International Archives
of
Allergy and Applied Immunology
Redactores: R. R. A. Combs-Cambridge - D. Harley-London - P. Kallós-Helsingborg - W. Kaufman-Bridgeport, Conn. - W. Löffler-Zürich.
BASEL (Schweiz) S. KARGER NEW YORK
Separatum *Vol. 12, No. 1–2* Printed in Switzerland

Doepfner, W. and *Cerletti, A.*: Int. Arch. Allergy *12*: 89—97 (1958)

Comparison of Lysergic Acid Derivatives and Antihistamines as Inhibitors of the Edema Provoked in the Rat's Paw by Serotonin

By W. Doepfner and A. Cerletti

Pharmacological Laboratory, Sandoz Ltd., Basel

During the last years extensive studies on 5-hydroxy-tryptamine (Serotonin) have shown that this amine exerts a number of peripheral effects quite similar to histamine. Of special importance seems the participation of serotonin in anaphylactic and allergic phenomena (1, 2, 3, 4, 5). For at least some animal species, serotonin can be considered to belong to the so-called H-substances. As shown by *Rowley* and *Benditt* (6) local injection of serotonin in the rat's paw provokes an edema similar to that obtained by using histamine. This serotonin-edema can be prevented according to *West* (7) by relatively large doses (4 mg/kg.) of 2-brom-lysergic acid diethylamide (BOL 148), a specific antiserotonin agent. The two antihistamines promethazine and mepyramine tested by the same author in similar doses had very little or no effect.

In previous studies (8) we tested about 50 lysergic acid derivatives in regard to their inhibitory qualities against serotonin-induced contractions of smooth muscles. We could show that certain substitutions on the ring complex of lysergic acid increase the antiserotonin potency of several semisynthetic amide derivatives of this acid. In other experiments (9) with a series of antihistamines some representatives of this class of drugs were found to exert strong serotonin inhibition in the isolated rat uterus preparation. It seemed to us therefore of interest to compare several lysergic acid derivatives and antihistamines concerning their influence on the development of the edema provoked by serotonin.

Methods

All the experiments were performed on albino Glaxo-rats, weighing 120–170 grams. After having verified that there was no difference in the reactivity of male and female animals, both sexes were used in equal portions for each test. All the animals were anesthetised by subcutaneous injection of a chloralose-urethane mixture which, according to our experience, is without influence on the development of the edema.

Fig. 1. Evaluation of edema by checking the thickness of the rat's paw.

The edema-producing substances, either serotonin or histamine, were injected into one hind-paw of each rat whereas the other, similarly treated by 0.85 % NaCl, served as control. A total volume of 0.1 ml. per injection was used, half of this amount being applied to the plantar and half to the dorsal side of the paw. The needle was inserted between the 3rd and 4th digits and pushed forward to approximately the midpoint of the paw. Each animal was used only once.

The degree of the edema was measured by checking the thickness of the paw in a sagittal diameter by means of a gauge with a scale unit of 0.01 mm., as illustrated in fig. 1. The difference in

thickness between test paw (serotonin, histamine) and the control paw (NaCl) was taken as edema-value. Measurements were made every 15 minutes during two hours. The maximal edema formation occurred within the first half hour and after one hour the values were found to remain practically constant as shown in fig. 2.

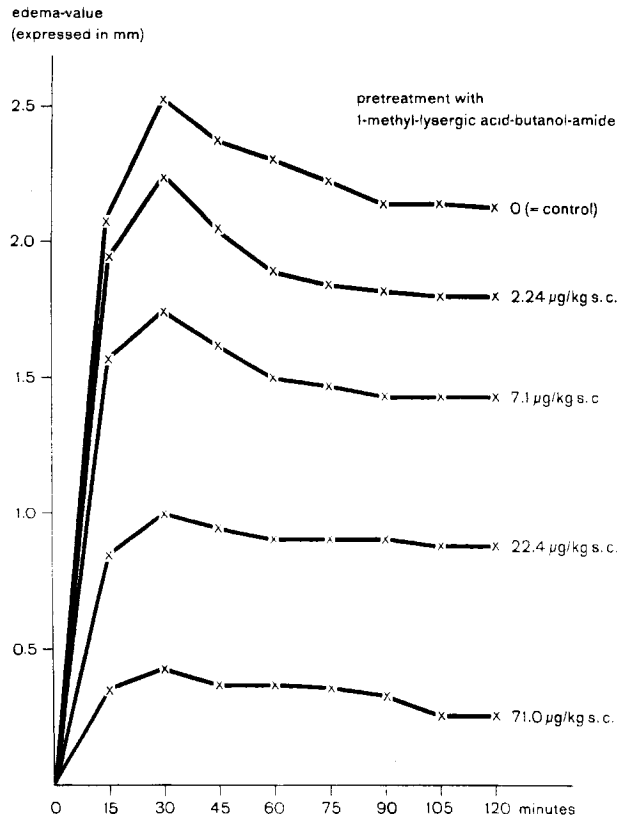

Fig. 2. Edema values (difference of thickness in mm. between the NaCl and serotonin treated paw) obtained during the 2 hours-observation period in a control experiment and in 4 experiments with successively increasing doses of a very potent antiserotonin compound (UML 491 or 1-methyl-lysergic acid butanolamide). Each curve represents the average values obtained from at least 10 animals.

For testing the inhibitory effect of the different compounds the lysergic acid derivatives were injected subcutaneously and the antihistamines intravenously 30 minutes prior to the edema provocation. Each dose was applied to at least 10 animals which were compared with twice as many controls treated by a subcutaneous

or intravenous injection of the NaCl-solution. Using the graphic representation as illustrated in fig. 2 edema-inhibition was estimated planimetrically. The 2 hours-surface value of the controls served as reference and the diminution of the corresponding planimetric values for different doses was expressed in percent. With the percent-values thus obtained regression lines were plotted for each preparation (see examples in fig. 4). These dose-response curves showed in most instances sufficient linearity between 20 and 80% to allow calculation of the ED_{50} (= dose inhibiting 50%) for the different compounds.

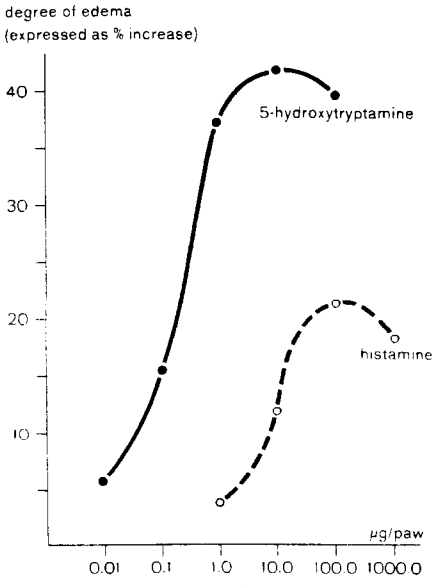

Fig. 3. Comparison of serotonin and histamine in regard to their potency as edema provoking agents in the rat's paw.

Results

1. Edema-producing potency of serotonin and histamine.

In a first experiment we have compared the edema-producing potency of histamine and serotonin at different dose levels, using 20 animals per dose. As shown in fig. 3 the dose-response-curves for serotonin and histamine are in their lower part parallel to each other. Related to the dose scale on the abscissa, they differ however, considerably, indicating an effectiveness of serotonin about 200

Table I
Antiserotonin Potency of Derivatives of D-lysergic Acid

group	name	ED_{50} (Base) in μg/kg	% relative activity (LSD = 100)
LSD-derivatives	D-lysergic acid diethylamide (LSD 25)	56.8	100.0
	dihydro-LSD	470.0	12.1
	1-methyl-LSD (MLD 41)	62.2	91.3
	2-brom-LSD (BOL 148)	196.5	28.9
	1-methyl-2-brom-LSD (MBL 61)	218.0	26.1
Other amide derivatives of D-lysergic acid	D-lysergic acid dimethylamide (DAM 57)	455.0	12.5
	D-lysergic acid ethylamide (LAE 32)	257.0	22.1
	1-methyl-D-lysergic acid ethylamide (MLA 74)	96.8	58.7
Ergotamine group	ergotamine	844.0	6.7
	dihydroergotamine (DHE 45)	833.0	6.8
	1-methylergotamine	430.0	13.2
Ergonovine group	D-lysergic acid propanol amide	122.0	46.8
	dihydro-D-lysergic acid propanolamide	895.0	6.4
	1-methyl-D-lysergic acid propanolamide (PML 946)	21.9	259.4
Methylergonovine group	D-lysergic acid butanolamide	37.4	151.9
	dihydro-D-lysergic acid butanolamide	271.0	21.0
	1-methyl-D-lysergic acid butanolamide (UML 491)	12.9	440.3

times higher than that of histamine. With higher doses only serotonin shows a further increase of effect, thus reaching a maximal value which is nearly twice that of histamine. In all further experiments the dose of 1 μg. serotonin per paw (10 μg./ml.) was used, which regularly results in a submaximal edema-reaction.

2. Inhibition of serotonin edema by lysergic acid derivatives.

Table I summarizes the results we have obtained with 17 compounds representing substances of different groups of lysergic acid derivatives, including ergot alkaloids. In the first column the

absolute values for the ED_{50} of each compound are recorded in terms of μg./kg. base injected subcutaneously. The second column gives the values for relative potency of each compound using the activity of LSD as the 100% reference. In the first two groups no substance shows higher activity than LSD itself. Hydrogenation of LSD leads to a loss of activity of nearly 90% and methylation is practically without influence in contrast to the findings on the isolated rat uterus preparation (8). BOL which was found on the uterus preparation as active as LSD is in this test about 4 times weaker. Methylation of BOL has also no effect in this test. The dimethylamide of lysergic acid could be tested only approximately because of its high toxicity. It is, however, interesting to note that the potency of the monoethylamide of lysergic acid (LAE), which is only 22% of LSD, can be increased more than twice by methylation.

Both ergotamine and dihydroergotamine are much less active and exert an edema-inhibiting effect only in a rather high dose range. Methylation of ergotamine however increases the activity by about 100%.

In the last two groups of table I the very interesting results are summarized which were obtained with the two well known oxytocic compounds ergonovine (= lysergic acid propanolamide) and methylergonovine (= lysergic acid butanolamide) as well as with their dihydro- and methyl-derivatives. Ergonovine itself is surprisingly effective against the serotonin edema, showing an activity only about 50% less than that of LSD. The potency of methylergonovine even exceeds that of LSD. Also in the assay on the rat uterus we had found methylergonovine significantly more active than ergonovine, but still weaker than LSD (8). Whereas hydrogenation of both compounds markedly reduces the activity, methylation leads to very potent derivatives. The preparation UML 491 or 1-methyl-lysergic acid butanolamide (= methylated methylergonovine) shows in 4–6 times smaller doses similar effects to LSD and is the most potent of all the compounds we have tested up to now. In fig. 4 the dose-response curves of the three preparations representing the so-called methylergonovine group are compared with the corresponding LSD-curve. In this as in many other examples hydrogenation of a compound is usually accompanied by a marked decrease of activity. Methylation of the indole N in the lysergic acid, however, seems in several instances to be a step which

can increase the potency from 2 to 6 times, an observation which is also confirmed in our experiments on the isolated rat uterus.

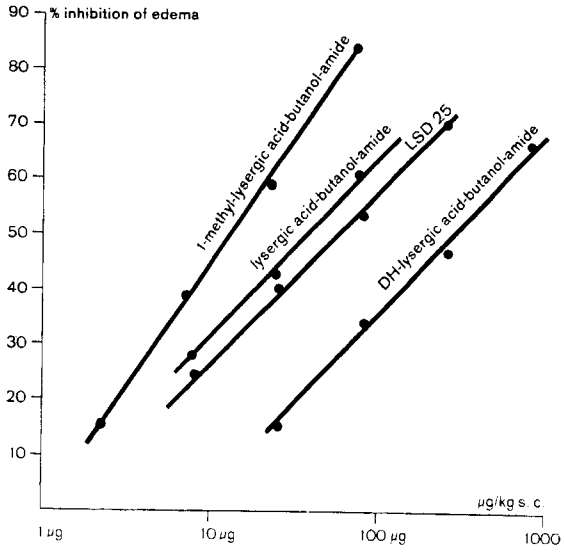

Fig. 4. Dose-response-curves for the edema-inhibiting effect of LSD as standard preparation, and for lysergic acid butanolamide (= Methylergonovine) respectively its hydrogenated and methylated derivatives.

With regard to the amount of serotonin required locally in the paw for provoking the edema reaction, it is interesting to note that this corresponds to approximately 0,5 mg./kg. free base of serotonin. Taking the results with the compound UML 491 as an example calculation of the drug ratio (agonist/antagonist) gives a value of about 40:1. This ratio has been checked by local injection of both agonist and antagonist, i.e. serotonin and UML 491, in the paw, and proved to be correct.

Although some other experimental studies with the two methylated compounds PML 946 and UML 491 are still under way, we have good reason to believe that these derivatives provide an interesting new tool for various investigations on clinical aspects of the serotonin-problem.

3. Inhibition of serotonin edema by antihistamines.

The same 8 compounds, belonging to chemically different groups, which we had previously studied on the isolated rat uterus (9) were also included in the present study. In contrast to the

activity found in the isolated organ, much weaker effects are observed when using the serotonin-edema test in vivo. Due to this fact, evaluation of an ED_{50} is for some preparations not possible and therefore the results are uniformly presented in table II as percent inhibition of the edema produced by i.v. doses of 5 and 10 mg./kg. antihistamine. As can be seen at a first glance, there are marked differences of the antiserotonin activity also within this series of compounds. From the three phenothiazine derivatives tested Chlor-

Table II
Antiserotonin Potency of 8 Antihistamine Compounds

group	name	inhibition of edema dose in mg/kg 5.0	10.0
Phenothiazine-Derivatives	Chlorpromazine	64.0 %	63.3 %
	Promethazine	37.6 %	56.9 %
	Diethazine	6.6 %	17.1 %
Piperidine-Derivatives	4-N-Benzylanilino-1-methyl-piperidine	36.4 %	48.7 %
	Thenophenopiperidine	25.7 %	53.2 %
Pyridindene-Derivative	Phenindamine	13.1 %	25.4 %
Imidazoline-Derivative	Antazoline	2.1 %	13.2 %
Ethylendiamine-Derivative	Tripelennamine	0 %	15.0 %

promazine and Promethazine are more potent serotonin inhibitors than Diethazine. Only slightly less active are the two piperidine derivatives, whereas from the remaining 3 preparations only Phenindamine shows an appreciable effect. This relative potency within the antihistamine series agrees well with our findings on the isolated rat uterus (9) and also with results of previous experiments concerning the effect of antihistamines on dextran- and egg white-edema (10), two forms of experimental edema, which must be attributed to the liberation of serotonin.

By comparing the doses of antihistamines in table II with those of the compounds in table I, it is evident that antihistamines are by far not as potent as the lysergic acid derivatives in inhibiting the serotonin edema. Using the in vitro assay (rat uterus) some antihistamines have shown antiserotonin potencies similar to LSD or LSD-derivatives. The in vivo test, as carried out in the present study on serotonin edema, modifies this picture considerably, since up to 1000 times larger amounts of antihistamines are required to

obtain responses similar to those observed with the most potent compounds from the group of lysergic acid derivatives.

Summary

The edema-provoking effects of locally injected serotonin and histamine in the rat's paw are compared. With 1 μg. serotonin per paw a more marked edema is obtained than with 200 times larger amounts of histamine. This serotonin edema can easily be blocked by various amide derivatives of lysergic acid. Two of these derivatives proved to be definitely stronger antiserotonin compounds than the well known hallucinogenic compound LSD, namely the methyl-derivatives of ergonovine and of methylergonovine. Among the series of 8 antihistamines included in this study, only representatives of the phenothiazine and piperidine group exert a marked inhibition of the serotonin edema, when used in a dose range which is between 100 and 1000 times higher than the effective doses of the most potent lysergic acid derivatives.

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Authors' address: Dr. W. Doepfner and Dr. A. Cerletti, Pharmacological Laboratory, Sandoz Ltd., *Basel* (Switzerland)