired aortic endothelial vasodilator function and aberrant aortic endothelial morphology raise this as a potential model for the study of hyperlipidemia (Fantappiè et al., 1992; Chinellato et al., 1994a,b). This animal model is derived from the Donryu rat by innoculation with Yoshida sarcoma cells (Omura et al., 1995). The present study characterizes, for the first time, responses of the mesenteric arterial and hepatic portal vasculature in the Donryu rat with and without a cholesterol-supplemented diet.

The increase in total serum cholesterol levels in cholesterol-fed Donryu rats was not uniform for the different lipid fractions; HDL was significantly decreased and LDL was significantly increased. HDL particles are antiatherogenic due to their participation in reverse cholesterol transport from peripheral tissues to the liver where it is catabolised. An increase in LDL is typically associated with the development of atherosclerotic lesions and it is possible that prolonged feeding of the rats with the high cholesterol diet may eventually result in such lesions. Changes in the endothelial content of endothelin-1 and different isoforms of nitric oxide synthase have been observed in the aorta from this model (unpublished observations). The decrease in HDL is in contrast to the cholesterol-fed Yoshida rat where HDL was increased (Chinellato et al., 1994b). A decrease in plasma HDL has also been noted in diet-induced atherosclerosis in rabbits (Thiery and Seidel, 1987) and the genetic model Watanabe heritable hyperlipidemic rabbits (Saku et al., 1989; Cirillo et al., 1992).

Fig. 5. Responses of the portally perfused hepatic vasculature from Donryu rats fed on either a control (●) or a cholesterol-supplemented (○) diet: (a) frequency-response curves showing constriction to electrical field stimulation (2–32 Hz, 1 ms, 90 V, 30 s); (b) dose-response curves to noradrenaline (NA); and (c) dose-response curves to α,β-methylene ATP (α,β-meATP). Data are presented as means with vertical bars indicating S.E.M.

The present study shows that endothelial function of the mesenteric arterial bed of the Donryu rat is unimpaired after dietary cholesterol supplementation. A relative resistance of mesenteric arteries to malfunction has previously been reported; hypercholesterolemia in rabbits was associated with atherosclerotic lesions and impaired endothelium-dependent relaxation in the aorta, whereas mesenteric and cerebral small arteries were largely unaffected (Simonsen et al., 1991). Impaired aortic endothelial function has also been observed in the Yoshida rat (Chinellato et al., 1994b). Freiman and coworkers noted that impaired endothelial function of the iliac artery of hypercholesterolemic dogs required the development of atherosclerosis (Freiman et al., 1986). It is possible that this could explain the different susceptibilities of mesenteric and other arteries to endothelial malfunction. Augmented constrictor responses to 5-hydroxytryptamine have been described in the mesenteric circulation of atherosclerotic monkeys, however, since this was selective for 5-hydroxytryptamine it is not likely to involve the endothelium (Toda et al., 1988; Lopez et al., 1989).

Although attention has focused on the effects of hyperlipidemia and atherosclerosis on endothelial function, there is increasing evidence that these conditions may also affect perivascular neurotransmission (for references, see Section 1). Hyperresponsiveness to electrical field stimulation and to exogenous noradrenaline of the mesenteric arterial bed was observed in rats fed an atherogenic diet (Al-Jubouri and Al-Bayati, 1981). However, in the present study, there was no evidence for enhancement of sympathetic neurotransmission, either in the mesenteric arterial or hepatic portal vasculature.

The main finding of the present study was that responses to the P_{2X} purinoceptor agonist α,β-methylene ATP were impaired in mesenteric arteries. This receptor mediates constrictor responses to ATP, which may be released as a cotransmitter from sympathetic nerves (Burnstock, 1990; Sjöblom-Widfeldt et al., 1990). The mechanism for this impairment is not clear and may involve changes in purine receptors or post-receptor mechanisms. The vascular P_{2X} purinoceptor rapidly desensitizes (Burnstock and Kennedy, 1985) and it is possible that prolonged cholesterol-feeding causes changes which facilitate this. Cholesterol-feeding had a profound effect on the body weight of the rats which may have been due to the combined effects of the mild hypothyroidism (thiouracil causes suppression of thyroid gland function) and hypercholesterolemia. Since contractile responses were not uniformly affected for all agents, it is unlikely that the smaller body weight and mesenteric bed size of the cholesterol-fed Donryu rats is the cause of the altered P_{2X} purinoceptor responsiveness. On the other hand, we cannot definitively exclude the possibility that the decrease in P_{2X} purinoceptor responsiveness occurs due to changes secondary to the increase in cholesterol. While it is intriguing and so far not clear why selective changes should occur in this particular receptor population in hyperlipidemia, this is not the first study to report such changes. Selectivity of effects of hyperlipidemia on purinoceptors has been suggested for endothelium-dependent relaxant responses in the Watanabe heritable hyperlipidemic rabbit, where responses to P_{2U} receptors were retained at a stage when those to P_{2Y} receptors were abolished (Chinellato and Ragazzi, 1995). In the mesenteric arterial bed of cirrhotic rats, there are selective changes in P_2 purinoceptors (Mathie et al., 1996).

Vascular reactivity of the hepatic portal vascular bed of hyperlipidemic rats has not been extensively examined. Changes in contractile function and in endothelium-dependent relaxation have been observed in the hepatic artery of the Watanabe heritable hyperlipidemic rabbit (Brizzolara et al., 1992). The present study suggests that constrictor function of the portal vasculature is unchanged in Donryu rats. Unlike those in mesenteric arteries, responses to α,β-methylene ATP were unimpaired. Despite the fact that the liver is a primary site for lipid metabolism morphological changes related to hyperlipidemia did not appear to take place in either the hepatic vasculature or mesenteric arteries (unpublished observations).

In conclusion, this study shows a reduction of responses to the P_{2X} purinoceptor agonist α,β-methylene ATP in the isolated perfused mesenteric arterial bed of cholesterol-fed Donryu rats. In contrast, there was no change in sympathetic constriction in the same preparation, or in P_{2X} purinoceptor-mediated responses in the perfused hepatic portal bed. It is suggested that high cholesterol in the Donryu rat induces hyperlipidemia and organ-specific changes in vasculature function.

Acknowledgements

This study was supported by the British Heart Foundation.

References

Al-Jubouri, H.H. and Z.A.F. Al-Bayati, 1981, Hyperresponsiveness of arteries from atherosclerotic rats to noradrenaline and to sympathetic electrical stimulation, IRCS Med. Sci. 9, 917.

Allain, C.C., L.S. Poon, C.S. Chan, W. Richmond and P.C. Fu, 1974, Enzymatic determination of total serum cholesterol, Clin. Chem. 20, 470.

Brizzolara, A.L., A. Tomlinson, J. Aberdeen, R.G. Gourdie and G. Burnstock, 1992, Sex and age as factors influencing the vascular reactivity in Watanabe heritable hyperlipidaemic (WHHL) rabbits: a pharmacological and morphological study of the hepatic artery, J. Cardiovasc. Pharmacol. 19, 86.

Burnstock, G., 1990, The Fifth Heymans Lecture, Ghent. Co-Transmission, Arch. Int. Pharmacodyn. Ther. 304, 7.

Burnstock, G. and C. Kennedy, 1985, Is there a basis for distinguishing two types of P_2-purinoceptor?, Gen. Pharmacol. 16, 433.

Chinellato, A. and E. Ragazzi, 1995, Receptor-mediated pathways of endothelium activity in experimental atherosclerosis, Pharmacol. Res. 31, 163.

Chinellato, A., E. Ragazzi, L. Pandolfo, A. Palermo Alvano, G. Froldi, M. De Biasi, L. Caparrotta, G. Aliev and G. Fassina, 1994a, Functional and morphological characterization of thoracic aorta in heritable hyperlipidemic Yoshida rats of different ages, J. Cardiovasc. Pharmacol. 24, 216.

Chinellato, A., E. Ragazzi, L. Petrelli, M. Paro, L. Pandolfo, G. Froldi, L. Caparrotta, G. Fassina, A. Mironov and G. Aliev, 1994b, Effect of cholesterol-supplemented diet in heritable hyperlipidemic Yoshida rats: functional and morphological characterization of thoracic aorta, Atherosclerosis 106, 51.

Cirillo, R., G. Aliev, G. Italiano and M. Prosdocimi, 1992, Functional responses of hindlimb circulation in aged normal and WHHL rabbits, Atherosclerosis 93, 133.

Cohen, R.A., K.M. Zitnay, C.C. Haudenschild and L.D. Cunningham, 1988, Loss of selective endothelial cell vasoactive functions caused by hypercholesterolemia in pig coronary arteries, Circ. Res. 63, 903.

Fantappié, S., A.L. Catapano, M. Cancillieri, L. Fasoli, E. De Fabiani, M. Bertolini and E. Bosisio, 1992, Plasma lipoproteins and cholesterol metabolism in Yoshida rats: an animal model of spontaneous hyperlipidemia, Life Sci. 50, 1913.

Förstermann, U., A. Mügge, U. Alheid, A. Haverich and J.C. Frölich, 1988, Selective attenuation of endothelium-mediated vasodilation in atherosclerotic human coronary arteries, Circ. Res. 62, 185.

Freiman, P.C., G.G. Mitchell, D.D. Heistad, M.L. Armstrong and D.G. Harrison, 1986, Atherosclerosis impairs endothelium-dependent vascular relaxation to acetylcholine and thrombin in primates, Circ. Res. 58, 783.

Heistad, D.D., M.L. Armstrong, M.L. Marcus, D.J. Piegors and A.L. Mark, 1984, Augmented responses to vasoconstrictor stimuli in hypercholesterolemic and atherosclerotic monkeys, Circ. Res. 54, 711.

Ibengwe, J.K. and H. Suzuki, 1986, Changes in mechanical responses of vascular smooth muscles to acetylcholine, noradrenaline and high potassium solution in hypercholesterolemic rabbits, Br. J. Pharmacol. 87, 395.

Joris, I., T. Zand, J. Nunnari, F.J. Krolikowski and G. Majno, 1983, Studies of the pathogenesis of atherosclerosis: adhesion and migration of mononuclear cells in the aorta of hypercholesterolemic rats, Am. J. Pathol. 113, 341.

Kang, S.Y., S.H. Kim, V.B. Schini and N.D. Kim, 1995, Dietary ginsenosides improve endothelium-dependent relaxation in the thoracic aorta of hypercholesterolemic rabbit, Gen. Pharmacol. 26, 483.

Lamping, K.G., D.J. Piegors, K.H. Benzuly, M.L. Armstrong and D.D. Heistad, 1994, Enhanced coronary vasoconstrictive response to serotonin subsides after removal of dietary cholesterol in atherosclerotic monkeys, Arteriosclerosis Thromb. 14, 951.

Lopez, J.A., B.P. Brown, M.L. Armstrong, D.J. Piegors and D.D. Heistad, 1989, Response of the mesenteric circulation to serotonin in normal and atherosclerotic monkeys: implications for the pathogenesis of non-occlusive intestinal ischemia, Cardiovasc. Res. 23, 117.

Mathie, R.T., V. Ralevic and G. Burnstock, 1996, Vasoconstrictor responsiveness of the rat portal venous vascular bed in cirrhosis, J. Hepatol. (in press).

Mathie, R.T., V. Ralevic, K.P. Moore and G. Burnstock, 1996, Mesenteric vascular responses in cirrhotic rats: role of nitric oxide, Hepatology 23, 130.

Megraw, R.E., D.E. Dunn and H.G. Biggs, 1979, Manual and continuous-flow colorimetry of triacylglycerides by a fully enzymatic method, Clin. Chem. 25, 273.

Omura, K., T. Misaki, T. Hashimoto, E. Kanehira, T. Watanabe, F. Ishida, Y. Watanabe and T. Shirasaka, 1995, Changes in folate concentration in Yoshida sarcoma after administration of leucovorin or cisplatin, Cancer Chemother. Pharmacol. 35, 183.

Osborne, J.A., M.J. Siegman, A.W. Sedar, S.U. Mooers and A.M. Lefer, 1989, Lack of endothelium-dependent relaxation in coronary resistance arteries of cholesterol-fed rabbits, Am. J. Physiol. 256, C591.

Panek, R.L., W.R. Dixon and C.O. Rutledge, 1985, Modification of sympathetic neuronal function in the rat tail artery by dietary lipid treatment, J. Pharmacol. Exp. Ther. 233, 578.

Ralevic, V., A. Belai and G. Burnstock, 1993, Impaired sensory-motor nerve function in the isolated mesenteric arterial bed of streptozotocin-diabetic and ganglioside-treated streptozotocin-diabetic rats, Br. J. Pharmacol. 110, 1105.

Saku, K., K. Yamamoto, T. Sakai, T. Yanagida, K. Hidaka, J. Sasaki and K. Arakawa, 1989, Kinetics of HDL-apo A-1 in the WHHL rabbit, an animal model of familial hypercholesterolemia, Atherosclerosis 79, 225.

Shimokawa, H., P. Kim and P.M. Vanhoutte, 1988, Endothelium-dependent relaxation to aggregating platelets in isolated basilar arteries of control and hypercholesterolemic pigs, Circ. Res. 63, 604.

Shimokawa, H. and P.M. Vanhoutte, 1989, Impaired endothelium-dependent relaxation to aggregating platelets and related vasoactive substances in porcine coronary arteries in hypercholesterolemia and atherosclerosis, Circ. Res. 64, 900.

Simonsen, U., E. Ehrnrooth, L.U. Gerdes, O. Faegemann, J. Buch, F. Andreasen and M.J. Mulvany, 1991, Functional properties in vitro of systemic small arteries fed a cholesterol-rich diet for 12 weeks, Clin. Sci. Colch. 80, 119.

Sjöblom-Widfeldt, N., H. Gustafsson and H. Nilsson, 1990, Transmitter characteristics of small mesenteric arteries from the rat, Acta Physiol. Scand. 138, 203.

Stewart-Lee, A.L., J. Aberdeen and G. Burnstock, 1991, Sympathetic neurotransmission in the Watanabe heritable hyperlipidaemic rabbit mesenteric artery, Cardiovasc. Res. 25, 1035.

Stewart-Lee, A.L., J. Aberdeen and G. Burnstock, 1992, The effect of atherosclerosis on neuromodulation of sympathetic neurotransmission by neuropeptide Y and calcitonin gene-related peptide in the rabbit mesenteric artery, Eur. J. Pharmacol. 216, 167.

Thiery, J. and D. Seidel, 1987, Fish oil feeding results in an enhancement of cholesterol-induced atherosclerosis in rabbits, Atherosclerosis 63, 53.

Toda, N., M. Miyazaki and F. Hazama, 1988, Functional and histological changes in mesenteric arteries and aortas from monkeys fed a high cholesterol diet, Jpn. J. Pharmacol. 48, 441.

Trzeciak, H.I., B. Okopien, A. Kozlowski, D. Chocilowska and A. Bara, 1993, Contractility of the tail artery in rats treated with bezafibrate and fed atherogenic diet, J. Cardiovasc. Pharmacol. 22, 761.

Verbeuren, T.J., F.H., Jordaens, L.L. Zonnekeyn, C.E. Van Hove, M.-C. Coene and A.G. Herman, 1986, Effect of hypercholesterolemia on vascular reactivity in the rabbit. I. Endothelium-dependent and endothelium-independent contractions and relaxations in isolated arteries of control and hypercholesterolemic rabbits, Circ. Res. 58, 552.