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Mediators of the Plasma Extravasation Response to Silver Nitrate in the Rat Skin, Subplantar Region and Ankle Joint

MARGARET C. FYFE AND LORIS A. CHAHL*

*Department of Physiotherapy, University of Queensland, St. Lucia, Qld. 4067, Australia, and *Faculty of Medicine, University of Newcastle, Newcastle, N.S.W. 2308, Australia*

Abstract—Plasma extravasation responses to silver nitrate ($AgNO_3$), histamine, 5-hydroxytryptamine (5-HT), bradykinin and prostaglandin E_1 (PGE_1) in the abdominal skin, hindpaw ankle joint and subplantar region of rats have been investigated using the Evans blue dye leakage technique. All substances tested produced plasma extravasation and combination of low doses (5×10^{-10}mol) of either histamine or bradykinin with PGE_1 (5×10^{-10}mol) exhibited potentiation of responses of all regions. Responses to $AgNO_3$ (1×10^{-6}mol) were significantly reduced by the H_1 receptor antagonist, mepyramine, only in the abdominal skin, but the H_2 receptor antagonist metiamide reduced the responses at subplantar and ankle joint regions. Indomethacin significantly reduced the $AgNO_3$ responses at the ankle joint only, but aprotinin reduced it at the other two regions. In rats pretreated with a combination of all antagonists the residual plasma extravasation response to $AgNO_3$ was very small, indicating that the response could be almost totally accounted for by the combined actions of mast cell amines, kinins and prostanoids. The finding that prostanoids played a major role in the plasma extravasation response of the rat ankle joint to $AgNO_3$ indicated that this model would be useful for the screening of non-steroidal anti-inflammatory drugs.

During an investigation of the effects of 'therapeutic' ultrasound on the inflammatory response in rats, silver nitrate ($AgNO_3$) was used to induce a plasma extravasation response in the ankle joint (Fyfe & Chahl 1985). This method provided a simple monoarticular model of inflammation in which plasma extravasation persisted for several days. Since this model appeared to offer the advantage of simplicity over other models such as adjuvant polyarthritis in rats for the study of anti-inflammatory agents, the pharmacology of the plasma extravasation response induced by $AgNO_3$ in the ankle joint of rats was investigated and compared with the pharmacology of $AgNO_3$-induced responses in rat abdominal skin and subplantar region.

Methods

Six week old Wistar rats, male unless stated otherwise, were used. At the start of each experiment rats were anaesthetized with ether and given Evans blue 50 mg kg^{-1} into a lateral tail vein. Inflammatory mediators (histamine, 5-hydroxytryptamine (5-HT), bradykinin and prostaglandin E_1 (PGE_1)) in the doses and combinations shown in Table 1, or $AgNO_3$ 1×10^{-6}mol were injected in 0·05 mL volumes into one side of the abdominal skin, or one ankle joint or at the subplantar region of one hindpaw. An injection of 0·05 mL of vehicle (Tyrode solution for the mediators and deionized water for $AgNO_3$) was made into the respective contralateral region. All injections were made with 26G needles. Antagonists of mediators were injected intraperitoneally (i.p.) or intravenously (i-v) before the experiments using the dose schedules given in Tables 1 and 2. Rats were killed either 0·5 or 1 h after injection of mediators or $AgNO_3$, by a blow to the head and stretching of the neck.

Plasma extravasation responses were quantified by the method described by Harada et al (1971). Tissues from the blued areas at the experimental sites were chopped into small pieces and placed into a mixture of sodium sulphate 0·5% (3 mL) and acetone (7 mL). A 20 mm diameter piece of skin from an area remote from the experimental site on each rat was treated similarly. After 24 h at room temperature (22°C) the tissues were removed from the reagents and the solutions of extracted dye were centrifuged for 20 min at 4°C and 600 *g*. The amounts of dye in the samples were measured spectrophotometrically at 620 nm, the supernatants from the 20 mm piece of control skin being used to zero the spectrophotometer.

Techniques particular to each site

Abdominal skin. The fur on the abdomen of each rat was shaved between sternum and pubis before making the intracutaneous injections of mediator or vehicle. The tissue specimens taken included the whole of the blued areas around the experimental sites.

Hindpaw ankle joint. For each intra-articular injection of mediator or vehicle, the rat was placed on its side with the ankle flexed to approximately a right angle. The needle was introduced from above and behind the lateral malleolus and was directed distally and inwards towards the heel. The rat foot and barrel of the syringe were held immobile while the injection was made and the needle was withdrawn. Tissue specimens taken were from the mid-tarsal joint to just above the ankle.

Correspondence to: L. A. Chahl, Faculty of Medicine, University of Newcastle, Newcastle, N.S.W. 2308, Australia.

Subplantar region. Injections were made by introducing the needle at an obtuse angle to the sole of the foot, and using the shaft of the needle near its tip to lift the skin gently to ensure injection of the fluid subcutaneously and not into the underlying muscle tissue. The tissue specimens were taken from the metatarsal heads to the subtalar joint and did not include the ankle. Experiments on the subplantar region were conducted in the same rats as those used for abdominal skin experiments.

Drugs and chemicals

Aprotinin 10 000 K.I.U. mL^{-1} (Trasylol, Bayer); bradykinin triacetate (Sigma); Evans blue (Difco or Gurr); histamine diphosphate (Sigma); 5-hydroxytryptamine creatinine sulphate (Sigma); indomethacin (Sigma); mepyramine maleate (May and Baker); methysergide maleate (Sandoz); metiamide (generously donated by Smith, Kline and French); prostaglandin E_1 tromethamine salt (Upjohn); Tris buffer, (tris-[hydroxymethyl] aminomethane) (Sigma 7–9) (Sigma). Indomethacin was made up in Tris buffer (0·05 M) immediately before use.

The composition of the Tyrode solution in mmol L^{-1} was: NaCl 136·9, KCl 2·7, $MgCl_2$ 1·05, $CaCl_2$ 1·8, NaH_2PO_4 0·42, $NaHCO_3$ 11·9.

Table 1. Drugs used to assess the effect of inflammatory mediators at three regions in the rat.

Inflammatory mediator and dose†	Antagonist and dose schedule	Duration of plasma extravasation (h)	No. of rats
Histamine 5×10^{-8}mol		1	15
Histamine 5×10^{-8}mol	Mepyramine $2{\cdot}5 \times 10^{-5}$mol kg^{-1}, i.p., 0·5 h before histamine	1	15
Histamine 5×10^{-8}mol		1	15
Histamine 5×10^{-8}mol	Metiamide $1{\cdot}4 \times 10^{-4}$mol kg^{-1}, i.p., 0·5 h before histamine	1	15
Histamine 5×10^{-8}mol	Metiamide $1{\cdot}4 \times 10^{-4}$mol kg^{-1} and mepyramine $2{\cdot}5 \times 10^{-5}$mol kg^{-1} both drugs i.p. 0·5 h before histamine	1	15
5-HT 5×10^{-9}mol		0·5	5
5-HT 5×10^{-9}mol	Methysergide 1×10^{-5}mol kg^{-1}, i.p., 0·5 h before 5-HT	0·5	5
Histamine 5×10^{-10}mol		1	10
PGE_1 5×10^{-10}mol		1	9
PGE_1 and histamine both 5×10^{-10}mol		1	10
Bradykinin 5×10^{-10}mol		1	10
PGE_1 5×10^{-10}mol		1	10
PGE_1 and bradykinin both 5×10^{-10}mol		1	10

† Doses used in abdominal skin were based on previous experiments where dose-response lines to mediators were obtained (Chahl 1976). Doses used at the other two regions were chosen following preliminary experiments.

Table 2. Antagonist drugs and dose schedules used in investigation of the mediators of the $AgNO_3$ response at three regions.*

(a) *Antagonists*	
Methysergide, 1×10^{-5}mol kg^{-1}	i.p. injection, 0·5 h before $AgNO_3$
Mepyramine, $2{\cdot}5 \times 10^{-5}$mol kg^{-1}	i.p. injection, 0·5 h before $AgNO_3$
Methysergide and mepyramine (doses as above)	as above
Aprotinin (Trasylol), 1000 Kallikrein inactivator units (K.I.U.)	i.v. injection (tail vein), 10 min before $AgNO_3$
Indomethacin, $5{\cdot}5 \times 10^{-5}$mol kg^{-1} in 0·05 M Tris buffer	i.p. injection, 30–40 min before $AgNO_3$
Tris buffer, the control for the indomethacin experiments	i.p. injection, 30–40 min before $AgNO_3$
(b) *Combinations of antagonists*	
Metiamide, $1{\cdot}4 \times 10^{-4}$mol kg^{-1}	i.p. injection, 0·5 h before $AgNO_3$
Metiamide, $1{\cdot}4 \times 10^{-4}$mol kg^{-1} and Mepyramine, $2{\cdot}5 \times 10^{-5}$mol kg^{-1}	as above
Metiamide + mepyramine (as above) and indomethacin, $5{\cdot}5 \times 10^{-5}$mol kg^{-1} in 0·5 M Tris buffer	i.p. injection, 0·5 h before $AgNO_3$ i.p. injection, 35–40 min before $AgNO_3$
Metiamide + mepyramine + indomethacin (as above) and methysergide, 1×10^{-5} mol kg^{-1}	as above i.p. injection, 0·5 h before $AgNO_3$
Metiamide + mepyramine + indomethacin + methysergide and aprotinin, 1000 K.I.U.	as above i.v. injection, 10 min before $AgNO_3$

* The duration of plasma extravasation was 1 h.

Table 3. The effect of mepyramine, metiamide and methysergide on plasma extravasation responses to histamine and 5-HT, respectively in the rat.

	Abdominal skin	Subplantar region	Ankle
Histamine			
Controls	0·071 ± 0·024	0·040 ± 0·024	0·110 ± 0·019
Mepyramine	*0·021 ± 0·016	0·015 ± 0·004	*0·034 ± 0·023
Controls	0·042 ± 0·003	0·021 ± 0·002	0·068 ± 0·025
Metiamide	0·039 ± 0·004	0·030 ± 0·003	0·044 ± 0·011
Mepyramine and metiamide	***0·001 ± 0·001	*0·013 ± 0·003	*0·015 ± 0·008
5-HT			
Controls			2·200 ± 0·176
Methysergide			***0·046 ± 0·014

Values are mean responses from 5 rats expressed as absorbance ± one standard error. The dose of histamine was 5×10^{-8}mol and of 5-HT, 5×10^{-9}mol. The value for the Tyrode control response was subtracted from each response to histamine or 5-HT. Antagonist doses were: mepyramine, $2{\cdot}5 \times 10^{-5}$mol kg^{-1}, metiamide $1{\cdot}4 \times 10^{-4}$mol kg^{-1} and methysergide 1×10^{-5}mol kg^{-1} given 0·5 h before histamine or 5-HT. Dye leakage was measured over 1 h (histamine) or 0·5 h (5-HT). Asterisks show significant differences between pretreated and control groups obtained in Student's *t*-test, * $P < 0{\cdot}05$, *** $P < 0{\cdot}001$.

Table 4. Plasma extravasation responses to 5×10^{-10}mol doses of histamine, bradykinin, and PGE_1 alone and in combination, at three regions in the rat.

Drug	Abdominal skin	Subplantar region	Ankle
Histamine	0·005 ± 0·002	0·005 ± 0·002	0·015 ± 0·003
PGE_1	0·004 ± 0·002	0·002 ± 0·003	0·030 ± 0·008
Histamine + PGE_1	***0·045 ± 0·008	***0·027 ± 0·004	*0·062 ± 0·004
Bradykinin	0·012 ± 0·005	0·051 ± 0·005	0·025 ± 0·004
PGE_1	0·010 ± 0·002	0·051 ± 0·008	0·042 ± 0·004 (n = 4)
Bradykinin + PGE_1	*0·039 ± 0·007	***0·175 ± 0·018	***0·152 ± 0·011

Values are mean responses and standard errors expressed as absorbance for groups of 5 rats except where shown. Asterisks represent significant differences from the sums of the mediator responses given separately obtained from paired *t*-test. * $P < 0{\cdot}05$; *** $P < 0{\cdot}001$.

Results

Responses to mediators of inflammation

The mean plasma extravasation responses induced by inflammatory mediators are shown in Tables 3 and 4. From the results in Table 3 it may be seen that the response to histamine 5×10^{-8}mol at all three sites was reduced by the H_1-receptor antagonist, mepyramine, although at the subplantar region the reduction did not reach statistical significance. The H_2-receptor antagonist, metiamide, given alone did not significantly reduce the responses at any of the sites, but a combination of mepyramine and metiamide virtually abolished the response in the abdominal skin and greatly reduced the responses at the other two sites (Table 3).

The effect of methysergide, a potent antagonist of the plasma extravasation response to 5-HT in rat abdominal skin (Chahl 1976), was tested in the present experiments on the 5-HT response at the ankle joint. Methysergide greatly reduced the response to 5-HT at this site also (Table 3).

When a low dose (5×10^{-10}mol) of either histamine or bradykinin was combined with PGE_1, 5×10^{-10}mol, responses at all three sites were produced with each combination which were significantly greater than the sums of the respective separate responses (see Table 4 for significance levels). Thus potentiation occurred between histamine and PGE_1 and between bradykinin and PGE_1.

Effect of antagonists on responses to $AgNO_3$

Responses at the three sites to the irritant $AgNO_3$, 1×10^{-6}mol, in control rats and in those pretreated with antagonists of inflammatory mediators, are shown in Figs 1 and 2.

Mepyramine significantly reduced the response to $AgNO_3$ in the abdominal skin only (Fig. 1), whereas metiamide significantly reduced the response in the subplantar and ankle joint sites but not at the abdominal skin (Fig. 2). A combination of both mepyramine and metiamide produced a significant reduction in the responses obtained at both the abdominal skin and the subplantar region, but not at the ankle joint (Fig. 2). Furthermore, methysergide alone did not affect the response to $AgNO_3$ at any of the three sites. However, when methysergide was given in combination with mepyramine significant reduction occurred not only of the response of the abdominal skin, which might be expected, but also of that of the subplantar region, which was not observed with mepyramine alone (Fig. 1).

Indomethacin, an inhibitor of prostanoid biosynthesis, significantly reduced the $AgNO_3$ response at the ankle joint but not at the other sites whereas aprotinin, a proteinase inhibitor which inhibits the biosynthesis of bradykinin, significantly reduced the $AgNO_3$ response at the abdominal skin and at the subplantar site but not at the ankle joint (Fig. 1). Indomethacin when given with both metiamide and mepyramine, resulted in a significant reduction in the $AgNO_3$ response at all three sites (Fig. 2). Further reductions were obtained by the addition to the pretreatment schedule of methysergide (Fig. 2). The $AgNO_3$ responses remaining at the three sites following pretreatment with these four drugs

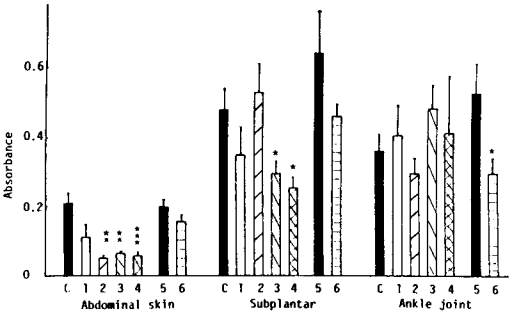

FIG. 1. The effect of antagonists of inflammatory mediators on plasma extravasation responses to $AgNO_3$, 1×10^{-6}mol, at three regions in the rat. Responses to $AgNO_3$ were obtained by subtracting the response to vehicle (H_2O) injected at the contralateral site from the response to $AgNO_3$. Histograms represent the mean responses, expressed as absorbance, from groups of 5 rats. Bars indicate the standard errors of the means. Results for abdominal skin and subplantar region were obtained from experiments on female rats. Doses are given in Table 2. Asterisks represent significance levels from Student's *t*-test between results obtained for control and treatment groups. * $P < 0{\cdot}05$; ** $P < 0{\cdot}01$; *** $P < 0{\cdot}001$. C = control, 1 = methysergide, 2 = mepyramine, 3 = methysergide + mepyramine, 4 = aprotinin, 5 = TRIS, 6 = indomethacin.

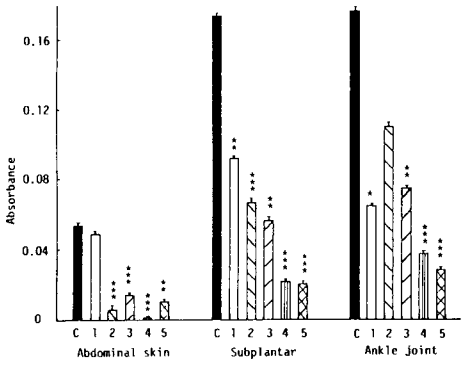

FIG. 2. The effect of combinations of antagonists of inflammatory mediators on plasma extravasation responses to $AgNO_3$, 1×10^{-6}mol in the rat. Responses to $AgNO_3$ were obtained by subtracting the response to vehicle (H_2O) injected at the contralateral site from the response to $AgNO_3$. Histograms represent the mean responses, expressed as absorbance, from groups of 5 rats, except for the control abdominal skin result where n = 4. Bars indicate the standard errors of the means. Abbreviations as for Fig. 1. Doses are given in Table 2. Asterisks represent significance levels from Student's *t*-test between results obtained for control and treatment groups. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. C = control, 1 = metiamide, 2 = metiamide + mepyramine, 3 = metiamide + mepyramine + indomethacin, 4 = metiamide + mepyramine + indomethacin + methysergide, 5 = metiamide + mepyramine + indomethacin + methysergide + aprotinin.

were very small and further reductions did not occur with the addition of aprotinin (Fig. 2).

Discussion

Several agents including formalin, dextran, turpentine and carrageenan have been used in models of acute inflammation. Although the models might not reproduce exactly all of the pathophysiological signs of inflammation they have played an important role in the study of physiological defence to injury and the assessment of anti-inflammatory drugs.

The present study has demonstrated that $AgNO_3$ produced a plasma extravasation response in which histamine, 5-HT, kinins and prostanoids played roles of varying importance in skin, subcutaneous (subplantar) tissues and joints. Histamine played an important role at all three sites although its action in the skin was mediated by H_1 receptors whereas that in subcutaneous tissues and joints was mediated by H_2 receptors. Kinins and 5-HT played little role alone in the ankle joint but kinins played an important role in the skin and subcutaneous tissues. A striking difference between the regions, however, was that prostanoids played a much more important role in joint plasma extravasation than at the other sites. Potentiation occurred between histamine and PGE_1 and bradykinin and PGE_1 at all three sites.

These results were not unexpected in light of previous studies on experimental inflammation (e.g. La Belle & Tornaben 1951; Di Rosa et al 1971; Moncada et al 1975; Chahl 1976; Rybák et al 1978). The importance of the study, however, is that it provides valuable background information on a model of joint damage and inflammation which might have certain advantages for the study of the mechanisms of pain arising from inflamed joints. The response to $AgNO_3$ is rapid in onset and lasts for several days (La Belle & Tornaben 1951; Fyfe & Chahl 1985), and, being restricted to a single joint, allows the contralateral joint to be used as a control.

A large number of mediators other than those which are currently susceptible to pharmacological manipulation are known to be involved in inflammatory states. Nevertheless, in the present experiments, the plasma extravasation response to $AgNO_3$ at 1 h could be almost totally accounted for by the combined actions of mast cell amines, kinins and prostanoids. The finding that prostanoids played a major role in the plasma extravasation response of the ankle joint to $AgNO_3$ indicated that this model would be useful for the screening of non-steroidal anti-inflammatory agents

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