The salvage of rabbit ischaemic epigastric free flaps using the vasodilator calcitonin gene-related peptide

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Summary—The rabbit epigastric free flap, subjected to 21 hours of warm (25°C) ischaemia, was used as an experimental model to test the ability of two endothelium-dependent vasodilators, calcitonin gene-related peptide (CGRP) and carbamyl β-methylcholine chloride (MCh, bethanechol chloride, the stable acetylcholine analogue) to improve flap viability. After the period of ischaemia, flaps were infused intra-arterially with either Hanks balanced salt solution (controls), CGRP or MCh for 30 minutes, and received additional intravascular boluses of these drugs at 2 and 32 minutes after revascularisation. The area of flap surviving improved significantly (p < 0.025) from 39.9% (n = 18) for controls to 70.2% (n = 14) for CGRP treatment at 2 μg/kg, but was unchanged at 47.1% (n = 14) for MCh treatment at 50 μg/kg. Both CGRP and MCh significantly increased blood flow (p < 0.05) resulting in 34% lower peripheral resistances compared with controls. These results suggest that CGRP has considerable clinical potential for the salvage of ischaemic flaps. CGRP must have several, as yet undefined, beneficial effects on the ischaemic tissue, since MCh invoked a vasodilatory response but failed to salvage ischaemic flaps.

A surgical flap subjected to ischaemia undergoes a sequence of pathological changes which may influence the flap’s survival after revascularisation. Changes during ischaemia include oedema, inflammation and rheological changes which may cause narrowing of the vascular lumen or vascular endothelial damage (May et al., 1978). At the time of revascularisation oxygen-derived free radicals may further damage the endothelial membranes (McCord, 1985), causing potential sites for thrombosis and failure of the microcirculation, known as the no-reflow phenomenon (Ames et al., 1968; May et al., 1978).

A number of agents have been used in an attempt to counteract the no-reflow phenomenon in flaps. The free radical scavenger superoxide dismutase has been effective (Manson et al., 1986), as have infusion of ATP-MgCl₂ (Zimmerman et al., 1987) which replaces the depleted endogenous energy of the cells. Other successful strategies are the therapeutic use of an antithrombotic agent like streptokinase (Puckett et al., 1983) or the use of the potent vasodilator prostacyclin (PGI₂) (Reus et al., 1984; Zachary et al., 1986). Several vasodilators such as isoxsuprine have also been tried, often with variable results (Finseth and Adelberg, 1978; Cherry, 1979; Sasaki and Harii, 1980).

A potentially useful agent is calcitonin gene-related peptide (CGRP). CGRP, a neurotransmitter peptide hormone, is a potent vasodilator of non-ischaemic microvessels in peripheral tissues (Brain et al., 1985) and skin flaps (Knight et al., 1988a). CGRP requires an intact endothelium for smooth muscle relaxation and vasodilatation (Brain et al., 1985; Grace et al., 1987) and stimulates cyclic AMP (cAMP) production in smooth muscle cells (Kubota et al., 1985). Agents such as acetylcholine, bradykinin and calcium ionophore A23187 also have an endothelium-dependent mode of vasodilatation (Furchgott and Zawadski, 1980; Kubota et al., 1985; Peach et al., 1985; Grace et al., 1987). These latter agents bind to endothelial receptors, initiating the release of endothelium-derived relaxing factor (EDRF) which results in increased cyclic GMP levels (cGMP) in smooth muscle cells. The latter mediates the smooth muscle relaxation (Rapoport et al., 1983). No changes in cGMP occur with CGRP (Grace et al., 1987). It is proposed (Grace et al., 1987) that vascular smooth muscle relaxation takes place when the CGRP-mediated increase in cAMP is superimposed on the tonic cGMP production in the presence of intact endothelium. CGRP has the advantage of being nearly as potent, yet chemically and metabolically more stable, than
PGI$_2$, the latter regarded as one of the most potent endogenous substances for treating ischaemic flaps (Knight et al., 1985, 1988a).

In this study the rabbit epigastric free flap has been used as the model to compare the ability of two endothelium-dependent vasodilators: human CGRP and carbamyl β-methylcholine chloride (bethanechol chloride, an acetylcholine analogue), to salvage failing ischaemic skin flaps after a period of warm ischaemia.

**Materials and methods**

**The rabbit epigastric free flap**

New Zealand white rabbits, approximately 2.2–2.8 kg, were used in this study. An oval 5 x 4 cm groin flap based on the right inferior epigastric blood vessels was raised under sterile conditions and under general anaesthesia (Knight et al., 1988b). This was induced with pentobarbitone and maintained with halothane, nitrous oxide and oxygen through a facial mask. The femoral artery and vein were ligated proximally and distally to the epigastric pedicle. The flap was stored in saline-moistened gauze in a sealed container in a 25°C water bath. After 21 hours, the flap was revascularised by microvascular anastomoses of the contralateral femoral artery and vein. These conditions of ischaemia were selected in order to produce substantial, but not irreversible, flap loss by day 7 post-ischaemia.

**Treatment with vasodilatory agents**

After the anastomoses and prior to clamp release, a catheter was placed in the distal femoral artery. At the time of clamp release, the test agent was infused by means of a Gilson pump at 0.02 ml/minute for 30 minutes (total approximately 0.5 ml) and diluted with arterial blood as previously reported (Knight et al., 1985). Bolus injections of 0.5 ml and 1.0 ml of the drug were administered by the ear vein at the start and end of this infusion respectively.

The treatment groups were as follows:

1. Controls (18 rabbits): 2 ml of Hanks buffered salt solution (BSS) obtained from Commonwealth Serum Laboratories, Melbourne, were infused and injected as described above.

2. CGRP (14 rabbits): Synthetic human calcitonin gene-related peptide purchased from Sigma Chemical Company, St Louis, MO, USA, was prepared as a 5 μg/2 ml Hanks BSS—0.1% bovine serum albumin solution. The dose chosen, 2 μg/kg, was based on the minimal dose necessary to achieve maximal increase in blood flow, as tested in non-ischaemic rabbit epigastric skin flaps (Knight et al., 1988a).

3. Carbamyl β-methylcholine chloride (Sigma) (14 rabbits): This agent, abbreviated hereafter as MCh, was prepared as a 125 μg/2 ml Hanks BSS solution. The final dose of MCh, 50 μg/kg, was based on tests similar to those described above for CGRP (unpublished data) and on past experiences in rabbits with acetylcholine infusion (Wright et al., 1987), taking into account that MCh is 10–20 times less potent but more stable in blood than acetylcholine.

**Monitoring and final assessment of flap survival**

Blood flow to the flap was measured for the first 40 minutes of revascularisation in all rabbits. A 1.0 mm diameter cuff (Peter Pohl Inc., Iowa City, USA) was placed on the femoral artery between the anastomosis site and the epigastric artery junction. The Doppler shift was measured by a 20 mHz ultrasonic Doppler flowmeter (Bioengineering Department, University of Iowa, Iowa City, USA) and compared with stepwise shifts 0–16 kHz from an oscillator calibration unit. This method has been previously described by others (Haywood et al., 1981; Parker et al., 1984; Wright et al., 1987).

In 4 rabbits per group the stump of the right femoral artery was catheterised. Blood pressure readings were taken during drug administration for the first 40 minutes of revascularisation by means of a Bentley Trantec pressure transducer and a Neotrace multichannel recorder (Neomedix Systems, Dee Why, NSW, Australia). The mean of 5 blood pressure readings evenly spaced over 40 minutes was recorded for each rabbit.

At day 7, 1 ml of 10% fluorescein was injected into the ear vein. The viable areas on the surface of the flap were visualised with the aid of an ultraviolet lamp and percentage survival was calculated by tracing the perfused (viable) and non-perfused (necrotic) dimensions of the flap onto a piece of paper and weighing the proportion of paper for each category. The patency of the arterial and venous anastomoses were noted (Hayhurst and O'Brien, 1975) and the rabbit then sacrificed with an overdose of Sagatal.

**Statistics**

The control percentage areas of flap survival were compared with treatment by Student's t test. Blood
flow and heart rates in controls were compared with treated groups by a randomised one-way analysis of variance (ANOVA) and individual means subjected to the Newman-Keuls studentised range statistic.

**Results**

CGRP produced significantly greater flap viability of 70.2% than Hanks BSS infused controls (39.9%), \( p < 0.025 \) by Student's t test analysis (Table 1). MCh, however, with 47.1% flap survival was not significantly different from controls.

Both test agents, CGRP and MCh, resulted in significantly increased mean blood flow \( (p < 0.05, \text{Newman Keuls analysis}) \). Heart rates in the rabbits with treated ischaemic flaps compared with rates in Hanks infused control rabbits also increased marginally during the period of drug infusion (Table 2) but the increases were not statistically significant. Blood pressure decreased by a mean of 0, 2 and 9 mm Hg for control, CGRP and MCh respectively in the first minute after bolus drug administration and recovered to normal usually within 5 minutes. During slow infusion of these drugs the blood pressure changes were indistinguishable from normal. Using the means of these three parameters, the peripheral resistances of the agents have been calculated (Table 2). Compared with a control value of 350 mm Hg/ml/min, treatment with both of the drugs led to large falls in peripheral resistance of 32% and 39% for CGRP and MCh respectively.

**Discussion**

The present study has demonstrated that CGRP is a vasodilator of the ischaemic flap vasculature, as has already been demonstrated in a pilot study with an acute non-ischaemic flap (Knight et al., 1988a). It seems likely that CGRP might have significant clinical potential for the salvage of ischaemic flaps and, by extrapolation, severed limbs which have undergone ischaemia. This agent is considerably more stable both metabolically and chemically than PGI\(_2\), even though its potency might be 10–20 times less than PGI\(_2\) (Knight et al., 1988a) and of similar potency to PGE\(_2\) (Brain et al., 1985).

There are several additional properties of CGRP which may be important in prolonging the viability of an ischaemic flap. Brain et al. (1985) found that topically applied CGRP induced dilatation of microvessels in the hamster cheek pouch. Brain and co-workers also found that CGRP (as well as acetylcholine) was capable of rapidly relieving noradrenaline-induced spasm in rat aortic rings. The ability to overcome spasm induced by ischaemia in skin flaps would certainly improve the chances of flap survival.

Two studies performed concurrently with our own have investigated the effect of human CGRP (\(\alpha\)-hCGRP) on experimental ischaemia using rat musculocutaneous flaps (Kjartansson and Dalsgaard, 1987) and rat epigastric flaps (Westin and Hedén, 1988). Kjartansson and Dalsgaard found

### Table 1 Percentage area of survival of rabbit epigastric skin flaps at day 7 post-ischaemia

<table>
<thead>
<tr>
<th>Infusion</th>
<th>N</th>
<th>Mean percentage area surviving (SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hanks BSS (control)</td>
<td>18</td>
<td>39.9 (9.1)</td>
</tr>
<tr>
<td>CGRP, 2 µg/kg</td>
<td>14</td>
<td>70.2 (10.6)*</td>
</tr>
<tr>
<td>MCh, 50 µg/kg</td>
<td>14</td>
<td>47.1 (11.9)</td>
</tr>
</tbody>
</table>

* Significantly different from control by Student’s t test at \( p < 0.025 \).

### Table 2 Changes in haemodynamic parameters at 20 minutes post-ischaemia

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Blood pressure (mm Hg)</th>
<th>Flap blood flow (ml/min)</th>
<th>Heart rate (beats/min)</th>
<th>Flap peripheral resistance (mmHg/ml/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hanks BSS</td>
<td>63</td>
<td>0.18 (0.04)</td>
<td>206 (8)</td>
<td>350</td>
</tr>
<tr>
<td>CGRP, 2 µg/kg</td>
<td>76</td>
<td>0.32 (0.04)*</td>
<td>223 (6)</td>
<td>238</td>
</tr>
<tr>
<td>MCh, 50 µg/kg</td>
<td>71</td>
<td>0.33 (0.04)*</td>
<td>224 (7)</td>
<td>215</td>
</tr>
</tbody>
</table>

Data are recorded as mean (SEM).

* Significant by one-way ANOVA at \( p < 0.05 \) compared with controls.

† Only 4 out of 14 rabbits per group were analysed for blood pressure.

Values in the Tables represent the average of the diastolic and systolic blood pressures.

‡ Converted from kHz to ml/min using a probe calibration factor of 1.25 for probes of 1.0 mm diameter (See Parker et al., 1984).

§ Peripheral resistance is defined as: \( \frac{\text{blood pressure}}{\text{blood flow}} \).
an increase in survival from 45% in controls to 90% in CGRP-treated flaps at doses “lower than those known to cause an increase in skin blood flow under normal conditions”. This implied that mechanisms for CGRP other than vasodilation alone may be important in salvaging ischaemic flaps. Our results show that MCh is an effective vasodilator (Table 2) without affecting flap survival (Table 1), which also suggests that factors other than increased blood flow are important for ischaemic flap survival.

Westin and Hedén (1988), on the other hand, found that a single $10^{-7}$ mol/l dose of CGRP increased flap survival from 18.4% (saline-treated controls) to 45.5%. Even better results were achieved with pre-ischaemic treatment (60.3% survival) and with both pre- and post-ischaemic treatment (66.3% survival) using $10^{-8}$ mol/l of CGRP. The dose given by Westin and Hedén (1988) was 0.05 µmol (for a $10^{-7}$ mol/l dose) or 0.18 µmol/kg in rats, compared with 1.28 µmol or 0.51 µmol/kg in rabbits in our study, i.e. approximately 3 times higher dose in our study. The improvement in flap survival for a single post-ischaemic dose: +27.1% in the Westin and Hedén (1988) study, +30.3% in our study, is also comparable.

Another agent with an endothelium-dependent vasodilatory mechanism is bradykinin. Morain and co-workers (1983) investigated the effect of this agent plus several other vasodilators, administered slowly over several days via an implantable pump, on the survival of rabbit island flaps with a random extension. Bradykinin was effective in significantly increasing the survival of random portions of the flap.

In conclusion, CGRP, a peptide neurotransmitter with an endothelium-dependent mechanism of vasodilation, with properties of overcoming spasm, plus other unknown beneficial effects on ischaemic tissues, has been shown to increase the survival of experimental ischaemic flaps in a single post-ischaemic dose. It is considered to have great clinical potential in the treatment of failing skin flaps. Further experiments will be necessary to optimise the dose, the timing and the mode of administration of CGRP.

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References


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