Alterations in regional blood flow in rats following sensitization to the nematode *Nippostrongylus brasiliensis*: effects of PAF antagonists

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1 Changes in tissue and organ blood flow associated with sensitization of rats to the nematode parasite, *Nippostrongylus brasiliensis*, were studied 30 to 35 days after infection, a time when very few worms remain in the animal.

2 Neither active nor passive sensitization modified heart rate, mean arterial blood pressure, cardiac output or total peripheral resistance. Passive sensitization and administration of non-immune sera did not modify blood flow to any of the tissues studied.

3 Active sensitization increased hepatic arterial blood flow, but decreased blood flow to the stomach, duodenum, jejunum and the submandibular glands. These effects cannot be attributed to residual nematode infections as treatment with the anthelmintic, thiabendazole, did not alter blood flow relative to untreated, actively sensitized rats.

4 The effects of active sensitization on blood flow were probably due to an action of platelet-activating factor (PAF) since treatment of actively sensitized animals with the selective antagonists, WEB-2086 and BN 52021, reversed the decrease in flow seen to the intestinal regions. The PAF antagonists increased blood flow to the kidneys and the trachea of sensitized animals.

5 These results suggest that the PAF released from undetermined sources in nematode-sensitized rats, produces altered blood flow, primarily to the stomach and proximal small bowel.

**Introduction**

Helminths are common infectious agents in man and other animals. Even though many of the parasites do not establish long-term residency in the host, sensitization to the parasites frequently develops and this is often expressed by type I hypersensitivity reactions (Keller, 1970; Jarrett & Miller, 1982). Animal models of these infections, such as *Nippostrongylus brasiliensis* in the rat, have been useful in understanding IgE synthesis and function, as well as mast cell biology (Miller et al., 1983; Heavey et al., 1988). Although immune responses are frequently studied in the infected animals (Egwang et al., 1984; McElroy & Befus, 1987; Ramaswamy & Befus, 1989), few attempts have been made to investigate pathophysiological alterations occurring in other organs consequent to sensitization, perhaps with the exception of some parameters in the intestine (King & Miller, 1984; Ramage et al., 1988). In this study we examined systemic and microcirculatory changes that occur 30 to 35 days following active sensitization of rats with the nematode, *N. brasiliensis*. Although the rats are sensitized by the infection at this time, the majority of worms have been expelled within 3 weeks after infection. To ensure that the circulatory changes associated with infection could not be attributed to residual worms in the intestine, some rats were treated with an anthelmintic prior to investigations of blood flow.

**Methods**

**Animals and sensitization**

Male Sprague-Dawley rats (Charles River Breeding Laboratories, Canada) of an initial weight of 200–250 g were maintained in filter top cages (2 to 3 per cage) to minimize the possibility of other infections. The rats were divided into three groups: untreated controls, infected (active sensitization) and passively sensitized.

For active sensitization, animals were infected in the scruff of the neck with 3000 third stage larvae of *N. brasiliensis*. The larvae were collected from faecal cultures and extensively washed prior to injection. The blood flow studies were performed 30 to 35 days following initial infection.

Passive sensitization was induced by intraperitoneal injection of 3 ml of 1:128 titre IgE-containing sera obtained from *N. brasiliensis*-infected rats. The IgE titres of the sensitizing sera were determined by passive cutaneous anaphylaxis (Befus et al., 1982). Regional blood flows were also determined in a group of animals that were injected with 3 ml of non-immune sera obtained from normal (non-infected) rats. Forty-eight hours after intraperitoneal injection of the sera (immune or non-immune) regional blood flows were studied. To evaluate the effectiveness of passive sensitization and non-immune sera in sensitizing the animals, blood flows were also determined 5 min after an i.v. injection of 100 μl of antigen containing 150 worm equivalents of homogenized *N. brasiliensis*. This dose of antigen induces anaphylaxis in actively sensitized rats (Mathison et al., 1990).

**Deworming procedures**

Even though previous observations (Woodbury et al., 1984) have shown that most of the worms are expelled from the animal by 2 weeks after infection we treated 5 rats with the anthelmintic agent thiabendazole (Thizbenzole; Merck, Sharp & Dohme Ltd) to control for possible effects of residual parasitic infections on regional blood flows. The drug was given at 80 mg kg⁻¹ in aqueous suspension by stomach tube (Jarrett & Stewart, 1973) and 7 days later blood flows were determined. In separate groups of rats (untreated and thiabendazole-treated) the number of worms present in the small bowel was determined.

**Treatment with platelet-activating factor (PAF) antagonists**

Actively sensitized rats were treated i.v. with PAF antagonists. Either 3 mg kg⁻¹ of the antagonist, WEB-2086 (3-(4-(2-chlo-
(4-morpholinyl) -1,2,4)-diazepine-2-yl) -3,4} fur-o-{3',2' -
have shown through

sodium (30mg infusion of I00 ng

microspheres

in a total

pressure transducer connected to

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meter;


Microsphere experiments

Anaesthesia was induced prior to surgery with pentobarbitone sodium (30mg kg-1 i.p.). To assess haemodynamic values and collect the reference blood sample the right femoral artery was cannulated with PE50 tubing. The left ventricle was cannulated through the right carotid artery with PE50 tubing. Verification of the ventricular cannula placement was obtained by monitoring the blood pressure until a characteristic left ventricular pressure tracing appeared and by post mortem examination. Mean arterial blood pressure (MABP) and mean left ventricular pressure were recorded with a Statham P23Db pressure transducer connected to a Beckman Dynograph and head rack prepared from the pressure trace.

Regional blood flow and cardiac output were determined with radioactive microspheres ([113Sn or 64 Nb, 15 ± 3µm diameter; New England Nuclear]. The microspheres were suspended in 0.9% NaCl containing 0.01% Tween-80. The microspheres (approximately 80000 to 100000) were injected in a total volume of 0.5 ml followed by 0.8 ml saline to flush them into the left ventricle. Starting 15 s before the microsphere injection, the reference blood sample was drawn into a motor-driven syringe at a rate of 0.68 ml min-1 for 75 s.

Upon completion of the experiment the animals were killed with an overdose of urethane. Individual tissues were removed, weighed and counted on a dual channel LKB gamma counter. Data reductions were performed with the programme described by Flaim et al. (1984), adapted and modified to run on a VAX computer.

Several requirements, as defined by Hadengue et al. (1989), were met before using the data from an individual animal: firstly, a difference of less than 10% between left and right kidneys which is indicative of adequate microsphere mixing; secondly, stability in MABP and heart rate between the pre- and post-injection periods.

Blood flow was expressed as net flow g-1 of wet tissue. Cardiac output (ml min-1) was calculated as radioactivity injected (c.p.m.)/reference sample radioactivity (c.p.m.) × 0.68 (ml min-1). Total peripheral resistance (TPR) was calculated according to the formula (Hadengue et al., 1989) where:

\[
TPR = \frac{MABP (mmHg) \times 80}{\text{cardiac output (ml min}^{-1})}
\]

Statistical analysis

Results are expressed as mean ± s.e.mean. In order to compare the variability between the groups of animals one-way analysis of variance was applied and the Student-Neuman-Keul procedure (Snedecor & Cochran, 1976) was used to determine significance at \(P < 0.05\). Statistical analyses were performed with the package (SPSS-PC+ SPSS Inc., Chicago, IL, U.S.A.).

Results

Macrovascular parameters including heart rate, MABP, cardiac output and TPR were not significantly different for the 7 groups of animals: unsensitized control rats (n = 8), unsensitized rats given non-immune serum (n = 4), passively sensitized rats (n = 3), actively sensitized rats (n = 11), and actively sensitized rats treated with thiabendazole (n = 5) or WEB-2086 (n = 6) or BN 52021 (n = 6). In unsensitized animals these measurements were heart rate 367 ± 9 beats min-1; MABP = 111 ± 6 mmHg; cardiac output 121 ± 17 ml min-1; TPR = 73.6 ± 160 dyn.s.cm-2.

Passive sensitization or administration of non-immune sera, when compared to unsensitized rats, did not modify blood flow to the kidneys, liver, trachea, salivary glands and all regions of the small intestine (Table 1). Passive sensitization was effective in sensitizing rats to antigen as decreases in blood flow to the gastrointestinal tract were observed 5 min after administration of allergen to passively sensitized rats (Table 2). In contrast, administration of antigen to rats treated with non-immune sera did not alter blood flow to any of the tissues. The depressive effects of anaphylaxis on blood flow to various tissues in actively sensitized rats have been described previously (Mathison et al., 1990).

Active sensitization decreased blood flows to the stomach and jejunum, but not the duodenum and ileum (Figure 1). Blood flow to the submandibular gland was also reduced in actively sensitized animals (Figure 2). Hepatic arterial blood flow was enhanced in the actively sensitized animals (Figure 3), whereas blood flows to all other tissues examined were not altered by the sensitization.

Blood flows in sensitized rats that had been treated 1 week previously with the deworming agent thiabendazole were similar to those observed in sensitized rats. Relative to the unsensitized controls, in anthelmintic treated and in untreated actively sensitized rats, blood flows were reduced in the stomach, jejunum (Figure 1), and submandibular gland (Figure 3), whereas hepatic arterial blood flow was enhanced (Figure 2). In 3 untreated rats a total of 4 nematodes were

Table 1 Blood flow in unsensitized, passively sensitized and non-immune serum treated rats

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Unsensitized</th>
<th>Passive</th>
<th>Non-Immune serum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stomach</td>
<td>0.93 ± 0.16</td>
<td>0.77 ± 0.17</td>
<td>0.79 ± 0.24</td>
</tr>
<tr>
<td>Duodenum</td>
<td>2.04 ± 0.38</td>
<td>2.17 ± 0.33</td>
<td>1.96 ± 0.37</td>
</tr>
<tr>
<td>Jejunum</td>
<td>1.94 ± 0.33</td>
<td>1.62 ± 0.03</td>
<td>1.80 ± 0.17</td>
</tr>
<tr>
<td>Ileum</td>
<td>1.73 ± 0.42</td>
<td>1.74 ± 0.22</td>
<td>1.83 ± 0.41</td>
</tr>
<tr>
<td>Kidney</td>
<td>3.56 ± 0.61</td>
<td>3.95 ± 0.21</td>
<td>3.97 ± 0.25</td>
</tr>
<tr>
<td>Trachea</td>
<td>1.94 ± 0.47</td>
<td>2.43 ± 0.04</td>
<td>2.18 ± 0.38</td>
</tr>
<tr>
<td>Liver</td>
<td>0.09 ± 0.02</td>
<td>0.13 ± 0.03</td>
<td>0.14 ± 0.04</td>
</tr>
<tr>
<td>Submandibular</td>
<td>0.77 ± 0.12</td>
<td>0.62 ± 0.10</td>
<td>0.51 ± 0.10</td>
</tr>
</tbody>
</table>

Number of experiments: unsensitized (n = 8), passively sensitized (n = 3) and non-immune sera (n = 4).

Table 2 Effects of antigen challenge on blood flow to several tissues in unsensitized, passively sensitized rats and non-immune sera treated rats

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Unsensitized</th>
<th>Passive</th>
<th>Non-Immune sera</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stomach</td>
<td>0.78 ± 0.11</td>
<td>0.42 ± 0.06*</td>
<td>0.77 ± 0.16</td>
</tr>
<tr>
<td>Duodenum</td>
<td>2.26 ± 0.38</td>
<td>1.02 ± 0.21*</td>
<td>1.96 ± 0.12</td>
</tr>
<tr>
<td>Jejunum</td>
<td>1.99 ± 0.35</td>
<td>0.59 ± 0.21*</td>
<td>1.80 ± 0.37</td>
</tr>
<tr>
<td>Ileum</td>
<td>2.30 ± 0.47</td>
<td>0.92 ± 0.36*</td>
<td>2.12 ± 0.33</td>
</tr>
<tr>
<td>Trachea</td>
<td>1.08 ± 0.28</td>
<td>1.29 ± 0.20</td>
<td>1.09 ± 0.29</td>
</tr>
<tr>
<td>Liver</td>
<td>0.13 ± 0.04</td>
<td>0.24 ± 0.03</td>
<td>0.15 ± 0.04</td>
</tr>
<tr>
<td>Submandibular</td>
<td>0.64 ± 0.12</td>
<td>0.69 ± 0.15</td>
<td>0.56 ± 0.09</td>
</tr>
</tbody>
</table>

* Significance (P < 0.05); passive < unsensitized and non-immune sera treated rats.

Number of experiments: unsensitized (n = 8), passively sensitized (n = 3) and non-immune sera (n = 4).
isolated from the small bowel, whereas none was found in 3 rats treated with the deworming agent.

Treatment with the PAF-antagonists, WEB-2086 and BN 52021, which on their own did not alter heart rate or MABP, reversed the reduced blood flows noted with sensitization in several tissues, especially to the stomach, duodenum and jejenum (Figure 1) where flows were increased to levels comparable to those seen in unsensitized animals, or even enhanced as in the duodenum. Hepatic arterial flow remained high to the liver in the WEB-2086-treated groups, whereas BN 52021 tended to reduce blood flows to rates seen in the unsensitized rat (Figure 2). Neither antagonist reversed the reduction in blood flow to the submandibular gland (Figure 3) that was seen in the other sensitized groups of animals, but treatment with either of them resulted in enhanced blood flow to the kidneys (Figure 2).

Discussion

Acute parasitic infection increases blood flow to the intestine in the mouse infected with Trichinella spiralis (Ottaway et al., 1980) and, in dogs infected with heart worm (Dirofilaria immitis) vascular reactivity to acetylcholine is altered (Kaiser et al., 1987). The dirofilariasis infection involves two endothelium-derived relaxing factors, one a prostaglandin and the other a non-prostaglandin metabolite (Kaiser et al., 1989).

The hypothesis was put forward in the latter study that adult Di. immitis release a factor that alters endothelial function. The present study, however, describes changes in blood flow 30–35 days after active sensitization, when the parasites have been expelled from the intestine (Woodbury et al., 1984). In fact, the changes in blood flow due to active sensitization cannot be due to residual infection as blood flows were similar in dewormed and untreated groups of animals. An intestinal mucosal mast cell hyperplasia is markedly developed and maintained for months after expulsion of the parasite N. brasiliensis from rats (Miller & Jarrett, 1971; Befus et al., 1979). The alterations in blood flow seen with active sensitization, nevertheless, are not a generalized phenomena as many tissues and organs are not affected. Furthermore, the changes in blood flow are not transmitted passively to the rats and thus probably do not solely involve an IgE-mediated mechanism.

We found that the PAF antagonists, WEB-2086 and BN 52021, reversed the reduced blood flows to primarily gastro-intestinal tissues in sensitized animals. Thus PAF appears to be a major contributor to sensitization-induced changes in vascular reactivity, although the role of arachidonic acid metabolites remains to be evaluated. Some of the other putative mediators of the vascular changes could be histamine (Church, 1975) and leukotrienes (Heavey et al., 1988) and they may be involved in sensitization-induced alterations in blood flow to the submandibular gland.

Other studies have described effects of PAF on mesenteric and intestinal blood flow. A low dose of PAF (0.3 nmol kg$^{-1}$) increases mesenteric blood flow, whereas higher doses (1.0 and 3.0 nmol kg$^{-1}$) decrease blood flow to the mesentery of the rat (Siren & Feuerstein, 1989), the former effect being associated with the vasodilator effect of PAF on mesenteric blood vessels (Lagente et al., 1989). Blood flow to the gastric mucosa is also reduced by PAF (Whittle et al., 1986). In contrast, PAF has vasoconstrictor actions on hamster cheek-pouch blood vessels that are antagonized by dexamethasone, indomethacin and kadsurenone, a thromboxane A$_2$ and PAF-antagonist (Dillon et al., 1988). PAF primarily causes stasis of flow, an action that might be related to the ability of PAF to stimulate platelet aggregation (Martins et al., 1988). One interesting observation in this study was the enhanced blood flows to the duodenum and kidneys following treatment with PAF-antagonists. This enhanced blood flow to the kidney may be related to the ability of the rat kidney to synthesize and release PAF (Pirotsky et al., 1984).

In conclusion, we have shown that sensitization of rats with N. brasiliensis results in reduced blood flow to several, if not primarily those of the gastrointestinal tract 30 to 35 days post-infection, a time when the parasites have been largely eliminated from the animal. These effects are not caused by
residual infection with the nematode and appear to involve increased actions of PAF in the infected animals.

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References


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