Assessment of Vascular Permeability Increase in the Mouse by Dye Leakage during Paw Edema

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Abstract—Vascular permeability increase induced by histamine, bradykinin, platelet-activating factor (PAF), or phorbol myristate acetate (PMA) in the mouse paw was assessed by the dye leakage method. The amount of dye extracted from the paw showed a clear dose-response relationship to the dose of each agonist injected into the paw. Among the autacoids used, PAF showed the most potent activity in the mouse paw. The results are consistent with those seen in the rat dorsal skin as previously reported. Involvement of histamine, 5-hydroxytryptamine and PAF is suggested in the vascular permeability increase induced by PMA in the mouse paw.

In acute inflammatory reactions, plasma exudation at the inflammatory site is an important parameter for evaluating the size and nature of the inflammatory reaction (1). To assess vascular permeability increases, we have measured the amount of dye leaked at the inflammatory site (2, 3). We found that there was significant species difference in the sensitivity of the vascular permeability increase induced by several autacoids (3). Since the mouse is commonly used as an experimental animal, we intend to develop a method to evaluate vascular permeability increases in mouse skin. Because the dorsal or abdominal skin of mice is too thin to examine dye leakage by the previous method, we therefore utilized the whole paw for this purpose.

Male 5-week-old ICR mice (Shizuoka Laboratory Animal Center, Hamamatsu) were kept in an air-conditioned room for one week before use. The mice were injected with 60 mg/kg pontamine sky blue (Tokyo Kasei) in saline solution into a tail vein under light pentobarbital sodium anesthesia (Sigma;

50 mg/kg, intraperitoneally). Ten minutes later, paw edema was induced by subcutaneous injection of 0.05 ml of Tyrode solution containing the indicated dose of each agonist into the right paw, and 0.05 ml of Tyrode solution alone was injected into the left paw as a control. In cases of platelet activating factor and phorbol myristate acetate, the vehicle contained final concentrations of 0.005% ethanol or 0.16% dimethylsulfoxide. Therefore, the vehicle was injected into the corresponding control paw. Mice were under the light anesthesia throughout the experiment and were exsanguinated to death 50 min after the agonist injection. Both paws were removed, and each paw was dipped in 1 ml of 1N KOH in a glass tube and kept overnight at 37°C. The solution was neutralized with 2.5 ml of 0.6 N phosphoric acid, and the dye was extracted with 6.5 ml of acetone as previously reported (2). Absorbance at 620 nm was measured, and the amount of dye leakage was calculated by subtraction of the amount found in the control paw extract from that found in the extract of the agonist-injected paw. The mean dye amount extracted from each control paw injected with Tyrode solution was 12.1 μ g/paw with a standard error of 1.1 (n=10). There was no significant difference among those that received the vehicles used.

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Figure 1A illustrates the dose-response relationship of the vascular permeability increase, as assessed by dye leakage at the mouse paw, to the dose of histamine (histamine maleate, Sigma), bradykinin (Peptide Institute, Minoh), platelet-activating factor (C_{16} -PAF, 1-O-hexadecyl-2-O-acetyl-sn-glycero-3-phosphocholine, Bachem Finechemicals, Switzerland), or phorbol myristate acetate (PMA, Sigma). In this model, C_{16} -PAF showed the most potent activity followed by bradykinin and then histamine; and this order of potency is similar to that seen previously in rat dorsal skin (3). Dye leakage caused by the above agonists had a short time course and mostly ended within 40 min. Therefore, we terminated the reaction at 50 min.

In the mouse paw, PAF (0.03 nmol) caused only additive dye leakage when injected simultaneously with histamine (14 nmol) or bradykinin (0.3 nmol), as shown in Fig. 1B (inset). A similar result was obtained when the dose of PAF was reduced to 0.01 nmol (data not shown). This result is different from

that obtained in rat dorsal skin, where PAF induced synergistic dye leakage with bradykinin or histamine (3).

Effects of some inhibitors on PMA-induced paw edema were also examined. Indomethacin (Sigma, 10 mg/kg) injected intraperitoneally 30 min before the agonist injection did not show any effect. Methysergide maleinate (a gift from Sandoz) injected intraperitoneally at the dose of 5 mg/kg 30 min before the PMA injection caused a significant inhibition of the dye leakage by $44.5 \pm 8.4\%$ ($n=7$). As shown in Table 1, mepyramine (mepyramine maleate, Sigma) at a dose of 5 mg/kg, significantly inhibited the dye leakage induced by 0.1 and by 1 nmol of PMA. The specific PAF antagonist CV-3988 (rac-3-(N-n-octadecyl-carbamoyloxy)-2-methoxy-propyl-2-thiazolioethyl phosphate, a gift from Takeda Pharmac. Ind.) (4), when injected intravenously at the dose of 10 mg/kg 5 min before the agonist, significantly inhibited the dye leakage induced by 1 nmol PMA, but not that by 0.1 nmol PMA (Table 1). CV-3988 either did not inhibit the dye leakage caused by

Fig. 1. Dye leakage in the mouse paw. A. Relationship between the extracted dye amount and doses of agonists. Histamine (□), bradykinin (○), C_{16} -PAF (●) and PMA (△) were subcutaneously injected into mouse paw as described in the text. Figures by the symbols indicate numbers of mice used, and standard errors were expressed with bars. B (inset). Dye leakage in the mouse paw induced by bradykinin, histamine, C_{16} -PAF or their mixture. Histamine (H, 14 nmol), bradykinin (B, 0.3 nmol), C_{16} -PAF (P, 0.03 nmol), histamine + C_{16} -PAF (H+P) or bradykinin + C_{16} -PAF (B+P) was injected into the mouse paw as described in the text. The number of mice used was 5 for each group with standard error.

Table 1. Effect of some inhibitors on PMA-induced dye leakage into the mouse paw

Treatment	Number of mice	Dye (μ g/paw)
PMA (0.1 nmol/site)		
No treatment	6	37.1 $\pm$ 5.9
Mepyramine (5 mg/kg)	4	8.8 $\pm$ 0.4**
CV-3988 (10 mg/kg)	6	52.8 $\pm$ 4.2
Mepyramine+CV-3988	4	8.3 $\pm$ 2.2**
PMA (1 nmol/site)		
No treatment	4	75.2 $\pm$ 7.2
Mepyramine (5 mg/kg)	5	26.5 $\pm$ 8.8**
CV-3988 (10 mg/kg)	5	37.7 $\pm$ 10.6*
Mepyramine+CV-3988	5	15.4 $\pm$ 6.1**
PAF (0.1 nmol/site)		
No treatment	6	66.4 $\pm$ 6.8
CV-3988 (10 mg/kg)	4	19.8 $\pm$ 3.0**

Values express means with standard errors. Mepyramine was intraperitoneally injected 30 min before and CV-3988, intravenously 5 min before, PMA or PAF injection. * $P < 0.05$ and ** $P < 0.01$ indicate the value is significantly different from that of each non-treated group by Student's *t*-test.

bradykinin or histamine (data not shown).

These results indicate that the PMA-induced vascular permeability increase in mouse skin is probably mediated mainly by histamine and serotonin, and at higher doses, by PAF in addition to these autacoids. Simultaneous treatment with mepyramine and CV-3988 suppressed the 1 nmol PMA-induced dye leakage, and this effect appeared to be additive effect as shown in Table 1, although the difference was statistically significant only between the control and each treated group, i.e., mepyramine, CV-3988, or mepyramine-CV-3988, but not between mepyramine-CV-3988 and each single treatment. In a previous study, we found that PMA induced mast cell degradation and release of histamine when injected into the rat pleural cavity (5) and that PMA also released PAF from resident mononuclear cells to cause plasma leakage into the pleural cavity (6). The above results with mouse paw are mostly consistent with the previous observations in rats.

There are some reports on the assessment of mouse paw edema induced by carrageenin by measurement of paw thickness (7) and of mouse ear edema by measurement of thickness after irritant application (8). The dye leakage method in the mouse paw is a convenient one for evaluating acute vascular leakage, especially in cases where paw swell-

ing in the mouse induced by some autacoids is too small to be measured by volume change or thickness. On the other hand, in the case of carrageenin-induced inflammation in the paw, the measurement of thickness may be superior to the dye leakage method, since carrageenin edema takes a longer time to develop.

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