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Noradrenergic and peptidergic sympathetic regulation of cutaneous microcirculation in the rat

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Abstract

Cutaneous microcirculatory changes were measured by laser-Doppler flowmetry in response to electrical stimulation of sympathetic efferent fibres of the rat's saphenous nerve. After perineural capsaicin (2%) pretreatment, electrical stimulation of the peripheral stump of the cut saphenous nerve evoked a reduction in blood flow (vasoconstriction) followed by a minimal enhancement. This late vasodilatation was further reduced by resiniferatoxin (1 μg/kg i.v.), and vasoconstriction was abolished by guanethidine (8 mg/kg i.v.), indicating the involvement of sensory and sympathetic fibres in the respective responses. The vasoconstrictor response was analysed after blockade of antidromic vasodilatation by combined capsaicin-resiniferatoxin pretreatment. α-Adrenoceptor antagonists (1 mg/kg phentolamine, 0.5 mg/kg prazosin and 1 mg/kg GYKI-12743 (*RS*-2-(3)*N*-(2-benzo;1,4*i*-dioxanyl)-methylamino(propyl)-3(2*H*)-piridazinone hydrochloride) inhibited, but did not eliminate the blood flow reduction evoked by 3 Hz stimulation. At 10 Hz stimulation significant inhibition was obtained only with GYKI-12743. No inhibition was observed with propranolol (10 μg/kg) on any occasion. A functional neuropeptide Y antagonist, α-trinositol (D-myo-inositol-1,2,6-trisphosphate, PP56; 50 mg/kg i.v.), markedly diminished the vasocontrictor response remaining after treatments with the α-adrenoceptor blocking agents. Inhibition was more pronounced at 10 Hz. Since 3 Hz corresponds to an average, and 10 Hz approaches the maximal firing rate of the sympathetic efferents, these results emphasise the significant role of neuropeptide Y in regulation of the cutaneous microcirculation by sympathetic fibres under physiological circumstances, particularly during high activity. © 1997 Elsevier Science B.V.

Keywords: Capsaicin-sensitive; Resiniferatoxin; Saphenous nerve; Neuropeptide Y; Vasoconstriction; Vasodilation; Laser-Doppler flowmetry

1. Introduction

The innervation of the cutaneous microvasculature includes sympathetic postganglionic fibres as well as sensory fibres able to release vasoactive substances. Sympathetic efferent neurones contain noradrenergic and non-adrenergic (peptidergic and purinergic) mediators. Noradrenaline is the main vasoconstrictor transmitter, but a potent vasoconstrictor peptide, neuropeptide Y, was shown in the last few years to be co-localised with noradrenaline in sympathetic nerve endings and to be released by electrical stimulation, particularly at high frequencies (Lundberg et al., 1982; Öhlén et al., 1990). This peptide causes vasoconstriction which is unrelated to α-adrenoceptors (Wahlestedt et al., 1985). Neuropeptide Y has a dual action on sympathetic neurotransmission: it is a vasoconstrictor mediator by itself (Lundberg and Tatemoto, 1982) and it also potentiates the noradrenaline-evoked vasoconstriction (Pernow et al., 1986; Nilsson, 1991; Dahlöf et al., 1985). Evidence has also been presented that ATP acts as an excitatory cotransmitter with noradrenaline released from sympathetic perivascular nerves and causes vasoconstriction via excitatory P_{2X} purinoceptors located on vascular smooth muscle (Burnstock and Kennedy, 1986).

Excitation of the distal stump of cut peripheral nerves evokes changes in cutaneous blood flow, which are due to neuromediators released from the activated sensory and sympathetic efferent fibres. Perineural capsaicin application strongly inhibits antidromic vasodilatation by functional blockade and loss of C and A-delta polymodal nociceptive afferents (Szolcsányi, 1988), but does not have any influence on the function of sympathetic efferents (Handwerker et al., 1984). The sympathetic vasoconstrictor effect can be eliminated by systemic guanethidine pretreatment (Lembeck and Holzer, 1979; Gamse and Saria, 1987;

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Escott and Brain, 1993), indicating that the release of all cotransmitters is prevented by the drug.

The aim of the present paper was to analyse the role of noradrenergic and peptidergic sympathetic mediators released by electrical stimulation of sympathetic efferent fibres in the regulation of the microvasculature of the skin. This was done by means of laser-Doppler flowmetry (Nilsson et al., 1980). Previous studies demonstrated that neuropeptide Y takes part in the changes in microcirculation evoked by high frequency (20 Hz) excitation (Lundberg, 1996; Lundberg and Modin, 1995). Nevertheless, this frequency exceeds the maximal firing rate recorded from sympathetic fibres; the activity of these fibres is 3–5 Hz under non-stimulated conditions and 10–15 Hz in maximal response to reflex activation (Jänig, 1988). Particular emphasis was put on examining the importance of neuropeptide Y in the mediation of cutaneous microcirculatory changes in the rat under physiological conditions.

2. Materials and methods

2.1. Animals

The experiments were performed on female Wistar rats (200–250 g). Animals were kept in a warm room (24–25°C) and provided with standard laboratory pellets and tap water ad libitum.

2.2. Experimental procedures

2.2.1. Perineural capsaicin pretreatment

Rats were anaesthetised with 40 mg/kg i.p. pentobarbital (Nembutal). The left saphenous nerve was exposed in the inguinal region. A small piece of fibrin sponge (Gelaspon), soaked with 2% capsaicin, was applied around the nerve for 30 min. An envelope of Parafilm placed around the sponge prevented the diffusion of the drug to the surrounding tissues. After removal of the cuff, the muscle and the skin were sutured and the animals recovered within the next 2 h (Szolcsányi, 1988).

2.2.2. Saphenous nerve stimulation

4–6 days after the perineural capsaicin pretreatment, the rats were anaesthetised with 100 mg/kg i.p. thiopental sodium (Trapanal). Body temperature was kept close to 37°C, using a heating pad controlled by a rectal thermistor probe. The animals were breathing spontaneously through a tracheal tube. One of the carotid arteries and one of the external jugular veins were also cannulated for recording arterial blood pressure and to inject drugs, respectively. The left saphenous nerve was re-exposed in the middle of the thigh and cut. The surrounding skin flaps were fixed to a metal ring to make a pool, which was filled with liquid paraffin. The peripheral stump of the nerve was placed on a pair of platinum-wire hook electrodes and stimulated electrically. Stimulation parameters were: 20 V, 0.5 ms, 3 or 10 Hz, 100 pulses. Cutaneous blood flux in the mediodorsal region of the left foot supplied by the saphenous nerve was measured by using a laser-Doppler flowmeter (Moor Instruments MBF3D, UK). Arterial blood pressure, heart rate, respiratory movements and respiration rate were continuously recorded through the arterial cannula and the T-tracheal tube by a polygraph (Grass, model 7). Both devices were connected to an IBM compatible personal computer. Resiniferatoxin (1 μg/kg) was injected intravenously after basal blood flow stabilised, in order to complete the inhibitory effect of perineural capsaicin pretreatment on antidromic vasodilatation (Szolcsányi, 1988). When resiniferatoxin caused apnoea, artificial ventilation was provided with a respirator (MTA-Kutesz, Hungary). When the acute cardiovascular changes evoked by this agent passed off, one of the sympathetic blocking agents (1 mg/kg phentolamine or 0.5 mg/kg prazosin or 1 mg/kg GYKI-12743 (*RS*-2-(3)*N*-(2-benzo;1,4*i*-dioxanyl)-methylamino(propyl)-3(2*H*)-piridazinone hydrochloride) or 0.01 mg/kg propranolol and 8 mg/kg guanethidine) was applied i.v. (Izumi et al., 1989; Koss et al., 1990; Rablóczky et al., 1991; Sun et al., 1991). In the second stage of the experiments 50 mg/kg α-trinositol was given i.v. after GYKI-12743 injection. The effect of α-trinositol was examined without α-adrenoceptor antagonist pretreatment as well. Electrical stimulations were performed before and a few minutes after drug administration, when the acute effects were over and the basal cutaneous blood flow was stabilised.

2.2.3. Testing the neuropeptide Y antagonistic effect of α-trinositol

Changes of cutaneous blood flow and systemic blood pressure evoked by neuropeptide Y (2.5 nmol/kg i.v. infusion, 0.25 ml/min) and noradrenaline (100 ng/rat i.v.) were compared before and after i.v. injection of 50 mg/kg α-trinositol.

2.3. Drugs

Pentobarbital (Nembutal) from May and Baker (UK) was used for general anaesthesia during the perineural capsaicin pretreatment. Capsaicin (Sigma) was dissolved in 10% ethanol, 10% Tween 80 (Reanal, Hungary) and 80% isotonic NaCl solution. The animals were anaesthetised with thiopental sodium (Trapanal; Byk, Germany) during the experimental procedure. Resiniferatoxin was obtained from Sigma, phentolamine from Ciba-Geigy (Switzerland), noradrenaline, prazosin and guanethidine from Sigma, propranolol from ICI Pharmaceutical (UK), GYKI-12743 (*RS*-2-(3)*N*-(2-benzo;1,4*i*-dioxanyl)-methylamino(propyl)-3(2*H*)-piridazinone hydrochloride) from Institute for Drug Research (Hungary), α-trinositol (D-myo-inositol-1,2,6-trisphosphate; PP56) from Perstorp Pharma (Sweden) and neuropeptide Y from Bachem (Switzerland).

2.4. Data analysis

Experimental data were evaluated by using a specific computer program (Geopolita, Hungary). As laser-Doppler flowmetry gives only relative flow values in arbitrary units (AU), for quantitative analysis the integrated blood flux responses (areas under the curve) were compared to the pre-stimulation control values and expressed as percent changes of cutaneous blood flux as described in other studies (Escott and Brain, 1993; Holzer and Jocic, 1994). Besides calculating these values, the amplitude of the peak vasoconstrictor responses was also measured and expressed as a percentage in relation to basal blood flow. The results are expressed as means ± S.E.M. Student's *t*-test for paired comparisons was used to evaluate statistical differences; $P < 0.05$ or lower was accepted to be significant.

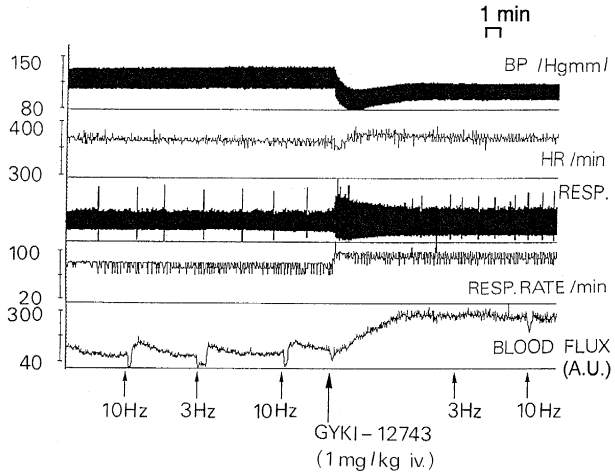

Fig. 2. Typical recording of systemic blood pressure (BP), heart rate (HR), respiratory movements (RESP.), respiration rate (RESP. RATE) and microvascular blood flow before and after GYKI-12743 (1 mg/kg, i.v.) treatment. Stimulations at 20 V, 0.5 ms, 3 Hz or 10 Hz frequencies (33 s or 10 s; 100 pulses) and GYKI-12743 injection are indicated with arrows.

3. Results

3.1. Effect of α-adrenoceptor antagonists on cutaneous blood flow reduction evoked by saphenous nerve stimulation

After perineural capsaicin pretreatment, electrical stimulation (3 Hz and 10 Hz) of the peripheral stump of the cut saphenous nerve elicited a biphasic blood flux change: a rapid (latency: 2–3 s) reduction followed by a long-lasting enhancement of blood flow. This vasodilatator response was almost completely abolished by resiniferatoxin (1 μg/kg i.v.) while the initial vasoconstrictor phase remained (Fig. 1). 8–18 s after the resiniferatoxin injection a small decrease of mean arterial blood pressure (25–35 mmHg) was observed for 60–80 s, which was followed by a prominent increase (70–110 mmHg) for 150–200 s and blood pressure gradually diminished to a lower baseline (from 123 ± 2.1 to 92 ± 3.8; −25%; $n = 17$). Parallel with the biphasic blood pressure changes, heart rate fell by 100–250/min. Marked intensification of respiration was followed by a progressive depression or in some cases apnoea (Fig. 1).

Intravenously applied α-adrenoceptor antagonists such as prazosin (0.5 mg/kg) or phentolamine (1 mg/kg) significantly inhibited but did not eliminate the vasoconstrictor response evoked by 3 Hz stimulation. These drugs did not influence the cutaneous microcirculatory alterations evoked by pulses delivered at 10 Hz frequency. A new, selective, postsynaptically acting α-adrenoceptor blocker, GYKI-12743 (1 mg/kg i.v.) (Horváth et al.,

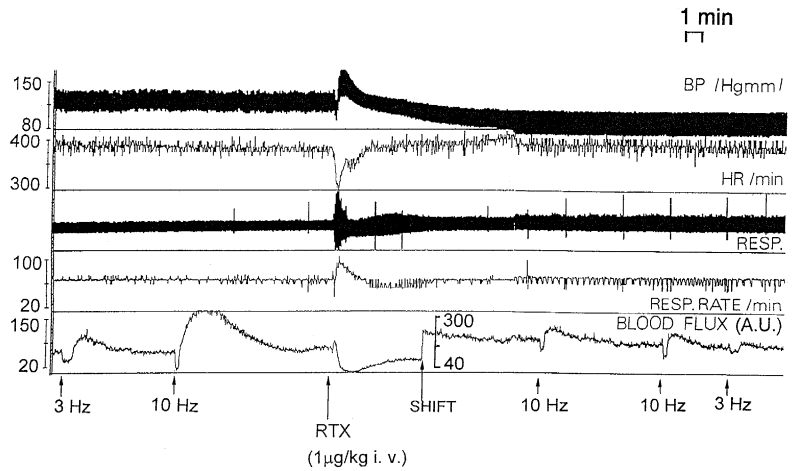

Fig. 1. Typical recording of systemic blood pressure (BP), heart rate (HR), respiratory movements (RESP.), respiration rate (RESP. RATE) and microvascular blood flow before and after intravenous administration of 1 μg/kg resiniferatoxin (RTX). Stimulation at 20 V, 0.5 ms, 3 Hz or 10 Hz frequencies (33 s or 10 s; 100 pulses) and resiniferatoxin injection are indicated with arrows. Because systemic blood pressure changes modified the cutaneous microcirculation, a shift was applied to the measuring interval of the laser-Doppler flowmeter.

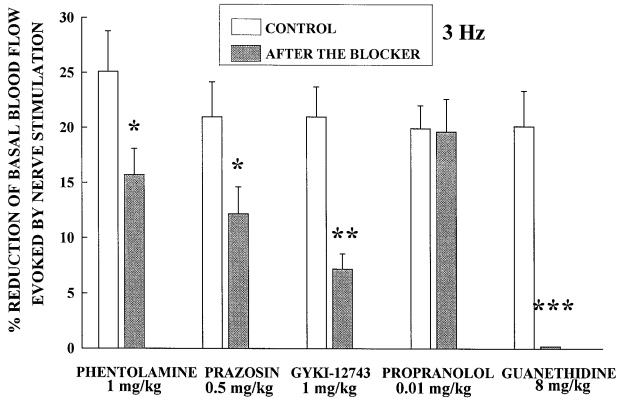

Fig. 3. Effect of various sympathetic blocking agents on the reduction of blood flux evoked by saphenous nerve stimulation at 3 Hz frequency. Values are calculated from areas under the curve and expressed as percentages of basal blood flow (mean ± S.E.M.). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ vs. control; $n = 5–7$/group.

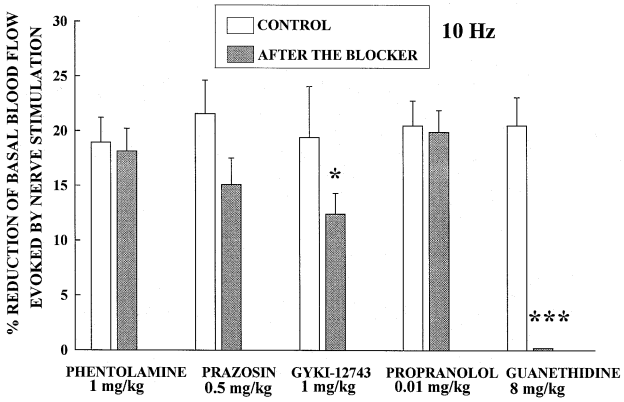

Fig. 4. Effect of different sympathetic blocking agents on the reduction of blood flux evoked by saphenous nerve stimulation at 10 Hz frequency. Values are calculated from areas under the curve and expressed as percentages of basal blood flow (mean ± S.E.M.). * $P < 0.05$, *** $P < 0.001$ vs. control; $n = 5–7$/group.

1990; Rablóczky et al., 1991), significantly reduced the vasoconstriction evoked by both 3 Hz and 10 Hz excitation (Fig. 2). Nevertheless even this compound was unable to completely reverse the blood flow reduction. With 3 Hz stimulation, phentolamine treatment decreased the vasoconstrictor response by 37.4%, prazosin by 42.2% and GYKI-12743 by 65.8%. In case of 10 Hz excitation, a 36.1% reduction of blood flux was observed after GYKI-12743 injection. The sympathetic neurone blocking agent guanethidine (8 mg/kg i.v.) totally abolished the rest of the responses (Figs. 3 and 4). Treatment with α-adrenoceptor blockers decreased mean arterial blood pressure: phentolamine caused a 36% ($n = 5$), prazosin 21% ($n = 5$) and GYKI-12743 a 35% ($n = 7$) reduction. Basal cutaneous blood flow increased by 30–100%. The α-adrenoceptor blocking agent propranolol (0.01 mg/kg i.v.) failed to influence the effect of either 3 Hz or 10 Hz excitation.

3.2. Effect of α-trinositol treatment on noradrenaline- and neuropeptide Y-induced vasculatory changes

Mean arterial blood pressure and cutaneous microcirculatory changes of the dorsum of the hindpaw evoked by i.v. injection of noradrenaline (100 ng/rat) and i.v. infusion of neuropeptide Y (2.5 nmol/kg, 0.25 ml/min) before and after i.v. α-trinositol (50 mg/kg) administration are demonstrated in Figs. 5 and 6 and Table 1.

3.3. Effect of α-trinositol on cutaneous blood flow reduction evoked by saphenous nerve stimulation

The vasoconstriction remaining after the most effective α-adrenoceptor blocker (GYKI-12743) was markedly diminished by α-trinositol (50 mg/kg i.v.), a selective, functional, non-peptide neuropeptide Y antagonist (Sun et

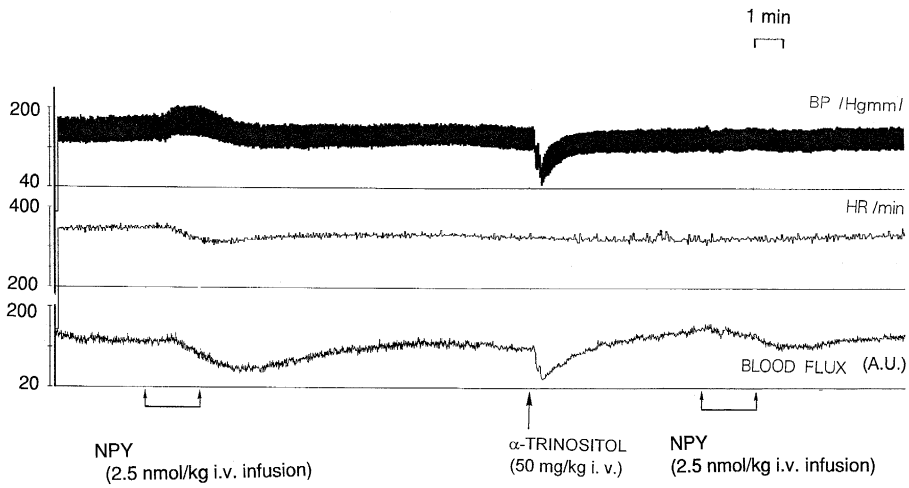

Fig. 5. Typical recording of systemic blood pressure (BP), heart rate (HR) and microvascular blood flow in response to exogenous neuropeptide Y (NPY) i.v. infusion (2.5 nmol/kg, 0.25 ml/min), before and after 50 mg/kg i.v. α-trinositol. Neuropeptide Y infusion and α-trinositol injection are indicated with arrows.

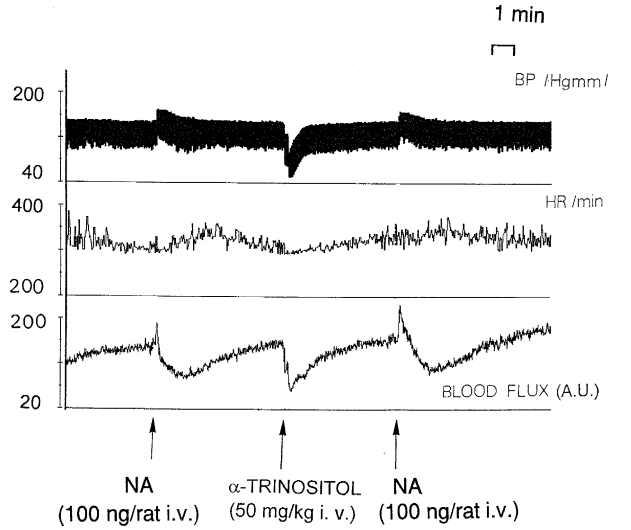

Fig. 6. Typical recording of systemic blood pressure (BP), heart rate (HR) and microvascular blood flow in response to exogenous noradrenaline (NA) i.v. injection (100 ng/rat), before and after 50 mg/kg i.v. α-trinositol. Noradrenaline and α-trinositol injections are indicated with arrows.

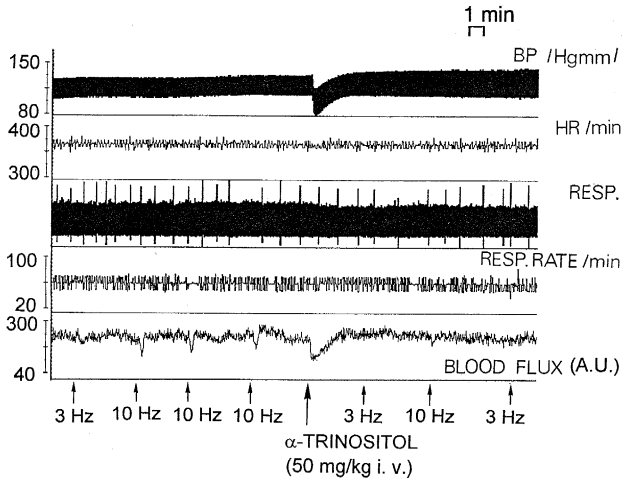

Fig. 7. Typical recording of systemic blood pressure (BP), heart rate (HR), respiratory movements (RESP.), respiration rate (RESP. RATE) and microvascular blood flow before and after 50 mg/kg i.v. α-trinositol. Stimulation at 20 V, 0.5 ms, 3 Hz or 10 Hz frequencies (33 s or 10 s; 100 pulses) and α-trinositol injection are indicated with arrows.

al., 1991, 1996; Adamsson et al., 1992; Feth et al., 1993; Yoo et al., 1994) (Fig. 7). Intravenous administration of α-trinositol had a transient hypotensive action, it lowered systemic blood pressure by 19% ($n = 7$) for 2–3 min. In case of 3 Hz stimulation, the 34.2% vasoconstrictor response remaining after GYKI-12743 administration was further inhibited by α-trinositol to 10.08%. With 10 Hz impulses, α-trinositol caused an additional decrease in the remaining blood flux reduction (from 63.9% to 17.43%) (Fig. 8).

When α-trinositol was applied without the α-adrenoceptor antagonist GYKI-12743, the vasoconstrictor responses evoked by 3 Hz and 10 Hz stimulation were inhibited by $38.62 \pm 3.19\%$ and $45.4 \pm 3.67\%$, respectively ($n = 4$).

Besides evaluating the total reduction of microcirculation by measuring areas under the curve, we determined the amplitude of the peak vasoconstrictor responses. After

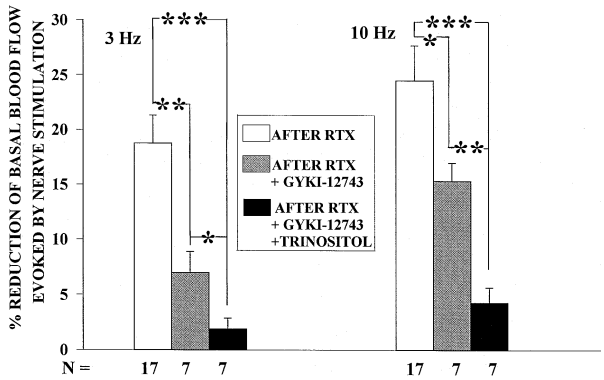

Fig. 8. Effects of resiniferatoxin (RTX) (1 μg/kg), resiniferatoxin + GYKI-12743 (1 mg/kg) and resiniferatoxin + GYKI-12743 + α-trinositol (50 mg/kg) on the reduction of integrated blood flow in the microcirculation of the dorsal skin of the hindpaw evoked by antidromic saphenous nerve stimulation. Values are calculated from areas under the curve and expressed as percentages of basal blood flow (mean ± S.E.M.). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs. control.

Table 1
Effect of exogenous neuropeptide Y and noradrenaline on systemic mean arterial blood pressure (MAP) and blood flow in the cutaneous microcirculation of the dorsum of the hindpaw before and after α-trinositol treatment

	Agonist	Change of MAP (maximum increase in % of control value)	Microcirculation (reduction of blood flow in % of control period)
Before α-trinositol	Neuropeptide Y	22.7 ± 0.91	26.18 ± 5.75
	Noradrenaline	28.75 ± 6.77	29.1 ± 4.62
After α-trinositol	Neuropeptide Y	10.19 ± 1.35 [a]	9.28 ± 1.97 [a]
	Noradrenaline	29.25 ± 4.49	32.18 ± 2.76

Responses were evoked by neuropeptide Y (2.5 nmol/kg i.v. infusion, 0.25 ml/min; 2 min) and noradrenaline (100 ng/rat i.v.) before and after α-trinositol (50 mg/kg i.v.) treatment (means ± S.E.M. of 5 animals). Blood flux changes were calculated as areas under the curve in response to stimulations. Periods of equal duration preceding the stimulation were taken as 100%. [a] $P < 0.01$ vs. control values before drug administration (Student *t*-test for paired comparison).

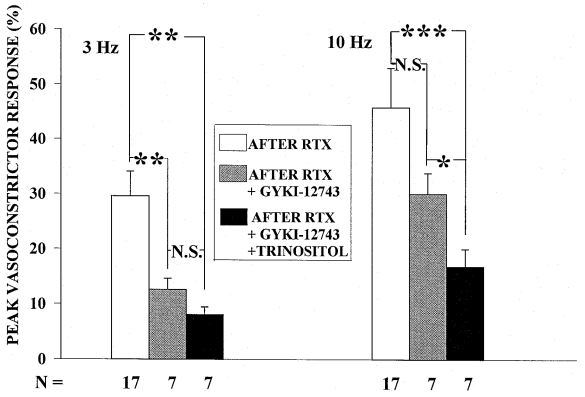

Fig. 9. Effects of resiniferatoxin (RTX) (1 μg/kg), resiniferatoxin + GYKI-12743 (1 mg/kg) and resiniferatoxin + GYKI-12743 + α-trinositol (50 mg/kg) on reduction of the amplitude of the peak vasoconstrictor response in the microvasculature of the dorsal skin of the hindpaw evoked by antidromic saphenous nerve stimulation. Values are expressed as percentages of basal blood flow (mean ± S.E.M.). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ vs. control.

resiniferatoxin 3-Hz pulses caused a maximal vasoconstriction of 29.6 ± 4.5% compared to the basal blood flow, which was significantly reduced after GYKI-12743 administration (12.6 ± 2.04%) and subsequent α-trinositol treatment (8.09 ± 1.35%). After resiniferatoxin 10 Hz stimulation elicited a maximum 45.8 ± 7.2% blood flux decrease, which was markedly but not significantly diminished after GYKI-12743 treatment (30.06 ± 3.8%) and strongly reduced after α-trinositol (16.8 ± 3.2%) application (Fig. 9).

The duration of the vasoconstrictor action was not influenced by the different drug treatments. In the case of 3 Hz stimulation, it was 58.86 ± 13.4 s after resiniferatoxin, 57.6 ± 2.58 s following GYKI-12743 and 53.53 ± 4.91 s after α-trinositol. In response to 10 Hz excitation, it was significantly shorter: 32.24 ± 3.55 s, 32.24 ± 3.81 s and 30.89 ± 3.99 s, respectively.

4. Discussion

The key role of noradrenergic mediation in the control of systemic blood pressure is well established, since hypertensive responses evoked by stimulation of sympathetic fibres can be abolished by α-adrenoceptor antagonists. In respect of the cutaneous microcirculation, however, the participation of adrenergic and non-adrenergic mediators in the sympathetic responses is far less clear. Some authors who used the laser-Doppler flowmetry technique obtained evidence for an exclusive role of noradrenergic neurotransmission in exteroceptive areas. The vasoconstrictor response evoked by electrical stimulation of the cervical sympathetic or the inferior alveolar nerve was completely abolished by pretreatment with phentolamine in the gingiva of the cat (Izumi et al., 1989). Koss et al. (1990) and Kawarai and Koss (1992) showed that the cutaneous microcirculation of the forepad, as well as of the digits in cats, is mainly under α_2-adrenoceptor-mediated neuronal control as sympathetic constrictor responses were clearly antagonised by phentolamine and yohimbine but only marginally by prazosin. Willette et al. (1991) could block the vasoconstrictor effect of sciatic nerve stimulation by phentolamine in the rat skin. In contrast, others emphasise the importance of non-adrenergic mediation as well, because the effects of sympathetic nerve stimulation could not be abolished by α-adrenoceptor antagonists (Pernow, 1988; Lundberg et al., 1990; Lacroix et al., 1990; Öhlén et al., 1990). The vasoconstrictor effect of sympathetic stimulation was only partially inhibited by phentolamine in the oral mucosa of cats when the radioactive ^{125}I tracer washout technique was used. The remaining effect of sympathetic stimulation was abolished by guanethidine (Edwall et al., 1985).

Evidence supporting a role for neuropeptide Y and ATP as co-transmitters at the sympathetic neuroeffector junction has been presented (Burnstock and Kennedy, 1986; Pernow, 1988; Lundberg et al., 1990; Nilsson, 1991; Brock and Cuanne, 1993; Lundberg, 1996).

Recently, it has been established by immunohistochemical techniques that neuropeptide Y co-exists with noradrenaline in sympathetic neurones (Tatemoto, 1982; Tatemoto et al., 1982; Pernow, 1988) and in perivascular plexuses of different organs including the skin (Lundberg and Hökfelt, 1986). Neuropeptide Y induces vasoconstriction upon systemic administration (Lundberg and Tatemoto, 1982). Lundberg and Modin (1995), using nerve stimulation combined with laser-Doppler flowmetry technique in the pig skin, found evidence for a role of neuropeptide Y in the microcirculatory responses, but only when a high (20 Hz) stimulation frequency was applied. This frequency, however, exceeds the physiological range of activity in sympathetic fibres (Jänig, 1988).

ATP can act both as a vasodilator, acting through P_{2Y} purinoceptors on endothelial cells, and as a vasoconstrictor, acting through P_{2X} purinoceptors on vascular smooth muscle. ATP is rapidly broken down to adenosine which is also a powerful vasodilator, acting on P_1 purinoceptors of the vascular smooth muscle (Burnstock and Kennedy, 1986). Taylor and Parsons (1989) demonstrated data for purinergic sympathetic neurotransmission in arterial resistance vessels in the cat intestine, although these vessels are predominantly under adrenergic control. After blockade of α-adrenoceptors, the initial rapid phase of the residual vasoconstrictor response was abolished by desensitization of P_{2X} purinoceptors with α,β-methylene ATP, while the sustained vasoconstriction still persisted, particularly in response to low-frequency nerve stimulation. They found, however, no evidence for purinergic transmission in the resistance vessels of the hindquarters and the kidney. The role of purinergic transmission in the reduction of microcirculation of the vessels supplied by the saphenous nerve was examined using laser-Doppler flowmetry (Khoshbaten and Ferrell, 1993). These authors

found no evidence for ATP being released from nerve endings and contributing towards the vasoconstriction elicited by saphenous nerve stimulation in the knee joint of the rabbit.

In the light of all these findings, the present investigations were performed to analyse the role of noradrenaline and neuropeptide Y in the reduction of blood flow evoked by saphenous nerve stimulation.

The new technique we developed makes it possible to analyse the cutaneous microvasculatory changes evoked by stimulation of sympathetic fibres under conditions when the released adrenergic and non-adrenergic transmitters could not act indirectly because of the inhibition of admixed peptidergic sensory fibres (Takaki and Nakayama, 1991; Grundemar et al., 1990).

Electrical stimulation of the peripheral stump of the cut saphenous nerve evokes antidromic vasodilatation, which is due to the vascular action of neuropeptides released from the endings of the activated sensory nerve fibres. Perineural treatment with 1% (Szolcsányi, 1988) and even with 2% (Pintér et al., 1992) capsaicin solution markedly diminished but did not completely abolish this vasodilatation. The remaining vasodilatator response was almost absent after subsequent administration of resiniferatoxin (1 μg/kg i.v). This agent – which is structurally related to capsaicin – desensitises capsaicin-sensitive afferents in the rat without provoking the pulmonary chemoreflex of bradycardia, fall in blood pressure and apnoea which are characteristic for i.v. injected capsaicin (Szolcsányi et al., 1990). The cardiorespiratory failure due to this reflex limits the use of capsaicin for producing acute desensitisation of primary afferents. For this reason, resiniferatoxin is a suitable pharmacological tool for inducing acute defunctionalisation of capsaicin-sensitive sensory neurones.

In accordance with earlier reports (Edwall et al., 1985; Öhlén et al., 1990), in our experiments phentolamine inhibited the vasoconstrictor response evoked by 3 Hz stimulation, but it was ineffective against the response elicited by 10 Hz. Similar results were obtained with prazosin injection and only GYKI-12743 had a considerable efficiency at the latter frequency. This compound is the first vasoselective, postsynaptic α_1- and α_2-adrenoceptor blocking agent (Horváth et al., 1990; Rablóczky et al., 1991), and it proved to be the most effective agent in the present study to inhibit the vasoconstrictor response of the cutaneous microvasculature.

Participation of neuropeptide Y in this response is suggested, since the vasoconstriction evoked by 10 Hz frequency stimulation was only partially affected by α-adrenoceptor blocking agents and the remaining response was significantly diminished by α-trinositol, a selective, non-peptide, functional neuropeptide Y antagonist. This compound inhibits the rise in intracellular Ca^{2+} as well as inositol-1,4,5-trisphosphate concentrations induced by neuropeptide Y (Sun et al., 1996). It also inhibited the cutaneous blood flow reduction induced by both 3 Hz and 10 Hz stimulation without α-adrenoceptor antagonist pretreatment. In agreement with earlier data (Dey et al., 1993; Schwieler and Hjemdahl, 1993; Sun et al., 1991), in our experiments α-trinositol antagonised the rise of blood pressure and decrease of cutaneous microcirculation evoked by neuropeptide Y but not that of elicited by noradrenaline.

The maximum discharge rate of sympathetic fibres in response to reflex activation is about 10-15 Hz (Jänig, 1988) and the optimum frequency for eliciting neuropeptide Y release is about 10 Hz (Pernow, 1988). In the light of all these findings, our results provide evidence for the transmitter role of neuropeptide Y in the cutaneous microvasculature under physiological conditions.

In conclusion, the results of the present investigation, obtained with laser-Doppler flowmetry, show that besides the classical sympathetic neuromediator noradrenaline, cotransmitters – particularly neuropeptide Y – contribute to the regulation of cutaneous blood flow. Therefore these experimental data form a functional basis for the possible usefulness of neuropeptide Y antagonists in the treatment of peripheral vascular diseases and microcirculatory disturbances.

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