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Serotonin-induced contractility in human saphenous vein is inhibited by naftidrofuryl

R. Vashisht, M. Sian, E. J. Sharp, H. L. Leathard* and M. K. O'Malley

*Departments of Surgery and *Pharmacology, Charing Cross and Westminster Medical School, London, UK*

Correspondence to:
Mr R. Vashisht, Department of Surgery, Charing Cross Hospital, Fulham Palace Road, London W6 8RF, UK

Vascular endothelial denudation contributes to vasospasm by causing platelet aggregation and the subsequent release of vasoconstrictors such as serotonin. It has recently been suggested that naftidrofuryl fumarate (NFT) may oppose serotonin-induced vasoconstriction. Fourteen rings of human saphenous vein from 14 patients undergoing varicose vein surgery were tested in standard organ bath experiments. Cumulative dose–response curves and maximal contraction in response to serotonin were recorded and this was repeated in the presence of NFT at 10^{-6} and 10^{-3} mol/l. The difference in maximal contractility between the three sets of curves was significant ($P < 0.0001$). Sensitivity to serotonin in each of the three curves was measured using the concentration for half-maximal response; differences were again significant ($P < 0.0001$). It is concluded that NFT reduces serotonin-induced contractility in a dose-dependent fashion in rings of human saphenous vein in vitro. *These results suggest a possible role for NFT in reducing vasospasm and support further investigation of this drug.*

Serotonin has long been recognized as an important vasoconstrictor[1,2]. It is stored in granules within circulating platelets. When these aggregate in response to endothelial trauma, the granules are discharged and the stored serotonin released. It is suggested that the released serotonin acts directly on 5-HT_2 receptors within smooth muscle cells in the vessel wall to induce vasoconstriction[3,4]. This vasoconstriction may be of clinical significance and has been implicated in the pathogenesis of both coronary and peripheral vein bypass graft spasm[5,6].

Naftidrofuryl fumarate (NFT) (Praxilene; Lipha Pharmaceuticals, West Drayton, UK) has been used to treat intermittent claudication because of its supposed ability to increase the efficiency of cellular metabolism in ischaemic tissue[7–10]. However, recent animal studies have also shown that NFT is a specific 5-HT_2 receptor antagonist and inhibits serotonin-induced vasoconstriction[4,11,12]. The present study was therefore designed to assess the ability of NFT to antagonize serotonin-induced contraction in human vessels *in vitro*.

Materials and methods

Studies were performed on human saphenous vein *in vitro*. The proximal segment of long saphenous vein was excised with minimum trauma from patients undergoing varicose vein surgery and placed immediately in Krebs' solution (122 mmol l^{-1} NaCl, 4·4 mmol l^{-1} KCl, 1·2 mmol l^{-1} $MgCl_2$, 2·5 mmol l^{-1} $CaCl_2$, 15·4 mmol l^{-1} $NaHCO_3$, 1·2 mmol l^{-1} KH_2PO_4, 5·5 mmol l^{-1} dextrose) at 37 °C. Veins were then cleared of adherent connective tissue and cut into rings of 4–5 mm in width. The rings were suspended in a conventional organ bath and connected to a force–displacement transducer linked to an isolated multichannel polygraph. The organ bath contained Krebs' solution at pH 7·4. The solution in the bath was changed every 15 min and continuously bubbled with a mixture of 95 per cent oxygen and 5 per cent carbon dioxide. The temperature of the bath was maintained at 37 °C.

Drugs

NFT was dissolved in distilled water and tested for antiserotonin activity at two concentrations: 10^{-6} and 10^{-3} mol/l. Serotonin (5-hydroxytryptamine creatinine sulphate complex; Sigma Chemicals, St Louis, Missouri, USA) was dissolved in normal saline.

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Rings were allowed to equilibrate at a constant tension of 1·5 g for 1 h. An optimal length–tension relationship for maximal contractility was established using 40 mmol/l KCl (in addition to the K^+ and Cl^- in the Krebs' solution).

The rings were then washed and equilibrated, and cumulative dose-response curves for serotonin recorded as a control (experiment 1). After recording the first curve, the rings were washed and re-equilibrated for 45 min, and the reproducibility of the contractile response confirmed using a submaximal dose of serotonin (5×10^{-5} mol/l). The rings were then incubated in 10^{-6} mol/l NFT for 30 min and a second dose-response curve recorded (experiment 2). The rings were again washed and allowed to equilibrate for 45 min, and the effective removal of the antagonist confirmed using the same submaximal dose of serotonin as before. Rings that retained the original responsiveness to serotonin were then incubated in 10^{-3} mol/l NFT for 30 min and a third dose–response curve to serotonin recorded (experiment 3). Reversibility of the antagonism by washing was again confirmed using the submaximal dose of serotonin. Only rings that remained responsive to serotonin throughout were included in the study. These rings were sent for histological examination to confirm the presence of endothelium.

Statistical analysis

Dose–response curves for each individual ring were analysed by probit analysis (SAS; SAS Institute, Cary, North Carolina, USA). The concentration at which 50 per cent of the maximal response for each ring was elicited (ED_{50}) was calculated for each curve separately. Maximal contractility was measured directly from the recorded dose–response curves and is expressed as tension in grams. All data are given as mean(s.e.m.). Differences in ED_{50} and maximal contractility were analysed using Student's paired *t* test.

Results from dose–response curves obtained after incubation with NFT (experiments 2 and 3) are expressed as percentages of the maximum contraction of the control curve (experiment 1).

Results

Histological examination confirmed the presence of endothelium in each of the vein rings used. Of the 31 rings initially tested, only 14 were used for the complete experiment. The remaining 17 were excluded because they failed to respond to serotonin initially ($n = 6$) or because the amplitude of the contraction elicited by the submaximal dose of serotonin progressively diminished during the experiment ($n = 11$).

Table 1 shows the maximal contractility for each of the three sets of curves ($n = 14$). In the control curves, mean(s.e.m.) maximal contractility was 62·1(4·1) g, whereas after preincuba-

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Table 1 *Response of human saphenous vein to serotonin alone and in the presence of naftidrofuryl*

	Maximal contractility (g)	ED_{50} (mol/l serotonin)
Serotonin	62·1(4·1)	$2·3(0·3) \times 10^{-7}$
Serotonin plus 10^{-6} mol/l NFT	20·9(1·4)	$8·7(1·0) \times 10^{-5}$
Serotonin plus 10^{-3} mol/l NFT	6·1(0·8)	$1·7(0·6) \times 10^{-4}$

Values are mean(s.e.m.). NFT, naftidrofuryl; ED_{50}, concentration for half-maximal response. $P < 0·0001$ between all groups (Student's t test)

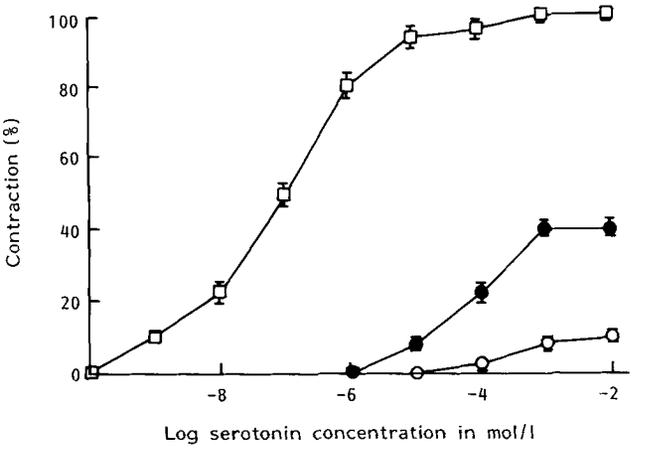

Figure 1 *Effects of increasing concentrations of naftidrofuryl (●, 10^{-6} mol/l; ○, 10^{-3} mol/l) on dose–response curves of human saphenous vein contracted by serotonin. Values are mean(s.e.m.) percentage of maximum contraction of the control curve (□)*

tion with 10^{-6} and 10^{-3} mol/l NFT it was 20·9(1·4) and 6·1(0·8) g respectively. The differences between any two groups were highly significant ($P < 0·0001$).

Standardized dose–response curves (*Figure 1*) showed a rightward shift with increase in concentration of NFT, suggesting a progressive decrease in sensitivity to serotonin with the increase in concentration of NFT in the bathing solution. The pattern of these plots, with suppression of the maximum response and a non-parallel shift of the second and third sets of curves, is suggestive of non-competitive antagonism.

The mean(s.e.m.) ED_{50} for serotonin increased progressively with addition of NFT (*Table 1*). It was $2·3(0·3) \times 10^{-7}$ mol/l for the control, increasing to $8·7(1·0) \times 10^{-5}$ mol/l for the lower and $1·7(0·6) \times 10^{-4}$ mol/l for the higher concentration of NFT. The differences between any two groups were again significant ($P < 0·0001$).

Discussion

This *in vitro* study demonstrates that NFT antagonizes serotonin-induced contraction of human saphenous vein in a dose-dependent and non-competitive manner. The maximal contractility of control rings was significantly reduced after preincubation with NFT, showing a reduction of more than 60 per cent with the physiological concentration[13] of 10^{-6} mol/l. However, the contractile response could not be completely abolished, even with the higher non-physiological concentration of NFT (10^{-3} mol/l) used in this study. Similar incomplete inhibition has been observed in animal experiments and is attributed to the intrinsic contractile activity of NFT[14]. This drug also blocks endogenous serotonin activity, as demonstrated by exposing the vessel preparation *in vitro* to human platelet-rich plasma[14].

NFT has been used for many years in patients with intermittent claudication. It is claimed to improve the efficiency of the Krebs cycle in ischaemic tissues by increasing oxygen uptake, improving glucose metabolism, increasing adenosine 5'-triphosphate production and lowering the lactate:pyruvate ratio[7–10]. The results from the present study support the growing evidence that NFT has antiserotonergic activity in humans and may have a clinical role in reducing serotonin-induced vasoconstriction.

In recent years, platelet-derived serotonin has been proposed as a major contributor in the pathogenesis of vasospasm. The vascular response to serotonin released from platelets will depend on the integrity of the endothelial cells, as they are responsible for its uptake and degradation[4]. Serotonin reaching 5-HT_1 receptors on the endothelial cells triggers the release of endothelium-derived relaxing factor (EDRF). However, if the endothelium is traumatized, this factor is not produced and platelet-derived serotonin induces vasoconstriction via the 5-HT_2 receptors on smooth muscle cells. Furthermore, the absence of endothelium allows platelet aggregation to persist[4,15–17]. Although the endothelium regenerates, it loses its ability to release EDRF[4]. The loss of this response favours abnormal vasoconstriction or spasm and platelet aggregation with release of platelet-derived growth factor, thus setting the stage for atherosclerosis and thrombosis[4,18,19].

It is of interest that atherosclerotic human vessels have increased sensitivity to serotonin[20], but it would be unwise to extrapolate the results of the present study to human arterial tissue. Nevertheless, NFT may have a valuable clinical role in reducing serotonin-induced vasospasm, particularly after intravascular manipulation such as angioplasty or vein bypass surgery.

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Case report

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Localized amyloid of the glans penis

V. Srinivasan, E. O. Kehinde, M. D. Harris* and A. G. Turner

*Departments of Urology, Edith Cavell Hospital and *Histopathology, Peterborough District Hospital, Peterborough, UK*

Correspondence to: Mr V. Srinivasan, Department of Urology, Edith Cavell Hospital, Peterborough PE3 9GX, UK

Case report

A 32-year-old caucasian man presented with an 8-week history of a painless mass (3 × 1 cm in size) on the glans penis. There were no urinary symptoms, and no past history of penile ulcers or trauma. The mass was neither tender nor ulcerated and did not extend into the urethra or over the shaft of the penis. There was no regional lymphadenopathy and general examination was unremarkable.

Excision biopsy was carried out. Light and electron microscopy confirmed the presence of large amounts of diffuse subcutaneous amyloid (*Figure 1*). Congo red staining and birefringence was abolished by prior treatment of sections with potassium permanganate, showing the material to be AA amyloid, the type usually associated with chronic inflammatory conditions. There was no histological evidence of epithelial dysplasia.

The patient's white cell count and differential count were normal; the erythrocyte sedimentation rate was 1 mm/h. Serum urea, creatinine and electrolyte levels, and results of liver function tests and electrophoresis, were all normal. Further investigations, including rectal biopsy, did not show any evidence of systemic amyloidosis. Findings at intravenous urography and cystoscopy were normal.

Discussion

Localized amyloidosis of the penis has been reported previously[1], but we are aware of only four cases involving the glans[2,3] and report what is believed to be the fifth. Amyloidosis is worth considering in the differential diagnosis of an apparent carcinoma of the glans, particularly in a young man. Local excision has been adequate, in most cases, although the patient described by Bodner *et al.*[2] showed locally extensive disease that required wider excision.

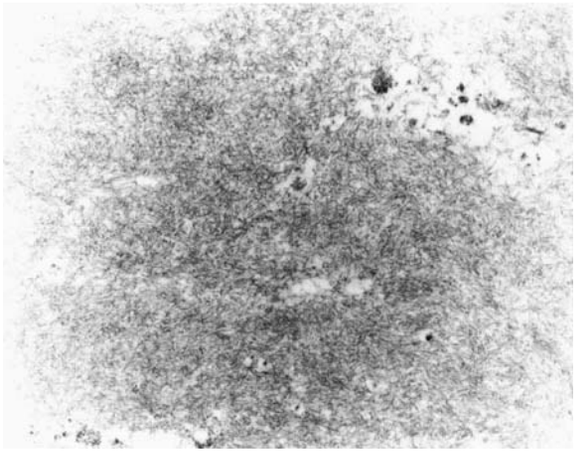

Figure 1 *Transmission electron micrograph showing numerous delicate fibrils, the classical appearance of amyloid. (Original magnification × 5800)*

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