Acta Dermatovener (Stockholm) 51: 50–54, 1971

CONTINUOUS SKIN PERFUSION IN VIVO AS A METHOD FOR STUDY OF PHARMACOLOGICAL AGENTS IN HUMAN SKIN

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Abstract. A quantitative study of in vivo skin perfusion as a method for study of pharmacological agents released in skin is described. Experiments have been carried out in which recovery of known amounts of agents infused or injected intradermally or subcutaneously into perfused skin has been determined. Intradermal or subcutaneous injection of microgram quantities of bradykinin or histamine resulted in recovery of only nanogram amounts of these agents in the perfusate. Recovery of infused bradykinin or histamine ranged from 25–35%. These findings, which indicate the low sensitivity of the technique as a method for recovery of pharmacological activity in skin, explain the low concentrations of histamine recovered in skin injected with high doses of compound 48/80.

Subcutaneous perfusion has been used to examine vasoactive substances in thermally stimulated eccrine sweating (4), axon reflex flares (2), wheal-and-flare reactions of different types (6, 12, 13) and delayed inflammation (7, 8). In some of these reports (4, 13) attempts were made to express pharmacological results in perfusates quantitatively and to draw conclusions from changes in concentration of agents in the perfusates without consideration of factors influencing these concentrations. No quantitative studies using this technique have previously been reported, and in an earlier study of whealing in urticaria pigmentosa (6) we noted that concentrations of histamine in the perfusates were surprisingly low despite the high skin histamine concentrations associated with this disease (14).

In the present paper we report determinations of recovery in the perfusate of pharmacological agents which have been infused subcutaneously, or injected subcutaneously or intradermally into perfused skin. These findings are then used to evaluate pharmacological data obtained in whealing skin due to injection of the chemical histamine liberator compound 48/80.

METHODS

Subjects. Thirty-five volunteer subjects were studied. These were patients with various localised non-inflammatory skin diseases, uninvolved skin being perfused in each case.

Perfusion. Subcutaneous perfusion was carried out by the method of Fox & Hilton (4) with certain modifications (6). Two needles (length 36 mm, internal diameter 1 mm) were inserted in parallel subdermally 10 mm apart pointing in opposite directions lengthwise in the flexor surface of the forearm. Both needles had 4 holes 0.635 mm diameter equally spaced along opposite sides of the shaft. Sterile Tyrode solution, warmed to 32–34°C, was infused through one needle and recovered through the other into siliconised glass tubes in an ice bath. Continuous and uniform withdrawal of the perfusate was obtained by applying suction ($^1/_2$ atmosphere pressure) with a peristaltic pump. To increase recovery the area of perfusion was confined by applying elastic bands proximal and distal to the needles. Tyrode solution was infused at 30 drops (2 ml) per minute, this being kept constant throughout the collection period. The volume of the perfusate recovered was 40–80% of the volume of the infused Tyrode solution. Visible oedema developed during the first few minutes of perfusion in most experiments and was limited to the area defined by the elastic bands, but after 15 min perfusion the volume of the forearm remained constant as shown by plethysmography.

In all experiments the following procedure was adopted. After beginning perfusion the first 15 min collection was discarded but all subsequent collections were retained for assay, all bloodstained samples being rejected.

Injections. Intradermal injections were carried out using a tuberculin syringe. All agents studied were injected in volume 0.1 ml using 0.154 M NaCl as a diluent.

Bioassay. All bioassays were carried out using a 2 ml or 5 ml organ bath gassed with oxygen, and an Automatic Bioassay apparatus (1). Kinin activity was assayed by the method of Gaddum et al. (5) using an oestrous rat uterus and duodenum mounted in a single 5 ml organ bath and bathed at 32°C in De Jalon solution containing atropine 2×10^{-6}, mepyramine 10^{-8} and bromlysergic acid diethylamide 5×10^{-7}. Contractions of the uterus or relaxation of the duodenum were recorded simultaneously on a kymograph. Synthetic bradykinin (Sandoz) was used as a standard, responses to as little as 0.1 ng being recorded with both preparations.

Table I. *Recovery of infused histamine*

Infusate		Perfusate		
Concentration (ng/ml)	Total (ng)	Concentration (ng/ml)	Total (ng)	Recovery (%)
7.0	945	4.0	232	24.5
5.0	620	4.5	216	35.5

Table II. *Recovery of 10 μg histamine injected intradermally*

Total histamine, ng Duration of perfusion, min				
0–15	15–30	30–45	45–60	Recovery (%)
90	74	34	5	2
62	98	12	0	1.7

Histamine assay was performed using a 2 ml organ bath and a strip of terminal guinea pig ileum bathed in Tyrode at 37°C containing atropine 2×10^{-6} and lysergic acid diethylamide (Sandoz) 5×10^{-7}. This preparation responds regularly to 1–2.5 ng/ml standard histamine solution. Histamine activity was confirmed using the specific competitive antagonist mepyramine maleate in concentration 10^{-8} and was expressed in terms of histamine base.

RESULTS

Control Experiments

In 16 subjects perfusion of duration 15–60 min was carried out with Tyrode solution. No histamine was detected in any of these subjects but kinin activity was found in 5. The highest concentration recorded was 4 ng/ml. In each case the identity of the kinin activity was confirmed by its ability to relax the rat duodenum.

Recovery Experiments

Recovery of histamine and bradykinin administered into perfused skin both by intradermal and subcutaneous injection and by addition to the infused Tyrode solution was studied. In the infusion experiments the agent under study was dissolved in Tyrode and the perfusate collected in successive 15 min aliquots over a period of 45–60 min. Both the warmed infusate and the corresponding perfusates were subjected to bioassay. It was noted that the concentration of histamine or bradykinin in the warmed infusate was consistently lower than that in the Tyrode reservoir. This is probably due to both the instability of these agents at alkaline pH and the effect of warming, since the Tyrode (pH 7.6) in which they were dissolved was heated to 46–48°C in order to achieve a temperature of 32–34°C on entering the skin. In the injection experiments successive 15 min aliquots of perfusate were collected for 60 min after injection. Whealing following intradermal injection of vasoactive substances into perfused skin was difficult to measure accurately owing to the oedema brought about by the perfusion. Thus only an approximate estimate of the diameter of the wheal was made.

Histamine

In studies on 2 patients histamine was infused into the skin and the perfusate collected in successive 15 min aliquots for 60 min. In the first experiment the total volume of infused Tyrode solution during the 60 min period was 135 ml and the total volume of the perfusate was 58 ml. In the second the corresponding volumes were 124 ml and 48 ml. The results (Table I) show that one-quarter and one-third of the infused histamine was recovered in the perfusate.

Assay for kinin activity was also carried out on all aliquots of the perfusates of Table I, but was present in only 1 aliquot in each of the two experiments the concentration in each case being 0.5 ng/ml. No other smooth muscle contracting activity was detected.

Table III. *Recovery of infused bradykinin*

Infusate		Perfusate		
Concentration (μg/ml)	Total (μg)	Concentration (μg/ml)	Total (μg)	Recovery (%)
0.6	77	0.25	18.0	23.4
0.25	31.5	0.15	10.0	32.0

Table IV. *Recovery of 10 μg bradykinin injected intradermally*

Total bradykinin, ng Duration of perfusion, min				
0–15	15–30	30–45	45–60	Recovery (%)
82	4	0	0	0.9
50	1	0	0	0.5

These findings were then compared with results obtained by injecting histamine intradermally into perfused skin. In 2 patients perfusion was continued for 15 min. Then the perfused skin was injected intradermally, with 10 μg histamine. In both patients a wheal of about 20 mm diameter developed surrounded by a flare. The perfusate was then collected for 60 min in successive 15 min aliquots. The results (Table II) show that only 2% or less of injected histamine is recovered in the perfusate over 60 min.

Bradykinin

Studies were carried out in 2 patients. Bradykinin was infused and successive 15 min aliquots of perfusate collected over a period of 45–60 min. In the first experiment collections of perfusate were made for 60 min, the total volume of the infused Tyrode solution being 128 ml and the total volume of the perfusate 72 ml. In the second, collections were made for 45 min and the corresponding volumes were 126 ml and 67 ml. The results (Table III) show that one-quarter to one-third of the infused bradykinin was recovered in the perfusate.

Bradykinin 10 μg was then injected intradermally into perfused skin in 2 subjects immediately after obtaining the first 15 min collection of perfusate. In both patients whealing of about 15 mm diameter developed. The perfusion was then continued for 60 min and 15 min aliquots retained for kinin assay. The results are shown in Table IV and show that less than 1% of this dose of bradykinin is recovered in the perfusate.

In 2 further subjects 10 μg bradykinin was given as a single subcutaneous injection immediately after obtaining the first 15 min collection of perfusate. The perfusion was then collected for 60 min, and 15 min aliquots retained for kinin assay. The results (Table V) show that only 3–4% of the injected bradykinin was recovered in the perfusate.

Compound 48/80

Preliminary studies with compound 48/80 showed that although as little as 1 μg injected intradermally would cause whealing, no histamine could be detected in the perfusate with doses below 100 μg injected into the perfused skin. Therefore in 9 subjects the effect of intradermal injection of 100 μg compound 48/80 on the histamine content of the perfusate was studied. This dose produced extensive whealing and a surrounding red flare.

Table V. *Recovery of 10 μg bradykinin injected subcutaneously*

Concentration of bradykinin, ng/ml Duration of perfusion, min				
0–15	15–30	30–45	45–60	Recovery (%)
292	16	2	0	3
356	28	0	0	4

In each experiment an initial 15 min aliquot of perfusate was obtained after which 48/80 was injected, the perfusion then being continued for 60 min. No histamine was present in the perfusates prior to injection of 48/80. The results of histamine and kinin assays on the perfusates after injection of 48/80 are given in Table VI.

Histamine was recovered after 48/80 in all but one of the 9 subjects, the highest concentration recorded being 9 ng/ml. Its identity was confirmed using the specific antagonist mepyramine. In two experiments it was found as long as 75 min after injection. Kinins were recovered in 5 of the 9 subjects in concentration up to 0.5 ng/ml. In only one of the 5 subjects was kinin activity present in more than one specimen of perfusate.

DISCUSSION

The results of the present experiments show that the subcutaneous perfusion technique used in both

Table VI. *Histamine release — compound 48/80 100 μg*

Patient	Histamine release, ng/ml Duration of perfusion, min 0–15	15–30	30–45	45–60	60–75	Kinin activity (ng/ml)
1	5	5	5	0	0	0.1
2	4	4	4	4	4	0.1
3	0	0	0	0	0	0.1–0.25
4	9	6	5	5	2.5	0
5	6	5	6	0	—	0
6	3.5	3.2	2.5	—	—	0
7	9	6	6	5	—	0.5
8	8	6	0	0	—	0
9	8	2	0	0	—	0.1

these and earlier experiments is likely to detect the presence of only large amounts of free pharmacological substances in skin. Thus the injection of microgram quantities of bradykinin or histamine intradermally or subcutaneously into perfused skin resulted in only nanogram quantities in the perfusate. By contrast if the same agents were continuously infused subcutaneously the recovery in the corresponding perfusates reached 23–35 %.

There are at least two possible explanations for these differences. Firstly, the infused Tyrode solution forms a subcutaneous pool which causes dilution of pharmacological activity in proportion to the volume of the pool. This would lead to dilution of histamine and bradykinin in the injection experiments. Secondly, diamine oxidase and peptidases present in the overlying skin could bring about enzymic degradation of intradermally or subcutaneously injected histamine and kinins. On the other hand enzymic degradation of continuously infused histamine or kinin would be less effective since the subcutaneous "pool" of Tyrode solution would cause dilution of the enzymes.

The values obtained for histamine and kinin activities in the perfusates may not represent true recovery of these agents since injected or infused vasoactive substances may themselves bring about release or formation of endogenous kinins or mast cell degranulation (3, 10, 15). The evidence in the present experiments on this point is incomplete but in the two experiments in which low concentrations of histamine were infused into the skin little or no kinin activity was detected in the perfusate. On the other hand whilst the nanogram concentrations of kinins found in less than one-third of subjects in control experiments do not in themselves alter the significance of the results obtained with microgram concentrations in the kinin experiments, they do raise the possibility that spontaneous kinin formation in skin may be enhanced by exogenous kinin. Infusion of radioactive labelled kinin and comparison of biological activity and radioactivity in the corresponding perfusates would clarify this point.

The concentrations of pharmacological agents recovered in the perfusate depend on several other factors. These include variation in diffusion through the perfused tissues and variation in uptake by lymphatic and blood vasculatures. The importance of these conditions, which are impossible to control experimentally, in determining the concentration of agents in the perfusate is uncertain.

The above observations may explain the striking discrepancy between the high dose of compound 48/80 injected into perfused skin and the concentration of histamine recovered in the perfusate. Whealing in human skin is brought about by as little as 1 μg compound 48/80 (11) but in the present experiments no histamine was recovered in the perfusate unless 100 μg was given. With this dose the concentration of histamine in the perfusate did not exceed 9 ng/ml. These low concentrations of histamine in response to a high intradermal dose of 48/80 agree with the findings of Horner & Winkelmann (9), using a similar subcutaneous perfusion technique.

Previous work with the subcutaneous perfusion technique has established its value in pharmacological analysis of inflammatory conditions of human skin. There is, however, clearly a need to make the method more sensitive and quantitative, and until further development of the existing method has been carried out its limitations should be borne in mind in evaluating results obtained.

ACKNOWLEDGEMENTS

We wish to thank Professor Sam Shuster for his valuable advice and criticism. Mrs V. Fairley, Miss M. Davison and Miss H. Heiligstadt gave valuable technical assistance. We are grateful to Dr Evans (Sandoz Pharmaceuticals Co.) for gifts of synthetic bradykinin and brom-lysergic acid diethylamide. The work reported in this paper was supported by a grant from the Nuffield Foundation. One of us (J. S.) is in receipt of a grant from Gillette Industries. Mr H. Poole, Chief Pharmacist, Royal Victoria Infirmary, Newcastle upon Tyne, kindly supplied Tyrode solution for infusion.

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Received June 23, 1970

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