Acupuncture and sensory neuropeptides increase cutaneous blood flow in rats

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The effect on blood flow of electro-acupuncture (EA) injection of substance P (SP) and calcitonin gene-related peptide (CGRP) was studied in musculocutaneous flaps in the rat, using laser Doppler flowmetry. The circulatory border was estimated before and after treatment. It was shown that treatment with EA increased the blood flow moving the circulatory border distally 66% after a treatment. Injection of NaCl into the dorsal central vein of the flap resulted in no increase in blood flow whereas SP 10⁻⁷ M and CGRP 10⁻⁷ M increased the blood flow so that the circulatory border moved distally 31% and 49%, respectively. It is suggested that the effect of EA on blood flow is similar to the effect achieved by injecting CGRP and SP.

In plastic surgery, reconstructive problems are often solved by surgical flaps. The main disadvantage of surgical flaps has been their tendency to develop ischemia and eventually necrosis of the distal part. This complication can in the end ruin, what postoperatively seemed to be an excellent result. Because of this, much research has been devoted to explain the mechanisms of surgical skin flap survival, and how to prevent and reverse established ischemia [5, 11, 12, 14, 23].

Adrenergic nerves and their effect on flap survival have been studied intensively and antiadrenergic drugs have been shown to increase experimental flap survival but the exact mechanisms are not known and they have not been useful in clinical situations [14].

In addition to vasoconstrictor fibers, vessels are innervated by sensory nerve fibers which are known to contain vasodilator neuropeptides [4]. Antidromic electric stimulation of sensory nerve fibers results in cutaneous vasodilation [9], which is assumed to involve substance P (SP) [18] and calcitonin gene-related peptide (CGRP) [1, 6]. It has been suggested that sensory nerve fibers have a physiological role in the regulation of blood flow to surgical flaps as capsaicin pretreatment, depleting the neuropeptide content of primary sensory neurons, markedly decreases the survival of the dorsal musculocutaneous flap in the rat [16, 17].

Thus, the circulatory effects of acupuncture on flap survival may be due to a release
of vaso dilatory substances from sensory neurons and/or an inhibition of sympathetic vasoconstriction. It is therefore of interest to examine whether or not acupuncture, CGRP and SP increase the blood flow in experimental flaps. Groups of 10 female albino rats (Sprague–Dawley, b.wt. 200 g) were used. The rats were anaesthetized with chloral hydrate (0.4 g/kg, Kebo, Sweden). They were shaved on the back and a flap was designed according to a standard pattern, 2 cm wide and 7 cm long, based on a line between the caudal part of the scapulae. The flap was raised from the deep fascia of the muscles and included the superficial fascia, panniculus carnosus, subcutaneous tissue and skin [15]. The groups of 10 rats were treated as follows: (1) pharmacologically untreated rats served as controls; (2) with retrograde injection of 0.2 ml isotonic NaCl into the central vein; (3) with retrograde injection of 0.2 ml synthetic SP $10^{-9}$ M into the central vein; (4) with retrograde injection into the central vein of 0.2 ml CGRP $10^{-9}$ M; (5) treatment with high-intensity (20 mA) 2 Hz electroacupuncture (EA) for 1 h after surgery. The EA stimulator (Enraf-Nonius) produced alternating square-wave pulses (pulse width 0.4 ms) in a pair of acupuncture needles (Acu Pur) placed at the base of the flap; (6) pretreatment with reserpine (Serpasil, Ciba-Geigy, Switzerland) (0.5 mg/kg for 4 consecutive days); (7) reserpine pretreatment plus treatment with high intensity (20 mA) 2 Hz EA for 1 h after surgery.

Blood flow was measured before and during treatment, and 24 h postoperatively, using a laser Doppler flowmeter (Perimed, Sweden) [20] with the flaps mounted in a frame to avoid movement. The standard probe PF2B of the laser Doppler was applied superficially on the skin [21, 22]. Flow was stabilized for 20 min before measuring along the midline from the base to the tip of the flap. The circulatory border was tattooed on the skin and the flap was sutured back in position. The circulatory border is defined as the distance in mm from the base of the flap where the blood flow is 5 laser doppler units (LDU, i.e. relative units of measured blood flow).

Blood flow was detected 30 mm from the base of the flap in the controls postoperatively (Fig. 1). Injection of saline into the central dorsal vein did not significantly change the blood flow level compared with the controls (Fig. 1).

Injection of SP or CGRP ($10^{-9}$ M) significantly shifted the circulatory border postoperatively (47 and 52 mm, respectively) (Fig. 1). In addition, an increased blood flow was also observed in the proximal parts of the flap (Fig. 2).

Treatment with EA induced an increase in blood flow (Fig. 1). The circulatory border was detected 58 mm from the base of the flap. Measurement of the blood flow proximal to the detection limit revealed a significant increase at all levels (Fig. 2). Continuous monitoring of blood flow during EA treatment showed that the increase started after about 5 min. Reserpine pretreatment alone did not alter the blood flow limit in comparison with the untreated controls postoperatively. With EA treatment also the blood flow limit increased to 50 mm, a significant increase compared with the controls but not significantly different from EA treatment without reserpin (Fig. 1).

The present study shows that treatment with EA (high-intensity, low-frequency, at the base of the flap) as well as SP and CGRP (given locally via the dorsal central vein) increase blood flow in the dorsal musculocutaneous flap in the rat.
Fig. 1. Laser Doppler flowmeter (LDF) measurement of circulatory border (mm from base) in the musculo-cutaneous flap of the rat 24 h after operation. Values are given as mean ± S.D. for 10 animals. Shown are the controls (7), the effects of isotonic NaCl (6), SP $10^{-9}$ M (5) or CGRP $10^{-9}$ M (4) (0.2 ml injected into the central vein of the flap), electro-acupuncture (3), systemic reserpine pretreatment (2) and systemic reserpine pretreatment combined with electro-acupuncture (1). *$P < 0.005$, **$P < 0.001$ versus control.

In primary sensory neurons many neuropeptides have been identified. These neuropeptides have been shown to have a modulatory effect on the quality of sensory information in the spinal cord [4]. A significant part of the produced peptides are transported to the periphery and not to the CNS [8]. It has been shown that nerve fibers containing SP, neurokinin A (NKA) and CGRP are found in the human skin [4].

Fig. 2. Measurement of blood flow at different localizations (mm from base) in the musculo-cutaneous flap of the rat after operation. The figure shows a comparison of blood flow between pharmacologically untreated controls, SP, CGRP and EA treated animals. Flow values are expressed as relative laser Doppler units (LDU). Values are given as mean ± S.D. for 10 animals. Blood flow values for SP ($P < 0.005$), CGRP and EA ($P < 0.001$) significantly increased as compared to controls.
These fibers are not only seen as free nerve endings or in connection with sensory corpuscles but have also been shown to innervate blood vessels, hair-follicles and sweat glands [4]. It is known that electrical stimulation of sensory nerves in a peripheral direction, which releases sensory peptides, leads to vasodilation and oedema in the skin [18]. This can also be accomplished by injecting the peptides SP and NKA. while CGRP leads only to vasodilation [2, 3, 7, 10, 18]. It has recently been shown that CGRP, found in connection with small vessels in the skin, is a very potent vasodilator and that it may have actions other than vasodilatory, i.e. metabolic or thrombolytic and/or altering the sensitivity in ischemic tissue [17].

Reserpine pretreatment did not alter the level of detectable blood flow in the flap. This is in line with the study by Kennedy et al. [13] which showed that the blood flow in the flap did not change even up to 24 h postoperatively. Although the level of detectable blood flow did not differ from that of untreated controls, reserpine treated rats have been shown to have a significantly higher flap survival [11].

The mechanism by which EA causes an increased blood flow and subsequent increased flap survival is not known. However, two possible ways have been suggested: (1) an inhibition of sympathetic vasoconstrictor fibers and/or (2) a release of vasodilator sensory neurotransmitter [15–17, 19]. From the present study it is suggested that EA shows more similarity to the effect achieved by injecting the sensory neurotransmitters SP or CGRP into the flap than to the effect of blocking the sympathetic vasoconstrictor neurons.

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