Local Increase of Vascular Permeability Induced by Pyridoxal-5'-Phosphate in the Rat Skin *

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(With 3 text-figures)

This work describes the activity of Pyridoxal-5'-phosphate (PLP) on the vascular permeability response measured in the abdominal wall of rats from the amount of extravasated Evans blue labelled with radioactive iodine 125 or 131, following intradermal injection of 0.1 ml of a solution of the active substance. Skin discs were cut with circular punch for external counting, the quantitative results being compared with control discs.

Mepyramine maleate and triprolidine hydrocloride considered among the most specific of the antihistaminics have a marked inhibitory effect on the local increase of vascular permeability induced by PLP in the rat skin. These results indicate that the effect of PLP is exerted through the histamine it releases.

INTRODUCTION

Pyridoxal-5'-phosphate (PLP) is a derivative of pyridoxal possessing vitamin B_6 activity. It is the coenzyme of a large group of specific enzymes catalysing reactions of amino group transfer, decarboxilation and other metabolic transformations.

In a previous work from our laboratory [1] PLP was used to block the alpha amino group of the kinins (Bradykinin, Kalidin and Methionyl Kalidin) followed by treatment with sodium borohydride. The phosphopyridoxil-kinins have shown a conspicuous reduction (90%) of the permeability increasing activity in the rat skin when compared with the three kinins.

Our main purpose in the present experiments is to give evidence that the simple injection of PLP intradermally induced the increase of vascular permeability in the skin of Wistar rats with a certain age and weight.

MATERIAL AND METHODS

Animals

Male Wistar rats from I.E.A. weighing 200-300g and 4-6 months old.

Drugs

Pyridoxal-HCl, Pyridoxal-5'-phosphate and Pyridoxamine-5'-phosphate were purchased from *Sigma Chemical Company*. Mepyramine maleate was a gift from *Merck Sharpe and Dohme, Rahway, New Jersey*. Triprolidine from *Burroughs Wellcome Co.*, was a gift from Dr. I. MOTA. Pyridoxol-5' phosphate was obtained by direct reduction of saline solution of Pyridoxal-5'-phosphate

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with solid borohydride *Merck* and the mixture adjusted to pH 7.0. Ethyl ether anesthetic obtained from *Rhodia*. All others reagents were of the purest commercially available grade. All drugs were dissolved in saline (NaCl 0.85%) and the pH adjusted to 7.0 when necessary. Evans blue bought from *Merck Darmstadt*. ^{131}I was prepared in the *Division of Production of Radio-isotopes* from the I.E.A. ^{125}I was furnished by *Farbwerke Hoechst AG. Frankfurt (Main)*.

Evans blue with Iodine-131 or Iodine-125 was labelled by the method described by MANI AND KULKARNI [2]with modifications; after diazotization of the dye followed by iodination of the resulting product by heating with $Na^{131}I$ the solution was thoroughly dialysed against saline. The radiochemical purity of aliquots of the labelled dye added with iodide carrier was confirmed by paper electrophoresis in strips of Whatman n.º 1 (30 × 2.5 cm), acetate buffer 0.1 M pH 5.5, 1.5 mA for each strip: after 45 minutes run the paper was dried and sprayed with 10% lead acetate to detect the zone of iodide for counting.

The radioactivity was measured in the *1185 Series Automatic Gamma Counting System (Nuclear Chicago Corporation)*.

EVANS BLUE RADIOACTIVE TEST

A pad of cotton sprinkled with ethylic ether was used in anesthesia of the rats. Conventional blue test was performed in the abdominal wall, injecting intravenously a 0.3% saline solution of Evans blue labelled with iodine 131 or 125 with 10μCi/ml in the dose of 0.1 ml per 100g of animal. After 10 minutes, 0.1 ml of saline and 0.1 ml of the experimental solutions were injected intradermically. The rats were decapitated after 15 minutes of the intradermal injection and the results were assessed at the inner face of the excised skin. Skin discs were cutted with circular punch of 15 mm diameter for external counting the quantitative results being compared with control discs from the basic activity of the skin and from the local of saline injection. The response on vascular permeability for each dose was calculated by the quocient of the maximal counts and the control value:

$$R = \frac{C - (C_{sal} - C_s)}{C_s}$$

C = counts of discs from the site of the tested drugs;
C_{sal} = counts of discs from the site of the saline injection;
C_s = counts of discs from the basic activity of the skin.

By using this method, satisfactory log dose-response curves were obtained that followed the general trend of S-shaped curve as indicated in Results.

To study the morphology of the mast cells, skin fragments of control and treated rats were fixed in 10% formaldehyde. Thick sections were then stained with a mixture of 1% toluidine blue in 10% formaldehyde acidified by 0.5% of acetic acid and observed by light microscopy [3]. The mast cells from the mesentery extended in a loop were fixed and stained by the same procedure.

EFFECT OF ANTIHISTAMINES

Rats were injected with Evans blue labelled with ^{131}I or ^{125}I and then received an intradermal injection of 0.1 ml saline contained 4,8 or 16 μg of PLP. With a delay of 10 minutes this same treatment was repeated on the other side of the abdominal skin 5 minutes after the intravenous injection of mepyramine 0.5 mg or triprolidine 50 μg per 100 g of body weight. The skin discs were cut 30 min after injection of PLP both before and after the the injection of the antihistamines [4].

RESULTS

As is evident from Figure 1 and TABLE I. pyridoxal-5'-phosphate increases the vascular (capillary) permeability in the skin of male Wistar rats about four months old and weighing around 200g.

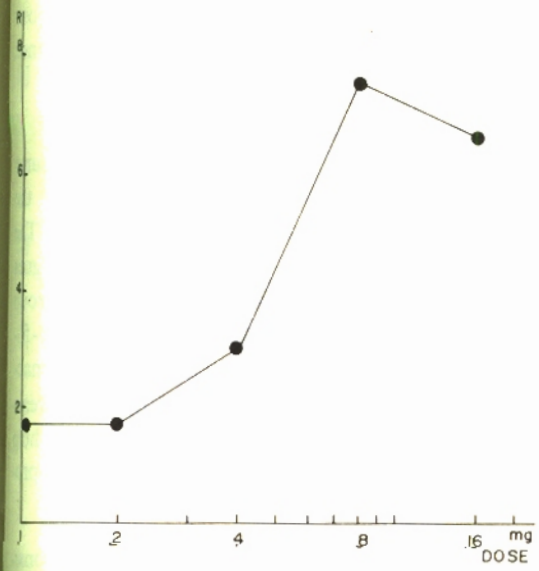

FIG. 1 — Log Dose-Response Curve of PLP in the Rat Skin.

TABLE I

Activity of Pyridoxal-5'-Phosphate (PLP) on Vascular Permeability in Rat Skin (Rats About Four Months Old Weighing Around 200g)

DOSE / RAT	1 µg	2 µg	4 µg	8 µg	16 µg
1	1.4	1.8	5.8	8.7	7.0
2	1.8	1.9	5.0	7.1	7.0
3	1.3	1.7	4.9	8.1	7.0
4	2.0	1.4	2.9	5.1	5.6
5	1.4	1.3	6.3	12.7	6.8
6	2.3	1.9	4.9	3.9	6.4
$\overline{M}$	1.7	1.7	5.0	7.6	6.6
±s	0.4	0.3	1.1	3.1	0.6

Microscopy examination of the dermis showed that while the section of control rats presented well preserved mast cells at the side of 0.1 ml saline injection, in the points of PLP injections (15 µg), in all cases, the mastocytes showed severe degranulation.

The mast cells from rat mesentery do not show the effect of PLP on degranulation observed in the skin.

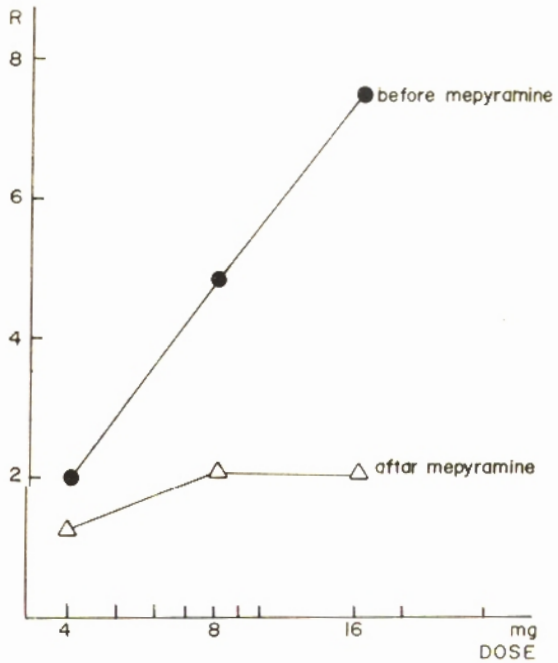

FIG. 2 — Log Dose-Response Curves of PLP Before and After Mepyramine Injection.

The results presented in the TABLE II and Figure 2, TABLE III and Figure 3 clearly indicate that the antihistamines principally used here — mepyramine and triprolidine — inhibited the permeability increasing effect of PLP.

TABLE II

Inhibition by Mepyramine (0.05 mg/100 g) of Permeability Increasing Effect in Rat Skin Produced by PLP. (Rats About Six Months Old Weighing Around 300g)

DOSE / RAT	4.0 µg		8.0 µg		16.0 µg	
	before	after	before	after	before	after
1	1.5	1.1	6.3	1.8	12.4	2.6
2	2.1	1.0	4.9	1.9	8.5	1.6
3	2.7	1.7	4.4	2.8	6.4	1.9
4	2.0	1.0	5.9	2.9	5.6	3.1
5	1.5	1.0	3.1	1.0	4.9	1.1
$\overline{M}$	2.0	1.2	4.9	2.1	7.6	2.1
± s	0.5	0.3	1.3	0.8	3.0	0.8
P	< 0.02		< 0.01		< 0.001	

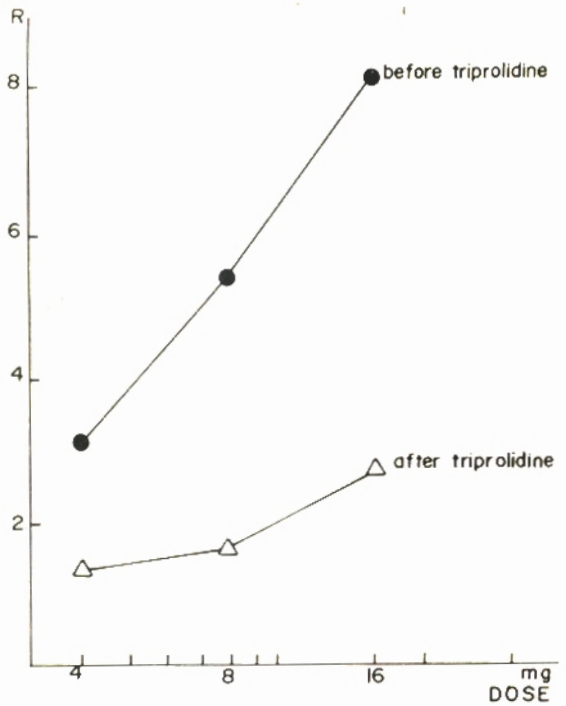

FIG. 3 — Log Dose-Response Curves of PLP Before and After Triprolidine Injection.

TABLE III

Inhibition by Triprolidine Hydrochloride (50μg/100g) of Permeability Increasing Effect in Rat Skin Produced by PLP (Rats About Six Months Old Weighing Around 300g)

DOSE / RAT	4.0 μg		8.0 μg		16.0 μg	
	before	after	before	after	before	after
1	2.5	1.0	3.2	1.0	4.2	1.6
2	3.4	1.8	7.7	2.5	11.2	4.2
3	3.9	1.0	6.4	1.0	8.8	2.2
4	2.2	1.5	3.0	1.0	4.7	1.3
5	3.3	1.0	5.8	2.3	10.9	3.6
6	3.4	1.2	6.5	1.7	8.7	3.5
$\overline{M}$	3.1	1.3	5.4	1.6	8.1	2.7
±s	0.6	0.3	1.9	0.7	3.0	1.2
P	< 0.01		< 0.01		< 0.02	

Different doses of PLP analogues tested (pyridoxal, pyridoxol-5'-phosphate, pyridoxamine-5'-phosphate) failed to increase the vascular permeability in the skin when compared to PLP for the level of significance.

DISCUSSION

From the above results one might assume a probable relationship of structure to the permeability increasing effect of PLP. The information obtained with PLP analogues shows that changes in the 4-carbonyl group (pyridoxol-5'-phosphate, pyridoxamine-5'-phosphate) result in derivatives that are inactive. The existence of pyridoxal in the unreactive internal hemi-acetal form [5] could help explain the failure of this substance in producing the effect noticed with PLP.

Triprolidine and Mepyramine are considered among the most specific of the antihistaminics [4]. The fact that they can prevent the permeability increasing effects of PLP indicate that the release of histamine, is involved in the process.

On the other hand a correlation seems to exist between histamine release and morphological changes in the mast cells [6]. Of interest in this connection is our finding that PLP causes degranulation of the mast cells of the dermis. The difference in behaviour of the mast cells of mesentery might be due to the occurrence in the dermis of particular requirements which are operating in the extrusion of secretory granules.

PLP is a coenzyme of a large group of enzymes and would be premature at the present stage of our information to draw further conclusions of the histamine release mechanism.

Of course, further experiments are in progress to clarify these phenomena.

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