Our objective in these experiments was to study actions of cryptolepine on α-adrenoceptors in rat intestine and to ascertain whether NO might be involved in its smooth-muscle-relaxant effects.

Methods Ileum segments derived from hooded Lister rats (250–350 g) were investigated under 1 g tension in Krebs solution (37°C, 95% O_2/5% CO_2) containing 100 μM hexamethonium bromide. Cryptolepine was synthesized as described by Wright et al (2001). Contractions of longitudinal muscle to electrical field stimulation (EFS; 40 V, 0.4–4 Hz for 30 seconds) were recorded isometrically in the absence or presence of atropine (0.01–0.1 μM) or cryptolepine (1–100 μM). To investigate involvement of adrenoceptors and NO in relaxation, experiments were repeated at 4 Hz in the absence or presence of one of the following: phentolamine (PHENT; a non-selective α-adrenoceptor antagonist, 1–30 μM), phenylephrine (PHENYL; an α1-adrenoceptor agonist, 1–10 μM), prazosin (PRAZ; an α1-adrenoceptor antagonist, 0.1–3 μM) or *N*-nitro-L-arginine methyl ester (L-NAME; a nitric oxide synthase (NOS) inhibitor, 100 μM), alone or in combination with cryptolepine (10–100 μM, n = 4–9).

Results Stimulation elicited frequency-dependent contraction, which was abolished by atropine (0.1 μM). Tissue responses at 4 Hz were biphasic, consisting of initial contraction, partial relaxation and a secondary contraction. Low concentrations of cryptolepine (1–10 μM) potentiated responses to EFS, while higher concentrations (30–100 μM) dose-dependently reduced both contractile components. Cryptolepine (1–10 μM), PHENT (1–30 μM) and PRAZ (0.1–3 μM) potentiated the secondary contraction. PHENYL (1–10 μM) reduced the contractile responses to EFS, an effect reversed by both PRAZ (3 μM) and cryptolepine (10 μM). L-NAME (100 μM) also potentiated the secondary contraction ($P < 0.001$), a substantial potentiation, which was unchanged by addition of cryptolepine (10–30 μM).

Conclusions Cryptolepine potentiated contraction elicited by EFS when administered at low concentration but inhibited contraction at high concentration. Contraction elicited by EFS was biphasic, the secondary contraction being most affected by α-adrenoceptor modulators. The effect of cryptolepine in potentiating contraction resembled actions of the α-antagonists, and was most likely to operate via α1-receptor antagonism at these low concentrations. At higher concentrations its anti-muscarinic action inhibited contractile activity. Cryptolepine at low concentrations did not affect the potentiation of contraction elicited by the NOS inhibitor L-NAME. We conclude that cryptolepine may possess α1-antagonist activity, which could moderate relaxant effects of the sympathetic system on the intestine, but an action on intestinal nitrergic transmission is unlikely.

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Vascular relaxant effects of testosterone

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Objectives Epidemiological evidence indicates that, until the menopause, women appear to be protected from coronary artery disease. High androgen levels are presumed to explain male predisposition to this condition. Although testosterone could act as a risk factor for cardiovascular disease, several studies suggest that testosterone is a vasorelaxant agent. Rosano et al (1999) showed that administering testosterone to men with coronary disease improved tolerance to exercise-induced cardiac ischaemia, which was attributed to a vasodilator action of testosterone. Much less is known about the vascular effects of testosterone than oestrogens, and the mechanism involved is controversial. An involvement of endothelial NO, L-type calcium channels and various potassium channels in testosterone-induced dilation has been suggested. In previous work we showed that neither endothelial nor tissue-derived NO accounted for testosterone's dilator action. Our present objective was to investigate the involvement of prostaglandin production, androgen receptor activation and aromatization in relaxant responses to testosterone, using longitudinal muscle from rat hepatic portal vein. This vascular muscle is unaffected by endothelium-derived nitric oxide as it is separated from endothelium by a substantial circular muscle layer.

Methods Portal veins from male hooded Lister rats (250–350 g) were placed under 0.5 g tension in Krebs solution (37°C, 95% O_2/5% CO_2). Concentration-response curves to phenylephrine (10–100 μM) were constructed in the absence or presence of testosterone (10 and 20 μM). These concentration-response curves were repeated following equilibration of the tissue with indomethacin (a cyclooxygenase inhibitor, 10 μM), flutamide (an androgen receptor antagonist, 10 μM) or aminoglutethimide (an aromatase inhibitor which prevents conversion of testosterone to oestrogen, 10 and 50 μM). These concentrations of inhibitor were shown to be effective in rabbit coronary arteries by Yue et al (1995). Testosterone was dissolved in 100% ethyl alcohol: no vehicle effects were observed (n = 4–9).

Results Testosterone (10 and 20 μM) decreased contractile responses to phenylephrine, reducing E_{max} to 43.2 ± 6 and 30.0 ± 2.9% for 10 and 20 μM respectively ($P < 0.001$). The decrease in contraction was unaltered by incubation of the tissue with indomethacin, flutamide or aminoglutethimide. In further experiments the portal vein was incubated in calcium-free Krebs solution: KCl (30 mM) plus increasing concentrations of calcium chloride were administered to construct a concentration-response curve to calcium in the absence or presence of testosterone. Testosterone (10 and 20 μM) caused a reduction in responses to calcium ions; E_{max} was reduced to 69.9 ± 10.9 and 49.7 ± 8.7% respectively. This reduction was significantly smaller than the relaxant effect of testosterone observed on phenylephrine-induced contraction in normal Krebs solution ($P < 0.05$).

Conclusions We conclude that synthesis of prostaglandins, androgen receptors and aromatization of testosterone to oestrogen are unlikely to be involved in the relaxant effects produced by testosterone on phenylephrine-induced contraction in portal vein. However, a proportion of the relaxant effect observed in these experiments can be attributed to an inhibition by testosterone of calcium entry into vascular smooth muscle cells.

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Tissue Engineering

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Polymeric fibres for bone regeneration: effect of collagen on fibre morphology and human osteoblast cell proliferation

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Objectives To develop novel biodegradable polymer fibres which will support the attachment and proliferation of human osteoblast (HOB) cells for bone regeneration. Previously, poly(ε-caprolactone) (PCL) films, with and without collagen, have been shown to support the growth of HOB cells *in vitro* (Coombes et al 2002). Therefore, here we assess the effect of collagen coating or incorporation on PCL fibre characteristics, morphology and cell-fibre interactions *in vitro*.

Methods 10% w/v PCL solution was wet spun by the gravity-spinning technique to generate 'as-spun' fibres. These 'as-spun' PCL fibres were coated with collagen at a concentration of either 0.01 or 0.1% w/v. Alternatively, collagen was mixed with the PCL solution (10% w/v) prior to being wet spun at a collagen concentration of either 0.005 or 0.01% w/v, to generate PCL fibres incorporating collagen. Scanning electron microscopy (SEM) was used to assess fibre morphology and confocal laser microscopy was used to visualize and confirm collagen distribution within the PCL fibres using rhodamine B. To each fibre platform, HOB cells were seeded at a density of 50,000 cells per well and incubated at 37°C in a humidified incubator with 5% CO_2. At day 56, cell attachment was analysed by SEM and a live/dead stain using confocal microscopy to visualize fibre morphology and cell-fibre interactions.

Results Incorporation of 0.01% w/v collagen results in a slower rate of fibre production, presumably due to the higher viscosity found for this PCL and collagen mixed solution (Table 1). With an increase in viscosity, the solution flows more slowly through the spinneret under gravity. Confocal laser microscopy images confirmed that rhodamine-labelled collagen was incorporated into the PCL fibres using this technique. After 56 days of incubation, SEM and confocal images showed that HOB cells were attached to all fibres tested in large numbers, showing a high degree of HOB cell spreading.

Table 1 Fibre-production rates and characteristics of PCL fibres incorporating various concentrations of collagen. Results denote mean ± SD, n = 6

Collagen concentration (% w/v)	Fibre production rate (m/minute)	Fibre diameter (μm)
0	1.7 ± 0.12	246 ± 14.5
0.005	1.6 ± 0.02	196 ± 7.1
0.01	1.4 ± 0.20*	178 ± 8.7

*Incorporation of 0.01% w/v collagen into PCL fibres significantly ($P < 0.05$, analysis of variance) reduces fibre production rate.

Conclusions 'As-spun' PCL fibres, with and without collagen, provide a promising biomaterial and scaffold system for the attachment, proliferation and support of human osteoblasts and bone regeneration.

Coombes, A. G. A. et al (2002) *Biomaterials* **23**: 2113–2118