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Chronic treatment with the angiotensin I converting enzyme inhibitor, perindopril, protects *in vitro* carbachol-induced vasorelaxation in a rat model of vascular calcium overload

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1 Treatment of young rats with vitamin D_3 plus nicotine produced 31 and 4 fold increases in the calcium content of the aorta and the mesenteric arterial bed, respectively.

2 Aortic rings and perfused mesenteric arterial beds from vitamin D_3/nicotine-treated animals showed a diminished contractile response to noradrenaline *in vitro*.

3 In vascular preparations from vitamin D_3/nicotine-treated animals, precontracted with noradrenaline, relaxation by the endothelium-dependent vasodilator, carbachol, was attenuated but responses to sodium nitroprusside were not modified.

4 Prolonged treatment with the angiotensin I converting enzyme inhibitor, perindopril, at a dose (1 mg kg^{-1}) which did not significantly modify blood pressure, failed to prevent vascular calcium overload.

5 Perindopril treatment diminished noradrenaline-evoked vasoconstrictor responses of aortic rings in both groups, but restored responses in mesenteric arterial beds of vitamin D_3/nicotine-treated rats.

6 Perindopril treatment also restored the maximal responses to carbachol of both aortic rings and mesenteric arterial beds of vitamin D_3/nicotine-treated rats.

7 In conclusion, in the vitamin D_3 plus nicotine model of calcium overload, reduced endothelial-mediated relaxation can be prevented by perindopril treatment.

Keywords: Calcium overload; rat aorta; endothelium; angiotensin I converting enzyme; ACE inhibitor; perindopril

Introduction

Treatment of spontaneously hypertensive rats for 18–20 months with high doses of angiotensin I converting enzyme inhibitor, captopril (up to 350 mg kg^{-1} per day, orally) has been shown to normalize blood pressure and prevent calcium overload of the aorta, and the mesenteric and other arteries (Fleckenstein *et al.*, 1987). Drugs which modify the evolution, and/or the consequences, of vascular calcium overload may be more effective in the treatment of vascular diseases, since, in addition to their haemodynamic action, they may correct the pathogenetic abnormalities of vascular calcium handling and consequences such as atherosclerosis and hypertension (Fleckenstein *et al.*, 1987). As vascular calcium overload develops very slowly in the spontaneously hypertensive rat and treatment aimed at modifying this natural process has to be applied for several months, a shorter-term model was developed (Fleckenstein, 1985).

Administration to young rats of vitamin D_3 followed by daily injections of nicotine produces large increases in the calcium content of arteries, followed by death within 6 days; simultaneous administration of large doses of calcium entry blockers attenuates the vascular calcium overload and protects the rats against the lethal effect of such treatment (Fleckenstein, 1985). Death may be due to calcium overload in many organs not only arteries. Immediately following treatment, hypercalcaemia and increases in the plasma levels of urea and creatinine are observed (Thorin *et al.*, 1990). We reduced the toxicity of the original model by (i) decreasing the total amount of nicotine administered, and (ii) allowing the rats to recover for 16 days following the treatment.

Changes in endothelial function have been described in both atherosclerosis (Parmley, 1990) and hypertension (Hongo *et al.*, 1988). The end result we chose, therefore, was evaluation of endothelium-dependent relaxation *in vitro*. As angiotensin I converting enzyme inhibitors prevent vascular calcium overload in the spontaneously hypertensive rat (see above) we evaluated the effects of perindopril in this model. Perindopril is approximately 10 times more potent than captopril (Muller *et al.*, 1990).

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Methods

Animals

One hundred and six male, outbred Wistar rats (220–250 g; Iffa-Credo SA, L'Arbresle, France) were given a standard diet (A04, UAR, Villemoisson sur Orge, France; calcium 150 mmol kg^{-1}) and mineral water (calcium 0.26 mM; Société de Eaux de Volvic, Volvic, Puy-de-Dôme) *ad libitum*.

Vitamin D_3 and nicotine treatment

Fifty-five of the rats were injected with vitamin D_3 (300 000 iu kg^{-1}, i.m.) and nicotine (25 mg kg^{-1}, orally) at 08 h 30 min, on day 1. The nicotine administration was repeated at 18 h 00 min. Rats were allowed 16 days to recover. The remaining 51 rats (controls) were administered distilled water (5 ml kg^{-1}, orally) and 0.15 M NaCl (2 × 1 ml kg^{-1}, i.m.).

Perindopril treatment

All rats were injected at 09 h 00 min on days 2 to 16 with perindopril (0.3, 1 or 3 mg kg^{-1}, i.p.) or sodium chloride (0.15 M, 0.5 ml kg^{-1}, i.p.).

Blood pressure and heart rate measurements

On the 17th day, the right carotid artery was cannulated following anaesthesia with sodium pentobarbitone (50 mg kg^{-1}, i.p.), and the cannula connected to a pressure transducer

linked to a polygraph recorder (Narco Biosystems, Houston, Texas, U.S.A.) for the measurement of mean arterial pressure (mmHg). Heart rate (b.p.m.) was recorded with a ratemeter driven by the pulse pressure signal.

Blood sampling

Following recording of blood pressure, 5 ml of blood were collected with heparin (10 iu ml^{-1}) used as anticoagulant. Samples were centrifuged for 10 min at 1000 *g* and 4°C. Calcium, phosphate, magnesium, sodium, potassium, creatinine, urea, cholesterol, triglycerides, glucose, protein, aspartate aminotransferase and alkaline phosphatase activities were determined by standard clinical chemistry methods.

Aortic ring preparation

Following blood sampling, a ring (3 mm) was dissected from the thoracic aorta and mounted under a tension of 0.8 g in Krebs bicarbonate solution (mM: NaCl 118, KCl 4.7, $MgCl_2 \cdot 6H_2O$ 1.2, NaH_2PO_4 1.0, $CaCl_2 \cdot 2H_2O$ 2.6, $NaHCO_3$ 25, glucose 11.1), at 37°C, oxygenated with 95% oxygen plus 5% carbon dioxide (pH 7.4 ± 0.1). Isometric tension was recorded by a force transducer (Ugo Basile, Comerio, Italy). After equilibration (90 min), cumulative doses of noradrenaline were added to contract the rings to approximately the same relative tension compared to the maximal response of the tissue in all groups. When contraction reached a plateau, carbachol (10^{-10} M to 10^{-4} M) was added in a cumulative manner. After several washes with Krebs bicarbonate and a 30 min recovery period, a cumulative dose-response curve was constructed for noradrenaline (10^{-9} M to 3×10^{-6} M). Responses to noradrenaline were evaluated on the basis of: (i) maximal increase in tension (mg), (ii) threshold sensitivity estimated from $ED_{0.1g}$, and (iii) midrange sensitivity estimated from $ED_{50\%}$.

After several washes with Krebs bicarbonate and a 30 min recovery period, cumulative doses of noradrenaline were added to precontract the ring as described above and sodium nitroprusside (10^{-11} M to 10^{-5} M) was added in a cumulative manner. Relaxation of the precontracted aortic ring by either carbachol or sodium nitroprusside is expressed as (i) maximal inhibition (%) of the noradrenaline-induced tension observed at the highest dose of carbachol or sodium nitroprusside used, and (ii) $IC_{50\%}$.

All experiments described in this paper were done in the presence of endothelium, thus the number of rats in each group is equivalent to that given in Table 1. In other experiments (not reported here), in the absence of endothelium carbachol had no effect, and the relaxant effect of sodium nitroprusside was slightly potentiated in all groups.

Mesenteric arterial bed preparation

Before removal of the thoracic aorta (for the aortic ring preparations, see above), the superior mesenteric artery was cannulated, the ileo-colonic branches tied off, the gut removed (McGregor, 1965), and the mesenteric arterial bed removed and perfused at 4 ml min^{-1} with Krebs bicarbonate. A bubble trap system, with a flow rate of 0.2 ml min^{-1}, continuously removed a small volume of perfusate plus any air bubbles. Vasoconstriction was estimated from the increase in perfusion pressure (mmHg) by use of a strain gauge transducer (Beckman, Palo Alto, California, U.S.A.). After a 15 min stabilization period, noradrenaline was injected (3 nmol in 0.33 ml, during 20 s) into the perfusate at 5 min intervals for 25 min, by which time a reproducible vasoconstrictor response was obtained. The following protocol was then used in half the rats. Firstly, a noradrenaline dose-response curve was constructed by injection of noradrenaline (0.33 ml in 20 s, 0.3 to 300 nmol) every 5 min. Vasoconstrictor responses to noradrenaline were evaluated on the basis of: (i) maximal increases in perfusion pressure (mmHg), (ii) threshold sensitivity estimated from ED_{10mmHg}, and (iii) midrange sensitivity estimated from $ED_{50\%}$. Secondly, following construction of the dose-response curve to noradrenaline, the mesenteric bed was perfused for 5 min with carbachol (10^{-9} M) then noradrenaline was injected, at a dose chosen on the basis of the noradrenaline dose-response curves, to produce an approximately equal increase in perfusion pressure relative to the maximal response in each group. The protocol was repeated with stepwise increases in the concentration of carbachol up to 10^{-4} M. Thirdly, following perfusion with Krebs bicarbonate for 15 min, the protocol was repeated with sodium nitroprusside (10^{-10} to 10^{-5} M). Relaxation is expressed as maximal attenuation (%) of noradrenaline-induced contraction, and $IC_{50\%}$.

The protocol was repeated in half the animals after the endothelium had been removed by passing air bubbles through the perfusion system (Story & Ziogas, 1986) before the construction of the noradrenaline dose-response curve.

Tissue calcium and magnesium levels

Samples of the left ventricle, thoracic aorta, abdominal aorta, carotid artery, mesenteric arterial bed, tail artery, femur, small intestine, cerebral cortex, eyes, kidneys and liver were weighed and heated to constant dry weight. Dry tissue samples were dissolved in nitric acid (14 N, 72 h) then centrifuged (2000 *g*, 10 min). Lanthanum chloride was added to the supernatant, and calcium and magnesium (μmol g^{-1} dry weight, except for femur: mmol g^{-1} dry weight) measured by atomic absorption spectrophotometry (Perkin-Elmer, Norwalk, Connecticut, U.S.A.).

Substances used

Vitamin D_3 (Vi-Dé) was a gift of Sandoz AG, Bern, Switzerland. Nicotine bitartrate, noradrenaline bitartrate, carbachol chloride, sodium nitroprusside and ascorbic acid were purchased from Sigma, St. Louis, Missouri, U.S.A. Noradrenaline bitartrate was dissolved in 0.1 mM ascorbic acid. Concentrations or doses are given as base. Sodium pentobarbitone was purchased from Sanofi SA, Paris, France. Other chemicals were purchased from Merck AG, Darmstadt, Germany or Biomérieux, Marcy l'Etoile, France. Perindopril was a gift of Servier Laboratories, Paris, France.

Statistics

Results are expressed as means ± s.e.mean. Significant differences between groups were determined by ANOVA and the Scheffé test. The probability level chosen was $P < 0.05$. Dose-response data were fitted to the sigmoid curve equation: response = c/(1 + exp ((*a* − $\log_{10}$ dose)/b)) + d, where response is expressed as 'mmHg' or 'mg', a = $\log_{10}$ ($ED_{50\%}$), b = slope, c = response at [dose] = ∞, d = response at [dose] = 0, using a computer programme written by M. Bordes, IMPC, Sophia-Antipolis, France, based on Bevington (1969). Dose-response data were also subjected to linear regression analysis following logit (response %) versus $\log_{10}$ [concentration] transformation. Threshold and midrange sensitivities were calculated from the regression lines obtained.

Results

Body weight and plasma profile

Control rats gained weight (4 to 6 g day^{-1}) throughout the experiment (Table 1). During the first 6 days following vitamin D_3 and nicotine, rats lost weight at a rate of 8 to 10 g day^{-1}. From day 7 onwards rats gained weight at a rate of 6 to 9 g day^{-1}. This growth rate was greater than that of control rats during the corresponding period. Perindopril had no

Table 1 Effect of chronic treatment with perindopril (Perind, mg kg^{-1} per day) on body weight in vitamin D/nicotine-treated and control rats

Group	n	Perind	Start (g)	Day 6 (g)	Day 17 (g)	Growth (g day^{-1})
Control	19	—	241 ± 8	269 ± 7	322 ± 6	5.6 & 5.3
Vitamin D/nicotine-treated rats	22	—	239 ± 9	189 ± 3	265 ± 9	−10.0 & 7.6
Control	8	0.3	231 ± 12	258 ± 13	315 ± 11	5.4 & 5.7
Vitamin D/nicotine-treated rats	8	0.3	219 ± 9	173 ± 5	264 ± 5	−9.2 & 9.1
Control	16	1	238 ± 9	269 ± 10	319 ± 10	6.2 & 5.0
Vitamin D/nicotine-treated rats	16	1	234 ± 8	193 ± 6	267 ± 5	−8.2 & 7.4
Control	8	3	221 ± 10	240 ± 9	296 ± 9	3.8 & 5.6
Vitamin D/nicotine-treated rats	9	3	220 ± 5	171 ± 5	233 ± 8	−9.8 & 6.2

Growth = growth rates (g day^{-1}) from day 1 to day 5, and from day 7 to day 17.

effect on growth rate of control or vitamin D_3/nicotine-treated rats. Neither vitamin D_3 plus nicotine nor perindopril had any effect on plasma ions, metabolites or enzymes (Table 2, results for perindopril: 1 mg kg^{-1} only).

Tissue calcium and magnesium contents

Treatment with vitamin D_3 plus nicotine increased the calcium content of blood vessels, heart and kidney. The calcium content of the femur decreased. Chronic treatment with perindopril had no effect on soft tissue calcium content in either vitamin D_3/nicotine treated or control rats, but prevented the fall in bone calcium following vitamin D_3 plus nicotine treatment (Table 3). Magnesium levels did not change (results not shown).

Blood pressure and heart rate

Mean arterial pressure (113 ± 6 and 113 ± 5 mmHg) and heart rate (409 ± 12 and 406 ± 11 b.p.m.) were similar in vitamin D_3/nicotine-treated and control rats. Lower doses (0.3 and 1 mg kg^{-1}, per day) of perindopril had no effect on either blood pressure or heart rate. Perindopril at a dose of 3 mg kg^{-1} per day had no effect on blood pressure (vitamin D_3/nicotine-treated rats 103 ± 4, and control rats 105 ± 3 mmHg) but lowered heart rate (vitamin D_3/nicotine-treated rats 342 ± 10, and control rats 360 ± 14 b.p.m., $P < 0.05$).

Aortic rings

Noradrenaline produced dose-related increases in tension in aortic rings; maximum and sensitivity were lower in aortic rings from vitamin D_3/nicotine-treated rats (Figure 1). In control rats, perindopril, at the lower dose of 0.3 mg kg^{-1} had no effect, but at the higher doses of 1 or 3 mg kg^{-1}, produced a decrease in maximum (see Figure 1 for results with 1 mg kg^{-1}). Perindopril treatment had no significant effect in vitamin D_3/nicotine-treated rats, although a similar tendency was observed.

Relaxation induced by carbachol was attenuated by treatment with vitamin D_3 and nicotine (Figure 2). The percentage relaxation induced by a concentration of carbachol of 1 μM was 73 ± 5 in controls and 35 ± 10% in vitamin D_3/nicotine-treated rats ($P < 0.5$). Treatment with higher doses of perindopril restored the maximal vasorelaxant response to carbachol in vitamin D_3/nicotine-treated rats (for 1 mg kg^{-1} see Figure 2; 3 mg kg^{-1}: 85 ± 3%). Relaxation at a concentration of carbachol of 1 μM was similar to that of controls. For example, following chronic treatment with 1 mg kg^{-1} perindopril, values were 62 ± 9 in controls and 59 ± 13% in vitamin D_3/nicotine-treated rats.

Although vitamin D_3 and nicotine treatment had no effect on the relaxation induced by sodium nitroprusside (Figure 3), chronic treatment with perindopril, albeit without any effect on the maximal response, produced dose-related increases ($P < 0.05$) in $IC_{50\%}$ values: 1.3 ± 0.3, 4.0 ± 0.4, 8.6 ± 0.7 and 15.0 ± 1.5 × 10^{-9} M in control rats, and 2.6 ± 0.6, 5.5 ± 0.7,

Table 2 Effect of chronic treatment with perindopril (1 mg kg^{-1} per day) on plasma ions, metabolites and enzymes in vitamin D/nicotine treated and control rats

	Controls	Vitamin D/nicotine	Controls	Vitamin D/nicotine
Perindopril	—	—	1	1
n	19	22	16	16
Calcium (mM)	2.45 ± 0.05	2.52 ± 0.01	2.41 ± 0.1	2.39 ± 0.09
Magnesium (mM)	1.02 ± 0.03	1.10 ± 0.03	1.10 ± 0.06	1.06 ± 0.04
Sodium (mM)	143 ± 3	144 ± 6	145 ± 5	138 ± 5
Potassium (mM)	3.93 ± 0.08	3.84 ± 0.08	3.83 ± 0.07	3.71 ± 0.09
Phosphate (mM)	1.84 ± 0.11	1.50 ± 0.09	1.79 ± 0.13	1.65 ± 0.14
Creatinine (μM)	82 ± 15	65 ± 6	66 ± 6	61 ± 3
Urea (mM)	6.2 ± 0.4	6.4 ± 0.8	6.9 ± 0.6	6.9 ± 0.4
Cholesterol (mM)	1.25 ± 0.08	1.43 ± 0.12	1.11 ± 0.05	1.26 ± 0.10
Triglycerides (mM)	0.78 ± 0.12	0.73 ± 0.18	0.72 ± 0.10	0.62 ± 0.03
Glucose (mM)	10.3 ± 0.7	8.7 ± 0.5	8.9 ± 0.9	8.7 ± 0.8
Protein (g l^{-1})	58 ± 3	53 ± 3	57 ± 2	54 ± 1
Aspartate aminotransferase activity (iu l^{-1})	75 ± 5	92 ± 10	85 ± 9	98 ± 7
Alkaline phosphatase activity (iu l^{-1})	304 ± 18	280 ± 37	272 ± 27	355 ± 48

Table 3 Effect of chronic treatment with perindopril (1 mg kg^{-1} per day) on tissue calcium content (μmol g^{-1} dry weight) in vitamin D/nicotine-treated and control rats

	Controls	*Vitamin D/ nicotine*	*Controls*	*Vitamin D/ nicotine*
Perindopril	—	—	1	1
n	19	22	16	16
Thoracic aorta	10.9 ± 0.9	307 ± 77*	12.5 ± 0.7	416 ± 143*
Abdominal aorta	14.8 ± 0.8	333 ± 44*	15.6 ± 1.8	232 ± 54*
Carotid artery	34 ± 5	543 ± 95*	33 ± 6	426 ± 99*
Tail artery	27 ± 3	67 ± 9*	27 ± 5	88 ± 7*
Mesenteric arterial bed	1.3 ± 0.2	5.1 ± 1.0*	1.1 ± 0.1	3.8 ± 0.7*
Myocardium	4.0 ± 0.1	38 ± 6*	4.5 ± 0.2	47 ± 5*
Kidney	6.2 ± 0.9	105 ± 33*	5.5 ± 0.5	103 ± 12*
Intestine	6.5 ± 0.4	9.0 ± 1.5	6.8 ± 0.4	10.9 ± 2.0
Liver	2.9 ± 0.2	2.6 ± 0.3	2.6 ± 0.3	2.3 ± 0.3
Cerebral cortex	5.5 ± 0.9	5.1 ± 0.7	5.3 ± 0.7	4.7 ± 0.6
Eye	9.3 ± 0.4	10.6 ± 0.4	8.8 ± 0.3	10.0 ± 0.3
Femur diaphysis (mmol g^{-1})	5.5 ± 0.2	4.7 ± 0.2*	5.2 ± 0.2	5.4 ± 0.3
Femur epiphysis (mmol g^{-1})	5.1 ± 0.1	4.6 ± 0.2*	5.3 ± 0.2	5.2 ± 0.3

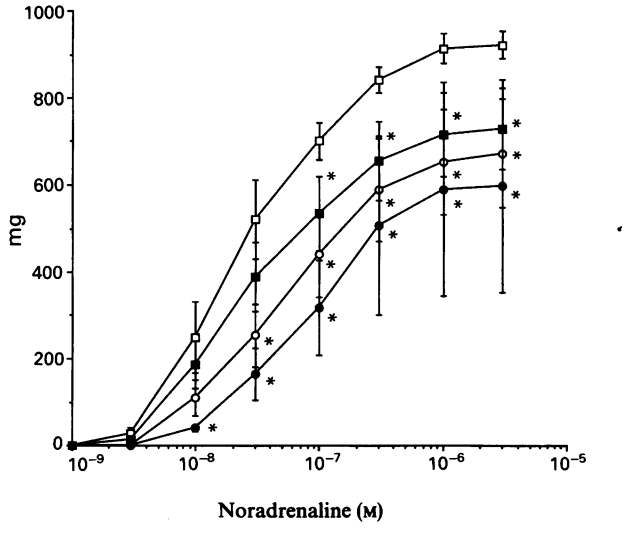
* $P < 0.05$ compared to means for control rats chronically treated with 0.5 M NaCl.

9.0 ± 1.5 and 12.5 ± 2.3 × 10^{-9} M in vitamin D_3/nicotine-treated rats, for doses of perindopril of 0, 0.3, 1 and 3 mg kg^{-1} per day, respectively.

Mesenteric arterial bed

Noradrenaline produced dose-related increases in perfusion pressure in mesenteric arterial beds perfused at a constant flow rate; maximum and sensitivity were lower in mesenteric arterial beds removed from rats previously treated with vitamin D_3 and nicotine (Figure 4). Perindopril had no effect in controls, but in mesenteric arterial beds from vitamin D_3/nicotine-treated rats the maximal response and sensitivity were increased at doses of 1 mg kg^{-1} (Figure 4) and 3 mg kg^{-1} per day. In all cases, removal of endothelium doubled mid-range sensitivity (for example in control rat preparations $ED_{50\%}$ decreased from 6.9 ± 0.8 to 2.9 ± 0.4 × 10^{-9} M, $n = 9$ per group, $P < 0.05$, following removal of endothelium), and slightly, but not significantly, increased the maximal response. The effects of chronic treatment with perindopril were proportionally similar to those observed in mesenteric arterial bed preparations with an intact endothelium.

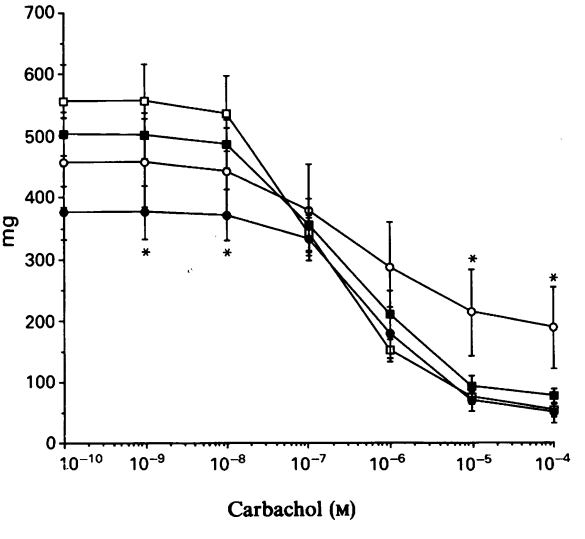
Relaxation induced by carbachol was decreased in vitamin D_3/nicotine-treated rats (Figure 5). Perindopril treatment had no effect in controls, but at doses of 1 (Figure 5) and 3 mg kg^{-1} per day, restored the maximal response to car-



Group	*Chronic treatment*	$ED_{50\%}$ (10^{-8} M)	*Maximum* (mg)
Control	0.15 M NaCl	1.6 ± 0.3	920 ± 30
Control	Perindopril	1.7 ± 0.3	730 ± 90*
Vitamin D/nicotine	0.15 M NaCl	4.3 ± 1.0*	670 ± 80*
Vitamin D/nicotine	Perindopril	8.4 ± 2.5*	600 ± 100*

* $P < 0.5$ compared to means for control rats chronically treated with 0.15 M NaCl (□).

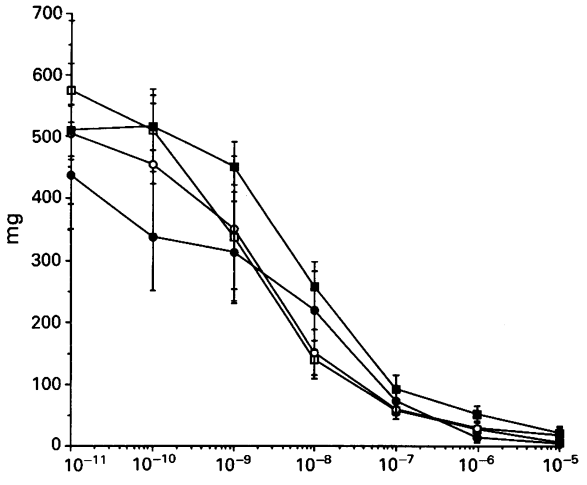
Figure 1 Effect of chronic treatment with perindopril (1 mg kg^{-1} per day, solid symbols) or 0.15 M NaCl (open symbols) on noradrenaline-induced contraction (mg) in aortic rings from control rats (squares) or vitamin D_3/nicotine-treated rats (circles).



Group	*Chronic treatment*	$IC_{50\%}$ (10^{-7} M)	*Maximum* (%)
Control	0.15 M NaCl	1.5 ± 0.3	90 ± 2
Control	Perindopril	3.0 ± 0.7	87 ± 2
Vitamin D/nicotine	0.15 M NaCl	4.3 ± 1.0*	61 ± 9*
Vitamin D/nicotine	Perindopril	6.7 ± 2.0*	92 ± 5

* $P < 0.05$ compared to means for control rats chronically treated with 0.15 M NaCl (□).

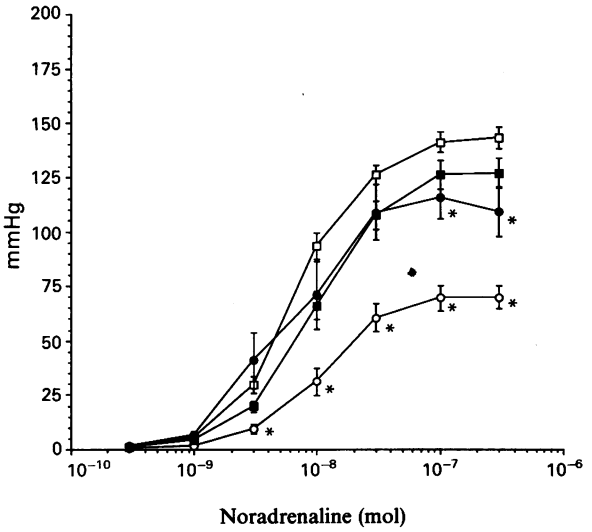
Figure 2 Effect of chronic treatment with perindopril (1 mg kg^{-1} per day, solid symbols) or 0.15 M NaCl (open symbols) on carbachol-induced relaxation of aortic rings precontracted with noradrenaline from control rats (squares) or vitamin D_3/nicotine-treated rats (circles).



Group	Chronic treatment	$IC_{50\%}$ (10^{-9} M)	Maximum (%)
Control	0.15 M NaCl	1.4 ± 0.3	99 ± 2
Control	Perindopril	8.6 ± 0.7*	96 ± 2
Vitamin D/nicotine	0.15 M NaCl	1.6 ± 0.6	96 ± 3
Vitamin D/nicotine	Perindropril	9.0 ± 1.5*	98 ± 2

* $P < 0.05$ compared to means for control rats chronically treated with 0.15 M NaCl (□).

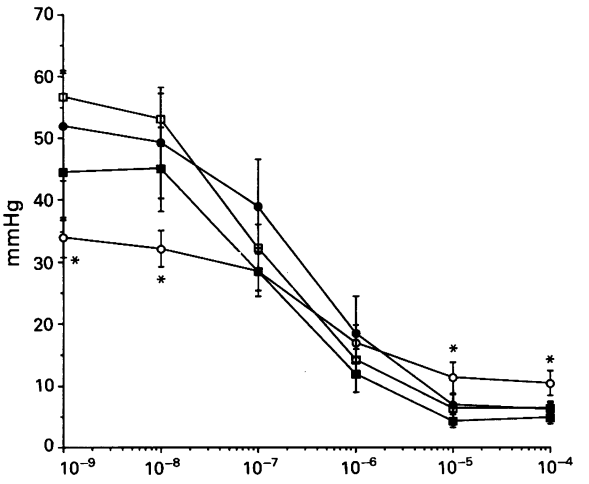
Figure 3 Effect of chronic treatment with perindopril (1 mg kg^{-1} per day, solid symbols) or 0.15 M NaCl (open symbols) on sodium nitroprusside-induced relaxation of aortic rings precontracted with noradrenaline from control rats (squares) or vitamin D_3/nicotine-treated rats (circles).



Group	Chronic treatment	$ED_{50\%}$ (10^{-9} M)	Maximum (mmHg)
Control	0.15 M NaCl	6.9 ± 0.8	143 ± 5
Control	Perindopril	9.7 ± 1.0	127 ± 7
Vitamin D/nicotine	0.15 M NaCl	11.4 ± 2.2*	70 ± 5*
Vitamin D/nicotine	Perindopril	6.5 ± 0.6	116 ± 10*

* $P < 0.05$ compared to means for control rats chronically treated with 0.15 M NaCl (□).

Figure 4 Effect of chronic treatment with perindopril (1 mg kg^{-1} per day, solid symbols) or 0.15 M NaCl (open symbols) on noradrenaline-induced vasoconstriction (mmHg) of mesenteric arterial beds removed from control rats (squares) or vitamin D_3/nicotine-treated rats (circles).



Group	Chronic treatment	$IC_{50\%}$ (10^{-7} M)	Maximum (%)
Control	0.15 M NaCl	1.1 ± 0.3	89 ± 3
Control	Perindopril	3.0 ± 0.8	89 ± 4
Vitamin D/nicotine	0.15 M NaCl	4.5 ± 0.8*	69 ± 4*
Vitamin D/nicotine	Perindopril	6.7 ± 1.0*	88 ± 3

* $P < 0.05$ compared to means for control rats chronically treated with 0.15 M NaCl (□).

Figure 5 Effect of chronic treatment with perindopril (1 mg kg^{-1} per day, solid symbols) or 0.15 M NaCl (open symbols) on carbachol-induced relaxation of mesenteric arterial bed preparations precontracted with noradrenaline from control rats (squares) or vitamin D_3/nicotine-treated rats (circles).

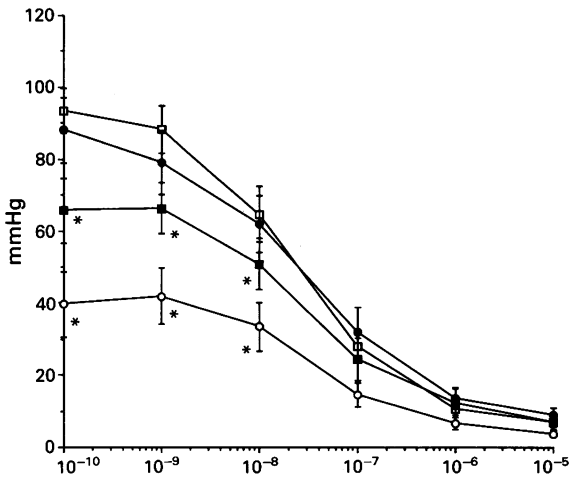
bachol in vitamin D_3/nicotine-treated rats. Following removal of endothelium, carbachol was without effect in mesenteric arterial bed preparations (results not shown).

Sodium nitroprusside-induced relaxation of mesenteric arterial bed preparations precontracted with noradrenaline was similar in controls and vitamin D_3/nicotine-treated rats (Figure 6). Perindopril treatment had no significant effect.

Discussion

Although body weight initially fell, vitamin D_3/nicotine-treated rats later showed a growth rate which was higher than that of controls. There were no signs of renal or other toxicity. Perindopril treatment had no effect on growth rate or measured plasma variables. Although the calcium content of the kidney increased 17 fold, plasma urea and creatinine levels in vitamin D_3/nicotine-treated rats were normal (Table 2; Waynforth, 1980). Endothelial function appeared to be modified in spite of normal plasma cholesterol levels, and this raises the possibility that vitamin D_3 plus nicotine treatment could provide one of the few models of altered endothelial function in the absence of any increase in the plasma level of cholesterol.

Calcium overloading of vessel walls presumably arises after the following sequence of events. Firstly, vitamin D, by increasing intestinal and renal calcium absorption (Guyton, 1991), and mobilizing bone calcium (present results) produces an initial hypercalcaemia (Thorin *et al.*, 1990). In the presence of increased extracellular calcium levels, nicotine causes intracellular accumulation of calcium, either directly (Nayler, 1963), or indirectly via release of catecholamines (Hass *et al.*, 1966). Intracellular accumulation of calcium is presumably lethal for the cell (Farber, 1981), and following cell death, extracellular deposition of calcium occurs (Seydewitz & Stau-



Group	Chronic treatment	$IC_{50\%}$ (10^{-8} M)	Maximum (%)
Control	0.15 M NaCl	2.4 ± 0.3	93 ± 3
Control	Perindopril	3.3 ± 0.3	89 ± 3
Vitamin D/nicotine	0.15 M NaCl	3.9 ± 0.5	90 ± 2
Vitamin D/nicotine	Perindopril	2.8 ± 0.4	90 ± 3

* $P < 0.05$ compared to means for control rats chronically treated with 0.15 M NaCl (□).

Figure 6 Effect of chronic treatment with perindopril (1 mg kg^{-1} per day, solid symbols) or 0.15 M NaCl (open symbols) on sodium nitroprusside-induced relaxation of mesenteric arterial bed preparations precontracted with noradrenaline from control rats (squares) or vitamin D_3/nicotine-treated rats (circles).

besand, 1988), especially in the media of the compliance vessels (Fleckenstein *et al.*, 1987; Henrion *et al.*, 1991). Calcium accumulation in the media may present a diffusion barrier for endothelium-derived relaxing factor (EDRF) and so explain the decrease in carbachol-induced relaxation. This hypothesis could be tested by superfusion experiments but appears unlikely as perindopril did not modify calcium accumulation and yet restored carbachol-induced relaxation.

The decrease in carbachol-induced relaxation could stem from a decrease of the affinity and/or number of the cholinoceptors on the endothelial cells following exposure to a high dose of nicotine. This appears unlikely as the relaxant properties of histamine are also diminished in aortic rings from vitamin D_3/nicotine-treated rats precontracted with noradrenaline ($IC_{50\%}$ 7.2 ± 0.3, controls 3.0 ± 0.2 μM, $n = 12$, $P < 0.05$, maximal relaxation 69 ± 5, controls 86 ± 1%, $P < 0.05$; Henrion *et al.*, unpublished results).

It is possible that calcium overloading of endothelial cells, produced by vitamin D_3 and nicotine, interferes with their intracellular calcium handling mechanisms and so diminishes release of EDRF, which is a calcium-dependent mechanism (Defeudis, 1985). Perindopril treatment could restore endothelial calcium handling mechanisms involved in carbachol-induced relaxation. Other changes in calcium handling are possible. Although vitamin D_3 plus nicotine produced calcium accumulation in small muscular arteries (tail artery) and in resistance vessels (mesenteric arterial bed) the rats did not become hypertensive. This raises the possibility that following vitamin D_3 plus nicotine treatment, calcium accumulates in smooth muscle cells at intracellular sites, such as the plasmalemma and the endoplasmic reticulum, and in some way interferes with the intracellular calcium handling. This may explain the decrease in the constrictor responses to noradrenaline in both the aortic rings and the mesenteric arterial bed preparations of vitamin D_3/nicotine-treated rats. Perindopril did not modify calcium accumulation in the mesenteric arterial bed of vitamin D_3/nicotine-treated rats but did restore noradrenaline-induced vasoconstriction (in both the presence and absence of endothelium). The effects of vitamin D_3 plus nicotine and/or perindopril treatment on calcium handling mechanisms could be investigated by following changes in intracellular free calcium levels with a fluorescent probe such as fura-2 (Thorin-Trescases *et al.*, 1990).

Converting enzyme inhibitors can modify prostanoid metabolism but the evidence as to whether or not such an effect is involved in cholinergic relaxation *in vitro* is contradictory. Indomethacin does not alter the potentiation by captopril of acetylcholine-induced relaxation of aortic rings (precontracted with noradrenaline) from normotensive rats (Schultz & Raij, 1989). Indomethacin did, however, decrease the ratio of 'relaxation divided by constriction' for 5-hydroxytryptamine in aortic rings from spontaneously hypertensive rats (Clozel *et al.*, 1990). The question as to whether or not chronic treatment with perindopril can prevent the changes in endothelial prostanoid metabolism induced by calcium overload could be investigated by using drugs such as indomethacin which interfere with prostanoid metabolism.

In conclusion, in both a compliance and a resistance vessel, treatment with vitamin D_3 and nicotine attenuated endothelial-dependent carbachol relaxation and perindopril treatment of these animals opposed this effect. This may be taken as evidence of a novel vascular action of angiotensin I converting enzyme inhibitors at the endothelial level.

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