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THE RELEASE OF HISTAMINE BY PETHIDINE, ATROPINE, QUININE, AND OTHER DRUGS

BY

M. SCHACHTER

From the National Institute for Medical Research, Mill Hill, London, N.W.7

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The administration of the potent and specific histamine releasing drug, compound 48/80 (Paton, 1951 ; Feldberg and Paton, 1951), produces a characteristic syndrome of erythema, pruritus, urticaria, and facial swelling in the unanaesthetized dog (Paton and Schachter, 1951). The similarity of this syndrome to the giant oedema and urticaria in so-called drug allergy, or drug idiosyncrasy, suggested the possibility that drugs which cause this acute type of cutaneous reaction in susceptible human subjects might possess primary histamine liberating properties. There is evidence in support of this, since curare is known to be a primary histamine liberator for various animal tissues, as first shown by Alam, Anrep, Barsoum, Talaat, and Wieninger (1939) and by Ambache and Barsoum (1939). These findings have since been confirmed for human skin (Comroe and Dripps, 1946 ; Grob, Lilienthal, and Harvey, 1947). Morphine had been shown to raise the blood histamine concentration in cats by Eichler and Speda (1940), although they failed to attribute this to the release of histamine from tissues. The actual release of histamine from skin and other tissues in this species by morphine and other opium alkaloids, however, has since been demonstrated (Nasmyth and Stewart, 1949 ; Feldberg and Paton, 1951). Atropine has recently been shown to release histamine from the liver of the dog (Burstein and Parrot, 1949). Curare, opium alkaloids, and atropine have been reported to produce cutaneous reactions of the type described above following systemic administration to humans (Bennett, 1940 ; Sollmann, 1948 ; Sutton and Sutton, 1939).

Drugs which are known to produce angio-oedema and urticaria in certain individuals were selected to be tested for histamine releasing properties, particularly in skin. Of these, neoarsphenamine, quinine, and atropine have long been known occasionally to produce dramatic cutaneous reactions after the systemic administration of therapeutic doses (Sutton and Sutton, 1939 ; Sollmann, 1948). More recently, reports have appeared of a similar response to first contact with pethidine (Butler, 1951), and generalized urticaria has been reported after the oral administration of small doses of priscol (Lindquist, 1943). All these drugs were found to release histamine from the isolated perfused skin of the cat, though some were more effective than others. Increased plasma histamine concentrations after injection of the drug into the blood circulation were demonstrable only with pethidine, atropine, and quinine.

Sulphanilamide and dinitrophenol, which are also known to cause similar cutaneous reactions (Sollmann, 1948), failed to release histamine. It is of interest that the clinical evidence indicates that these two drugs appear never to provoke the reaction on first administration (Hageman and Blake, 1937 ; Tainter, Stockton, and Cutting, 1935).

Sodium bromide was tested because of the skin lesions which occur after prolonged bromide therapy. The bromide eruption, however, is considered a toxic effect of bromide accumulation and is not of the " allergic " type (Sollmann, 1948). This drug also failed to release histamine.

The bile salt mixture, sodium tauroglycocholate, was tested because pruritus frequently occurs in obstructive jaundice. This released some histamine from the perfused skin, but much less than the others.

Drugs such as caffeine and alcohol which to our knowledge do not provoke angio-oedema and urticaria were without effect. Hydrochloric acid, isotonic saline, and anoxia likewise failed to release histamine. Distilled water was either ineffective or released barely detectable amounts from the perfused skin.

A preliminary report of some of these results has been published (Schachter, 1951).

METHODS

Cats were anaesthetized with chloralose intravenously, preceded by ethyl chloride-ether induction. Blood samples taken from the femoral artery were heparinized and centrifuged and the plasma tested for histamine. Dogs were anaesthetized with intravenous nembutal. Caffeine was used as base, atropine as sulphate, and quinine as the hydrochloride. Histamine values are given as the base.

Isolated skin preparations.—These were prepared according to the method of Feldberg and Paton (1951), as modified by Feldberg and Schachter (1952). Cat skin preparations weighed 4–8 g. and dog skin preparations 15–20 g. Drugs were injected into the saphenous artery in 0.5 ml. volumes and the perfusate collected from the corresponding vein. The venous outflow ranged between 2 and 3 ml. per min. The infusion was arrested for 10–20 sec. during the injection, permitting the drug to remain in contact with the tissues for this period.

The histamine retained in the subcutaneous oedema fluid of the skin preparation at the end of the experiment was determined by cutting into the oedematous tissue, draining off the fluid, and assaying its histamine content. Since the weight increase after perfusion was known by comparing the weight of perfused skin with that of a similar flap from the normal side, the total histamine in the oedema fluid could be calculated.

Intra-arterial injection in the eviscerated cat.—Drugs were injected into the aorta through a cannula tied into the right renal artery. This assured a high drug concentration in the tissues of the lower part of the animal. The ovarian or testicular vessels were tied and in some cats the adrenal vessels as well. During the arterial injection the inferior vena cava was obstructed for 20 sec. by a loose ligature placed around it above the entrance of the left renal vein. This permitted the injected drug to remain in contact with the tissues for a short time. The left renal vein was tied near the kidney and blood samples for histamine assay obtained with a syringe by inserting a needle through the vein to its junction with the vena cava, which was obstructed by tension on the ligature during this interval. Samples could be obtained repeatedly in this way.

Histamine assay.—All the drugs used, except neoarsphenamine, depressed the response of the guinea-pig ileum to histamine. Their action in this respect roughly paralleled

their histamine releasing ability. Thus the amounts of the various drugs which began to depress the histamine contraction on addition to a 15 ml. bath were, in mg.: pethidine, 0.001 ; atropine, 0.01–0.03 ; quinine, 0.1 ; priscol, 0.1 ; sodium tauroglycocholate, 0.1. The test solution added to the bath in most instances did not contain a sufficient concentration of any of these substances to interfere with the histamine assay. However, when the amount of drug injected was great enough to raise its concentration in the test sample beyond the above values, the standard histamine solutions were made up in the maximum concentration of the specific drug which could be present in the test solution. Histamine assay was performed on the arterial blood pressure of the eviscerated cat when the drug concentration was too great to permit satisfactory assay on the guinea-pig ileum.

Neoarsphenamine increased the response of the guinea-pig ileum to histamine by 20–50 per cent when added to the bath in amounts of 1 mg. or more. It also caused a similar potentiation of the response to acetylcholine.

The fact that the active substance released in each experiment was histamine was substantiated by the abolition of its action on either the isolated guinea-pig ileum or blood pressure of the cat by mepyramine.

RESULTS

Histamine release from isolated perfused skin preparations

Cat skin.—Table I shows the amounts of histamine released from skin preparations by intra-arterial injections of 0.5 ml. volumes of the various drugs. Comparison of histamine releasing potency shows pethidine to be the most effective, followed by atropine, quinine, priscol, neoarsphenamine, and bile salt in this order. Neoarsphenamine occasionally released only small amounts of histamine after a single intra-arterial injection ; on these occasions, a second injection produced the usual release. The possibility that the histamine releasing property of neoarsphenamine was due to oxidized derivatives was excluded by demonstrating that this effect was not less if the drug was carefully dissolved in nitrogenated saline or distilled water and injected immediately, than if the solution was oxygenated and allowed to stand for several hours. Injection of 0.5 ml. volumes of the following substances failed to release any histamine: 10 per cent alcohol (v/v), 5 per cent caffeine, 0.9 per cent sodium bromide, 0.9 per cent sodium chloride, 0.4 per cent sulphanilamide, 0.2 per cent dinitrophenol, and 0.15 per cent hydrochloric acid (w/v). Injection of distilled water occasionally released barely detectable amounts. Anoxia, produced by arresting the inflow of Locke's solution for several minutes, was without effect.

Fig. 1 shows the histamine output from the skin in typical experiments with each drug. The failure of caffeine and ethyl alcohol to release histamine is also shown. The characteristics of the release were identical for pethidine, atropine, quinine, and priscol, in that the venous outflow was temporarily reduced after intra-arterial injection ; by far the greatest histamine concentration was present in the first 10 min. sample and considerable oedema of the preparation was apparent after one hour. The distinctive feature following bile salt injection was the great reduction or arrest of venous outflow, although the inflow from the reservoir continued. Thus, despite the release of lesser amounts of histamine, the development of oedema was rapid and dramatic. The time course of the change in histamine concentration in the perfusate after injection of neoarsphenamine differed from that of the other drugs in so far as the greatest concentrations frequently appeared in later samples, both the initial increase and subsequent decrease in concentration being gradual

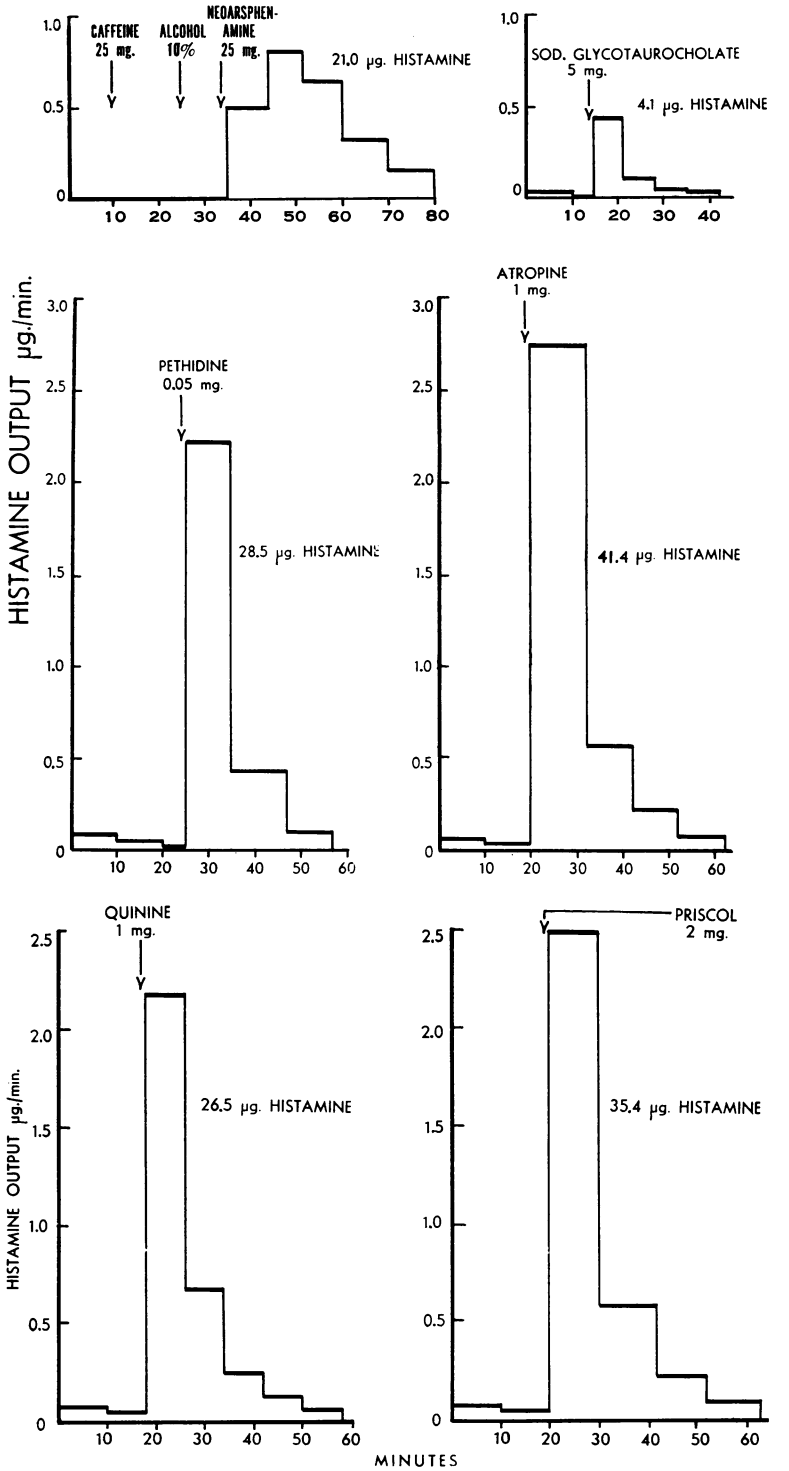

FIG. 1.—Output of histamine from isolated Locke-perfused cat skin preparations following intra-arterial injection of various drugs. Ordinates: histamine output in µg./min. Abscissae: time in 10 min. intervals.

TABLE I

HISTAMINE RELEASE ON INTRA-ARTERIAL INJECTION OF VARIOUS DRUGS IN THE ISOLATED PERFUSED SKIN PREPARATION OF THE CAT

Drug	Amount of drug injected (mg. in. 0.5 ml.)	Total histamine collected in perfusate (μg.)	Total histamine in subcutaneous oedema fluid of isolated preparation (μg.)
Pethidine	25.0	21.0	
	5.0	73.7	6.0
	0.5	55.8	
	0.2	38.3	
	0.05	28.5	2.7
	0.02	0	
	0.01	0	
Atropine	1.0 (2 exps.)	41.4; 30.3	2.8; 3.1
	0.5 (2 exps.)	62.9; 21.8	
	0.1 (2 exps.)	2.9; 2.4	
	0.025	traces	
Quinine	4.0	19.3	4.5
	2.0	36.2	
	1.0	26.5	
	0.1	5.8	
	0.01	traces	
Priscol	2.0	35.4	
	0.5	15.8	
Neoarsphenamine ..	50.0	19.3	19.6
	25.0 (10 exps.)	12.9 (Mean) (S.D.=6.5)	8.5 (Mean) (S.D.=6.6)
Sod. glycotaurocholate ..	5.0 (2 exps.)	4.8; 4.1	7.9; 6.5
	4.0	0.6	
	3.0	0.2	3.5

rather than abrupt. Furthermore, the concentrations and total amounts of histamine present in the subcutaneous oedema fluid of the preparation at the end of the experiment were considerably greater after bile salt and neoarsphenamine injections than after the other drugs (Table I).

Dog skin.—Pethidine and atropine, the two most effective drugs for the cat skin, were tested and found effective in releasing histamine from the isolated dog skin preparation. Pethidine was again the more active. The intra-arterial injection of 0.5 mg. pethidine released 21.6 μg. histamine from the skin preparation of one leg. Two mg. atropine released 16.1 μg. from the skin of the opposite leg. The results of these experiments are shown in Fig. 2.

Histamine release by intra-arterial injections in the cat

The substances tested were injected retrogradely into the renal artery of eviscerated cats and blood was collected from the renal vein 2 min. after injection. The drugs thus reached the skin and muscles of the lower half of the animal in higher concentrations than if they were injected intravenously. Pethidine, atropine, and quinine, in this order of effectiveness, raised the plasma histamine concentration

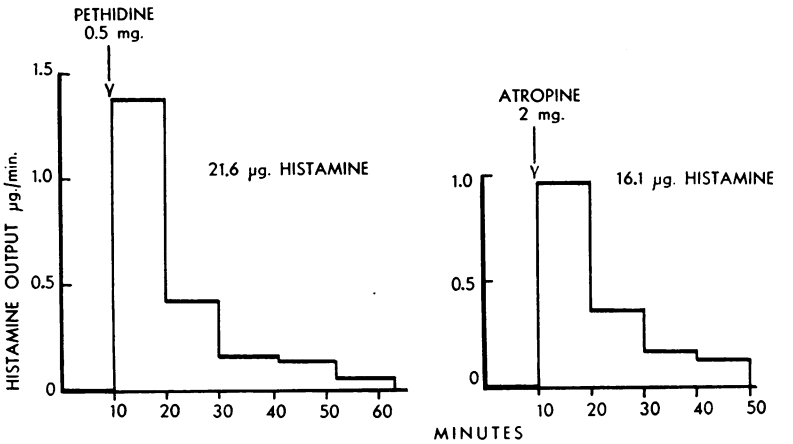

FIG. 2.—Output of histamine by isolated dog skin following intra-arterial injection of pethidine and of atropine.

although greater amounts were required than in the isolated perfused preparation (Table II). Priscol, neoarsphenamine, and bile salt, even with large doses, failed to increase the plasma histamine concentration. This is in contrast to the ready demonstration of histamine release from the isolated Locke-perfused cat skin preparation by these compounds. Plasma histamine assays after pethidine and atropine injections were performed on the blood pressure of the eviscerated cat.

TABLE II

PLASMA HISTAMINE CONCENTRATIONS OF VENOUS BLOOD (INFERIOR VENA CAVA) BEFORE AND 2 MIN. AFTER INJECTION OF PETHIDINE, ATROPINE, OR QUININE INTO THE RENAL ARTERY OF THE EVISCERATED CAT

Drug	Amount of drug injected		Plasma histamine concentration before injection (μg./ml.)	Plasma histamine concentration 2 min. after injection (μg./ml.)
	mg./kg.	mg./ml.		
Pethidine ..	15	15	<0.05	1.4
	15	15	<0.02	0.8
Atropine ..	15	15	<0.03	0.8
	15	15	<0.02	0.15
	15	15	<0.02	<0.02
Quinine ..	50	30	0.01	0.09
	50	30	0.01	0.07
	50	30	0.02	0.02
	25	20	<0.01	0.02
	25	20	<0.01	<0.01
	10	10	<0.02	<0.02

Histamine release by intravenous injections in the cat

Both pethidine and atropine caused an increase in plasma histamine concentration on intravenous injection in the cat. The dosage required, however, produced severe toxic effects, most of which could not be attributed to released histamine.

Pethidine (15 mg./kg.) regularly raised the plasma histamine concentration (Table III), but this dose was sufficient to arrest breathing and artificial ventilation was required. Atropine (20 mg./kg.), after 3 min., raised the plasma histamine concentration from less than 0.01 μg./ml. to 0.05 μg./ml. in one experiment and failed to produce any change in another. These histamine assays were performed on the blood pressure of the eviscerated cat, since the amounts of pethidine or atropine in the plasma were too great to permit assay on the guinea-pig ileum.

TABLE III

PLASMA HISTAMINE CONCENTRATIONS (μG./ML.) BEFORE AND AT VARIOUS INTERVALS AFTER THE INTRAVENOUS INJECTION OF PETHIDINE (15 MG./KG.) IN THE CAT

Exp. No.	Before injection	Minutes after injection			
		3	7	15	40
I	<0.01	0.10	0.10	0.04	0.02
II	<0.01	0.18	0.16	—	0.08
III	<0.04	0.25	0.15	—	0.05

The other drugs failed to raise the plasma histamine concentration under these conditions, even with very large doses. Indeed, a distinct reduction or disappearance of plasma histamine regularly occurred after the intravenous injection of neoarsphenamine (200 mg./kg.) and bile salt (50 mg./kg.). This was associated with a gross increase in size of the buffy cell layer.

DISCUSSION

It has long been known clinically that many drugs, diverse in their pharmacological properties, could evoke a common syndrome bearing considerable resemblance to experimental protein anaphylaxis. This led to the inclusion of this type of drug reaction in the broad category of allergy and its designation as drug allergy. Theoretical objections to this classification were the frequent reactions on first contact, the non-protein nature of the substances, and failure to detect antibodies in reacting subjects. The objection to the non-protein nature of the antigen was subsequently overcome to some extent by the demonstration that the introduction of specific chemical groups into protein molecules did not interfere with the antigenic properties of the protein, and endowed it with a serological specificity related to the nature of the chemical group introduced. This has been shown for many chemical modifications of protein such as iodination (Obermayer and Pick, 1906), nitration (Mutsaars and Gregoire, 1936), and diazotization (Landsteiner, 1945). These experiments led to the concept of haptens or partial antigens, and the hypothesis that if some drugs are repeatedly brought into contact with the tissues a compound antigen is formed which consists of the drug or a part thereof in combination with tissue protein. Such a compound would theoretically derive its specificity from the drug or drug radical and its antigenicity from the combined protein. This hypothesis still fails to explain the facts that there is frequently no evidence of previous contact with the drug incitants and that antibodies are, in general, not demonstrable even by passive transfer (Zinsser and Bayne-Jones, 1939 ; Landsteiner, 1945 ; Sodeman, 1950). The finding, however, that many of these " anaphylactoid "

drugs are primary histamine liberators modifies these theoretical difficulties in so far as prior sensitization need not be regarded as a *sine qua non* of the human reaction. Thus, in addition to reactions in which the subject becomes sensitized to the drug acting as a partial antigen, reactions to first contact could occur on the basis of the primary histamine releasing properties of the drug. It is possible that drugs such as sulphanilamide and dinitrophenol, which do not possess primary histamine releasing properties, evoke reactions only after sensitization. Drugs which possess the primary histamine releasing property might, however, act either on first contact or after sensitization, since experimental animal sensitization to arsenicals (Frei, 1928 ; Sulzberger and Simon, 1934) and quinine (Landsteiner and Chase, 1941) has been demonstrated. The problem why only certain individuals react by releasing histamine from skin on first contact with small doses of these drugs remains. However, the theoretical emphasis could now be placed on the nature of histamine " fixation " in the tissue. At present little is known of the physical or chemical mechanisms stabilizing tissue histamine or permitting its rapid release. Such information might explain the possible " lability " of the skin histamine of individuals who react on first contact with these drugs.

Histamine release was more readily demonstrable from the Locke perfused skin than under conditions of natural blood circulation. The relative effectiveness of the drugs was the same, however, in all three types of preparation employed. The failure to demonstrate increased plasma histamine concentrations in the intact animal with some of the drugs is probably due to a variety of factors such as the histaminase activity of blood, removal or destruction of histamine by various organs, faster circulation rate, or possibly other conditions elevating the threshold for the release mechanism in the presence of blood.

These drugs differ from some other histamine liberators, such as various amines and amidines whose main pharmacological effects are due to the released histamine (MacIntosh and Paton, 1947, 1949 ; Parrot, 1948). In the drugs considered in this analysis, the ability to release histamine is a property incidental to their main pharmacological actions. This is also true of curare and morphine. It is of interest that Burn (1950) has pointed out the overlap in the various pharmacological properties of pethidine, atropine, quinine, and priscol, and suggests that the basis of their common properties is related to their ability to depress the effects of acetylcholine, histamine, and adrenaline. The ability to release histamine can now be added to their common properties.

SUMMARY

1. Pethidine, atropine, quinine, priscol, neoarsphenamine, and bile salt, in this order of effectiveness, release histamine from the isolated perfused cat skin. The characteristics of this release are identical for pethidine, atropine, quinine, and priscol, but differ in some respects for neoarsphenamine and bile salt. Pethidine and atropine were also shown to release histamine from the isolated perfused dog skin.

2. Injection of pethidine, atropine, or quinine into the blood circulation of the anaesthetized cat causes an increase in plasma histamine concentration.

3. The possible significance of these observations to the giant oedema-urticaria type of cutaneous drug reactions in humans is discussed.

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