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HISTAMINE RELEASE AND PAIN PRODUCTION BY XANTHOSINE AND RELATED COMPOUNDS

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Xanthosine (5 to 50 μg.) caused pain on intradermal injection in all human subjects tested: in about half the subjects it also gave a flare. Inosinic acid, uric acid, allantoin, alloxan and theophylline caused pain, but not a flare by intradermal injection in man. Xanthine caused both pain and flare. A number of related compounds in similar doses caused neither pain nor flare. Inosine, inosinic acid, and guanosine, but not xanthosine, were powerful histamine liberators in animals. The lowering of the cat blood pressure, depression of the activity of the guinea-pig ileum and stimulation of guinea-pig uterus by adenosine were confirmed.

The purine nucleosides have recently been found effective in "reviving" stored blood (Prankerd and Altman, 1954; Gabrio, Donohue, and Finch, 1955; Prankerd, 1956a). Our interest in these substances arose from the need to decide which was the least pharmacologically active and hence most suitable for "reviving" blood intended for transfusion into patients. We concluded that guanosine was the most satisfactory, and it has in fact been used (Prankerd, 1956b).

In addition to repeating some of the earlier work, as reviewed by Drury (1936), on the cat blood pressure preparation, the isolated guinea-pig ileum and the isolated guinea-pig uterus, we tested the histamine-liberating properties of these compounds. Some of the nucleosides, especially inosine, were found to be potent histamine liberators. This suggested an investigation into their ability to increase capillary permeabiltiy and in most cases the results obtained paralleled those from estimation of histamine release: a full report of the capillary permeability experiments is given elsewhere (Spector and Willoughby, 1957).

An intradermal injection technique was then used to investigate the histamine liberating properties of these substances in man. We were surprised to find that xanthosine, which is only a very weak histamine liberator in animals, was a potent liberator, as judged by the production of a flare and weal, in about half the fifty subjects tested. Inosine, on the other hand, which liberated much histamine in animals, was inactive when injected intradermally in man.

Another unexpected finding was that xanthosine, on intradermal injection, caused a sharp pain in all subjects tested, and that this was not antagonized by an antihistamine (mepyramine maleate).

We conclude that xanthosine and some related compounds must be added to the list of naturally occurring pain-producing substances. The pain-producing properties are not antagonized by mepyramine maleate, although many of these compounds are histamine liberators in certain species. Some of our results were demonstrated to the British Pharmacological Society in January, 1957.

METHODS

The following preparations used for the present work were set up in the usual manner: (*a*) Cat blood pressure preparation. Anaesthetic: pentobarbitone. (*b*) Isolated guinea-pig ileum preparation. 18 ml. bath of Tyrode solution at 35°. (*c*) Isolated virgin guinea-pig uterus preparation. 18 ml. bath of Tyrode solution at 35°. (*d*) Rat hind-quarter preparation, perfused with Locke solution (Rocha e Silva and Schild, 1949). Full technical details of this preparation are given elsewhere (Willoughby, 1957). (*e*) Animals pretreated with trypan blue as described by Spector (1951). A 1% solution of trypan blue was injected intravenously into rats (1 ml.), rabbits (5 ml.), guinea-pigs (2 ml.), and mice (0.25 ml.).

In man, an intradermal injection technique was used in which the assessment of pain production was necessarily subjective, but conditions were kept as constant as possible. Injections were made intradermally with a 1-ml. tuberculin syringe through a 26-gauge

needle. All injections were made into the flexor aspect of the forearm. The volume of each injection was kept constant (0.05 ml.) and all solutions were at neutral *p*H and were made isotonic with 0.85% (w/v) NaCl. The procedure suggested by Armstrong, Dry, Keele, and Markham (1953) was used in which the needle was inserted for 15 sec. before the solution was injected. This eliminated the initial sensation of the insertion from the pain assessment. An initial injection of normal saline was made and the subject told that this was only saline and was to be compared with subsequent injections. All injections, except the first one of saline, were made in random order and the subject was not told what was being injected. Three or four injections after the first were made: these consisted of normal saline, histamine (0.05 μg.), xanthosine and sometimes other substances under test. After each injection the subject was asked if it had caused pain, and if so whether it was more or less severe than that produced by normal saline. The injection sites were observed after 10 min. and the presence or absence of a flare was noted. The criterion "a flare after 10 min." was adopted because: (*a*) An immediate flare, possibly associated with trauma, followed the injections in some subjects, but this rapidly faded over 5 min. Reading at 10 min. excluded this effect from the results. (*b*) Although a flare at 10 min. was usually associated with a weal obviously larger than that seen when saline had been injected, any intradermal injection led to formation of a small bleb which is occasionally difficult to distinguish from a weal. This difficulty was avoided by adopting the presence or absence of a flare as the criterion. (*c*) The response to the injection of histamine, and in some subjects to xanthosine and xanthine, was maximal after about 10 min.

A few subjects (five out of the first hundred) gave a very small flare at 10 min. to the control saline injection, but the response to xanthosine and xanthine was either the same as that to saline or obviously much larger.

A small number of additional experiments were performed in which injections were made into areas of skin pre-treated in various ways. The effect of local antihistamine was tested on six occasions by preparing the skin with mepyramine maleate (Anthisan) cream at intervals for 48 hr. before injecting xanthosine. On one occasion an area of skin depleted of histamine was produced by daily injections with 48/80 (1 μg.) until this no longer produced a triple response. On four subjects the antagonistic effects of procaine (1 ml. of 0.5% solution) were tested by subcutaneous infiltration a few minutes before the xanthosine was injected. In all experiments involving pre-treatment with drugs the untreated arm was used as control.

Chemicals Used.—The values for histamine are expressed as base, but the doses given for the other substances refer to the following: adenosine, cytosine, guanosine, inosine, adenine, cytidine nitrate, guanidine hydrochloride, hypoxanthine, guanylic acid sodium salt, theophylline, caffeine, alloxan, allantoin, uric acid (all the foregoing were obtained from Light); theobromine (British Drug Houses); mepyramine maleate (Anthisan, May and Baker). Compound 48/80 as chloride was kindly given to us by Burroughs Wellcome, and inosinic acid by Sigma Chemical Co., U.S.A. Paper chromatography of the nucleosides did not reveal any impurity other than a 10% contamination by the corresponding purine base.

Results

Effects on Cat's Blood Pressure Preparation.—Adenosine (1 mg./kg.) gave either a depressor or biphasic type of response which was not abolished by hexamethonium (25 mg./kg.) or atropine (0.1 mg./kg.). This depressor response was not antagonized by mepyramine maleate (5 mg./kg.) and this is evidence against the possibility that histamine liberation is responsible for the fall in blood pressure. An infusion of 5 mg. of adenosine over 5 min. produced a drop in blood pressure of 25 mm. of mercury.

In similar doses inosine had no effect, but the response to doses of 10 mg./kg. was very similar to that of 1 mg./kg. adenosine. This agreed with the results of Green and Stoner (1950).

Guanosine, infused in doses of 2.5 mg./kg. and 25 mg./kg., did not alter the blood pressure. Single doses of 20 mg./kg. had no effect. Xanthosine, cytidine, guanylic acid, hypoxanthine and cytosine were all without effect on the cat blood pressure in doses up to 5 mg./kg.

In one experiment an electrocardiogram was taken and the effect of the purine nucleosides was tested in doses of 2.5 mg./kg. Adenosine, the only nucleoside to affect the blood pressure in this dosage, caused a nodal rhythm. The remainder had no effect.

Effects on Guinea-pig Ileum.—The purine bases, nucleosides and nucleotides were tested on the isolated guinea-pig ileum. Adenosine had a relaxant action on the isolated gut and antagonized histamine-produced contractions. In doses of 0.1 mg. adenosine reduced the response to 0.05 μg. histamine by at least 50%. Contractions produced by acetylcholine were also antagonized by doses of 0.1 mg. adenosine and recovery was slow after washing. Guinea-pig ileum does not normally show spontaneous activity, though it may occasionally do so. Adenosine was added to a spontaneously active preparation on one occasion and caused relaxation with inhibition of the contractions.

Guanosine, guanidine, guanylic acid, adenine, inosine, xanthine, xanthosine, hypoxanthine, cytidine or cytosine all had no effect on the isolated ileum in doses up to 0.25 mg.

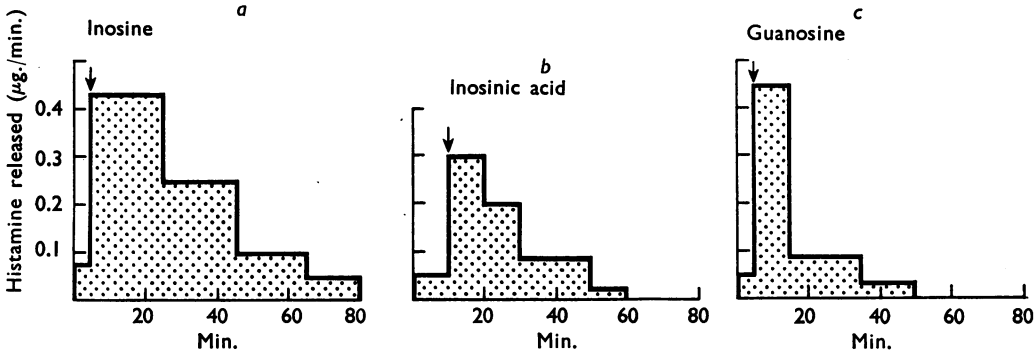

FIG. 1.—Histamine release (ordinates) from the rat hind-quarters preparation. (*a*), 0.25 mg. of inosine liberated a total of 14.9 μg. of histamine. (*b*), 0.25 mg. of inosinic acid liberated a total of 5.0 μg. of histamine. (*c*), 0.25 mg. of guanosine liberated a total of 5.0 μg. of histamine.

Effect on Guinea-pig Uterus.—The nucleosides were tested on the isolated guinea-pig uterus preparation. 0.25 mg. of adenosine produced a contraction approximately equal to that following 0.01 μg. of acetylcholine. Xanthosine, inosine and guanosine had no effect in doses of up to 1 mg.; they did not affect contractions elicited by acetylcholine. These results are in agreement with the findings of von Euler and Gaddum (1931) and Flossner (1934).

Rat Hind-quarters Perfusion.—The histamine liberated was assayed on the guinea-pig ileum preparation and its identity as histamine was established by parallel inhibition experiments using mepyramine maleate on both the isolated guinea-pig ileum and cat blood pressure preparations. Inosine was found to be a fairly potent histamine liberator: 0.25 mg. always released 12 to 15 μg. of histamine. The release was of an explosive type (Fig. 1) similar to that reported with other liberators (MacIntosh and Paton, 1949; Feldberg and Paton, 1951). The release of histamine by inosine appears to be greater than that by (+)-tubocurarine as reported by Rocha e Silva and Schild (1949) using a similar preparation. Guanosine was less active, 0.25 mg. of guanosine releasing only 5.0 μg. of histamine. Xanthosine, adenosine and cytosine in the same dosage released negligible quantities of histamine (<1 μg.) from this preparation. A larger dose of xanthosine (10 mg.) liberated 5.2 μg. of histamine. Inosinic acid (0.25 mg.), which is the nucleotide corresponding to inosine, liberated 4.5 μg. histamine. Guanylic acid gave no liberation, nor did doses of 0.25 mg. of the bases guanidine, xanthine, adenine, hypoxanthine or cytidine. Hypoxanthine (0.25 mg.), which is the base corresponding to inosine, gave a small liberation (0 to 2.5 μg. histamine). All the substances tested were injected at least twice on separate preparations at approximately neutral *p*H. After any compound which released little or no histamine, an injection of 10 μg. of 48/80 always resulted in a large release, proving the ability of the preparation to liberate histamine. Preparations which became grossly oedematous were discarded, since the oedema fluid pressed out of such a preparation after a large dose of 48/80 was found to contain the same quantity of histamine as the perfusate.

Trypan Blue Method for Capillary Permeability.—Table I shows the effect of intradermal injection of the nucleosides on capillary permeability in animals. Inosine was very active on all the species tested; guanosine and adenosine had some effect, but xanthosine was without effect. This method has been used in the rat to test a large number of substances related to inflammation, including the nucleic acid derivatives, for the property of increasing capillary permeability (Spector and Willoughby, 1957).

TABLE I

THE EFFECT OF PURINE NUCLEOSIDES INCREASING CAPILLARY PERMEABILITY IN THE SKIN OF LABORATORY ANIMALS

In this and subsequent tables, the presence of a response is denoted by +. The more intense the response, the more symbols + are recorded. 0 denotes the absence of a response. ± indicates a threshold response.

Substance	Increase in Capillary Permeability following Intradermal Injection of 25 μg. of Nucleoside			
	Rat	Mouse	Guinea-pig	Rabbit
Xanthosine	None	None	None	None
Inosine ..	+++	+++	+++	+++
Guanosine	+	±	+	±
Adenosine	+	±	+	+

Intradermal Injections in Man

Flare Production.—Twenty-four of an initial group of fifty subjects tested with 50 μg. of xanthosine gave a marked flare round the injection site equal to or larger than that from 0.05 μg. of histamine. Subjects giving a flare to xanthosine will be referred to as " reactors " and those not giving one as " non-reactors."

The response of a reactor to xanthosine (50 μg.) was indistinguishable from that to histamine (0.05 μg.), but the weal component of the response has been disregarded for reasons given in the method. Four doses of xanthosine was tested in a group of 15 subjects (Table II). Seven reactors gave a flare

TABLE II

RESPONSE IN MAN TO INTRADERMAL INJECTIONS OF XANTHOSINE

For assessment of responses see Table I.

Dose (μg./0·05 ml.)	Reactors to Xanthosine		Non-reactors to Xanthosine	
	Flare	Pain	Flare	Pain
5	+	+	0	+
20	+	++	0	++
30	+	+++	0	+++
50	+	++++	0	++++
No. of subjects	7		8	

but eight non-reactors gave no flare with any dose tested. The size of flare in the reactors increased with increasing dose. The division into reactors and non-reactors was sharp: a reactor always gave a typical flare even with 5 μg., whilst a non-reactor did not, even with 50 μg. Xanthine has only been tested in a dose of 50 μg.

The effect of pre-treatment of the skin with an antihistamine or a local anaesthetic is shown in Table III. In six reactors, mepyramine maleate cream was found to prevent the appearance of the flare to 50 μg. xanthosine. One reactor had an area of skin depleted of histamine by injections of 48/80; subsequent injections of xanthosine into the area then failed to produce a flare. The pre-treatment with 48/80 was very painful and has not been repeated. Procaine infiltration prevented the flare component of the response, but the weal was not affected.

The nucleosides adenosine, inosine and guanosine did not give a flare even when the dose was increased to 250 μg. (Neither did 0.05 ml. of a saturated solution of guanosine, 20°.) Xanthinic acid was not available; guanylic and inosinic acids gave no triple response. The base xanthine gave results identical to those obtained with xanthosine, but adenine, guanine and hypoxanthine were inactive. None of the related compounds shown in Table IV gave a flare on intradermal injection. Dosage of many compounds was limited by solubility.

Pain Production.—All of the fifty subjects given intradermal injections of 50 μg. of xanthosine complained of pain. This was a sharp, " bright " pain of immediate onset, which lasted only a few seconds and which began to decline in severity as soon as the injection was completed. With thirty of these subjects the procedure was repeated using 5 μg. of xanthosine. All the subjects reported that injection of normal saline was painless when compared with 50 μg. of xanthosine and that 5 μg. of xanthosine was definitely more painful than the saline. The fifteen subjects given doses ranging from 5 to 50 μg. (Table II) were asked to place the injections in order of increasing pain. Three of them placed the 20 and 30 μg. injections in reversed order, but otherwise the placing according to severity of pain corresponded with the dose.

Pain production was quite independent of the presence or absence of a flare. Non-reactors seemed to suffer just as much pain as reactors. The results are shown in Table IV.

The effects of various pre-treatments are shown in Table III. Local anaesthesia with procaine made subsequent injections of 50 μg. of xanthosine completely painless, although in reactors a weal was still produced. Inunction with sufficient antihistamine to prevent the formation of a flare in reactors had no effect whatever on the pain

TABLE III

MODIFICATION OF THE RESPONSE TO INTRADERMAL INJECTION OF 50 μG. OF XANTHOSINE IN MAN BY PROCAINE, BY 48/80 AND BY MEPYRAMINE

For assessment of responses see Table I.

Pre-treatment	No. of Expts.		Flare		Pain	
	Reactors	Non-reactors	Reactor	Non-reactor	Reactor	Non-reactor
None	24	26	++++	0	++++	++++
Procaine infiltration	2	2	Weal only	0	0	0
Histamine depletion	1	0	0		++++	
Mepyramine maleate	6	0	0		++++	

TABLE IV

RESPONSE IN MAN TO INTRADERMAL INJECTIONS OF THE NUCLEOSIDES AND SOME RELATED COMPOUNDS

For assessment of responses see Table I.

Substance	Dose (μg./0·05 ml.)	Reactors to Xanthosine		Non-reactors to Xanthosine		No. of Subjects		
		Flare	Pain	Flare	Pain	Total	Reactors	Non-reactors
Xanthosine	50	+	++++	0	++++	50	24	26
Adenosine	50	0	0	0	0	15	8	7
,,	250	0	0	0	0	6	3	3
Inosine	50	0	0	0	0	15	7	8
,,	250	0	0	0	0	6	3	3
Guanosine	50	0	0	0	0	15	8	7
,,	Saturated solution	0	0	0	0	6	3	3
Xanthine	50	+	++++	0	++++	6	3	3
Adenine	50	0	0	0	0	6	3	3
Hypoxanthine	50	0	0	0	0	6	3	3
Guanine	50	0	0	0	0	6	3	3
Guanylic acid	50	0	0	0	0	6	3	3
Inosinic ,,	50	0	+++	0	+++	6	3	3
Uric acid	Saturated solution	0	+++	0	+++	12	6	6
Allantoin	50	0	+	0	+	6	3	3
Alloxan	50	0	+++	0	+++	6	3	3
Uracil	250	0	+	0	+	6	3	3
Caffeine	50	0	0	0	0	6	3	3
Theobromine	50	0	0	0	0	12	6	6
Theophylline	50	0	+	0	+	6	3	3
,,	250	0	+++	0	+++	9	6	3
Histamine	0·05	+	Itch	+	Itch	50	24	26

from xanthosine. Similarly in the single histamine depletion experiment subsequent xanthosine injections were just as painful as before.

The other nucleosides did not cause pain in any dose tested (up to 250 μg.). Of the bases, only xanthine caused pain on intradermal injection, and this pain was just as severe, and of a similar quality to that from xanthosine. Adenine, guanine, and hypoxanthine did not cause pain. Alloxan (50 μg.), theophylline (250 μg.), inosinic acid (250 μg.) and a saturated solution of uric acid (approximately 2.5 μg.) all caused less pain than 50 μg. of xanthosine. Allantoin (50 μg.) and uracil (250 μg.) only caused slight pain. Guanylic acid (50 μg.), caffeine (50 μg.), and theobromine (50 μg.) were not painful on intradermal injection.

With none of the substances tested was there any correlation between the production of a flare and the sensation of pain. In the concentration used, histamine caused itching rather than pain. No itching was felt after injections of any of the nucleosides or related compounds.

DISCUSSION

The depression of the cat blood pressure by inosine and guanosine was unlikely to be due to histamine release as it was not affected by mepyramine. Although inosine and, to a lesser extent, guanosine were powerful histamine liberators in animals, but not in man, xanthosine was only a very weak liberator in animals but gave a flare in about half of the human subjects. This suggests that if drugs for use in man are only tested on laboratory animals, it is possible for a histamine liberator to be overlooked. In about half the human subjects, xanthosine (50 μg.) gave a response indistinguishable from that to histamine (0.05 μg.). This response was antagonized by mepyramine and in a single experiment did not appear after histamine depletion. Hence it is concluded that the flare and weal produced by xanthosine in some subjects probably result from histamine liberation. In 50 experiments we have not found a reactor change to a non-reactor or vice versa when injections of xanthosine were repeated. Reactors and non-reactors have been classified on the basis of their reaction to xanthosine, but an identical response was obtained with xanthine in a smaller group of subjects.

Xanthosine, xanthine and uric acid are all naturally occurring, powerful pain-producing substances. The pain seems very unlikely to be dependent upon histamine release, since they hurt reactors and non-reactors equally and the pain produced by histamine is of a quite different quality. In a preliminary experiment, local pre-treatment with subcutaneous injection of diphenhydramine hydrochloride (10 mg.) was performed, but this was very painful and was replaced by inunction with mepyramine maleate. Pre-treatment with these antihistamines never gave any decrease in the pain following xanthosine injections, and this is further evidence against the pain being dependent upon histamine release. The effect of pre-treating the skin with procaine

and mepyramine on the pain produced by substances other than xanthosine has not been investigated.

Some of these compounds might be involved in pain production following tissue damage, and their abnormal metabolism and accumulation could account for some of the pain in gout.

We cannot offer an adequate explanation of the division into reactors and non-reactors. A natural or acquired hypersensitivity to xanthosine or xanthine on the part of the reactors seems the likeliest explanation which is supported by the highly antigenic properties of many compounds derived from a pyrimidine nucleus (Alexander, 1955). The genetical aspects of the division are at present being investigated.

We are unwilling to attempt to relate pain production in this group of substances to their chemical formulae as thorough investigation of the group by this technique requires injection of many subjects with a wide range of doses. However, it is of interest that six out of the eight compounds causing pain on intradermal injection have oxygen atoms linked to carbon 2. On the other hand, of the nine substances tested which did not cause pain, only two have this grouping.

The estimation of pain is necessarily subjective, but the pain due to xanthosine is of a different quality to that from acetylcholine, histamine or 5-hydroxytryptamine, none of which gave such an intense "bright" pain. The differences in quality make comparison of dose responses very difficult and this has not been attempted.

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