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Histamine release inhibition in anti-inflammatory mechanism

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Histamine release inhibition in anti-inflammatory mechanism*

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Abstract

Rats were depleted of skin histamine by more than 80 % by intraperitoneal injections of sinomenine with daily increasing doses for 6 days. In these rats, egg-white edema induced in the hind paws was inhibited by 68 % of control. The weight of the wall of granuloma pouch made by croton oil was also evidently smaller in the rat treated similarly with sinomenine than that of control. This suggests an important role of histamine participating in the inflammation. It has been observed that a variety of non-steroidal anti-inflammatory drugs inhibited both degranulation and histamine release induced by compound 48/80 of mast cells isolated from rat peritoneal fluid. The degranulation inhibiting actions of anti-inflammatory drugs were markedly decreased in the presence of glucose as in cases of dinitrophenol, dicumarol and warfarin which are known uncouplers of oxidative phosphorylation. Also, prevention of edema provoked by anti-rat serum is roughly correlated to a potency of degranulation inhibiting effect of anti-inflammatory agents. These observations suggest that there is a common mechanism between these two phenomena, and the prevention of mast cell degranulation by the anti-inflammatory agents is, at least, partially due to their uncoupling effects. A working hypothesis explaining the process of edema formation at the inflammatory site has. been made based on the data of the present experiment and other ob3ervations: a leakage of plasma into the tissue space from the gap between two adjacent endothelial cells which are contracted by released histamine may activate a kinin-forming system in the plasma, and kinin(s) may further aggravate a leakage. The mechanism of action of anti-inflammatory agents, which interfere with the histamine effect in inflammation, should be understood in twofold: one is prevention of histamine release from the tissue, mainly by inhibiting mast-cell degranulation, and the other is prevention of the contraction of endothial cells by their uncoupling activities.

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HISTAMINE RELEASE INHIBITION IN ANTI-INFLAMMATORY MECHANISM

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Inflammation is a complex process involving many biochemical changes with the participation of several mediators. Since a number of factors should be considered, it is not easy to elucidate the role of a single mediator in the development of inflammation. Although it has been suggested that histamine plays an important role in inflammation, it is not possible to explain the whole set of events only by histamine action.

In recent years, much attention has been turned to the other factors participating in the inflammation, such as serotonin (1) and bradykinin (2, 3). However, the degree of participation of each mediator is not the same regarding the etiology of inflammation and also varies with the species of animals (4). It has been mentioned that the common feature in various types of experimental inflammation is in delayed, prolonged permeability response, and histamine acts only in immediate, transient response. This immediate response of histamine can be seen both in rabbits and guinea pigs but not in rats (5). The present report concerns the experiments performed by using the rat to see the degree of histamine participation in experimental inflammation.

METHODS

Measurement of egg-white edema and its inhibition: Male albino rats weighing 100-150 g were used in this experiment because histamine content of the skin is relatively constant in this weight range (6) and males are more sensitive to egg white than females (7).

The egg-white edema was induced by the subcutaneous injection of 0.1 ml of fresh egg white diluted ten times with 0.85% saline solution into the dorsa of the hind paw. The intensity of edema was measured with a micrometer gauge with weakened spring and was represented as the difference of the dorso-planter thickness in mm of hind paw before and after the injection of egg white. As the edema could be produced in the same degree on both paws of one rat, one side

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was used as control. On the day after the control edema was measured, edema was produced on the other hind paw of the same rat administered with drug to be tested. The percentage inhibition was calculated from the following equation: $(En-Et)\ 100/En$, where En represents intensity of control edema (mm), and Et (mm) is intensity of edema after drug administration.

Measurement of histamine release from the skin and its inhibition: A small piece (100—200 mg) of the skin on one side of the abdomen was excised aseptically under light ether anesthesia. This was performed three days before the drug administration because this period is required for the recovery of the skin histamine contents from the remote injury (8). Similar excision on the corresponding side was made between 24 and 30 hr after the drug administration to determine the histamine release from the skin. To determine the effect of drugs in inhibiting the histamine release, 3600 mg/kg of dextran was injected intraperitoneally in one group, and in another group a drug to be tested was administered before dextran injection. The percentage reduction of skin histamine in these two groups was compared. Extraction and bioassay of histamine were made by the methods described by SANUKI (9). The histamine content was expressed by the weight of the base.

Granuloma pouch: Fifteen milliliters of air was injected into the subcutaneous connective tissue between the shoulder blades of male albino rats weighing 100–130 g and 1 ml of 0.5 % croton oil dissolved in corn oil was injected into a resulting air space. For the measurement of intensity of inflammation, total weight of the pouch was measured. The pouch wall was stripped off from the skin and underlying tissues cautiously, and the oval pouch containing an exudate was extirpated. After an exudate was removed, the pouch was weighed. To determine histamine content, 0.2–0.5 g of the tissue specimen was taken from the pouch wall.

Anti-inflammatory assay: Evaluation of anti-inflammatory effect was made by the punch method devised by UNGAR *et al.* (10), in which anti-inflammatory effect of drug is determined by measuring the inhibition of cutaneous edema provoked by antiserum. Male Wistar rats weighing about 150 g were used in this experiment. Each animal was injected with the lyophilized anti-rat rabbit serum 5 mg in 0.05 ml normal saline, adjusted to pH 7.2, per site intracutaneously on the shaved skin of the back, 4 sites on one side, while in corresponding 4 sites on the other side saline injection of the same quantity was made. Two hr after the injection of antiserum, when the edema reached its maxium, the animal was stunned and exsanguinated. The removed back skin was pinned on a board and injected sites were cut with a steel punch. Each piece of skin, 112 mm^2, was weighed immediately after removal. Intensity of inflammation was expressed as follows: $(Wi-Ws)\ 100/Ws$, where Wi is the weight of the inflamed site and Ws is the weight of the saline injected site. Sixty-four experiments on sixteen control animals gave a mean of 89 ± 0.68 (S. E.). Anti-inflammatory activity was expressed as per cent inhibition of inflammation: $(Ic-It)\ 100/Ic$, where Ic is intensity of inflammation in the control animal and It is the intensity in the animal treated with the drug. Drugs

were usually administered intramuscularly 2 or 3 hr before the injection of antiserum and animal was killed 2 hr later. In cases of testing the effects of metabolic inhibitors, antiserum injection was given 30 min after adminstration of inhibitors.

Experiments on mast cells: After exsanguination of Wistar rats of either sex weighing 150–300 g, 5 ml of buffered physiological solution containing 0.1 % w/v bovine serum albumin were injected into the peritoneal cavity and gentle massage of the abdominal wall was done for 90 sec. The mast cells were isolated from peritoneal fluid by differential centrifugation using a 37 % (w/w) solution of bovine serum albumin. They were suspended in a physiological buffer solution and kept on ice before use. Mast cells ($0.5–1 \times 10^5$) were suspended in 0.95 ml of the buffer solution either in the presence or absence of the drug and incubated for 15 min at 37°C. After addition of 0.05 ml of compound 48/80 to make the concentration of 0.5 μg/ml, incubation was continued for another 15 min. At the termination of incubation, mast cells were fixed with 4 % formaldehyde. Morphological changes were observed by means of the invert-type phase contrast microscope, and percentage of degranulated mast cells was estimated. Inhibitory effect of the drug was calculated from (A-B) 100/A, where A and B are the percentages of degranulated mast cells in the media with and without drug, respectively.

To determine histamine release from mast cells, the mast cell suspension incubated with compound 48/80 was centrifuged for 10 min at $5000 \times g$ in a refrigerated centrifuge. A few drops of water were added to the precipitate containing mast cells. After freezing and thawing it was diluted with buffer solution. Histamine in the supernatant and the precipitate was determined on atropinized guinea-pig ileum. None of the drugs tested interfered with histamine assay at the concentrations used in the present experiment.

RESULTS

1. *Histamine depletion and inhibition of egg-white edema*

Sinomenine is an alkaloid derived from the roots of *Sinomenium acutum* REHDER *et* WILSON. A single intraperitoneal injection of this compound caused a marked reduction of histamine content in the abdominal skin of the rat, especially in the inner layer of the skin. When sinomenine was injected intraperitoneally to rats for 6 consecutive days, twice a day starting with 50 mg/kg and increasing daily dose by 100 mg/kg, an average of 88.2 % reduction of histamine content in the abdominal skin was achieved. Symptoms attributable to the released histamine, such as reddening around the ears, snout, sole of paws and genital areas, itching of the face, sometimes cyanosis accompanied by severe dyspnea and prostration, were observed. But, during the repeated injections appearance of these symptoms decreased gradually in spite of increasing dose.

In those animals depleted the skin of histamine anaphylactoid edema

TABLE 1 INHIBITION OF EGG-WHITE EDEMA OF THE HIND-PAW AND REDUCTION OF HISTAMINE OF THE ABDOMINAL SKIN OF RAT BY HISTAMINE RELEASERS. EGG-WHITE EDEMA WAS INDUCED 24 HR AFTER THE END OF INJECTIONS OF SINOMENINE, 48/80 AND DEXTRAN

Drug	mg/kg (i. p.)	No. of rats	Edema inhibition (%)	Histamine reduction (%)
Sinomenine	50	6	45.6±1.6	32.5±3.7
Sinomenine	500*	6	62.9±2.6	39.8±2.7
Sinomenine	2100†	6	68.4±1.3	88.2±2.0
48/80	1	6	24.3±2.0	18.4±0.78
Dextran	3600	6	43.0±2.9	30.4±3.5

* 50 mg/kg×2, for 5 days.

† 50 to 300 mg/kg×2, daily increasing for 6 days.

induced by egg white in the hind paws was markedly inhibited. Table 1 shows histamine depletion and inhibition of egg-white edema in rats receiving a single intraperitoneal injection of 50 mg/kg of sinomenine, 1 mg/kg of compound 48/80 and of 3600 mg/kg of dextran and also after consecutive injections of sinomenine to the total doses of 500 mg/kg and 2100 mg/kg. In the latter two groups, inhibition of edema was more than 60 %. As far as these three compounds are concerned, there is a rough correlation between histamine depletion and edema inhibition.

2. *Edema inhibition in association with prevention of histamine release by some anti-inflammatory drugs*

Table 2 shows the effects of some anti-inflammatory drugs on the histamine content and the inhibition of edema when these drugs were given intraperitoneally one hr before the egg-white injection to the hind paw. Although all of them had some inhibitory effects in greater or lesser extent on the edema formation, none of them showed a significant reduction of the histamine content. Similar experiments performed with dextran alone and simultaneous administration with some anti-inflammatory agents

TABLE 2 INHIBITION OF EGG-WHITE EDEMA OF THE HIND-PAW AND CHANGE IN HISTAMINE CONTENT OF THE ABDOMINAL SKIN BY ANTI-INFLAMMATORY DRUGS. DRUGS WERE INJECTED 1 HR BEFORE EDEMA PRODUCTION

Drug	mg/kg (i. p.)	No. of rats	Edema inhibition (%)	Change of histamine content (%)
Sod. salicylate	250	6	26.2±1.2	0±1.1
Aminopyrine	100	6	28.1±2.4	0.6±0.97 (increase)
Butazolidine sod.	100	6	26.0±1.7	0.4±0.72 (increase)
Cortisone	100*	6	18.2±1.4	6.6±1.4 (increase)
Guaiazulene	200	6	51.6±1.6	4.0±2.3 (increase)
Guaiazulene	100	6	24.9±2.3	—
Guaiazulene	50	6	18.1±2.3	—

* 50 mg/kg (i. m.) 24 hr and 1 hr before edema.

TABLE 3 EFFECTS OF ANTI-INFLAMMATORY DRUGS ON THE EDEMA INHIBITION (HIND PAWS) AND THE HISTAMINE REDUCTION (ABDOMINAL SKIN) INDUCED BY DEXTRAN

Drug (mg/kg)*	No. of rats †	Edema inhibition (%)	Histamine reduction (%)	No. of rats ‡	Histamine reduction (%)
Dextran (3600) alone	6	43.0±3.0	30.4 ±4.2	6	30.4±4.2
Sod. salicylate (250)+Dextran (3600)	6	57.2±2.8	4.3 ±1.8	6	10.2±1.6
Aminopyrine (100) + 〃	5	54.9±3.3	18.5 ±3.5	6	20.8±1.2
Cortisone (100) + 〃	6	59.6±2.2	13.9 ±2.5	6	15.9±1.2
Guaiazulene (100) + 〃	5	51.5±3.7 §	0.27±2.7	6	17.3±2.1
Phenergan (5) + 〃	6	46.1±1.9 §	15.9±1.9	6	21.7±2.5 §

* Dextran was injected intraperitoneally.

† In these series of experiments sodium salicylate, aminopyrine and guaiazulene were given intraperitoneally 1 hr before, phenergan subcutaneously 30 min before dextran, cortisone intramuscularly 50 mg/kg, 24 hr and also 1 hr before dextran.

‡ In these series cortisone was given 50 mg/kg×2, 48 and 24 hr, and the other drugs 24 hr before dextran by similar routes in the † series.

§ No significant difference from the effect of dextran alone at the value of P as 0.05.

are shown in Table 3. Histamine depletion by dextran alone was evidently reduced when these anti-inflammatory agents were given simultaneously. On the contrary, edema inhibition of dextran was clearly enhanced by simultaneous administrations of these drugs. The results so far mentioned suggest that there are at least two mechanisms in inhibiting edema formation; one is the histamine depletion from the tissue and the other is due to the prevention of histamine release.

3. *Granuloma pouch*

Granuloma pouch technique described by SELYE was employed as the other means of studying acute inflammation. Since croton oil disrupts mast cells of the rat skin more drastically than egg white (11), this material may be suitable for studying the role of histamine in a severe inflammation. To know the detailed histamine participation in this inflammation, following experimental schedules have been made. The experiment was carried out on 5 groups, each with 2-5 animals. Experimental schedule in each group is shown in Fig. 1: (a) is non-treated control, (b) is sinomenine-treated, (c) is sinomenine and cortisone-treated, (d) is histamine-treated and (e) is aminoguanidine-treated group. The dosage and period of the drug administration in each group are shown in Fig. 1.

Histamine contents in the abdominal skin after the pouch formation are shown in Fig. 2. The mean value of 37 μg/g in the control group was almost the same as that obtained from normal rats. Both in histamine-treated and aminoguanidine-treated groups, histamine contents of the skin were almost twice of the normal value. On the contrary, in the

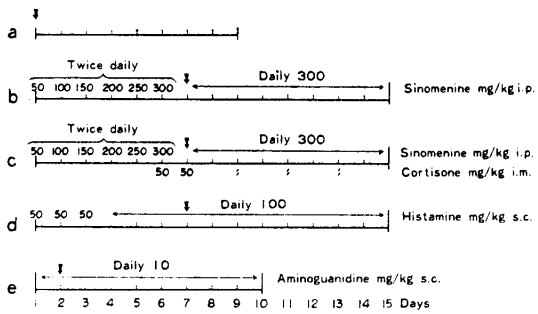

Fig. 1 Scheme of experimental design. a) control, b) sinomenine-treated, c) sinomenine and cortisone-treated, d) histamine-treated, and e) aminoguanidine-treated. At arrow, granuloma pouch was made.

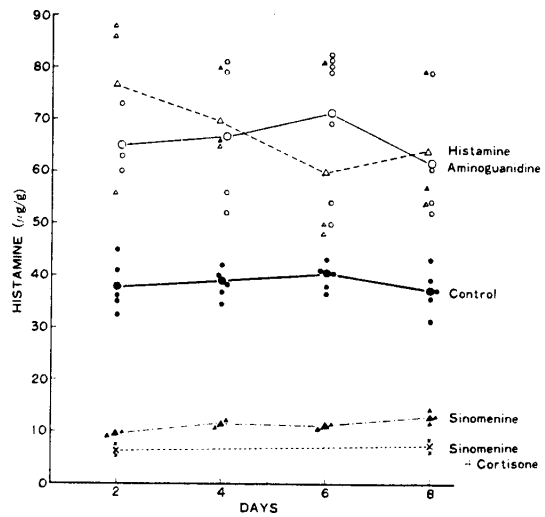

Fig. 2 Effects of repeated daily injections of sinomenine, sinomenine plus cortisone, histamine and aminoguanidine on the histamine content of the abdominal skin after granuloma pouch formation.

sinomenine-treated and in the sinomenine and cortisone-treated groups, the values were less than one third of the control value. It is noticed that the changes of mean values in all groups dropped in fairly narrow range after the formation of granuloma pouches. Especially, the fact that the mean value of histamine content in the control group differed not much from the normal value indicates that formation of the pouch did not change the skin histamine remarkably.

Fig. 3 shows the time course of changes in histamine content of the pouch wall in different groups. These changes, in general, are similar to

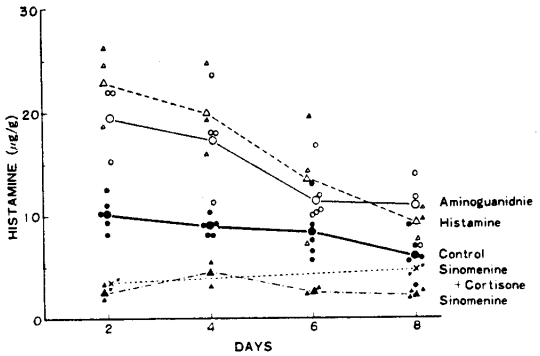

Fig. 3 Time course of changes in histamine content of the pouch wall during the repeated daily administrations of sinomenine, sinomenine plus cortisone, histamine and aminoguanidine

those of the skin histamine contents. However, after the fourth day the values declined gradually in both the histamine- and aminoguanidine-treated groups and also in the control group. The reason for such a decrement may simply be due to edematous swelling, not causing decrease in the total histamine amount (*cf.* Fig. 4).

The variation in the weight of the pouch wall in different groups is shown in Fig. 4. In the control group, the high values were shown from the 4th to the 6th day. The weights of the pouch wall both in the sinomenine-treated and sinomenine and cortisone-groups were remarkably smaller

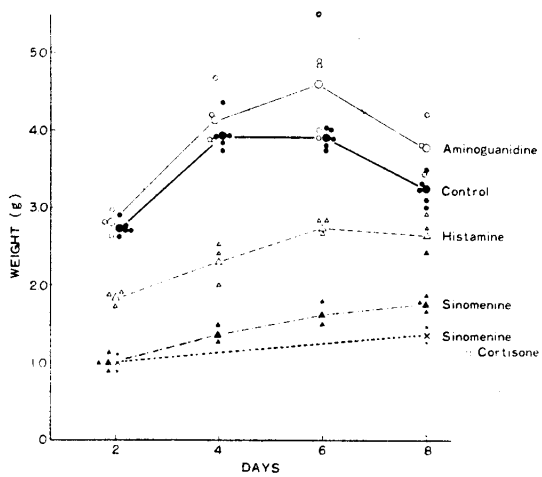

Fig. 4 Changes in weight of the granuloma pouch wall during the consecutive daily administrations of sinomenine, sinomenine plus cortisone, histamine and aminoguanidine.

than that of control. The weight of the pouch wall in histamine-treated group was also less than that of the control group. A decreased reactivity to histamine after its desensitization may be the reason for inhibition of edema formation.

4. *Inhibition of mast-cell degranulation*

a. *Anti-inflammatory agents and related compounds*

Dose-response relationship of the inhibitory effect of some anti-inflammatory agents on mast-cell degranulation induced by compound 48/80 is shown in Fig. 5. Oxyphenbutazone was the most potent inhibitor in

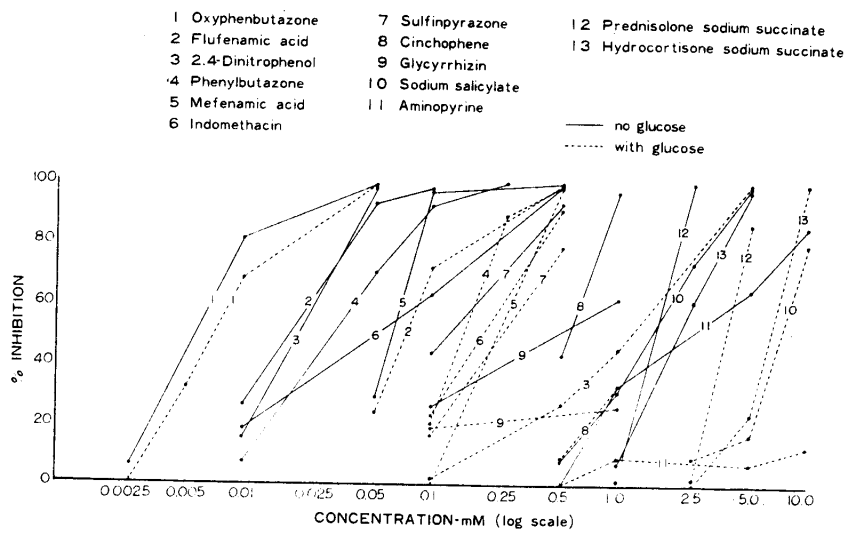

Fig. 5 Dose-response relationship of inhibitory activity of some anti-inflammatory drugs on mast-cell degranulation in the presence and absence of glucose (5.6 mM). Each point is the mean of at least 4 determinations.

this experiment either with or without glucose (5.6 mM). Marked inhibitory effects were also seen in flufenamic acid, phenylbutazone, mefenamic acid and indomethacin decreasing in potency in that order. Especially in indomethacin, superior inhibition was noticed rather in lower concentrations. In general, the presence of glucose in the medium lowered the inhibitory effect of the anti-inflammatory agents.

b. *Inhibition of histamine release from mast cells*

Fig. 6 shows both mast-cell degranulation and histamine release induced by compound 48/80 are being inhibited by pretreatment with phenylbutazone and sodium salicylate. Percentages of inhibitions of histamine release and of degranulation run parallel with the variance of drug con-

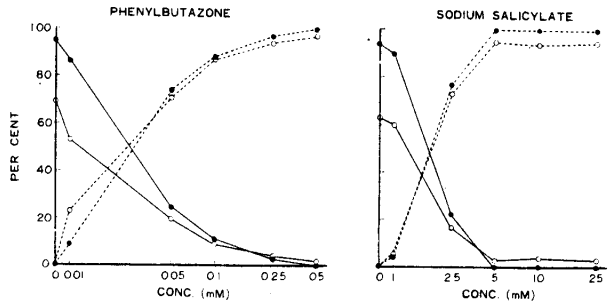

Fig. 6 Parallel inhibition by phenylbutazone and sodium salicylate of mast-cell degranulation and histamine release induced by compound 48/80 (0.5 μg/ml) in the absence of glucose.
Degranulation (●——●); histamine release (○——○); inhibition of degranulation (●·····●); inhibition of histamine release (○·····○).

centration, indicating that these drugs inhibit the morphological changes in mast cells leading to the release of histamine.

c. *Metabolic inhibitors*

See Table 4. Mast-cell degranulation by compound 48/80 was

TABLE 4 EFFECTS OF METABOLIC INHIBITORS ON MAST-CELL DEGRANULATION BY COMPOUND 48/80 AND ON INFLAMMATORY EDEMA BY ANTISERUM

Drug	Conc. of 50% inhibition of M. C. degranulation		Anti-inflammatory effect	
	No glucose	With glucose*	Dose	Reduction of weight increase
	mM	mM	mg/kg	%±S. E.
a. Inhibitors of respiration or oxidative phosphorylation				
2,4-Dinitrophenol	0.02	1.2	30	26±0.9 (38±1.1†)
Dicumarol	0.02	0.5	30	15±1.4 (32±1.0†)
Warfarin sodium	0.16	6.0	30	33±0.8 (38±0.5†)
Amytal sodium	0.5	2.6	30	30±1.1
b. Inhibitors of glycolysis or glucose transport				
Phlorizin		<1.0‡	30	50±1.0
2-Deoxyglucose		5.6‡	250	39±1.1†
Iodoacetate	0.5	0.5	30	42±0.8
Sodium fluoride		10§	30	50±0.9

* 5.6 mM.
† Injected s. c. 30 min before antiserum, while all the others were injected i. m. 2 hr before.
‡ Under anaerobic condition.
§ Under anaerobic condition, in Ca^{2+}-free medium.

inhibited when pretreated with low concentrations of 2, 4-dinitrophenol, dicumarol and warfarin which are known as uncouplers of oxidative phosphorylation. Amytal, a respiratory inhibitor, also produced a moderate inhibition. These inhibitions were largely enervated in the presence of glucose as in the cases of inhibition by anti-inflammatory agents (Fig. 5). Mast cells cannot be degranulated by compound 48/80 when oxygen is lacking, but this inability is reversed when glucose is present in the medium. This indicates that glycolysis is one of the suppliers of energy required for the mechanism of mast-cell degranulation. Some of the inhibitors of glycolysis or glucose transport could also inhibit mast-cell degranulation by 48/80 in the glucose containing medium in anoxia (Table 4, b). These results imply that at least a part of the inhibitory effect, abolished in the presence of glucose, of anti-inflammatory agents on mast-cell degranulation may concern the glucose dependent action which escaped likewise from the inhibitory action of uncouplers or respiratory inhibitors by the presence of glucose. Actually, some anti-inflammatory drugs such as salicylate, indomethacin and phenylbutazone are known as inhibitors of oxidative phosphorylation as reported by ADAMS and COBB (12) and also by WHITEHOUSE (13).

5. *Anti-inflammatory effect*

a. *Anti-inflammatory agents and related compounds*

Among salicylates and related compounds in the present test, sodium salicylate, acetylsalicylic acid and 2, 5-dihydroxybenzoic acid were effective in inhibiting mast-cell degranulation. These compounds also moderately inhibited the inflammatory edema induced by anti-rat serum by 43, 33 and 30 %, respectively as shown in Table 5. To our interest, while salicylic acid and 2, 5-dihydroxybenzoic acid were effective inhibitors of mast-cell degranulation and of inflammatory edema, *m*- and *p*-isomers of salicylic acid, and 2, 4-, 2, 6- and 3, 5-dihydroxybenzoic acids were all less effective in inhibiting either reaction.

Of the pyrazolone derivatives, sulfinpyrazone, oxphenbutazone, phenylbutazone and aminopyrine showed marked anti-inflammatory effects, while antipyrine and 4-aminoantipyrine were ineffective. Among non-steroidal agents, indomethacin had the most potent anti-inflammatory activity followed in order by mefenamic acid, glycyrrhizin and chloroquine phosphate. Prednisolone was the most effective anti-inflammatory agent tested in the present experiment. These results show that there is a gross correlation between anti-inflammatory effect and degranulation-inhibiting effect in many drugs tested, except chloroquine, aminopyrine and the glucocorticoids which showed disproportionally strong anti-edema effect compared to the weak inhibition on mast-cell degranulation.

TABLE 5 EFFECTS OF ANTI-INFLAMMATORY AGENTS AND RELATED COMPOUNDS ON MAST-CELL DEGRANULATION BY COMPOUND 48/80 AND ON INFLAMMATORY EDEMA BY ANTISERUM

Drug	Conc. of 50% inhibition of M. C. degranulation		Anti-inflammatory effect	
	No glucose	With glucose*	Dose	Reduction of weight increase
	mM	mM	mg/kg	%±S. E.
a. Salicylates and related compounds				
Sodium salicylate	1.5	7.2	250	43±0.5
Acetylsalicylic acid	1.1	2.8	250	33±1.0
Salicylamide	1.0	5.0	250	20±0.6
m-Hydroxybenzoic acid	16	30	250	23±1.5
p-Hydroxybenzoic acid	17	18	250†	19±1.1
2,5-Dihydroxybenzoic acid	0.9	2.8	250	30±1.9
2,4-Dihydroxybenzoic acid	7.8	29	250	13±1.3
2,6-Dihydroxybenzoic acid	2.5	5.2	250	23±1.0
3,5-Dihydroxybenzoic acid	5.2	29	250	14±1.7
5-Sulfosalicylic acid	17	17	250	11±0.7
p-Aminosalicylic acid	2.0	2.3	250	9±1.3
Thiosalicylic acid	0.7	2.0	30	9±0.7
Salicylaldehyde	0.07	1.9	250	18±0.7
Benzoic acid	11	16	250†	16±1.4
b. Pyrazolone derivatives				
Antipyrine	16	16	30	7±1.1
			250	22±0.9
Aminopyrine	2.4	15	30	38±0.4
4-Aminoantipyrine	5.4	17	30	9±1.0
Phenylbutazone	0.03	0.15	30	48±1.1
Oxyphenbutazone	0.006	0.007	30†	52±0.5
Sulfinpyrazone	0.12	0.24	30†	36±1.3
c. Other non-steroid agents				
Anthranilic acid	7.8	28	30	27±2.0
Flufenamic acid	0.018	0.07	30	40±2.0
Mefenamic acid	0.062	0.23	30	49±1.3
Indomethacin	0.05	0.19	30†	57±1.3
Glycyrrhizin	0.46	〃	30	46±2.2
Cinchophen	0.55	1.6	250	30±1.4
Chloroquine phosphate	3	3.6	25†	42±1.2
d. Glucocorticoids				
Hydrocortisone sodium succinate	2.1	6.4	30	47±1.1
Prednisolone sodium succinate	1.6	3.8	5	47±0.7

* 5.6 mM.

† Injected i. m. 3 hr before antiserum, while all the others were injected 2 hr before.

b. *Effect of metabolic inhibitors*

In relation to the assumption that the effects of some anti-inflammatory agents may be due partially to the property as uncoupler of oxidative phosphorylation, the anti-inflammatory effect of some typical uncouplers was investigated. As shown in Table 4, DNP, dicumarol, warfarin and amytal sodium produced a considerable anti-inflammatory effect at the dose of 30 mg/kg. Fig. 7 shows the time course of the anti-inflammatory

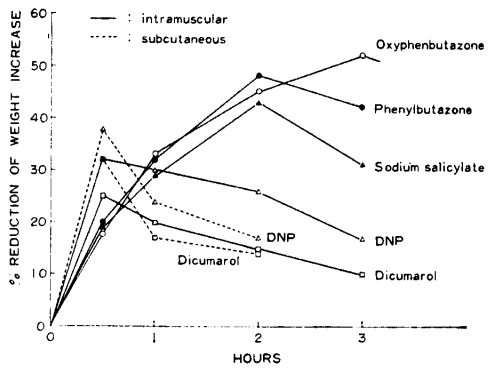

Fig. 7 Time course of inhibitory effect of some metabolic inhibitors and anti-inflammatory drugs on inflammatory edema induced by antiserum. Ordinate: Per cent reduction in weight increase of skin induced by antiserum. Abscissa: Time intervals at which antiserum was injected after intramuscular or subcutaneous administration of drugs.

effect of some metabolic inhibitors on the edema induced by antiserum injection. The effects of DNP and dicumarol are short lived both in subcutaneous and intramuscular injections, reaching maximum effects within 30 min after injection. The transient effect of DNP may be due to the rapid biotransformation to inactive metabolites such as 2-amino-4-nitrophenol, which are weaker uncoupling agents, in the body (14).

DISCUSSION

From the results of experiment in egg-white edema, it is suggested that there are at least two mechanisms in inhibiting egg-white edema: one is the histamine depletion and the other is the prevention of histamine release from the tissue. With a single dose of sinomenine, compound 48/80 and dextran, edema inhibition was effected roughly in parallel with histamine depletion from the skin; and consecutive sinomenine injections gave more than 60 % inhibition. Edema inhibition after histamine depletion from the tissue seems to provide circumstantial evidence for histamine participa-

tion in the inflammation, even though it may not be free from some critical points as follows: 1) it is very difficult to release the whole tissue histamine and this hinders clear cut understanding of the role of histamine in inflammation; 2) some other mediators such as serotonin may be released at the inflammatory site, although this seems to be minor possibility as discussed later; 3) the non-specific action of histamine releaser itself can not be dismissed.

It has been shown that in the sinomenine-treated group the weight of the granuloma pouch was remarkably smaller than that of the control group. In the histological examination, vasodilatation and exudation were strongly inhibited in the pouch wall accompanied by a slight leukocyte emigration in sinomenine-treated group (15). This fact suggests an important role of histamine in producing these inflammatory reactions. Sinomenine may also release serotonin, because compound 48/80 which is a strong basic histamine liberator like sinomenine is known to liberate these two amines simultaneously (16). However, the fact that leaking of blue dye at the site of intradermal injection of sinomenine was almost identical even after reserpinization in rats, while it was weakened in the case of 48/80 and dextran (17), seems to contradict such a possibility. This agrees with the finding of STERN and NIKULIN (18) who observed that the histological structure of the granuloma pouch remained unchanged after depletion of serotonin, while depletion of histamine caused marked change. From this observation, they emphasized principally the active role of histamine in inflammatory process, especially in dilatation and permeability increase of the vessels. The data presented in the experiment on isolated rat mast cells show that there are at least two different types of anti-inflammatory effects in anti-inflammatory agents. One is prevention of mast-cell degranulation induced by compound 48/80 and the other is inhibition of edema induced by anti-rat serum. Since these two effects are roughly correlated in most of the drugs tested and also uncouplers of oxidative phosphorylation simulate these two effects of anti-inflammatory agents, it is suggested that there is a mechanism common to these two effects which are both energy dependent. Actually, some anti-inflammatory agents such as salicylate, indomethacin and phenylbutazone are reported as inhibitors of oxidative phosphorylation. However, there are a few exceptional drugs showing disproportionally potent edema inhibition compared to their weak degranulation-preventing effects. Even in such drugs, inhibition of histamine release was remarkably noticed. Marked inhibition of histamine release provoked by dextran was shown under the effects of cortisone, as well as prednisolone, hydrocortisone and dexamethasone, in the rat (19). It is known that histamine

release caused by an exposure to antigen of sensitized tissues was inhibited by sodium salicylate (20, 21). The mechanism of anti-edema effect of chloroquine phosphate is possibly, at least in part, related to its anti-histaminic action (22) in interfering with the contraction of the endothelial cells by released histamine. There are microscopic and electron microscopic findings (23, 24) showing a wide opening of intercellular space between two adjacent endothelial cells which become globular in form responding to histamine and some other injurious stimulations. It may not be unreasonable to assume that this morphological change of endothelial cells is energy dependent. SPECTOR (25) has pointed out the fact that all pharmacologically active agents increasing the permeability of capillary, contract the smooth muscle. SPECTOR (25) and MCLEAN, AHMED and JUDAH (26) speculated that these active substances contract the endothelial cells and provoke a leakage of the plasma. It is known that all the materials necessary for a kinin formation are contained in the plasma and simple matters such as dilution of the plasma or its contact to foreign bodies activate the Hageman factor and this motivates a kinin formation.

On the basis of speculation referring to the data so far presented and the proposal of other investigators a scheme shown in Fig. 8 is postulated.

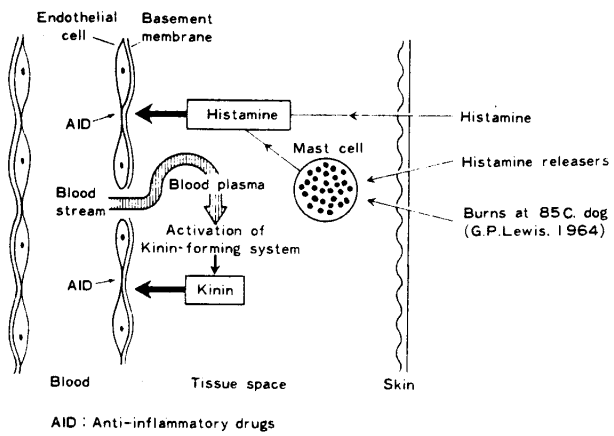

Fig. 8 Presumed mechanisms of acute inflammatory responses and their inhibition by anti-inflammatory drugs of uncoupler type.

At the primary stage of inflammation, histamine, and some other possible factors such as serotonin, are released from the tissue and histamine acts upon the endothelium of the capillary and widens the gap between two adjacent endothelial cells. This may cause a leakage of blood plasma into the tissue space and a kinin-forming system in the plasma may be activated

in the tissue space. If this were the case, a kinin formed in such a sequence further acts upon the capillary and produces an additional leaking. Thus, a vicious circle starts. If these events really do occur, then histamine is a primary mediator initiating a kinin formation as proposed by LEWIS (27) in his demonstration with a dog foot. From these considerations, it is clear that an inhibition of histamine release by anti-inflammatory agents will be one of the important mechanisms in displaying their effects. Uncoupler type anti-inflammatory agents may exert their effects by preventing the contraction of the endothelial cells in a manner to lessen an energy supply.

SUMMARY

Rats were depleted of skin histamine by more than 80 % by intraperitoneal injections of sinomenine with daily increasing doses for 6 days. In these rats, egg-white edema induced in the hind paws was inhibited by 68 % of control. The weight of the wall of granuloma pouch made by croton oil was also evidently smaller in the rat treated similarly with sinomenine than that of control. This suggests an important role of histamine participating in the inflammation.

It has been observed that a variety of non-steroidal anti-inflammatory drugs inhibited both degranulation and histamine release induced by compound 48/80 of mast cells isolated from rat peritoneal fluid. The degranulation inhibiting actions of anti-inflammatory drugs were markedly decreased in the presence of glucose as in cases of dinitrophenol, dicumarol and warfarin which are known uncouplers of oxidative phosphorylation. Also, prevention of edema provoked by anti-rat serum is roughly correlated to a potency of degranulation inhibiting effect of anti-inflammatory agents. These observations suggest that there is a common mechanism between these two phenomena, and the prevention of mast cell degranulation by the anti-inflammatory agents is, at least, partially due to their uncoupling effects.

A working hypothesis explaining the process of edema formation at the inflammatory site has been made based on the data of the present experiment and other observations: a leakage of plasma into the tissue space from the gap between two adjacent endothelial cells which are contracted by released histamine may activate a kinin-forming system in the plasma, and kinin(s) may further aggravate a leakage. The mechanism of action of anti-inflammatory agents, which interfere with the histamine effect in inflammation, should be understood in twofold: one is preven-

tion of histamine release from the tissue, mainly by inhibiting mast-cell degranulation, and the other is prevention of the contraction of endothial cells by their uncoupling activities.

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