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THE EFFECT OF NON-STEROIDAL ANTI-INFLAMMATORY DRUGS ON HISTAMINE FORMATION IN THE RAT

BY

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Histamine has been implicated as a mediator of the inflammatory process ever since the description of the "triple response" by Lewis in 1927. It is released after burns, chemical injury, bacterial invasion, and X-ray injury (Spector, 1958) as well as in anaphylactic shock in dogs (Dragstedt & Gebauer-Fuelnegg, 1932) and guinea-pigs (Code, 1939). Histamine was recently found to be formed at a high rate in tissues of mice subjected to burns (Schayer & Ganley, 1959), endotoxin shock (Schayer, 1960) and tourniquet shock (Schayer, 1962), as well as in rat lung after endotoxin shock (Schauer, Menzinger & Gielow, 1966) and in different tissues of rat and guinea-pig after anaphylactic shock (Kahlson, Rosengren & Thunberg, 1964, 1966; Radwin & West, 1967a).

Whitehouse & Skidmore (1965) found that some non-steroidal anti-inflammatory drugs inhibit the histidine decarboxylase activity of rat pyloric stomach and foetal liver and they suggested that these drugs exert their anti-inflammatory action through this effect. The present study is an extension of this work, the action of non-steroidal anti-inflammatory drugs being tested on the histidine decarboxylases of different sources.

METHODS

Groups of four male Sprague-Dawley rats (body weight 120–150 g) obtained from Fisons Pharmaceuticals Ltd. (Holmes Chapel) were used in most experiments. The foetal material was obtained from female rats 17–19 days after mating. All rats were fed on a cube diet (No. 41B, Associated London Flour Millers Ltd.), allowed free access to drinking water, and housed at 21° ± 0.5° C.

Preparation of tissue extracts

Pooled tissues from freshly killed animals were cut into small pieces and homogenized in 0.9% w/v saline (5 ml./g) in a glass homogenizer. Foetuses were cut up whole. The homogenate was then centrifuged at 5,000 *g* for 15 min in a refrigerated centrifuge, and aliquots of the supernatant (equivalent to 50 or 100 mg tissue) were removed for the incubation experiments.

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Bacterial histidine decarboxylase

This was obtained as a dry powder from Nutritional Biochemicals Corporation (Cleveland, Ohio) and was dissolved in isotonic saline (0.9% w/v sodium chloride) to provide a solution containing 10 μg/ml. Aliquots of 0.5 ml. were used for the incubation experiments.

Histidine decarboxylase activity of tissue extracts

This was estimated using the method developed by Kobayashi (1963), in which the tissue extract (or the bacterial enzyme) is incubated with carboxyl-labelled ^{14}C-histidine under standard conditions and the $^{14}CO_2$ formed is estimated in a Packard Tricarb liquid scintillation counter. Details of the method have been described by Radwan & West (1967b). The pH value of the solution of the anti-inflammatory agent under test was always adjusted to the optimal for the enzyme being studied before the enzyme was added (in aliquots of 0.1 ml.) to the incubation mixture. The optimal pH values for the enzymes used were: 5.6 and 7.6 for the fundic and pyloric portions of rat stomach respectively (Radwan & West, 1967b), 6.6 for the rat foetal extract (Burkhalter, 1962) and 4.6 for the bacterial enzyme (Radwan, 1967). Incubations of the drugs without enzyme as well as incubations of the enzyme without drugs were also carried out in similar conditions. All experiments were made in duplicate and results have always been corrected for the blank values and expressed as percentages of the control values. Differences of 20% or more in the readings are significant ($P=0.05$).

Drugs

Molar solutions of the following drugs were prepared with 0.9% saline and diluted as required: salicylic acid, sodium salicylate, indomethacin, phenylbutazone, flufenamic acid, mefenamic acid, meclofenamic acid, 4-bromo-3-hydroxy-benzyloxyamine (NSD-1055), 2,5-dihydroxybenzoic acid and benzoic acid.

RESULTS

Effect of non-steroidal anti-inflammatory drugs on different rat histidine decarboxylase

Fundic portion of stomach

The histidine decarboxylase of the fundic portion was significantly inhibited when NSD-1055 was present in the incubation mixture, a concentration of 1×10^{-3}M, for example, producing 79% inhibition (see Fig. 1). All the other anti-inflammatory agents did not significantly alter the fundic enzyme activity (Table 1).

Pyloric portion of stomach

As shown in Fig. 1 and Table 1, the most active inhibitor of the histidine decarboxylase activity of the pyloric stomach was 2,5-dihydroxybenzoic acid, a concentration of 1×10^{-3}M, for example, producing 97% inhibition. NSD-1055 (1×10^{-3}M) produced 54% inhibition but six other non-steroidal anti-inflammatory drugs (Table 1) had no effect on the enzyme in the three concentrations tested. Indomethacin produced marked inhibition only when the highest concentration (1×10^{-2}M) was used. Benzoic acid, which has no anti-inflammatory activity, was also inactive against this enzyme preparation.

Whole foetus

All the non-steroidal anti-inflammatory drugs inhibited the histidine decarboxylase activity of rat foetal extract, the extent of inhibition depending on the concentrations (Fig. 1 and Table 2). NSD-1055 was by far the most powerful inhibitor of the enzyme

activity, 100% inhibition being produced by 1×10^{-4}M and 80% inhibition by 1×10^{-6}M. Both benzoic acid and 2,5-dihydroxybenzoic acid, in high concentration, also inhibited this enzyme.

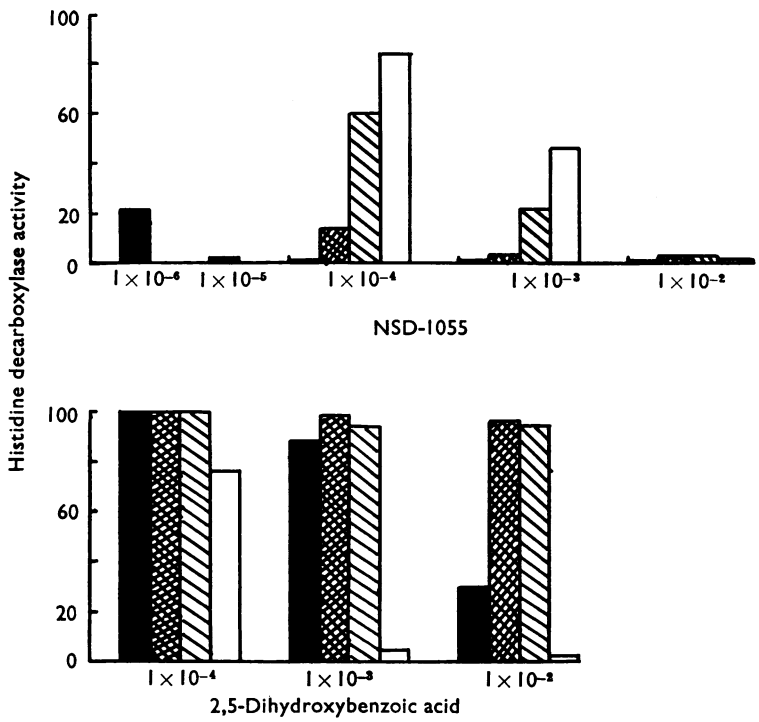

Fig. 1. Effect of different molar concentrations of NSD-1055 (upper figure) and 2,5-dihydroxybenzoic acid (lower figure) on the histidine decarboxylase activity of rat foetus (■), the bacterial enzyme (⛌), and the fundic (▨) and pyloric (□) portions of rat stomach. Enzyme activities are expressed as percentages of those of control incubations without inhibitor.

TABLE 1

EFFECT OF DIFFERENT CONCENTRATIONS OF NON-STEROIDAL ANTI-INFLAMMATORY DRUGS ON THE HISTIDINE DECARBOXYLASE ACTIVITIES OF THE FUNDIC AND PYLORIC PORTIONS OF RAT STOMACH, AS DETERMINED BY THE $^{14}CO_2$ METHOD USING OPTIMAL pH VALUES

Results are expressed as percentages of those obtained from incubations without inhibitor.

	Fundic enzyme			Pyloric enzyme		
Drug	1×10^{-4}M	1×10^{-3}M	1×10^{-2}M	1×10^{-4}M	1×10^{-3}M	1×10^{-2}M
Salicylic acid	85	84	88	96	98	95
Sodium salicylate	88	97	85	102	99	103
Indomethacin	98	106	100	102	93	16
Phenylbutazone	—	—	—	119	117	104
Flufenamic acid	98	90	89	87	87	93
Mefenamic acid	100	102	84	92	96	105
Meclofenamic acid	100	96	97	94	110	105
NSD-1055	60	21	0	85	46	0
2,5-Dihydroxybenzoic acid	100	95	94	76	3	0
Benzoic acid	89	85	79	96	94	75

Effect of non-steroidal anti-inflammatory drugs on bacterial histidine decarboxylase

The bacterial enzyme was not affected by salicylic, flufenamic, mefenamic or 2,5-dihydroxybenzoic acids, but was inhibited by NSD-1055, a concentration of 1×10^{-4}M producing 86% inhibition (see Fig. 1 and Table 2).

TABLE 2

EFFECT OF DIFFERENT CONCENTRATIONS OF NON-STEROIDAL ANTI-INFLAMMATORY DRUGS ON THE HISTIDINE DECARBOXYLASE ACTIVITIES OF RAT FOETAL AND BACTERIAL ENZYMES, AS DETERMINED BY THE $^{14}CO_2$ METHOD USING OPTIMAL pH VALUES

Results are expressed as percentages of those obtained from incubations without inhibitor.

	Rat foetus			Bacterial enzyme		
Drugs	1×10^{-4}M	1×10^{-3}M	1×10^{-2}M	1×10^{-4}M	1×10^{-3}M	1×10^{-2}M
Salicylic acid	92	96	2	102	101	100
Sodium salicylate	97	59	7	—	—	—
Indomethacin	66	13	0	—	—	—
Phenylbutazone	95	55	0	—	—	—
Flufenamic acid	64	0	0	98	100	99
Mefenamic acid	—	—	—	99	101	100
NSD-1055	0	0	0	14	2	0
2,5-Dihydroxybenzoic acid	100	88	29	100	98	96
Benzoic acid	100	89	31	—	—	—

Effect of pyridoxal on the inhibitory action of NSD-1055 and 2,5-dihydroxybenzoic acid

The inhibitory effect of NSD-1055 on the rat fundic histidine decarboxylase was prevented by increasing the concentration of pyridoxal-5-phosphate in the incubation mixture (Fig. 2). A similar result was also obtained when the rat pyloric enzyme was used. The inhibitory effect of 2,5-hydroxybenzoic acid (1×10^{-3}M) on the rat pyloric stomach enzyme was also prevented by pyridoxal-5-phosphate (1×10^{-3}M).

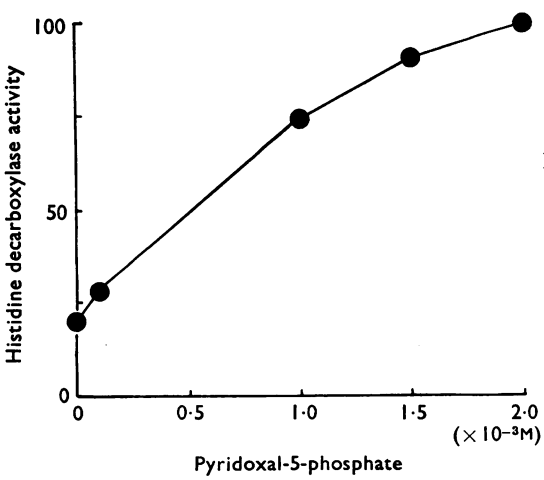

Fig. 2. Effect of different molar concentrations of pyridoxal-5-phosphate on the action of NSD-1055 ($\times 10^{-3}$M) in inhibiting the histidine decarboxylase activity of rat fundic stomach. Enzyme activity is expressed as a percentage of that found in incubations without inhibitor.

DISCUSSION

Whitehouse & Skidmore (1965) postulated that the lysyl ε-amino-groups of some proteins are important binding sites for acidic anti-inflammatory drugs and that enzymic

reactions which depend on the availability of these amino-groups, for example, for pyridoxal phosphate binding are inhibited by these drugs. The results of the present study are in line with those of Skidmore & Whitehouse (1966), some acidic non-steroidal anti-inflammatory drugs inhibiting the specific histidine decarboxylase of rat foetal origin; however, most of them had little or no effect on the fundic and pyloric histidine decarboxylases. Moreover, the inhibitory potency of these drugs on the rat foetal enzyme did not correspond with their activities as anti-inflammatory agents; for example, flufenamic acid is as potent as phenylbutazone in inhibiting the ultraviolet erythema test in guinea-pigs (Whitehouse, 1965), a test which correlates well with the clinical anti-inflammatory action (Winder, Wax, Burr, Been & Rosiere, 1958), and yet it is twice as active as phenylbutazone in inhibiting the histidine decarboxylase enzyme. Furthermore, both 2,5-dihydroxybenzoic acid, which has no effect on ultraviolet erythema test in guinea-pigs (Adams & Cobb, 1958; Winder *et al.*, 1958) and only slight anti-rheumatic activity (Rosenberg, Krevsky & Kagan, 1952; Bywaters, 1963), and benzoic acid, which has no anti-inflammatory activity (Adams & Cobb, 1958), inhibit the rat foetal histidine decarboxylase when used in appropriate concentrations. Phenylbutazone has a more powerful anti-inflammatory action than salicylic acid (Adams & Cobb, 1958; Adams, 1960) and yet it inhibits the rat foetal enzyme to about the same extent as do salicylates. Thus, the anti-inflammatory activity of these drugs is little related to their action on the enzyme histidine decarboxylase.

The present results also show that not only the acidic anti-inflammatory drugs but also the basic compound NSD-1055 (Levine, Sato & Sjoerdsma, 1965) inhibit the rat foetal histidine decarboxylase. The inhibition of enzyme activity by NSD-1055 and by 2,5-dihydroxybenzoic acid probably depends on an interaction of the inhibitor with the coenzyme. For example, the degree of inhibition produced by each of these two compounds *in vitro* was reduced in the presence of pyridoxal phosphate, and this inhibitory action may therefore be caused by competition with the coenzyme for the active enzyme centre or it may be through a chemical combination with the coenzyme, rendering it inactive. In fact, it has been recently shown that NSD-1055 chemically combines with pyridoxal phosphate (Reid & Shepherd, 1966).

The conclusion that the anti-inflammatory activity of non-steroidal compounds is not related to inhibition of histamine formation has been based on *in vitro* experiments and needs confirmation by *in vivo* tests.

SUMMARY

1. The action of nine non-steroidal anti-inflammatory drugs on the activity of histidine decarboxylase of different origin has been investigated.

2. Whereas the rat foetal histidine decarboxylase is inhibited by all drugs to differing degrees, the fundic stomach enzyme is only inhibited by NSD-1055, and the pyloric stomach enzyme is only inhibited by NSD-1055 (a basic compound) and 2,5-dihydroxybenzoic acid.

3. The inhibitory action of NSD-1055 on the fundic and pyloric enzymes and of 2,5-dihydroxybenzoic acid on the pyloric preparation is overcome when excess pyridoxal-5-phosphate is present in the incubation mixture.

4. It is concluded from *in vitro* experiments that the anti-inflammatory activity of the drugs investigated is not related to their action on mammalian histidine decarboxylase.

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