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Reduced sympathetic noradrenergic neurotransmission in the tail artery of Donryu rats fed with high cholesterol-supplemented diet

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1 Sympathetic neurotransmission and noradrenaline content of the tail artery of Donryu rats fed for 2 months with a cholesterol-supplemented diet enriched with 4% cholesterol, 1% cholic acid, 0.5% thiouracil (CCT), were examined.

2 Total serum cholesterol level of CCT fed rats (7.05 ± 1.77 mg ml^{-1}, $n=8$) was significantly greater than lab-chow fed controls (2.58 ± 0.32 mg ml^{-1}, $n=8$). Low density lipoprotein level was also significantly increased in CCT-fed (1.79 ± 0.26 mg ml^{-1}, $n=8$) compared with control fed rats (1.35 ± 0.25 mg ml^{-1}, $n=8$) but plasma levels of triglyceride and high density lipoproteins did not differ significantly between the two groups.

3 Contractile responses of the arterial rings to transmural nerve stimulation (65 V, 0.1 ms, 4–64 Hz, 1 s), were markedly attenuated in the CCT fed animals compared with the controls. This reduction involved the noradrenergic rather than purinergic component of sympathetic transmission.

4 Vasoconstrictor responses to exogenous noradrenaline (0.01–300 μM) and adenosine 5′-triphosphate (0.3–1000 μM) were unaffected by CCT diet, indicating prejunctional alteration of sympathetic neurotransmission during CCT-induced hyperlipidaemia.

5 The noradrenaline content of the tail arteries of CCT fed animals (2.64 ± 0.36 ng mg^{-1}, $n=6$) was significantly lower than that of controls (3.82 ± 0.32 ng mg^{-1}, $n=6$).

6 These findings show that chronic treatment of Donryu rats with a cholesterol-supplemented diet led to altered levels of circulating lipid fractions accompanied by attenuated sympathetic noradrenergic neurotransmission and reduced noradrenaline content of the rat tail artery.

Keywords: CCT; induced hyperlipidaemia; rat tail artery; sympathetic neurotransmission

Introduction

Perivascular neurotransmission is thought to be affected in hyperlipidaemia and consequent atherosclerosis (Panek *et al*., 1985; Lichtor *et al*., 1987; Burnstock *et al*., 1991; Stewart-Lee *et al*., 1991a; 1992). Few animal models of endogenous hyperlipidaemia without formation of atherosclerotic lesions have been developed so far to enable the study of the effects of hyperlipidaemia. Inoculation of the Donryu rats with Yoshida sarcoma cells has been used to develop inbred hyperlipidaemic rats that do not develop typical atheromatous lesions or functional and morphological damage of smooth muscle cells, despite high serum cholesterol levels (Fantappie *et al*., 1992; Chinellato *et al*., 1994a,b; Omura *et al*., 1995). Hyperlipidaemia in this strain of rat is attributed to hepatic overproduction of lipoproteins (Fantappie *et al*., 1992). However, a direct effect of a cholesterol-supplemented diet on vascular reactivity has not been studied in Donryu rats, nor has perivascular sympathetic neurotransmission been examined in this model.

The rat isolated tail artery preparation has been used by several investigators to study release of noradrenaline (NA) evoked by periarterial nerve stimulation (Bonaccorsi *et al*., 1977; Panek *et al*., 1985). Furthermore, the sympathetic neurotransmission in the rat tail artery is known to be mediated by the co-transmitters NA and adenosine 5′-triphosphate (ATP) (Sneddon & Burnstock, 1984; Bao, 1993).

In the present study, we have investigated the effects of dietary lipids on adrenergic-purinergic perivascular co-transmission, as well as neurotransmitter content of sympathetic perivascular nerves in the tail artery of the Donryu rats. To this effect, vascular responses to transmural nerve stimulation (TNS) and exogenous application of co-transmitters NA and ATP were evaluated in the tail artery of Donryu rats with and without cholesterol-supplemented diet. The tissue content of NA was also measured.

Methods

Animal model

Male Donryu rats 8 weeks old, were commercially obtained from Charles River, Japan. One group of rats (referred to as 'control') were fed a standard laboratory diet and water *ad libitum*. Another group (referred to as 'CCT fed') received the same diet supplemented with 4% cholesterol, 1% cholic acid, 0.5% thiouracil (CCT diet) (Joris *et al*., 1983; Chinellato *et al*., 1994a,b). After 2 months on the appropriate diet the rats were killed by CO_2 asphyxiation.

Cholesterol and triglyceride levels

Blood was removed from all rats immediately after death. Total and high density lipoprotein (HDL) serum cholesterol levels were determined by the cholesterol oxidase/peroxidase

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method (Allain *et al*., 1974) and the low density lipoprotein (LDL) levels determined by calculation. HDL was determined on serum samples after removal of LDL and very low density lipoprotein fractions with phosphotungstic acid and $MgCl_2$. Serum triglyceride levels were determined enzymatically (Megraw *et al*., 1979).

Pharmacology

Tail arteries were dissected and cleaned of excess tissue. Ring segments of 3–4 mm were cut and mounted in 5 ml organ baths containing (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. Rings were left to equilibrate for 60–90 min under a resting tension of 1 g. Isometric tension was recorded by a Grass FT03C transducer and displayed on a Grass ink-writing polygraph (model 79). Transmural nerve stimulation (TNS) was achieved by passing a current between two electrodes parallel to the arterial rings (65 V, 0.1 ms, 4–64 Hz, for 1 s). Cumulative concentration-responses curves to NA and ATP were established. Vascular responses were evaluated as percentage of maximal tension developed by 120 mM KCl.

Noradrenaline assay

The proximal end of tail artery was dissected out and cleaned of excess tissue. Segments of 3–4 cm were rapidly frozen and stored in liquid nitrogen until assay. After measurements of weight and length of the segments. NA levels were measured by use of high-performance liquid chromatography with electrochemical detection. Tissue samples were homogenized in 500 μl of 0.1 M perchloric acid containing 0.4 M sodium bisulphate and 25 ng of dihydroxybenzylamine by a motor-driven glass homogenizer. After 2.5 min of centrifugation at 13000 r.p.m., the supernatants were subject to alumina extraction. Noradrenaline and dihydroxybenzylamine were separated on a 10-μ Bondapak C-18 reverse-phase column (Waters Associated, Norwich, U.K.) with a mobile phase of 0.1 M sodium dihydrogen phosphate (pH 5.0) containing 5 nM heptan sulphonate, 0.1 mM ethylenediaminetetraacetic acid and 13% (v/v) methanol. Quantification was performed with a glassy carbon-paste electrode set at a potential of +0.72 V. Noradrenaline levels were corrected for recovery by use of dihydroxybenzylamine as an internal standard.

Drugs

(−)-Noradrenaline bitartrate, adenosine 5′-triphosphate (ATP) disodium salt, α,β-methylene ATP sodium salt, prazosin, yohimbine and tetrodotoxin were obtained from Sigma Chemical (Poole, U.K.). Sodium dihydroxybenzylamine and heptan sulphonate were obtained from Aldrich (Gillingham, England). Monoamines were dissolved in ascorbic acid (0.1 mM), yohimbine was initially dissolved in 25% ethanol and further diluted in distilled water. All other drugs were dissolved in distilled water.

Statistical analysis

In the text and figures, all results are expressed as means ±s.e.mean, n referring to the number of animals used. Statistical analysis was performed by use of Student's unpaired t test and a value of $P<0.05$ was considered statistically significant.

Table 1 Circulating lipid levels (mg ml^{-1}) in Donryu rats fed with control and cholesterol-supplemented (CCT) diet

	Control diet	*CCT diet*
Total serum cholesterol	2.58±0.32	7.05±1.77*
Low density lipoprotein	0.59±0.14	6.17±1.58**
Triglyceride	0.61±0.12	0.48±0.10
High density lipoprotein	1.79±0.26	1.35±0.25

All results are expressed as mean±s.e.mean ($n=7$ animals). Significant difference from control, *$P<0.05$, **$P<0.01$.

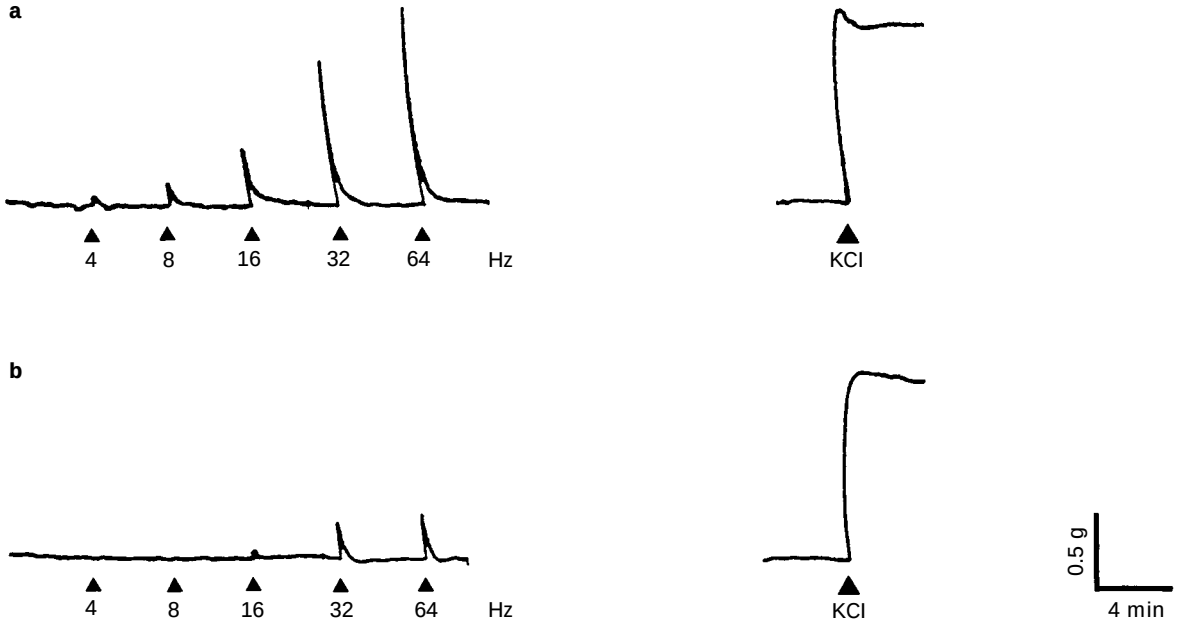

Figure 1 Recording of a typical experiment showing vasoconstrictor responses to transmural nerve stimulation (65 V, 0.1 ms, 4–64 Hz, for 1 s) and KCl (120 mM) in rat tail arteries taken from controls (a) and CCT fed animals (b).

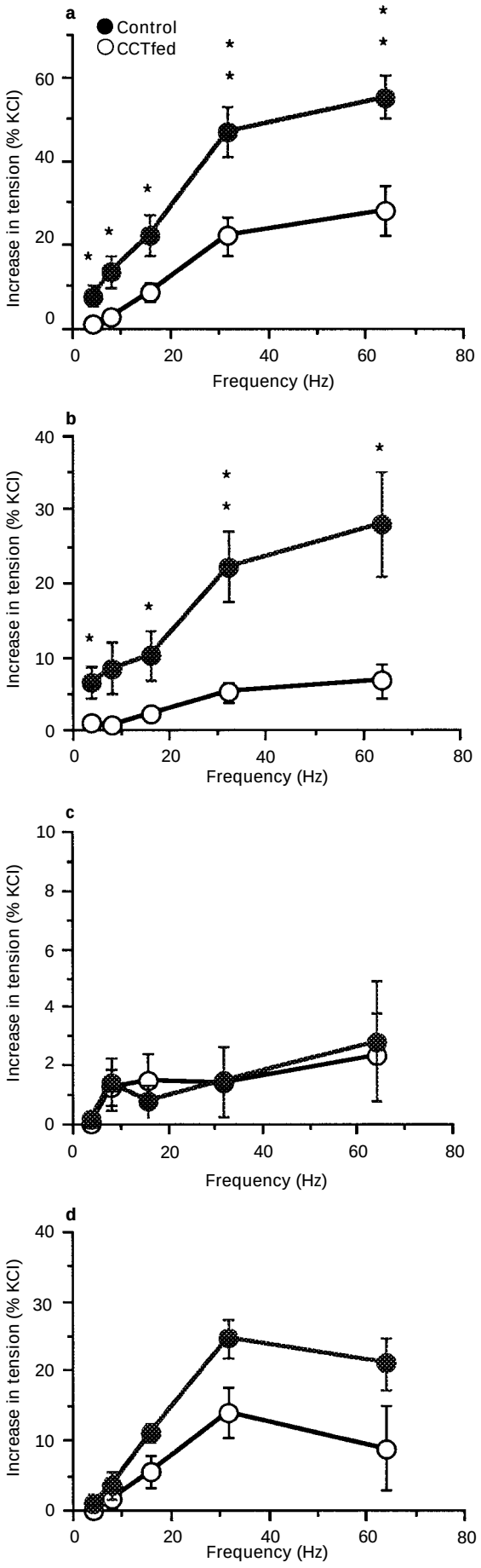

Figure 2 Frequency-response curves to transmural nerve stimulation (65 V, 0.1 ms, 4–64 Hz, for 1 s) in rat tail arteries taken from controls and CCT fed animals : (a) in the absence of all antagonists. (b) Noradrenergic component of response blocked by α_1-adrenoceptor antagonist prazosin (1 μM). (c) Purinergic component of response blocked by α,β-methylene ATP (1 μM) in presence of prazosin. (d) Noradrenergic component of response blocked by α_2-adrenoceptor antagonist, yohimbine (1 μM) in presence of α,β-methylene ATP and prazosin. Antagonists were applied sequentially in the order of: prazosin, α,β-methylene ATP and yohimbine. Vasoconstriction responses are expressed as a percentage of the response to KCl (120 mM) and points represent the mean ($n=6$) with vertical lines showing s.e.mean. $^*P<0.02$ and $^{**}P<0.01$.

Results

Animal model

Body weights of the animals were significantly reduced from 470.0 ± 1.3 g ($n=7$) in controls to 259.2 ± 1.6 g ($n=7$) in CCT fed animals. Circulating cholesterol and LDL levels were significantly increased in CCT fed compared with control fed animals (Table 1). In contrast triglyceride and HDL levels did not differ between the animal groups (Table 1).

Pharmacology

In rat tail arterial rings, TNS (4–64 Hz) evoked frequency-dependent contractile responses. These responses were abolished by application of tetrodotoxin (1 μM), thus revealing their neuronal nature. The increase in contractile tension induced by TNS was significantly attenuated in rings from CCT fed animals compared with controls (Figure 1). The responses to all frequencies examined were significantly reduced by at least 2 fold in CCT fed compared with control rats. At 64 Hz vascular responses were 28.0 ± 5.9 ($n=5$) and 55.0 ± 5.2 ($n=5$) % of maximal response to 120 mM KCl, in arterial rings from CCT and normally fed rats, respectively (Figure 2a).

In the presence of 1 μM prazosin, vascular responses to TNS were partially reduced. The α_1-mediated component of noradrenergic response to TNS was calculated as the difference between contractile responses in the absence and presence of prazosin. In CCT fed animals the noradrenergic component of perivascular sympathetic neurotransmission was significantly lower than in control animals at 4, 16, 32 and 64 Hz (Figure 2b). Treatment of the preparation with α,β-methylene ATP (1 μM, in the presence of prazosin), further reduced the vascular response to TNS. The purinergic component of the vascular response to TNS was calculated as the difference between contractile responses in the absence and presence of prazosin after correction for the residual part of the response. In CCT fed animals the purinergic component of perivascular sympathetic neurotransmission was not significantly altered compared with the control animals (Figure 2c).

The residual part of the contractile response to TNS which was resistant to both prazosin and α,β-methylene ATP, was abolished by application of the α_2-adrenoceptor antagonist, yohimbine (1 μM) and did not significantly differ between CCT fed and control animals (Figure 2d).

Application of exogenous NA (0.01–300 μM) evoked concentration-dependent contractile responses which were similar in both groups of animals (Figure 3a). Similarly, vascular responses to ATP (0.3–1000 μM) did not significantly differ in arterial rings from CCT fed and control animals (Figure 3b).

Tension developed by application of 120 mM KCl at the end of each experiment, was significantly lower in arterial rings from the CCT fed arterial rings compared with control

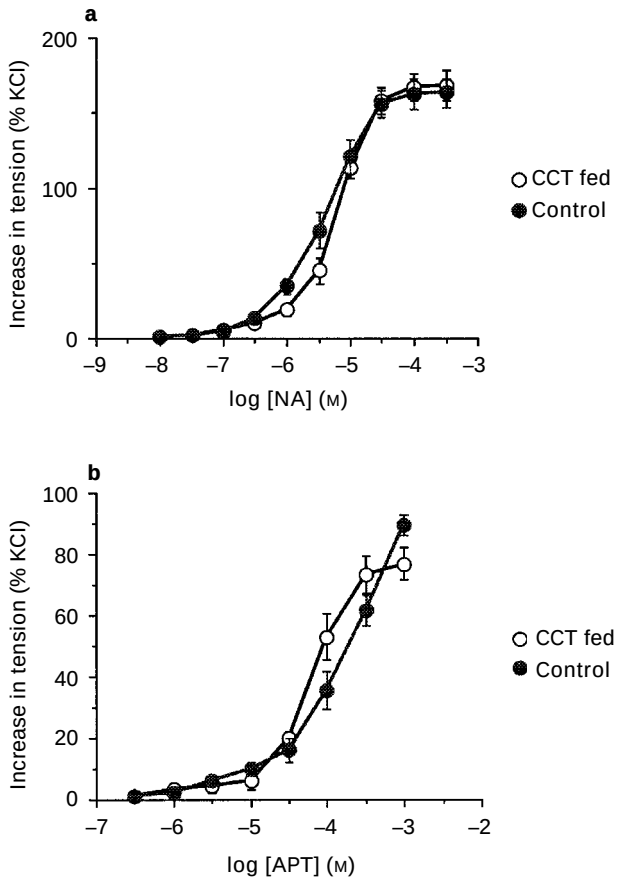

Figure 3 Concentration-response curves to (a) noradrenaline (NA) and (b) and adenosine 5′-triphosphate (ATP) of rat tail arteries taken from controls and CCT fed animals. Vasoconstriction responses are expressed as a percentage of the response to KCl (120 mM) and points represent the mean ($n=6$) with vertical lines showing s.e.mean.

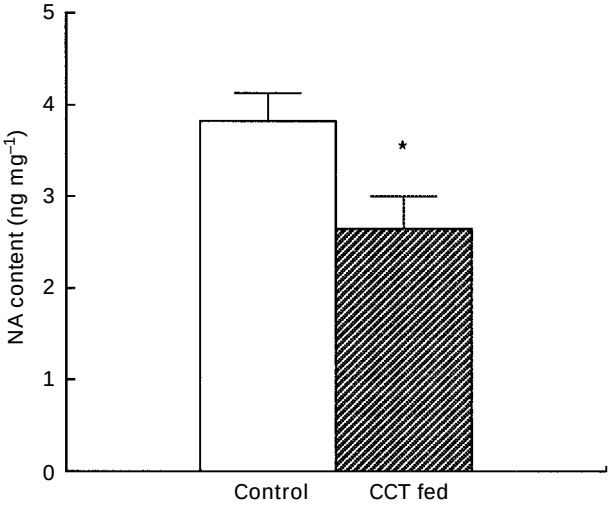

Figure 4 Tissue content (ng mg^{-1}) of noradrenaline (NA) in rat tail arteries taken from control ($n=6$) and CCT fed ($n=6$) animals. Columns show means and vertical lines represent s.e.mean. *$P<0.05$.

preparations, reaching maximal tension of 1.06 ± 0.09 g ($n=6$) and 1.45 ± 0.12 g ($n=6$), respectively.

Noradrenaline content

The NA content of the proximal tail artery after 2 months of CCT treatment (2.64 ± 0.36 ng mg^{-1}, $n=6$) was significantly reduced compared with levels in the artery of control animals, (3.82 ± 0.32 ng mg^{-1}, $n=6$), as shown in Figure 4.

Discussion

In the present study chronic treatment of Donryu rats with a cholesterol-supplemented diet led to altered levels of circulating lipid fractions: total serum cholesterol and LDL levels were significantly increased during cholesterol-supplemented feeding with no change in triglyceride and HDL levels. The absence of any effect of HDL levels is in contrast to the CCT fed Yoshida rats where HDL was increased (Chinellato *et al*., 1994a). A decrease in HDL level has also been found in a diet-induced atherosclerosis and genetic model of WHHL rabbit (Thiery & Seidel, 1987; Saku *et al*., 1989; Cirillo *et al*., 1992).

In this study the vasoconstriction evoked by TNS in arterial rings was sensitive to antagonism of the α_1- and α_2-adrenoceptors by prazosin and yohimbine, respectively, as well as to desensitization of the P2X-purinoceptor by α,β-methylene ATP, and were completely abolished by tetrodotoxin. These data describe classical sympathetic co-transmission of NA and ATP in the rat tail artery (Sneddon & Burnstock, 1984; Bao, 1993). Heterogeneity of smooth muscle α-adrenoceptors in rat tail artery is well established. Medgett & Langer (1984) demonstrated a predominance of α_1-adrenoceptors in addition to a subpopulation of α_2 which also contribute to vasoconstrictor responses of vascular smooth muscle to NA. Supporting evidence has since been accumulating (Medgett, 1985; Xiao *et al*., 1987; Szabo & Hardebo, 1990; Rajanayagam *et al*., 1990), in line with our finding that a component of the noradrenergic vasoconstrictor response was due to activation of postjunctional α_2-receptors.

Contractile responses to TNS were significantly lower in the CCT fed animals. When noradrenergic and purinergic components of perivascular sympathetic neurotransmission were evaluated separately, the noradrenergic component appeared to be attenuated following CCT diet. In contrast, in the presence of the α-adrenoceptor blockers prazosin and yohimbine, which unmasked the purinergic component of perivascular neurotransmission, vascular responses to TNS were unaltered. Attenuation of the noradrenergic transmission but lack of change in the purinergic component of perivascular neurotransmission observed in the present study is consistent with previous findings showing plasticity of expression of the different components of perivascular sympathetic neurotransmission and pattern of innervation in pathophysiological conditions. However, in contrast to the present findings in rat tail artery with hyperlipidaemia, a single dose of X-irradiation or chronic electrical nerve stimulation predominantly altered the purinergic rather than noradrenergic component of sympathetic co-transmission of the rabbit ear artery (Stewart-Lee *et al*., 1991b; Maynard *et al*., 1992). In 1,3-dipropyl-8-sulphophenylxanthine-induced hypertension, both purinergic and noradrenergic components of the sympathetic nerve mediated contractile responses in rat tail artery were increased (Karoon *et al*., 1995).

While vascular responses to sympathetic nerve stimulation were significantly attenuated in CCT fed animals, vasoconstriction to exogenous sympathetic co-transmitters NA and ATP did not differ betwen CCT fed and control animals, thus indicating that pre- rather than post-junctional modifications

account for the attenuated neurotransmission seen in the CCT fed animals.

Attenuation of sympathetic neurotransmission, as observed in this study after long-term treatment with cholesterol-enriched diet, has also been described in preparations taken from animals on a high cholesterol diet with saturated fat. Panek and co-workers have shown that the tail artery of rats receiving a diet enriched in saturated fats has reduced perfusion pressure responses to transmural nerve stimulation (Panek *et al*., 1985). There are conflicting findings concerning the changes that occur in the response of the tail artery of rats to exogenous NA during hyperlipidaemia; no change (Panek *et al*., 1985) and elevation (Trzeciak *et al*., 1993) of responses to NA have both been obtained. However, there is no record of a previous investigation of the response to exogenous ATP in the tail artery of hyperlipidaemic rats.

The pharmacological evidence from this study indicates that long-term treatment with cholesterol-enriched diet selectively affects the noradrenergic component of sympathetic neurotransmission at the prejunctional level. This is clearly indicated by a lack of effect of high cholesterol diet on vascular responses to the exogenous neurotransmitter NA.

The contractile responses to KCl were reduced in CCT fed animals indicating a general hyporesponsiveness of vascular smooth muscle. This alteration could be due to morphological and/or functional modification of vascular smooth muscle during hyperlipidaemia and is open to further investigation. However, appropriate correction was applied by calculating all contractile responses to TNS and exogenous neurotransmitters as a percentage of maximal response to KCl. Therefore the observed attenuation of the vascular responses to TNS indicates a selective modification of adrenergic transmission.

The finding of a significant reduction in the NA content of tail arteries in CCT fed animals compared with controls is consistent with the pharmacological observations and further supports the evidence for neuronal changes associated with hyperlipidaemic diet. The reduced NA content observed after CCT feeding could be due to a reduction in the number of sympathetic nerves or to an alteration in their transmitter synthesis and storage.

Chronic exposure to dietary lipids may have induced long-term alterations in adrenergic innervation, thus leading to functional modification of neurotransmission. In contrast to our findings, the NA content in the tail artery of saturated fatty acid-treated rats was shown to be increased due to impaired neuronal storage and reduced release of NA (Panek *et al*., 1985).

A significant reduction in body weight was noted in the CCT fed animals, which has also been found in rats and rabbits subjected to other high-cholesterol diets (Reyes *et al*., 1990; Verbeuren *et al*., 1994). The possibility of altered sympathetic function due to the impaired growth of CCT fed rats at this stage cannot be ruled out and is open to further investigation.

In conclusion, our results indicate that chronic treatment of Donryu rats with a cholesterol-supplemented diet leads to altered levels of circulating lipid fractions associated with attenuation of sympathetic neurotransmission. This reduction involves the noradrenergic component of sympathetic neurotransmission and occurs at the prejunctional level. The fall in NA content of the rat tail artery further indicates the possible alteration in innervation density, enzymatic activity or storage mechanisms in sympathetic perivascular nerves after chronic cholesterol-supplemented feeding.

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