Clinical and experimental efforts to reduce tissue necrosis following skin-flap surgery have been focused mainly on pharmacological improvement of skin blood flow. Although decreased blood perfusion is one likely reason for flap necrosis, another mechanism with the potential to damage flap tissue is inflammatory recruitment of leukocytes, a well-established feature of physical tissue injury. The main purpose of such phagocyte recruitment is host defence against pathogen invasion; however, the microbicidal toxins (e.g. oxidants and proteolytic enzymes) released from granulocytes may also damage host tissues. Accordingly, neutrophils have been shown to reduce wound margin strength in intestinal anastomoses. Moreover, flap surgery has been shown to be associated with substantial neutrophil accumulation and the involvement of neutrophils in flap necrosis has been implicated in some skin-flap studies. Further in support of inflammatory processes being involved in tissue necrosis following flap surgery are studies showing that pretreatment with certain anti-inflammatory agents (e.g. glucocorticoids, leukotriene-synthesis inhibitors, oxygen-radical scavengers) may improve skin-flap survival. Another therapy known to reduce necrosis formation in surgical flaps is sensory-nerve activation using acupuncture or transcutaneous electrical nerve stimulation (TENS). Such activation of sensory nerves in the skin is known to liberate predominantly calcitonin gene-related peptide, a potent vasodilator known to improve skin-flap viability. One likely mechanism by which exogenous CGRP or sensory-nerve activation reduces flap necrosis is arteriolar dilation and increased blood flow. However, it is also possible that CGRP and sensory-nerve stimulation improve flap survival by interfering with inflammatory processes. The objective of the present study was to examine whether intraperitoneal CGRP treatment might improve survival and reduce neutrophil accumulation in surgical skin flaps in the rat.
2 x 7 cm, cranially based, dorsal musculocutaneous flap with the base at the lower scapular angle was raised and sutured back as previously described.23,24 Six days after surgery, the animals were killed with an overdose of pentobarbital, and the percentage flap survival was estimated using computer-assisted planimetry of schematic drawings of surviving and necrotic flap areas. In all animals, a clear demarcation line separated vital and necrotic skin areas. Arterial blood samples were collected from a tail artery (at the time for tissue sampling) for systemic leukocyte counts.

Rats used for the determination of skin-flap viability were randomly divided into groups of six animals and treated i.p. with saline, 10^-18, 10^-15, 10^-12, 10^-9, or 10^-6 mol CGRP (0.5 ml) 20 min before flap construction. All solutions of drugs and vehicle were kept coded until the degree of flap survival had been evaluated at the end of the experiments.

**Myeloperoxidase assay**

The enzyme myeloperoxidase (MPO) is abundant in neutrophil leukocytes and has been found to be a reliable marker for the detection of neutrophil accumulation in inflamed skin in vivo.25 To determine flap neutrophil recruitment, the proximal and distal halves of the flap were collected, weighed, homogenised in 10 ml 0.5% hexadecyltrimethyl-ammonium bromide, and freeze-thawed. The MPO activity of the supernatant was determined spectrophotometrically as the change in absorbance at 650 nm (25°C) occurring in the redox reaction of H_2O_2–tetramethylbenzidine catalysed by MPO. Values are expressed as MPO units/mg tissue. Rats used for the determination of skin-flap MPO content (at 24 h after surgery) were divided into two groups and treated i.p. with saline (n = 9) or 5 x 10^-9 mol CGRP (n = 6) 20 min before flap construction.

**Arterial blood pressure and flap blood flow**

As previously described,26,27 blood flow/flux in the flap and arterial blood pressure were measured in anaesthetised animals (n = 3 in each group) before and at 10 min intervals for 60 min after i.p. injection of CGRP (10^-12, 10^-9, or 10^-6 mol CGRP). The animals were kept on a heating pad and their core temperature was repeatedly controlled to avoid temperature loss. Briefly, a polythene catheter was inserted into the carotid artery and mean arterial blood pressure (MAP) was displayed continuously on a chart recorder (Grass Polygraph 79D). The blood flow/flux in the flap was simultaneously measured using a dual-channel Periflux PF 4001 laser-Doppler flowmeter (Perimed AB, Sweden) connected to an A/D converter box (PF 472, Perimed AB, Sweden). The flap was mounted in a frame to avoid movement, and a standard probe (PF2B) of the laser was applied superficially on the skin in the distal half of the flap. Flow/flux was stabilised at 5 perfusion units (PU) for 15 min before actual measurement. Flow/flux values were expressed in PU, representing relative changes. The PeriSoft software (Perimed AB, Sweden) was used to store, retrieve and analyse data.

**Drugs and chemicals**

Chloral hydrate (Merck, Germany); CGRP (Peninsula Laboratories, Inc., UK); MPO standard (The Green Cross Corp., Japan).

**Statistical analysis**

For statistical analysis, the Mann–Whitney rank sum test or the Kruskal–Wallis one-way analysis of variance on ranks was used. P-values of < 0.05 were considered significant. All values are expressed as mean ± SE. n represents number of animals in each group.

**Results**

**Skin-flap survival**

The skin-flap survival in vehicle-treated animals was approximately 40% (Fig. 1), with the necrosis formation consistently occurring in the distal portion of the flap. Pretreatment with CGRP (10^-18–10^-6 mol i.p., n = 6 per dose), given 20 min prior to surgery, dose-dependently increased the degree of flap survival up to 73% (Fig. 1). In this regard, the lowest dose of CGRP that significantly improved survival was 10^-12 mol (Fig. 1).

**Arterial blood pressure and flap blood flow**

Intraperitoneal treatment with 10^-12, 10^-9, or 10^-6 mol CGRP for 1 h did not cause detectable changes in either arterial blood pressure (Fig. 2) or flap blood flow/flux (Fig. 3).

**Myeloperoxidase accumulation**

In saline-treated rats, the average flap neutrophil content immediately after surgery was low, as indicated by a skin MPO concentration of 0.20 ± 0.08 units/g tissue (n = 6). However, 24 h after surgery, the MPO accum-

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**Figure 1** — Skin-flap survival in untreated rats (1) and in rats treated i.p. with saline (2), 10^-18 (3), 10^-15 (4), 10^-12 (5), 10^-9 (6), or 10^-6 (7) mol CGRP administered 20 min before surgery. Mean values ± SE, n = 6 in each group. *P < 0.05 versus saline.
mulation (i.e. neutrophil recruitment) increased almost 100-fold in the distal half of the flap and 40-fold in the proximal half (Fig. 4). As compared with the saline treatment (\(n = 9\)), \(5 \times 10^{-9}\) mol CGRP (i.p. 20 min prior to surgery) significantly reduced (by 66%, \(n = 6\)) the 24 h MPO accumulation in the distal half of the flap, with no inhibition in the proximal half (Fig. 4). The CGRP treatment (\(5 \times 10^{-9}\)) did not affect the number of circulating neutrophils, i.e. at 24 h after surgery, the blood polymorphonuclear leukocyte (PMNL) count was \(34.8 \pm 5.5 \times 10^5/ml\) blood in the saline group (\(n = 9\)) and \(31.5 \pm 4.4 \times 10^5/ml\) blood in the CGRP group (\(n = 6\)).

Discussion

In this study, we have shown that intraperitoneal pretreatment with low doses of CGRP (\(\geq 1\) pmol per animal) greatly improved the survival of surgical flaps in the rat. This effect on tissue survival of CGRP was dose-dependent and required only a single injection of CGRP prior to surgery. These findings are in line with observations by Kjartansson and Dalsgaard, who used the same model to show that very low doses of CGRP injected locally into the venous circulation of the flap caused a similar improvement in tissue survival. Yet, the mode of action by which such low doses of CGRP increase flap survival is still unknown. One possibility is that CGRP reduces tissue ischaemia by causing vasodilatation. However, as shown previously and confirmed in this study, the lowest active doses of CGRP used in the present study and by Kjartansson and co-workers are below the established vasodilator concentrations of CGRP. An alternative mechanism of action by CGRP in this regard may be inhibition of leukocyte recruitment, a well-known feature of mechanical trauma with potential to cause further tissue injury. In fact, we found an apparent spatial correlation between the necrosis formation and the striking increase in skin flap MPO, and that CGRP treatment (5 nmol i.p.) markedly reduced the accumulation of neutrophil MPO in the flap. At present, we cannot fully explain why CGRP decreased flap MPO only in the distal half of the flap.

While several investigators have suggested that CGRP may be an important proinflammatory mediator, our present finding that very low concentrations of CGRP inhibit dermal neutrophil recruitment indicate that CGRP may also act to control inflammation. This is in line with previous studies demonstrating that CGRP can inhibit inflammatory oedema formation in several different species.

The mechanism by which CGRP inhibited the flap neutrophil accumulation was not directly addressed in this study. However, one possibility is that CGRP may suppress the expression or activation of leukocyte adhesion molecules such as the endothelial selectins and/or leukocytic integrins (for review, see Ley). Additional documented actions by CGRP that may contribute to the beneficial effects of this neuropeptide on skin-flap survival include promotion of endothelial cell growth and inhibition of cell types.
such as lymphocytes, macrophages and epidermal Langerhan cells.3–10

Taken together, we have shown that i.p. treatment with low doses of CGRP markedly improves the survival of surgical flaps in the rat. Our findings indicate that this beneficial effect by CGRP may be related to a reduction in the surgically induced neutrophil recruitment into the flap.

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