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Development of Enhanced Blood Flow Responses to Prostaglandin E_1 in Carrageenan-Induced Granulation Tissue

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Abstract

The distribution of cardiac output (c.o.) was measured by the radioactive microsphere method in rats at different time intervals after the implantation of carrageenan-impregnated sponges. The amount of blood distributed to the developing granulomata increased from day 5 after sponge implantation to day 7, but showed no further increase at day 10. A similar pattern in blood flow was observed in the skin covering the granulomata. Injection of PGE_1 (100 ng) into the sponges led to an increase in blood flow, the magnitude of which became gradually larger between days 5 and 10. A similar, though less marked increase in sensitivity to PGE_1 was observed in the skin covering the granulomata, PGE_1 causing a significant increase in blood flow to the skin on day 10. These changes in sensitivity to exogenous PGE_1 may be due to decreasing levels of endogenous PGE and/or maturation of the newly formed blood vessels in the granulation tissue.

Introduction

Newly formed blood vessels in granulation tissues are abnormally permeable to plasma constituents, apparently due to gaps between endothelial cells [1]. However, when the inflammatory mediators, histamine, 5-hydroxytryptamine (5-HT) or bradykinin are injected locally to granulation tissues, only the more mature vessels exhibit increased permeability, but not the newly formed terminal capillaries [2, 3]. In fact, in granulation tissue formed around wounds made in the rat cremaster muscle, histamine only elicited an increase in permeability in vessels which were at least 2–3 weeks old [3]. Similarly, while histamine induced increased permeability in skin vessels during acute pregranulomatous carrageenan pouch inflammation in rats, the same compound was ineffective after 7 days, presumably because it was acting on the vessels of the newly established granuloma [4]. In contrast, under the same experimental conditions, prostaglandins (PGs) E_1 and E_2 were ineffective in increasing vascular permeability during the pregranulomatous phase, but were effective on day 7 of the granulomatous pouch inflammation [4], possibly because new vessels become sensitive to PGE more rapidly than to histamine.

Although the actions of inflammatory mediators on vascular permeability in granulation tissue have been the subject of only a few studies, even less is known about either basal flow or the actions of these compounds on blood flow in granulation tissue. Blood flow through granulation tissue in bipedicle tube flaps in rabbits has been shown to increase with time [5] and in a not unrelated study, levels of endogenous PGE_2, $PGF_{2\alpha}$ and histamine were found to parallel certain phases of increased blood flow in rabbit skin grafts [6]. Several recent reports have demonstrated that PGE_1 and PGE_2 increase blood flow in normal vessels of rabbit and guinea-pig skin [7–9] and of the dog knee joint [10, 11]. Furthermore, vasodilatation induced by PGE may be responsible for the associated enhancement of plasma exudation [8]. In the light of these observations, we investigated the local blood flow changes produced by PGE_1 at different stages of carrageenan-impregnated sponge-induced granuloma formation, in order to compare alterations in the magnitude and time course of these changes with those in vascular permeability observed by CHANG and TSURUFUJI [4]. A preliminary report of our results has been presented to the British Pharmacological Society [12].

Materials and methods

(a) Sponge implantation

The method used was that described by BONTA et al. [13]. Briefly, polyether sponges, with indwelling polyethylene cannulae, were soaked in 1 ml 2% carrageenan and dried. The impregnated sponges were implanted s.c. into the backs of male Wistar rats (170–250 g) with one sponge on either side of the central incision. The two cannulae were drawn out under the skin and secured at the base of the neck. The cannulae permitted injections of PGE_1 to be made into the sponges at any desired period after implantation.

(b) Microsphere and drug administration

Rats were anaesthetized with urethane (i.p.) 5, 7 or 10 days after sponge implantation. An incision was made in the neck and the muscle dissected medially to locate the right carotid artery. A polyvinyl catheter (i.d. 0.5 mm) was inserted into the artery and attached to a Statham P23 DC transducer from which blood pressure was recorded on a pen recorder. The catheter was slowly pushed along the artery until the pulse pressure broadened and diastolic pressure was zero. This indicated that the tip of the catheter lay in the left ventricle of the heart and in each case the situation of the catheter was confirmed on subsequent dissection.

An injection of radioactively labelled microspheres (15 ± 5 (s.d.) μm dia.) in 0.1 ml of 0.9% (w/v) saline, was then given into the heart through this catheter (over a 15–20 sec period) and was followed after 15 min by an injection of 100 ng PGE_1 in 0.5 ml saline into one sponge (via the indwelling cannula), simultaneously with an injection of 0.5 ml saline into the other sponge. Further injections of 0.1 ml of microspheres were given into the heart 1.5 and 15 min after the intra-sponge injections. Microsphere injections consisted of 20,000–150,000 microspheres and each injection involved a different isotope. All rats were killed with an overdose of sodium pentobarbitone 15 min after the third and final microsphere injection.

(c) Assessment of blood flow

The basis of the microsphere method for measurement of blood flow is that an increase in flow to any particular anatomical region will lead to a greater distribution of the number of microspheres injected into the heart. Since the diameter of capillaries (1–8 μm) is less than that of the microspheres, the latter become trapped in the tissue and the radioactivity in that particular tissue represents the amount of cardiac output which it has received [14]. By using three different isotopes, in our study, for each of the three microsphere injections per experiment (i.e. 15 min before, 1.5 and 15 min after intra-sponge injections), the radioactivities at each time point could all be determined from the same tissue. This was carried out by dissecting the rats into various organs and tissues which were weighed and counted in a Packard γ-scintillation counter equipped with a multichannel analyser using suitable windows to discriminate between the various isotopes used [15]. Blood flow to a particular tissue (e.g. granulation tissue) was expressed as per cent distribution of cardiac output (c.o.)/100 g tissue and was calculated as:

$$\frac{\text{radioactivity in tissue} \times 100}{\text{radioactivity in whole body}} \times \frac{100}{\text{weight of tissue (g)}}$$

Since absolute c.o. was not measured, the effect of PGE_1 on blood flow was determined by subtracting the values for per cent distribution of c.o./100 g in saline-treated tissues from the values in the respective PGE_1-treated tissues for each microsphere injection.

(d) Materials

Prostaglandin E_1 was obtained from Unilever Research Labs., Vlaardingen, The Netherlands; carrageenan sodium (Viscarin®) from Marine Colloids, Springfield, USA; urethane from E. Merck, Darmstadt, W. Germany; sodium pentobarbitone (Nembutal®) from Abott, Ottignies, Belgium and ^{125}I, ^{141}Ce, ^{85}Sr and ^{46}Sc-labelled plastic microspheres from 3M Company, St. Paul, Minn, USA.

(e) Statistical analysis

Significance of differences between baseline values and those following PGE_1 injection and between values obtained on different days after sponge implantation was assessed by the Mann-Whitney U test. The limit of significance was taken as $P \leqslant 0.05$.

Results

(a) Stability of distribution of cardiac output

To test whether the general distribution of cardiac output changed during the course of the experiment (either due to leakage of PGE_1 from the sponges or to cardiovascular damage), data from several selected organs (Table 1) were inspected for each rat. Any animals with organs which showed variation in distribution of cardiac output, between microsphere injections, of 50% or more of that of the baseline values were discarded. This ensured that, in the remaining animals, the local changes induced by PGE_1 in blood flow to granulation tissue would not be complicated by simultaneous systemic changes in cardiac output. As shown in Table 1, the per cent distribution of cardiac output to the heart, gut, kidney, liver and lungs did not alter significantly during the course of the experiment in the rats chosen for analysis.

(b) Baseline distribution of cardiac output to granulation tissue

Basal values for per cent distribution of c.o./100 g to granulomata showed a significant increase between days 5 and 7 after sponge implantation and then stabilized between days 7 and 10 (Fig. 1). This change in the basal amount of blood entering the tissue was also reflected, to a lesser extent, by values obtained from the skin covering the granulomata (Fig. 1). There was no difference in baseline values for per cent distribution of c.o./100 g between tissues which were later treated with either saline or PGE_1. This applied to both granulomata and skin.

Table 1
Stability of per cent distribution of cardiac output per 100 g tissue to some selected organs during local injections of PGE_1 into granulomata.

Organ	[a]Time after PGE_1 injection (min)	% distribution of c.o./100 g		
		day 5 ($n = 4$)	day 7 ($n = 7$)	day 10 ($n = 5$)
Heart	−15.0	1378 ± 497	543 ± 156	557 ± 148
	1.5	1568 ± 654	646 ± 160	649 ± 144
	15.0	1456 ± 580	580 ± 108	687 ± 147
Gut[b]	−15.0	115 ± 31	66 ± 12	81 ± 15
	1.5	117 ± 31	67 ± 10	84 ± 14
	15.0	116 ± 32	69 ± 11	77 ± 13
Kidney	−15.0	410 ± 60	347 ± 63	342 ± 58
	1.5	463 ± 58	338 ± 62	341 ± 77
	15.0	459 ± 52	321 ± 69	337 ± 67
Liver	−15.0	58 ± 18	44 ± 6	37 ± 7
	1.5	61 ± 21	43 ± 7	34 ± 6
	15.0	63 ± 21	44 ± 7	39 ± 9
Lungs	−15.0	74 ± 15	72 ± 14	70 ± 25
	1.5	90 ± 19	71 ± 12	92 ± 27
	15.0	113 ± 24	74 ± 12	79 ± 23

Values given are means ± S.E. mean.
[a] The times shown correspond to the successive injections of differently labelled radioactive microspheres.
[b] Including stomach, small and large intestines.
There were no significant differences from baseline ($t = -15.0$) values.

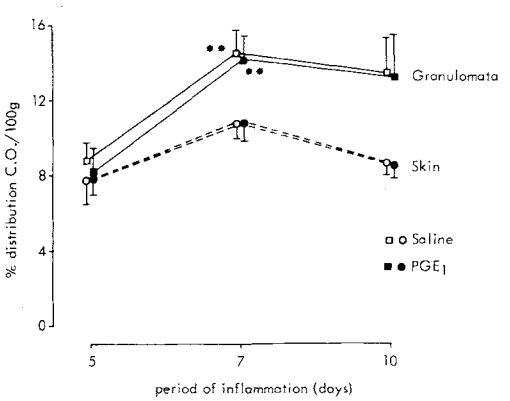

Figure 1
Baseline values for per cent distribution of c.o./100 g to granulomata and skin covering granulomata. Values shown are the means ± S.E. mean obtained from the first microsphere injection (i.e. 15 min before intrasponge injections) on day 5 ($n = 4$), day 7 ($n = 7$) and day 10 ($n = 5$). Data from tissues which later received treatment with either saline or PGE_1 are plotted separately. ** $p < 0.01$ (v day 5).

(c) Effect of PGE_1 on blood flow to granulomata

After subtraction of values for blood flow in saline-treated granulomata from those in PGE_1-treated granulomata, it is clear that the magnitude of the increase in blood flow produced by PGE_1 (100 ng/sponge) became larger as the inflammatory response progressed (Fig. 2). While blood flow 1.5 and 15 min after PGE_1 injection was significantly enhanced above baseline on days 7 and 10 after sponge implantation, blood flow 1.5 min after PGE_1 injection on day 10 was also significantly higher than that 1.5 min after PGE_1 injection on day 5. There was no significant increase in blood flow following treatment with PGE_1 on day 5. However, per cent distribution of c.o./100 g to *saline*-treated granulomata on this day increased from 8.9 ± 0.9 (s.e. mean), 15 min before saline injection, to 12.5 ± 1.9, 15 min after saline injection, though this increase was not significant. This increase probably reflected slight diffusion of PGE_1 from the contralateral sponge and may have prevented the day 5 value 15 min after PGE_1 injection, shown in Figure 2, from achieving a significant difference from baseline.

(d) Effect of PGE_1 on blood blow to skin covering granulomata

A similar enhancement of the PGE_1-induced increase in blood flow, as inflammation progressed, was also observed in the skin covering

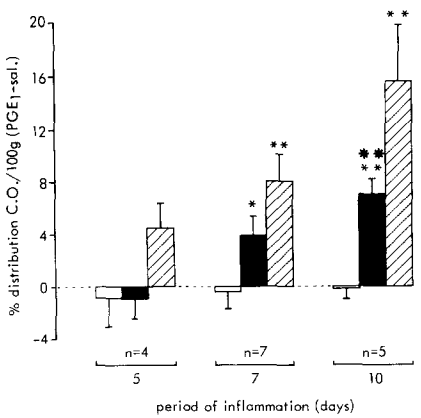

Figure 2
Increased blood flow to granulomata produced by PGE_1 (100 ng) on different days after sponge implantation. Values are means ± S.E. mean of the number of observations shown, obtained after subtraction of values in saline-treated tissues from the respective values in the contralateral PGE_1-treated tissues. Open columns represent values obtained from microspheres injected 15 min before PGE_1; filled columns represent values obtained from microspheres injected 1.5 min after PGE_1; hatched columns represent values obtained from microspheres injected 15 min after PGE_1. * $p < 0.05$; ** $p < 0.01$ (small asterisks: v baseline values; large asterisks: v value 1.5 min after PGE_1 on day 5).

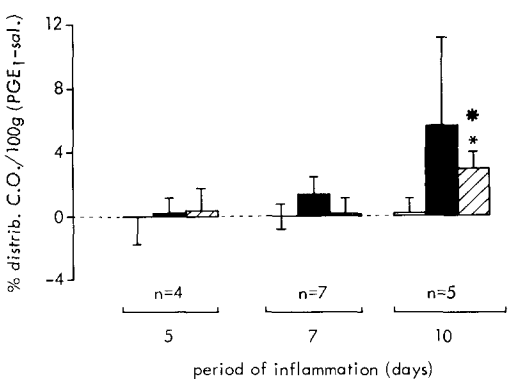

Figure 3
Increased blood flow to skin covering granulomata produced by PGE_1 (100 ng) on different days after sponge implantation. The explanation of the figure is the same as that given in the legend to Figure 2. * $p < 0.05$ (small asterisk: v baseline value; large asterisk: v value 15 min after PGE_1 on day 7).

the granulomata (Fig. 3). However, with the skin, the PGE_1 only produced a significant increase in blood flow on day 10. As with the granulomata, on day 5 per cent distribution of c.o./100 g to saline-treated tissues showed a slight, non-significant increase from 8.0 ± 1.5 (s.e. mean), 15 min before saline injection, to 11.2 ± 3.1, 15 min after saline injection. Thus, values for day 5, shown in Figure 3, may be slightly less than for the 'true' effect of PGE_1.

Discussion

The results presented in this paper show that the blood flow response to PGE_1 in carrageenan-induced granulation tissue, measured with radioactive microspheres, increases with time, as appears to be the case for PGE-induced vascular permeability increases in the same tissue [4].

The sources of error and requirements for local blood flow measurements with radioactive microspheres have been carefully studied by VADAS and HAY [9] following injections of inflammatory mediators into the skin of rabbits. These authors showed that the relationship between the number of microspheres injected and the number of microspheres accumulated per injection site is linear over the range 2–50 × 10^6 microspheres per 2.5–3.0 kg rabbit. This corresponds to 20,000–500,000 microspheres per 250 g, which is the range we used. Vadas and Hay also showed that even with more than 15,000 microspheres present in a single injection site (removed with a 15.9 mm diameter cork borer), there was no evidence of a compromised microcirculation. Since the doses of microspheres and the size of the sponges we used would have given a much smaller density of locally impacted microspheres, a compromised microcirculation is not expected to have been a source of error, even after three separate microsphere doses. Other suggestions, made by Vadas and Hay, which were also incorporated into our study were: urethane anaesthesia, expression of blood flow as a percentage of cardiac output and the use of a saline-injected site as a within-rat control. Because of the nature of our sponge model, we did not use four to six replicate injection sites per animal, as suggested by Vadas and Hay. Thus, differences in the intensity of the inflammatory response between individual sponges and rats probably accounted for most of the variation which remained in systemic and baseline values for distribution of c.o. (Table 1; Fig. 1).

By determining the arithmetic difference between blood flow in saline-treated sponges and that in PGE_1-treated sponges, rather than a per cent change in flow, the effects of any underlying intersponge variation in baseline flow on the measured responses to PGE_1 would have been

minimized. Furthermore, the observed increase in sensitivity to PGE_1 could not be attributable to changes in systemic distribution of c.o., since at no stage during any of the experiments did the PGE_1 or the surgical procedures alter distribution of c.o. to several major organs (Table 1). However, slight diffusion of PGE_1 into the contralateral saline-treated tissues on day 5 after sponge implantation may have artificially amplified the increased sensitivity to PGE_1 between days 5 and 7.

Since the increased sensitivity of the granulation tissue vasculature to PGE_1 exhibited a continuous slope from day 5 to day 10 (Fig. 2), it seems unlikely that this gradual change was wholly related to the change in basal distribution of c.o., which only increased between 5 and 7 (Fig. 1). HOLMSTRÖM et al. [5] have shown that, in granulation tissue growing in bipedicle tube flaps in rabbits, blood flow increases in parallel with capillary density, both reaching a plateau after 2 weeks. In addition, other authors have shown that capillary density increases for up to 15 days in developing BCG tubercles in rabbits [16]. The increase in basal amount of blood entering the granulomata which we observed between days 5 and 7 may, thus, have been due to an increase in vessel density. If this were so then the increased sensitivity to blood flow changes induced by PGE_1 between days 5 and 7 may simply have been due to the fact that on day 7 there were more vessels to be dilated by PGE_1 than on day 5. Alternatively, the increase in baseline flow between days 5 and 7 may have been due to greater dilatation of the vessels on day 7. Although we have no evidence for this alternative explanation it cannot be excluded, in which case the change in baseline flow would not account for the associated increase in sensitivity to PGE_1.

The further increase, between days 7 and 10, in sensitivity of granulomata to blood flow changes induced by PGE_1 is open to three interpretations. Firstly, there may have been a gradual decrease in the activity of the PG metabolizing enzyme 15α-hydroxy dehydrogenase. However, OHUCHI et al. [17] found very little PG metabolizing activity in carrageenan granuloma in rats after 1 and 3 days. Thus, any further decrease in enzyme activity is unlikely to have accounted for our findings. Secondly, the increased sensitivity to exogenous PGE_1 may have been due to a gradual reduction in endogenous PGE production, since under conditions of reduced endogenous PG production blood vessels appear to become hypersensitive to exogenous PGs [11, 18–20]. Furthermore, levels of PGE-like material in exudates of carrageenan granulomata reach a peak within 24 h and decline thereafter [17, 21]. Such an explanation may be feasible, though this can only be applied to the change in sensitivity of blood flow to PGE_1 between days 7 and 10, since a decrease in endogenous PGE from day 1 would be expected to lead to a decrease in basal flow between days 5 and 7. Because basal flow increased between days 5 and 7, the increase in capillary density would appear to be more important in controlling blood flow at this stage. The third possible explanation for the increased sensitivity of blood flow in granulomata to PGE_1 between days 7 and 10 is that a larger number of mature blood vessels were present in the granulation tissue on day 10. Only mature blood vessels in granulation tissue exhibit increased vascular permeability responses to inflammatory mediators [2, 3]. Furthermore, it has been suggested that the action of PGE in potentiating plasma exudation induced by other mediators is due to PGE-induced vasodilatation [8]. It does not seem unreasonable, therefore, to assume that, as with exudation, only mature vessels exhibit appreciable vasodilatation and increased blood flow responses to mediators such as PGE_1.

Apart from the granulation tissue itself, the vessels of the skin covering the granulomata also showed a slight increase in sensitivity to blood flow changes induced by PGE_1 as the inflammatory response progressed (Fig. 3). Using the microsphere method, PGE_1 has been shown to cause long-lasting increases in blood flow to normal skin [7–9]. Thus, a decrease in endogenous PGE production, as discussed above, is probably the most likely explanation for the changes in sensitivity in the skin, rather than a change in blood vessel morphology. It is worth noting that a reduced cutaneous vascular response to PGE_1 was found in rheumatoid arthritis patients and overproduction of endogenous PGs was one explanation put forward by the authors for their findings [22].

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