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INHIBITION BY VARIOUS SUBSTANCES OF OEDEMA FORMATION IN THE HIND-PAW OF THE RAT INDUCED BY 5-HYDROXYTRYPTAMINE, HISTAMINE, DEXTRAN, EGGWHITE AND COMPOUND 48/80

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Of the substances studied for their inhibitory action of the oedema formation in the hind-paw of the rat, the most active was a new derivative of phenothiazine, related to chlorpromazine. It is methotrimeprazine {10-(3-dimethylamino-2-methylpropyl)-2-methoxyphenothiazine ; 7044 RP}. This substance possesses potent antihistamine and anti-5-hydroxytryptamine properties, and doses of 0.25 mg./kg. inhibited the blueing and swelling of the foot after injections of histamine, 5-hydroxytryptamine, eggwhite, dextran or compound 48/80 into the feet of rats with sulphan blue (Evan's blue) dye in their circulation. The results indicate that both histamine and 5-hydroxytryptamine play rôles in increasing capillary permeability in the rat.

It has been shown by Rowley and Benditt (1956) and Parratt and West (1957) that the anaphylactoid reaction produced in rats by injections of eggwhite and dextran is mediated chiefly through the release of 5-hydroxytryptamine. This has been achieved by using drugs which deplete the tissues only of their 5-hydroxytryptamine or block specifically the action of 5-hydroxytryptamine. The purpose of the present experiments is to show that a number of other drugs, which are antagonists of 5-hydroxytryptamine, reduce or prevent the oedema induced by eggwhite and dextran in the rat hind-paw. Antagonism of the oedema induced by compound 48/80, histamine and 5-hydroxytryptamine has also been studied.

METHODS

Groups of 4 to 6 female albino rats (100 to 150 g.) were used in all experiments. Subcutaneous injections of 0.1 ml. of each oedema-producing substance were made into the hind-paws to study the vascular changes after each animal had received an intravenous dose of sulphan blue (Evan's blue) dye (2 mg.) 30 min. previously. Drugs used to inhibit the oedema formation were given intravenously with the dye, except for adrenaline and noradrenaline which were administered subcutaneously at the same time as the intravenous dye. The following solutions of the oedema-producing substances were used: 5-hydroxytryptamine (5 μg./ml.), histamine (1 mg./ml.), dextran (60 μg./ml.), fresh eggwhite (0.5% v/v solution), and compound 48/80 (10 μg./ml.). These concentrations produced nearly maximal oedema of approximately equal severity. Injections of normal saline into some paws served as controls, but the very slight swelling and blueing which were produced in these areas subsided in about 1 hr. when the degree of oedema was assessed. The measurement of the oedema reaction has been described in detail (Parratt and West, 1957). Briefly, we have recorded the degree of swelling (oedema) and of blueing (leakage of plasma proteins into the tissue spaces) on a relative scale from 0 to +++. By allotting marks to each degree (+=2, ++=4, +++=6), the results may be expressed as % of the maximal possible effect (+++) for the dose chosen for each substance. On this system of measurement, a difference of 20% is within the error of the test. The values shown in the Tables are the means of 3 different experiments, separate records of the oedema and blueing being assessed to each rat and the scores then averaged. Usually, oedema and blueing go hand in hand, though this is not always so.

The following drugs have been tested for inhibitory action: 2-bromolysergic acid diethylamide (BOL 148), mepyramine maleate, phenindamine, thenalidine tartrate (1-methyl-4-*N*-then-2′-ylanilinopiperidine tartrate), 5-methoxy-2-methyltryptamine, 1-benzyl-5-methoxy-2-methyltryptamine, adrenaline tartrate and noradrenaline tartrate, and the phenothiazine derivatives—promethazine hydrochloride (3277 RP), chlorpromazine hydrochloride (4560 RP), methylpromazine (4627 RP), 4670 RP {2-chloro-10-(3-pyrrolidin-1′-ylpropyl)phenothiazine}, trimeprazine

(6549 RP) and methotrimeprazine (7044 RP). All the values in the Tables refer to the bases.

RESULTS

BOL 148.—We have previously shown that intravenous doses of 4 mg./kg. of this substance completely prevented oedema formation by 5-hydroxytryptamine, dextran, eggwhite and compound 48/80, leaving that by histamine unchanged. When the dose was reduced to 1 mg./kg., the 5-hydroxytryptamine response was still completely inhibited, but those of the other three substances were only reduced (Table I). This result indicates that BOL 148 is an effective antagonist of exogenous 5-hydroxytryptamine when doses of 1 mg./kg. are used, but larger doses are needed to prevent the action of released 5-hydroxytryptamine.

Mepyramine and Phenindamine.—These two antihistamine drugs were weak in inhibiting the oedema formation by most agents. For example, doses of 10 mg./kg. of mepyramine did not affect the 5-hydroxytryptamine response and only reduced the responses of the other agents (Table I). This reduction, however, probably indicates an inhibitory action on released histamine in the cases of dextran, eggwhite and compound 48/80, since these three substances are capable of releasing both histamine and 5-hydroxytryptamine. Phenindamine was a slightly stronger inhibitor of the oedema response than was mepyramine, yet it is generally considered to be a less potent antihistamine drug. On the other hand, phenindamine is a more potent antagonist of 5-hydroxytryptamine on isolated tissue than is mepyramine, and the combined antagonistic actions of phenindamine probably account for the full inhibition of the dextran and eggwhite responses found at dose levels of 8 mg./kg.

TABLE I

THE EFFECTS OF INTRAVENOUS DOSES OF ANTAGONISTS ON THE LOCAL OEDEMA REACTION PRODUCED IN RATS BY 5-HYDROXYTRYPTAMINE (5-HT), HISTAMINE, DEXTRAN, EGGWHITE, AND COMPOUND 48/80

Responses measured in terms of the maximal (100%) found in saline-treated rats.

Antagonist		5-HT	Histamine	Dextran	Eggwhite	Compound 48/80
Name	Dose (mg./kg.)					
BOL 148 ..	1	13	100	39	41	42
	2	16	100	17	21	42
	4	10	100	9	20	18
Mepyramine ..	4	100	70	100	100	100
	10	82	42	42	42	42
Phenindamine	4	100	70	75	87	100
	8	70	30	10	20	65
Thenalidine ..	5	100	80	42	100	85
	10	54	52	13	37	85
	15	33	54	4	8	35

Thenalidine.—This substance has been shown by Rothlin and Cerletti (1955) to possess antihistaminic properties as well as marked inhibitory effects on increased capillary permeability shown in various forms of experimental oedema produced by dextran or eggwhite. In the present experiments, it was more effective against dextran and eggwhite than it was against either 5-hydroxytryptamine, histamine or compound 48/80 (Table I). Thenalidine, like phenindamine, possesses some anti-5-hydroxytryptamine properties.

Antimetabolites of 5-Hydroxytryptamine.—The two agents, 5-methoxy-2-methyltryptamine and 1-benzyl-5-methoxy-2-methyltryptamine, used are the most powerful antagonists of 5-hydroxytryptamine known to be effective orally in dogs. Yet, with one exception, maximally tolerated doses (10 mg./kg.) of these substances did not markedly influence the oedema production by either of the agents. The exception was the oedema induced by

TABLE II

THE EFFECTS OF DOSES OF ANTIMETABOLITES OF 5-HYDROXYTRYPTAMINE (5-HT) AND CERTAIN SYMPATHOMIMETIC AMINES ON THE LOCAL OEDEMA REACTION PRODUCED IN RATS BY 5-HYDROXYTRYPTAMINE, HISTAMINE, DEXTRAN, EGGWHITE AND COMPOUND 48/80

Responses measured in terms of the maximal (100%) found in saline-treated rats.

Antagonist			5-HT	Histamine	Dextran	Eggwhite	Compound 48/80
Name	Route	Dose (mg./kg.)					
5-Methoxy-2-methyltryptamine	Intravenous	4	100	100	100	100	83
	,,	10	70	100	90	76	76
1-Benzyl-5-methoxy-2-methyltryptamine ..	,,	4	88	100	72	84	96
	,,	10	78	84	52	84	84
Adrenaline	Subcutaneous	1	54	84	41	39	65
	,,	2	54	42	18	8	50
	,,	4	20	55	20	8	20
Noradrenaline	,,	10	38	80	38	58	75

dextran which was reduced by the benzyl derivative to about 50% of the maximal response (see Table II).

Sympathomimetic Amines. — Adrenaline was found to be a potent inhibitor of the anaphylactoid reaction of dextran and eggwhite, doses of 2 mg./kg. completely preventing the response. Yet such doses were not sufficient to antagonize completely the effects of 5-hydroxytryptamine, histamine or compound 48/80. If this difference was due to the vasoconstrictor action of adrenaline, then noradrenaline should be more active than, or at least as active as, adrenaline. But the results shown in Table II indicate that noradrenaline is only about 1/10 as active as adrenaline in reducing the oedema production.

Phenothiazine Derivatives. — The chemical structures of the derivatives tested are shown in Fig. 1. Promethazine has been tested previously at a dose of 4 mg./kg. and found to reduce considerably the responses of dextran and histamine.

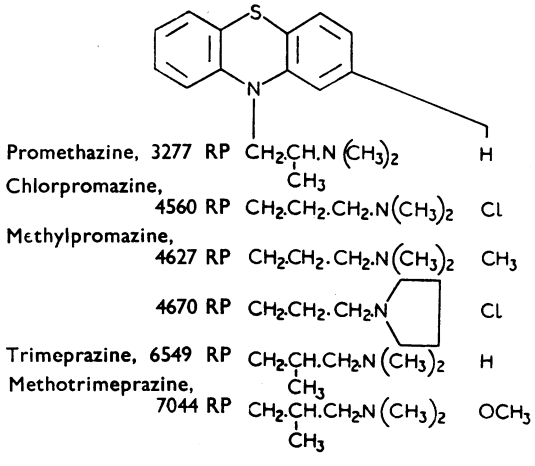

FIG. 1.—Chemical structure of derivatives of phenothiazine.

Chlorpromazine, methylpromazine and 4670 RP were included in this study since Viaud (1954) has reported that these three substances are very effective in protecting rats against dextran-induced oedema. This author linked this protective action of these substances with their antihistamine action. Trimeprazine and methotrimeprazine are newer derivatives which have also been found in screening tests to be very potent in this action.

All six derivatives were tested at various dose levels against oedema induced by the five agents (Table III). Promethazine in a dose of 8 mg./kg. completely prevented all the responses whereas 4 mg./kg. markedly reduced the oedema produced by dextran, eggwhite, compound 48/80 and histamine. Chlorpromazine was effective against dextran and eggwhite at much smaller doses (0.5 mg./kg.), so confirming the results of Benditt and Rowley (1956). Larger doses, however, were required in our experiments to reduce the responses of 5-hydroxytryptamine, histamine and compound 48/80. Methylpromazine was effective at a dose of 1 mg./kg. against histamine, dextran, eggwhite, and compound 48/80, but much less so against 5-hydroxytryptamine. This latter result shows that histamine plays a rôle (even if only a minor one) in the anaphylactoid reaction. A similar set of results was obtained with trimeprazine, methotrimeprazine and 4670 RP, which were more effective against histamine than against 5-hydroxytryptamine. Doses as low as 0.1 mg./kg. of methotrimeprazine prevented the oedema produced by histamine, eggwhite, dextran and compound 48/80, and this new derivative of phenothiazine is the most active antagonist of the locally-induced anaphylactoid reaction that we have so far tested. The approximate effectiveness of these derivatives in inhibiting the oedema responses is shown in Table IV, where the activity of the most potent (methotrimeprazine) is taken as 100%. It is of interest that with the more potent

TABLE III

THE EFFECTS OF INTRAVENOUS DOSES OF DERIVATIVES OF PHENOTHIAZINE ON THE LOCAL OEDEMA REACTION PRODUCED IN RATS BY 5-HYDROXYTRYPTAMINE (5-HT), HISTAMINE, DEXTRAN, EGGWHITE AND COMPOUND 48/80

Responses measured in terms of the maximal (100%) found in saline-treated rats.

Derivative						
Name or No.	Dose (mg./kg.)	5-HT	Histamine	Dextran	Eggwhite	Compound 48/80
Promethazine (3277 RP)	2	100	40	32	75	75
	4	60	33	29	28	29
	8	16	16	0	0	12
Chlorpromazine (4560 RP)	0·5	60	60	22	22	90
	1	30	60	0	0	55
	2	30	30	0	0	60
	4	15	15	0	0	20
Methylpromazine (4627 RP)	0·5	92	42	25	29	21
	1	66	12	4	12	25
	2	34	8	8	1?	4
	4	20	2	10	10	0
4670 RP	0·5	83	33	29	28	68
	1	67	17	5	15	25
	2	17	0	2	4	17
Trimeprazine (6549 RP)	0·25	100	14	50	41	59
	0·5	50	18	25	17	25
	1	54	2	4	0	4
Methotrimeprazine (7044 RP)	0·1	62	16	19	23	23
	0·25	25	8	4	4	6
	1	0	5	0	0	0

TABLE IV

APPROXIMATE EFFECTIVENESS OF SOME DERIVATIVES OF PHENOTHIAZINE IN INHIBITING THE OEDEMA PRODUCTION BY 5-HYDROXYTRYPTAMINE (5-HT), HISTAMINE, DEXTRAN, EGGWHITE AND COMPOUND 48/80

Activity of methotrimeprazine is taken as 100%.

Compound	5-HT	Histamine	Dextran	Eggwhite	Compound 48/80
Promethazine	8	2	2	2	2
Chlorpromazine ..	16	3	20	20	3
Methylpromazine ..	12	10	10	10	10
4670 RP	25	10	10	10	10
Trimeprazine	25	30	20	20	20
Methotrimeprazine ..	100	100	100	100	100

compounds in this series blueing of the foot was usually easier to inhibit than was swelling.

On the isolated guinea-pig ileum, methotrimeprazine exerted its antihistamine and anti-5-hydroxytryptamine action in concentrations as low as 0.1 ng./ml. One hundred times this concentration did not alter the acetylcholine response (Fig. 2). The 5-hydroxytryptamine response recovered slightly quicker than the histamine response when methotrimeprazine was washed out (Fig. 3).

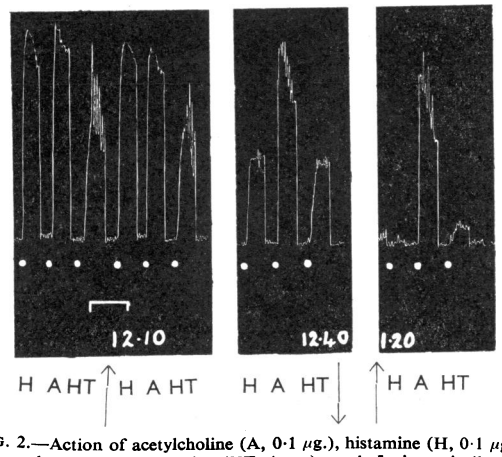

FIG. 2.—Action of acetylcholine (A, 0·1 μg.), histamine (H, 0·1 μg.) and 5-hydroxytryptamine (HT, 1 μg.) on the guinea-pig ileum. Bath vol., 15 ml. Time in min. From 12·10 to 12.40 p.m., methotrimeprazine (0·1 ng./ml.) was in the bath; from 1.20 p.m., this concentration was increased to 10 ng./ml.

Inhibition of the Formaldehyde Reaction.—In a few of the experiments of the present series, the inhibitory action of the antagonists was tested against the oedema reaction produced by the local injection of 4 mg. formaldehyde into the dorsum of the foot. This reaction is known to be different from that of the other oedema-producing agents, since swelling and blueing are much more diffuse and spread well above the knee. Further, the reaction occurs when the tissue histamine or 5-hydroxytryptamine has been depleted, and it is unaffected by pretreatment with BOL 148 (Parratt and West, 1957).

Both adrenaline (4 mg./kg.) and noradrenaline (10 mg./kg.) prevented the swelling and blueing produced by injections of formaldehyde. However, it was only a temporary inhibition, since the response had become maximal when measurements were again made 4 hr. after injection. The most active of the phenothiazine derivatives in preventing the formaldehyde reaction was methotrimeprazine, which was effective in doses of 1 mg./kg. Again the inhibition was of a temporary nature, the formaldehyde response being maximal about 4 hr. after injection.

DISCUSSION

The results of the present experiments using the specific anti-5-hydroxytryptamine substance, BOL 148, add further support to the hypothesis that 5-hydroxytryptamine plays a major rôle in the anaphylactoid reaction in the rat. Small doses are effective in preventing the oedema reaction of egg-white, dextran, compound 48/80 and 5-hydroxy-

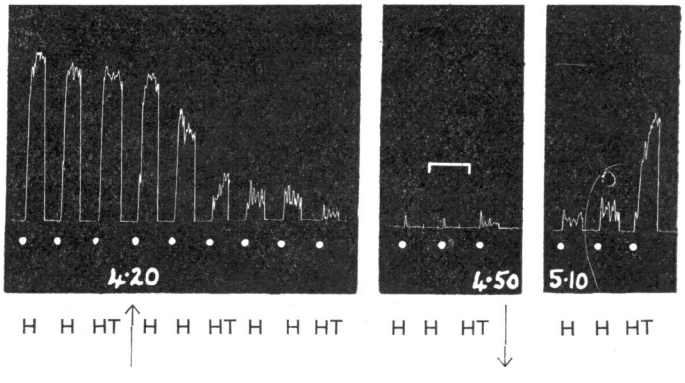

FIG. 3.—Action of histamine (H, 0·1 μg.) and 5-hydroxytryptamine (HT, 1 μg.) on the guinea-pig ileum. Bath vol., 15 ml. Time in min. From 4.20 to 4.50 p.m., methotrimeprazine (1 ng./ml.) in the bath.

tryptamine, leaving that of histamine unchanged. On the other hand, larger doses of the antihistamine substances are generally required and even then the relative effectiveness of such compounds appears to depend not so much on their antihistamine potency as on their ability to antagonize the actions of 5-hydroxytryptamine. Thus promethazine is much more effective than mepyramine in preventing the anaphylactoid reaction since the former possesses greater anti-5-hydroxytryptamine activity than does the latter. Phenindamine and thenalidine likewise are superior to mepyramine in this respect. Halpern and Briot (1950) have screened many such antihistamine substances for their effectiveness in reducing the oedema induced in rats by injections of eggwhite.

The results of the experiments using the two antimetabolites of 5-hydroxytryptamine were disappointing. Most of this type of substance are active *in vitro* against 5-hydroxytryptamine but weakly active in living animals. However, the two used in the present experiments are water-soluble and active orally when given to dogs (Shaw and Woolley, 1956). Yet intravenous doses were virtually devoid of any anti-5-hydroxytryptamine action in the oedema reaction and did not markedly influence the local response of any of the other agents. It is possible that the conditions used in the present experiments were not optimal for the antagonistic action and further work along these lines is in progress.

Much work has already been carried out with sympathomimetic amines and the anaphylactoid reaction in rats. For example, Clark and MacKay (1949) showed that adrenaline, noradrenaline and isoprenaline were capable of modifying the eggwhite response, though the relative effectiveness of these compounds varied greatly. In the present experiments, vasoconstriction does not appear to be the chief reason for the inhibition of the responses by adrenaline since noradrenaline, a more potent vasoconstrictor amine, is much less active. A metabolic action of adrenaline may thus be involved, though we have as yet no proof of this.

The phenothiazine derivatives, first studied for their chemotherapeutic properties, were later shown to possess antihistamine properties. Further work indicated that they were potent substances in the protection of rats against dextran oedema, a property which was linked until very recently with their antihistamine potency. It is now certain that the anti-5-hydroxytryptamine potency of a compound is also important when considering inhibition of the anaphylactoid reaction. Methotrimeprazine is the most potent inhibitor we have used as yet, and experiments have shown that it is also active *in vitro* against both histamine and 5-hydroxytryptamine in extremely low concentrations. Chlorpromazine is about one-fifth as active as methotrimeprazine in inhibiting the oedema induced by local injections of dextran or eggwhite and its anti-5-hydroxytryptamine and antihistamine properties are likewise weaker. It is clear from Table IV that an increase in the length of the side chain in this series of compounds results in increased activity against oedema production (compare promethazine with chlorpromazine), and further any substitution in the side chain also increases activity (compare trimeprazine with methylpromazine).

Measurement of the oedema reaction has involved an assessment of both the swelling and blueing of the foot. Visual records have been made and the results averaged. Usually oedema and blueing go hand in hand, though the mechanisms involved may be different. Gözsy and Kato (1956), for example, have suggested that blueing of the foot is proportional to the phagocytic power of the endothelial cells of the blood vessels, whereas swelling is simply the increase in tissue permeability. Thus the two criteria may be quite separate, and the more active phenothiazine derivatives have occasionally been found to prevent the blueing, but not the swelling, of the foot when histamine or 5-hydroxytryptamine has been injected locally. More recently, Sparrow and Wilhelm (1957) have shown that mepyramine reduces the intensity of colour, but not the area of blueing, of the lesions induced by compound 48/80 or by 5-hydroxytryptamine.

The inhibition of the oedema reaction in the hind-paw of the rat can be used as a useful screening test for new antagonists of 5-hydroxytryptamine and histamine. The drug can be given intravenously 30 min. before the local injections of 5-hydroxytryptamine, histamine, eggwhite or dextran and the responses measured 1 to 2 hr. later. It is possible that this test is more useful for screening new compounds than is the *in vitro* test using antagonism on the guinea-pig ileum.

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