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SOME INHIBITORS OF HISTAMINE-INDUCED AND FORMALDEHYDE-INDUCED INFLAMMATION IN MICE

BY

B. J. NORTHOVER AND G. SUBRAMANIAN

From the Department of Pharmacology, Christian Medical College, Vellore, S. India

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The swelling and capillary hyperpermeability of the mouse foot in response to an injection of formaldehyde, and the increased capillary permeability to an intradermal injection of histamine, have been investigated. Cortisone, mepyramine and sodium salicylate were effective in reducing histamine-induced inflammation, sodium salicylate being less active in the adrenalectomized animal. In formaldehyde-induced inflammation, however, cortisone was ineffective whereas sodium salicylate was effective in the intact mouse, but not in the adrenalectomized animal. Certain aryloxypropionates and anti-esterases were also active in reducing the severity of the formaldehyde-induced inflammation.

Formaldehyde will induce an inflammatory reaction at the site of injection, and this has been used by Parratt & West (1957) for studying inflammation quantitatively in the rat foot. Selye (1949) first described the effect of injected formaldehyde in the rat foot as an "arthritic" reaction, but this has been criticized by Bourne (1951) as inaccurate. Various authors are agreed, however, upon the main point, that formaldehyde produces a local inflammation.

The reaction of the rat foot to formaldehyde has been investigated pharmacologically by Parratt & West (1958), who showed that the reaction was not antagonized by inhibitors of either histamine or 5-hydroxytryptamine, with the exception of the compound methotrimeprazine, which is probably active because of some action separate from its antihistaminic action. Selye (1949) has shown that in the adrenalectomized rat both cortisone and adrenocorticotrophic hormone are effective in antagonizing the reaction to formaldehyde. It has been reported by Setnikar, Salvaterra & Temelcou (1959) that the swelling of the rat foot caused by an injection of formaldehyde can be partially prevented by iproniazid, phenylbutazone and salicylamide. Recently, Buch (1959) has shown that various phenylpropylcarbamates are effective in reducing the reaction to formaldehyde.

Histamine, having been implicated in the mediation of inflammation for many years, has been extensively studied. Lockett & Jarman (1958) have devised a satisfactory method for relating the response quantitatively to an intradermal injection of histamine in the mouse.

The present work shows that formaldehyde-induced inflammation in the mouse foot can be inhibited by a number of substances, some of which may also inhibit the reaction to an intradermal injection of histamine.

METHODS

Formaldehyde-induced inflammation in the mouse foot. Preliminary experiments showed that 0.05 ml. of a 3.5% w/v solution of formaldehyde in 0.9% sodium chloride produced a submaximal degree of swelling which was very considerably greater than that produced by the same vol. of 0.9% sodium chloride alone. Since there was slight tissue damage from the injection of 0.05 ml. of 0.9% sodium chloride one hind foot was injected with this and the opposite foot injected with the formaldehyde solution, so that the relative degrees of swelling could be compared in each animal. The injections were made subcutaneously into the dorsum of the foot, as this was found to produce less mechanical trauma than an injection beneath the plantar aponeurosis.

Adult mice weighing between 25 and 35 g, of both sexes, were arranged in groups of 5 animals. Those animals receiving pretreatment were given daily subcutaneous injections of the drug into the forearm in a dose vol. of 0.1 ml., except those receiving anti-esterases, which were given intramuscularly daily into the leg on the same side as was to be used for the injection of formaldehyde. The pretreatment was continued for 3 days, and the final injection was given 30 min before the injection of formaldehyde. Immediately before the injection of formaldehyde each mouse was given into the tail vein 0.1 ml. of a 1% w/v solution of Evans blue in 0.9% sodium chloride. The extent of the swelling and the leakage of blue dye were noted by two observers at the end of 1 hr from the time of giving the formaldehyde, and given a subjectively assigned score of from 0 to 3. The mean response of the members of each group was then calculated for both the swelling and the blue dye leakage, and expressed in each case as a percentage of that seen in a control group which was given pretreatment with 0.9% sodium chloride for 3 days.

Since it was doubtful whether these subjectively assigned values for foot swelling could be related to objectively made measurements such as foot volume or ankle joint diameter, we made some observations using a fluid displacement method for determining the foot vol. and found that the results were comparable with the subjectively assigned results.

Histamine-induced inflammation in the abdominal skin of mice. Mice were arranged in groups as above and an intradermal injection of 2 μg of histamine in 0.1 ml. of 0.9% sodium chloride was made into ventral abdominal skin. The procedure was that of Lockett & Jarman (1958), with the difference that we did not anaesthetize the animal to give the histamine. Not only is this more convenient, but it removes the objection that the anaesthesia may alter capillary permeability, as in fact was demonstrated by Lockett & Jarman (1958). After 30 min the mice were killed, the ventral abdominal skin reflected, and the intensity of the blue dye leakage on the inside of the skin noted. For each animal the coloration was given a subjectively assigned score of from 0 to 3, so that the mean response for each group could be calculated and the result expressed as a percentage of the response in the control group of animals pretreated with normal saline.

Adrenalectomized mice. Mice were subjected to a bilateral adrenalectomy under ether anaesthesia, using the dorsal approach. The animals were maintained post-operatively by administration of 2 mg/kg/day of cortisone acetate given intramuscularly. The mice were also given 0.9% sodium chloride in place of drinking water.

Chemicals. Formaldehyde was reagent grade material protected from light and contained no free acid. Other drugs used were histamine diphosphate, mepyramine maleate, a microcrystalline suspension of cortisone acetate, atropine sulphate, adrenaline bitartrate, physostigmine salicylate, edrophonium chloride, neostigmine bromide, di*iso*propylfluorophosphonate in arachis oil, sodium salicylate, phenylbutazone, sodium 2:3-dihydroxybenzoate (sodium gentisate), a suspension of 3-(*o*-methoxyphenoxy)-2-hydroxypropyl carbamate (methocarbamol), sodium alpha-4-*sec*-butylphenoxypropionate (B.C. 7586), sodium alpha-4-phenylphenoxypropionate (B.C. 8402), sodium alpha-4-carboxyphenoxypropionate (B.C. 8424s), sodium alpha-4-hydroxyphenoxypropionate (B.C. 8422s), tolazoline hydrochloride, ephedrine hydrochloride, freshly prepared sodium acetylsalicylate, a suspension of amidopyrine and a suspension of N-acetyl-p-aminophenol.

RESULTS

Formaldehyde-induced inflammation in the mouse foot. The results of the experiments which were performed to determine the effectiveness of antagonists to formaldehyde-induced inflammation are presented in Tables 1 and 2. Sodium

TABLE 1

ANTAGONISTS OF FORMALDEHYDE-INDUCED INFLAMMATION

(Normal intact mice)

An asterisk denotes that the difference between the response of a group and that of the control group is statistically significant ($P<0.05$)

Drug	Daily dose mg/kg	Mean inflammatory response at 1 hr as a % of the control: Blue colour	Swelling
Normal saline	—	100	100
Phenylbutazone	75	28*	33*
Cortisone acetate	40	86	99
Sodium gentisate	75	87	91
Methocarbamol	75	23*	25*
B.C. 8402	75	67*	54*
B.C. 8422s	75	46*	58*
B.C. 8424s	75	68*	50*
Sodium acetylsalicylate	150	70*	66*
N-Acetyl-p-aminophenol	150	89	77*
Amidopyrine	75	71*	57*
Atropine sulphate	40	104	95
Atropine sulphate + Physostigmine	40 + 0·4	86	74*
Atropine sulphate + Neostigmine	40 + 0·1	111	96
Atropine sulphate + Edrophonium	40 + 4	113	107
Tolazoline	0·1	73*	72*
Tolazoline + B.C. 7586	0·1 + 75	68*	56*
Adrenaline	0·4 (single dose)	109	91
Ephedrine	2	83	75*

TABLE 2

ANTAGONISTS OF FORMALDEHYDE-INDUCED INFLAMMATION

(Adrenalectomized mice=A. Normal intact mice=N)

An asterisk denotes that the difference between the response of a group and that of the control group is statistically significant ($P<0.05$)

Drug	Daily dose mg/kg		Mean inflammatory response at 1 hr as a % of the control: Blue colour	Swelling
Sodium salicylate	150	N.	75*	73*
Sodium salicylate	150	A.	96	102
B.C. 7586	75	N.	53*	61*
B.C. 7586	75	A.	79*	82*
Atropine sulphate + Di*iso*propylfluorophosphonate	40 + 2	N.	32*	48*
Atropine sulphate + Di*iso*propylfluorophosphonate	40 + 2	A.	41*	67*
Normal saline	—	A.	127*	115*

salicylate and N-acetylaminophenol were slightly active, whereas cortisone acetate was inactive. Methocarbamol, amidopyrine and phenylbutazone, however, were highly active, whereas the four aryloxypropionates tested and sodium acetylsalicylate were moderately active. The activity of phenylbutazone confirmed the observations of Setnikar, Salvaterra & Temelcou (1959). In the cortisone-maintained adrenalectomized mice both sodium salicylate and B.C. 7586 were less effective than in the intact animals.

Several compounds with anti-esterase properties were tested for anti-inflammatory activity in fully atropinized mice, since they were administered in a dose which would otherwise cause considerable disturbance from the inhibition of cholinesterase. Atropine in the dose used had no anti-inflammatory action of its own. Di*iso*propylfluorophosphonate had a very marked inhibitory effect against the reaction caused by formaldehyde, as also had physostigmine, but to a less extent. Both neostigmine and edrophonium were inactive in this test in the doses used, which were also approximately the maximum tolerated doses. Spector & Willoughby (1960) have reported that di*iso*propylfluorophosphonate will antagonize the production of a turpentine pleurisy in rats, but in their test physostigmine was inactive. Since both the species and the nature of the inflammation were different in their work it is not possible to make a valid comparison between their results and ours.

Adrenaline has been reported by Geschickter, O'Malley & Rubacky (1960) to inhibit the inflammatory reaction to the injection of egg-white, and as there is some evidence (Smith, 1955) that salicylates can cause a stimulation of the adrenal medulla, it seemed worth while attempting to investigate the possibility that the aryloxypropionates and the other analgesic-antipyretics were acting through the adrenal medulla.

TABLE 3

ANTAGONISTS OF HISTAMINE-INDUCED CAPILLARY HYPERPERMEABILITY
(Normal = N; adrenalectomized = A)

An asterisk denotes that the difference between the response of a group and that of the control group is statistically significant ($P<0.05$)

Drug	Daily dose mg/kg		Mean inflammatory response at 30 min as a % of the control
Normal saline	Control	N.	100
Normal saline	—	A.	96
Sodium salicylate	150	N.	36*
Sodium salicylate	150	A.	79*
B.C. 7586	75	N.	98
B.C. 8402	75	N.	105
Cortisone acetate	40	N.	47*
Mepyramine maleate	5 (single dose)	N.	23*

Tolazoline was chosen as an antagonist to the peripheral actions of adrenaline, but instead of blocking the action of B.C. 7586 it can be seen (Table 1) that tolazoline had some inhibitory action to formaldehyde-induced inflammation. This point is being investigated further. Adrenaline was not an effective antagonist, but since we have shown some activity by ephedrine it seems probable that the failure of adrenaline is due to its being more rapidly inactivated in the body than ephedrine.

Histamine-induced inflammation in the mouse skin. The results shown in Table 3 indicate that sodium salicylate is capable of inhibiting the capillary hyperpermeability produced by the intradermal injection of histamine. The fact that sodium salicylate was considerably less active in the adrenalectomized animal recalls the observation of Keleman (1957) that sodium salicylate was less active in the adrenalectomized rat than in the intact animal in antagonizing the oedema of the rat foot in response to an injection of 5-hydroxytryptamine.

It is noteworthy that cortisone acetate was active in preventing the capillary hyperpermeability reaction, since this is in contrast to its ineffectiveness against the formaldehyde reaction. The two aryloxypropionates tested here were inactive in a dose which afforded considerable protection against the effects of formaldehyde.

Anti-inflammatory agents in the adrenalectomized mouse. In the present experiments three anti-inflammatory agents proved to be less effective in the adrenalectomized animal than in its intact counterpart. This could be interpreted as meaning that all these agents stimulate the secretion of adrenal hormones which are, in fact, the active inflammatory agents. However, since cortisone and adrenaline were inactive in our test against formaldehyde-induced inflammation, this seems unlikely. It is more likely that the adrenal hormones play the part which Ingle (1952) has termed the " permissive " role of the adrenals. On this basis it is convenient to think of the adrenal secretion as acting upon the capillaries to render them sufficiently responsive to anti-inflammatory agents for the latter compounds to show their inherent activity. In the absence of the adrenal secretion, however, the capillaries are rendered refractory to any attempt to protect them from chemical damage.

DISCUSSION

Many compounds have been shown to cause an increased permeability of the capillaries when introduced into the tissues. These compounds may be classified into those which act upon the capillary endothelium to render it more permeable to water and plasma protein, and those which are more or less indiscriminately injurious to cells and release from such damaged cells substances which bring about capillary hyperpermeability. There can be little doubt that histamine belongs to the former category and has a direct action upon the capillary endothelium, rendering it more permeable than normal to both water and plasma proteins. There is also reason to suppose that formaldehyde, along with many other " irritant " substances, causes the liberation or activation of some substance or substances within the tissues, which in their turn act upon the capillaries to render them more permeable. It is not possible to say with any certainty what is the nature of the mediator or mediators which are involved in formaldehyde-induced inflammation, but the pharmacological evidence is already interesting and suggestive, and may eventually give us an answer to the problem.

Ungar (1952) has shown that it is possible to explain the anti-inflammatory action of the antipyretic-analgesic class of drugs on the basis of their anti-fibrinolysin activity, and it could be postulated that the ability of the various analgesic-antipyretic drugs capable of inhibiting formaldehyde-induced inflammation in the mouse foot

is due to their anti-fibrinolysin action. Recently, however, Collier, Holgate, Schachter & Shorley (1960) have shown that salicylate is capable of completely inhibiting the action of bradykinin on bronchial smooth muscle *in vivo*. Since this peptide also causes an increase in capillary permeability, it is possible that the inhibition of formaldehyde-induced inflammation which we have observed is due to an inhibition of the action of bradykinin released by the damaged tissues. Spector & Willoughby (1959) have investigated the increased permeability of the small blood vessels in response to turpentine, and have shown that it is due to the activation of a serum globulin system. This was inhibited by salicylate *in vitro*, as also was the increased permeability of the blood vessels *in vivo*. Besides these proposed direct actions of the analgesic-antipyretic drugs in antagonizing the production of inflammation, many workers have suggested that the salicylates, since they stimulate the pituitary-adrenal system, have an anti-inflammatory action due to released adrenocortical steroids. The evidence for this assertion has been critically reviewed by Smith (1953), who came to the conclusion that this mechanism, whilst it no doubt occurs, is not sufficient to explain all the anti-inflammatory actions of salicylates, notably their established effectiveness in rheumatoid diseases. It is probable that the analgesic-antipyretic class of drugs exert their effect by more than one mechanism.

We have shown that although salicylate is capable of antagonizing the inflammation produced by both histamine and formaldehyde, cortisone is only effective against histamine and the four aryloxypropionates tested are only effective against formaldehyde. Consequently any explanation of the mode of action of these inflammatory agents will have to take into account the selective effect of some antagonists. The inactivity of cortisone is in accord with its inactivity against thermal inflammation reported by Sevitt (1957), and its inactivity to the inflammation resulting from various bacterial toxins, which was shown by Lattes, Blunt, Rose, Jessor, Vaillencourt & Ragan (1953). Cortisone is also without any action against an ultra-violet radiation-induced erythema in guinea-pigs (Adams, personal communication). At present it is impossible to predict which substances, in the broad category of analgesic-antipyretics, will be active as antagonists to formaldehyde-induced inflammation.

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