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Baseline Blood Flow and Antidromic Vasodilation in Normal and Inflamed Rat Skin

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ABSTRACT. Blood flow in the rat skin was measured by laser Doppler flowmeter under normal conditions and after mild inflammation. Skin inflammation was induced either by a single subcutaneous injection (50μl) of a 2% carrageenan solution or by repeated topical treatment with chloroform over three days (once daily). In normal skin, the mean baseline blood flow is 57.7 ± 3.7 arbitrary units (n=96), but has increased to 78.5 ± 8.7 arbitrary units (n = 48) in the carrageenan inflamed skin, and to 144.6 ± 16.9 arbitrary units (n = 48) in chloroform inflamed skin. Antidromic stimulation of the saphenous nerve (0.2-5.0 mA, 0.5 ms, 1 Hz) for 10 seconds has increased the blood flow in normal skin to 77.3 ± 9.0% (n = 25), and in carrageenan inflamed skin to 104.8 ± 9.6% (n = 57). The results show that the cutaneous neurovascular responses are elevated under the conditions of acute inflammation.

Antidromic stimulation of the cutaneous nerves was shown to cause vasodilation and increased permeability (Jancso *et al.* 1967, Lembeck and Holzer 1979 and Gamse *et al.* 1980). This neurogenic inflammatory response is mediated by the small unmyelinated, peptide-containing nerve fibers (Gamse *et al.* 1980, 1981,. Lembeck and Donnerer 1981 and Hukkanen *et al.*1991) of the C-polymodal nociceptors (Kenins 1981). This group of nerve fibers was shown to be sensitized during acute skin inflammation (Kocher *et al.* 1987), which might affect the blood flow in the area innervated by them as demonstrated by the enhancement of plasma protein extravasation (Scott *et al.* 1992) and vasodilation (Lam and Ferrell 1993) in response to application of exogenous neuropeptides. However, the skin blood flow has not yet been studied under such neurovascular inflammatory conditions. Hence, in the present study, the skin blood flow has been monitored using a laser Doppler flowmeter to determine the cutaneous microvascular changes due to antidromic nerve stimulation in normal, as well as during mild neurovascular inflammation in rat skin.

Materials and Methods

Animal preparation

Experiments were performed on male Sprague-Dawley rats weighing 200-490 g anesthetized initially by the intraperitoneal injection of 30 mg kg^{-1} body weight of pentobarbitone sodium (Sagatal, May and Baker Ltd., England). Deep anesthesia was maintained by additional doses (20 mg kg^{-1}. hr) administered through the cannulated left jugular vein. The animal body temperature was maintained around 37 °C by a heating pad under its body and controlled by a rectal thermistor probe. The animal blood pressure was monitored through a cannula inserted in the left common carotid artery. Blood flow measurements were recorded when the systolic blood pressure was above 80 mm Hg.

Skin inflammation and measurements of blood flow

In a series of experiments, the skin inflammation was induced by the subcutaneous injection of 50μl of a 2% carrageenan solution (Sigma Ltd., U.S.A.) and 10% Evans blue (Kocher *et al.* 1987, and Joris *et al.* 1990) into three sites within the area innervated by the saphenous nerve medially on the right leg, 3-5 hrs before measuring blood flow. In another series of experiments, inflammation was induced by repeated topical treatment of the medial aspect of the saphenous field of the right leg with chloroform (Leeh and Zimmermann 1988). The skin blood flow was monitored using two laser Doppler flowmeters, one with two laser probes (Moor Instruments, MBF30. England) and the other with a single one (Moor Instruments, MBF2). The laser probes were firmly fixed at 1-2 mm above the skin, and the output of the flowmeters and the blood pressure transducer were plotted by a chart recorder (Dash IV, Astro, Med., U.S.A.). The baseline blood flow was simultaneously measured (for 10 seconds) at two identical locations, one on normal skin (contralateral left leg) and the second on inflamed skin (ipsilateral right leg). In each case, 6-14 locations were randomly selected and their baseline blood flow was recorded.

Antidromic vasodilation

The saphenous nerve was gently exposed, cut in the upper thigh and covered with a pool of liquid paraffin made from skin flaps sutured to a brass ring. Antidromic stimulation was done at the proximal cut end using a pair of platinum electrodes, and the compound action potential was recorded through a similar pair of electrodes placed distally above the knee. Antidromic vasodilation (ADV) was evoked by applying fixed electric stimuli sufficient to excite unmyelinated C-fibers at 0.2-5.0 mA, 0.5 ms (millisecond) duration and 1 Hz frequency for 10 seconds. In each preparation, 4-10 antidromic vasodilatory responses were evoked (at 3 sites, simultaneously) from both normal and inflamed skin. The ADV was measured from peak blood flow change and expressed as a percentage above the resting level.

Statistical methods

The data were analyzed statistically by Student's t-test for unpaired data. Values are expressed as means ± standard error (S.E.M.).

Results

Baseline blood flow in normal and inflamed rat skin

The basal blood flow in normal skin was compared with that in skin inflamed either by carrageenan injection or by chloroform treatment (Fig. 1). In normal skin, the mean baseline blood flow was 57.7 ± 3.7 arbitrary units (n = 96, where n is the total number of locations randomly selected from normal skin in the left leg). In the carrageenan inflamed skin, the mean baseline blood flow was 78.5 ± 8.7 arbitrary units (n = 48), which is significantly higher ($P < 0.01$) than that in normal skin. However, the increase in the mean baseline blood flow in the chloroform inflamed skin (144.6 ± 16.9 arbitrary units, n = 48) was highly significant ($P < 0.001$) when compared to that of the carrageenan inflamed skin.

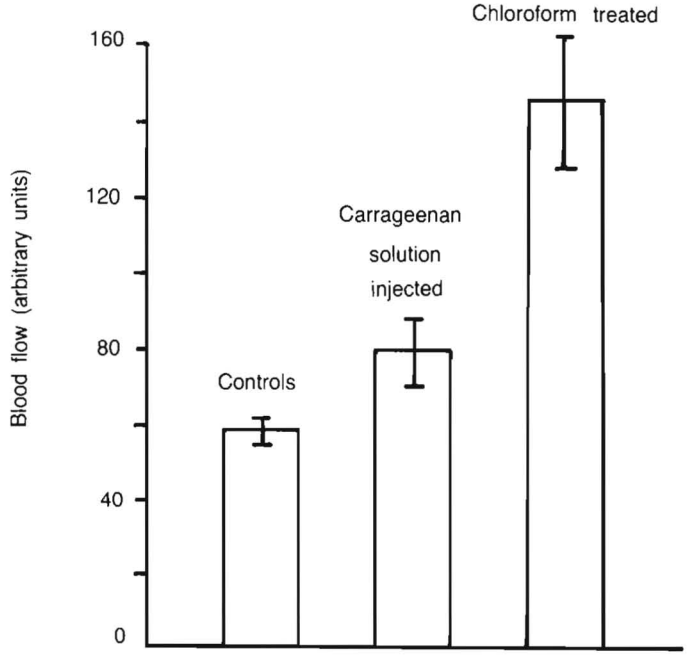

Fig. 1. The effect of inflammation on the blood flow in the rat skin. Bar indicates average ± standard error (S.E.M.).

Antidromic vasodilation in normal and inflamed rat skin

Antidromic stimulation of the rat saphenous nerve at maximum C-fiber potentials for 10 seconds caused an increase in blood flow lasting 2-6 minutes (Fig. 2a). This response was greatly enhanced after the acute skin inflammation induced by the subcutaneous injection of 2% carrageenan solution (Figs. 2b and 3). In normal skin, the mean percentage increase in blood flow to antidromic nerve stimulation was 77.3 $\pm$ 9.0% (n = 25), but has significantly ($P < 0.02$) risen to 104.8 $\pm$ 9.6% (n = 57) in the carrageenan inflamed skin.

Discussion

The present study examined the blood flow in rat skin under normal conditions and after mild local inflammation induced by chemical irritants, which are known to activate a specific class of unmyelinated cutaneous afferent nerve fibers of the C-polymodal nociceptors, resulting in the release of neuropeptides, such as substance P (Lembeck *et al.* 1982, Foreman 1987, Szolcsanyi 1988, Holzer 1988, Yaksh 1988, and Lisney and Bharali 1989). Following acute skin inflammation, this neurovascular response has enhanced as indicated by the increase in both of the baseline blood flow and antidromic vasodilation compared with that in normal rat skin. The overall increase in the antidromic vasodilation observed in inflamed rat skin was about 35% more than the percentage increase in the same response in normal skin. A value that is also comparable to the rise in the baseline blood flow following induction of inflammation. This might indicate that the whole response is already enhanced at the resting level. The shift in the baseline blood flow in the inflamed skin is in agreement with the recent findings on another model of inflammation in the rat knee joint (Lam and Ferrell 1993). This is probably due to the increase in the activity of the nociceptive afferent nerve fibers (C–polymodal nociceptors), as has previously been reported in the rat (Kocher *et al.* 1987) as well as in the cat (Russell *et al.* 1987). This could well be due to an increase in the level of the neuropeptide substance P (Lembeck and Holzer 1979, and Gamse *et al.* 1980) and to the upregulation of the neuropeptide receptors in the inflamed tissue (Lam and Ferrell 1993). It might also be indirectly facilitated through degranulation of mast cells by substance P with the subsequent release of endogenous mediators of inflammation such as histamine (Lembeck and Holzer 1979, and Barnes *et al.* 1986).

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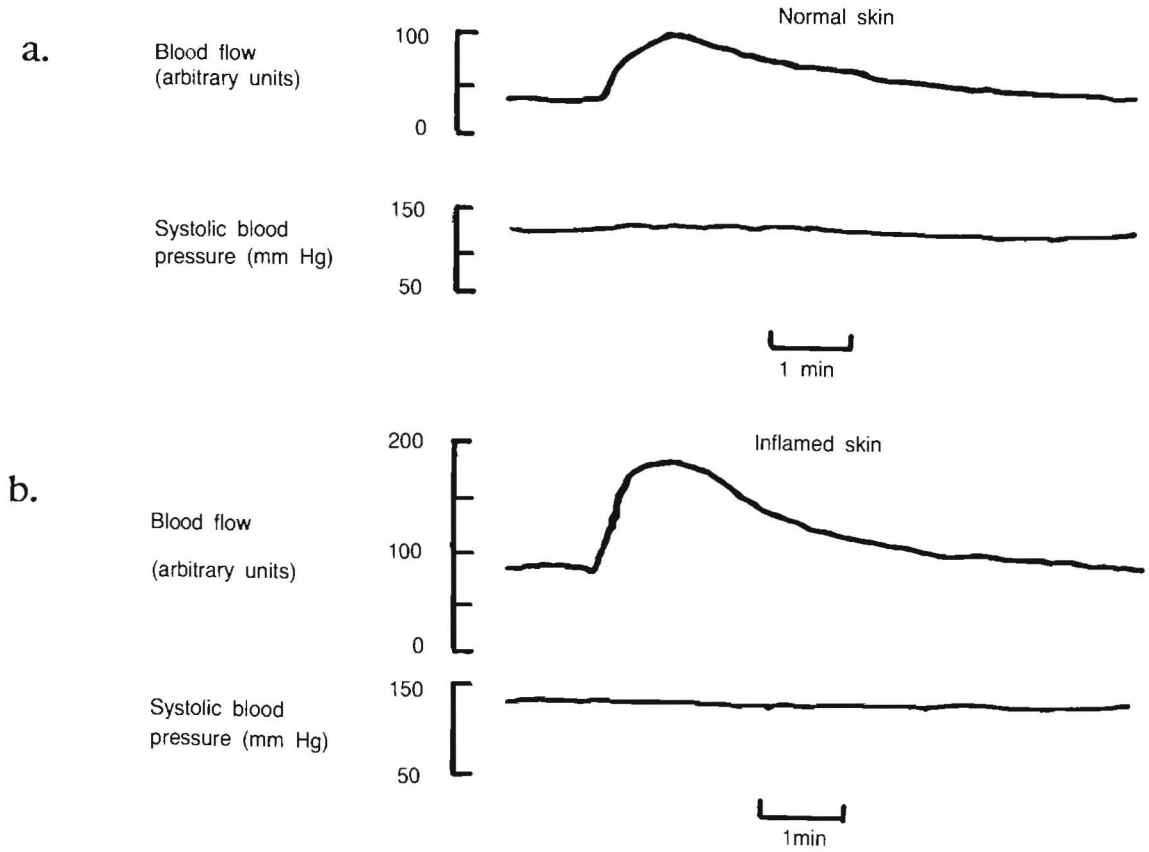

Fig. 2. Simultaneous recording of skin blood flow and systolic blood pressure following antidromic stimulation of the rat saphenous nerve at 2.0 mA. 0.5 ms., 1 Hz for 10 seconds in normal skin (a), and at 0.8 mA, 0.5 ms, 1 Hz for 10 seconds in carrageenan inflamed skin (b).

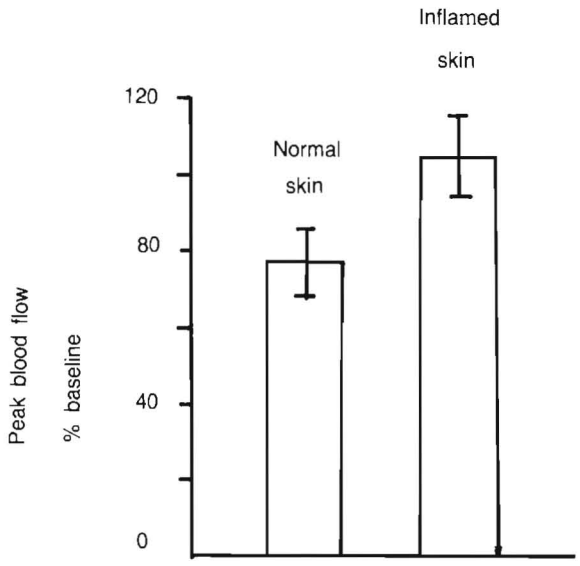

Fig. 3. The effect of carrageenan induced inflammation on skin blood flow to antidromic stimulation of rat saphenous nerve. Bar indicates average ± standard error (S.E.M.).

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الخط القاعدي لجريان الدم والتوسع الوعائي الدموي الناتج عن الإثارة الكهربائية المعاكسة في جلد الجرذ الطبيعي والملتهب

جمعة الشخانبة

قسم العلوم الحياتية ـ كلية العلوم ـ جامعة مؤتة
ص.ب: ٧ ـ الكرك ـ المملكة الاردنية الهاشمية

لقد تمّ قياس جريان الدم في جلد الجرذ بمقياس جريان دوبلر اللّيزري في الظروف الطبيعية وخلال وجود إلتهابات طفيفة. لقد أحدثت الإلتهابات الجلديّة إما بواسطة حُقنة واحدة تحت جلديّة (٥٠ ميكروليتر) من ٢٪ من محلول الكاراجينان، أو بمعاملة سطحيّة متكررة بالكلوروفورم لمدة ثلاثة أيام (مرّة واحدة يومياً). في الجلد الطبيعي فقد كان معدل الخط القاعدي للجريان الدموي هو ٥٧,٧ ± ٣,٧ وحدة أعتباطية (ن = ٩٦). ولقد أزداد معدل الخط القاعدي للجريان الدموي إلى ٧٨,٥ ± ٨,٧ وحدة أعتباطية (ن = ٤٨) في الجلد الملتهب بمحاولة الكاراجينان، وإلى ١٤٤,٦ ± ١٦,٩ وحدة أعتباطية (ن = ٤٨) في الجلد الملتهب بالكلوروفورم. كما وجد أيضاً أن إثارة العصب الأسفيني كهربائياً معاكساً (٠,٢ - ٥,٠ ميليمبير، ٠,٥ ميليثانية، ١ هيرتز) لمدة عشرة ثواني أدّت إلى زيادة نسبة الجريان الدموي في الجلد الطبيعي إلى ٧٧,٣ ± ٩,٠٪ (ن = ٢٥)، وفي الجلد الملتهب بمحلول الكاراجينان إلى ١٠٤,٨ ± ٩,٦٪ (ن = ٥٧). تبيّن النتائج أن الظاهرة الوعائية الدموية العصبية في الجلد تزداد بوجود إلتهابات طفيفة.