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INHIBITION OF ULCER FORMATION BY URINE EXTRACTS WITH ANTIHISTAMINE ACTIVITY

BY

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The presence of an antihistamine-like substance(s) in extracts of mammalian urine and tissues has previously been reported (Kovacs & Melville, 1962, 1963; Kovacs, Pelletier & Rose, 1963; Francis, Melville & Douglas, 1963). It has also been shown that these extracts exert a wide range of activity in antagonizing the *in vitro* effects of histamine, serotonin, acetylcholine and bradykinin as well as the *in vivo* effects of histamine and bradykinin (Kovacs & Melville, 1963). Since the active principle is a naturally occurring substance with a wide range of activity, it seemed of interest to investigate its possible effect, if any, on gastric acid secretion.

The results presented in this paper were obtained with partially purified urine extracts which uniformly contained the antihistamine principle(s). When injected into guinea-pigs, these extracts antagonized the histamine-induced gastric hypersecretion. Furthermore, the administration of the extracts into rats strongly hindered or prevented the development of experimentally induced gastric ulcer.

METHODS

Preparation of urine extracts:

(a) Horse urine

Fractions of pregnant mare urine, obtained as byproducts in the course of the isolation of oestrons, were tested for antihistamine activity. One of the fractions was found to contain the antihistamine-like principle(s) in fairly large quantities. This partially purified extract is a dark brown, viscous substance and each g of this preparation corresponds to approximately 5 l. horse urine. The fraction is insoluble in water but is readily soluble in organic solvents, such as chloroform, petroleum, ether, etc. In order to eliminate simple phenols (phenol, cresol, etc), 1 g residue was dissolved in 50 ml. chloroform and extracted four times with 100 ml. 0.1 N NaOH then four times with 100 ml. distilled water. The chloroform fraction containing the active principle(s) was concentrated to dryness under reduced pressure and kept at $-10°$ C under argon. Immediately before use, it was dissolved in isopropylmyristate (100 mg extract/0.2 ml.), and injected in this form.

(b) Human urine

Urine was freshly collected from healthy male subjects and extracted with chloroform using the method previously described (Kovacs & Melville, 1962): 100 l. urine yielded 26.6 g crude chloroform extract. This extract was further purified according to the method described for horse urine residue.

Biological methods

Guinea-pig ileum preparation:

The method previously described (Kovacs & Melville, 1962) was used with the slight modification that in the present experiments the gut was suspended not in a 20 ml., but in a 50 ml. bath. Since the extract was insoluble in water, it was dissolved in ethanol (10 mg/0.2 ml.). The ethanol solution was first diluted to 1.0 ml. with Tyrode, and, from this stock solution, a further dilution of the extract was made resulting in a final concentration of 0.5 mg/ml. From this solution 0.1 to 1.0 ml. was added to the bath. Similarly prepared controls containing the same amount of ethanol were invariably without effect.

Histamine-induced gastric acid hypersecretion

A multi-coloured, short-haired variety of guinea-pigs obtained from the Quebec Breeders Association (Canada) was used. Male guinea-pigs weighing between 280 and 380 g were purchased one week prior to experimentation and kept on a steady diet consisting of Purine guinea-pig pellets, hay and water *ad lib.* Before the experiment they were fasted for 24 hr: water was allowed at all times.

To induce anaesthesia, a mixture of 10% urethane and 5% sodium pentobarbitone (nembutal) solution (ratio 19:1) was used, 4 ml./kg being administered from the mixture intraperitoneally 20 min prior to the operation. When necessary, this dosage was supplemented with additional quantities. The method used for collecting and analyzing the gastric juice was similar to that described by Herr & Porszasz (1951). Through a midline incision the stomach was gently pulled out, following which the duodenum was ligated about 2 cm distal from the pylorus, carefully avoiding all near-by vessels. Through a small incision 1 cm proximal to the pylorus a polyethylene tube (3 mm diameter, 10 cm long) was inserted into the stomach. The position of the tube was carefully fixed by a thread in such a way that its tip penetrated no deeper than 2 to 3 mm into the cavity of the stomach. The animals were then placed on an elevated holder, face down, and remained in this position throughout the experiment. Soft rubber catheters (Ingram–Bell, No. 8) were inserted orally and the stomach was washed with 0.85% NaCl solution, kept at 37° C until all food particles were removed. By washing the stomach with 3 ml. water (37° C) the gastric juice was collected at 15-min intervals. A complete recovery of the fluid was achieved by gently pushing 2 ml. air after the fluid, taking care not to cause any mechanical distension. The samples for analysis were collected directly from the polyethylene cannulae into a graduated cylinder and measured, the actual volume of gastric juice then being obtained by subtracting the 3 ml. washing fluid from the total volume measured. Using Töpfer's reagent and phenolphthalein as indicators, "free" and total acidity were determined by titrating the samples with 0.01N NaOH. Urine extract or the corresponding volume of the vehicle was injected intraperitoneally 60 min prior to histamine administration. Histamine was given subcutaneously in a dose of 0.4 mg/kg (calculated as base) and gastric juice was collected for the subsequent two hr. Total acidity, considered a truer reflection of parietal cell secretion than free acidity (Shay, Komarov & Berk, 1950), was chosen to illustrate the acid output during the entire procedure.

Shay method

Male hooded rats weighing between 150 to 170 g were fasted 48 hr prior to experimentation. Water was allowed at all times. Urine extract was administered either orally (2 to 3 g/kg) 6 or 24 hr prior to pylorus ligation or intraduodenally (0.5 to 1.5 g/kg) immediately after ligation. Intraduodenal injections were given with a 23-gauge needle distal to ligation. Control animals received the corresponding volumes of the vehicle. The pylorus was ligated under light ether anaesthesia and all animals were sacrificed 17 hr later. The severity of ulceration was evaluated and expressed according to Pauls, Wick & Mackay (1947). From each animal the gastric juice was collected and centrifuged. Those samples that contained 0.5 ml. or more of solid particles were discarded. Free and total acidity were determined as described previously.

Restraint (stress) method

Female albino rats weighing 170 to 190 g were fasted for 24 hr before experimentation: water was allowed at all times. The urine extract was administered either intraperitoneally (0.75 to 1.0 g/kg) or intramuscularly (1 to 1.5 g/kg) immediately prior to immobilization. Control animals

received the corresponding volume of the vehicle. Both control and treated animals were immobilized for 24 hr according to the method of Bonfils and Lambling (1963), following which they were killed. The stomachs were examined macroscopically and ulceration was evaluated according to the "all or nothing" method of Bonfils and Lambling (1963).

Blood pressure

Guinea-pigs weighing 280 to 380 g and rats weighing 150 to 180 g were anaesthetized with urethane (1 g/kg given intraperitoneally) 30 min prior to operation. Blood pressure was recorded from the carotid artery on a Gilson polygraph maintaining artificial respiration. Urine extracts were administered intraperitoneally in a dose of 1.0 to 2.0 g/kg and records were taken at 15-min intervals for 2 to 3 hr following the administration of the extract.

In the statistical evaluation of the results, Student's "t" was used as a significance test, except in the "restraint" method, where the results were evaluated according to the Chi Square test.

RESULTS

Antihistamine activity

As a standard procedure, every extract was tested on isolated guinea-pig ileum preparation in order to establish its antihistamine activity. A typical example of the results obtained by the extracts on histamine-induced contractions is shown in Fig. 1.

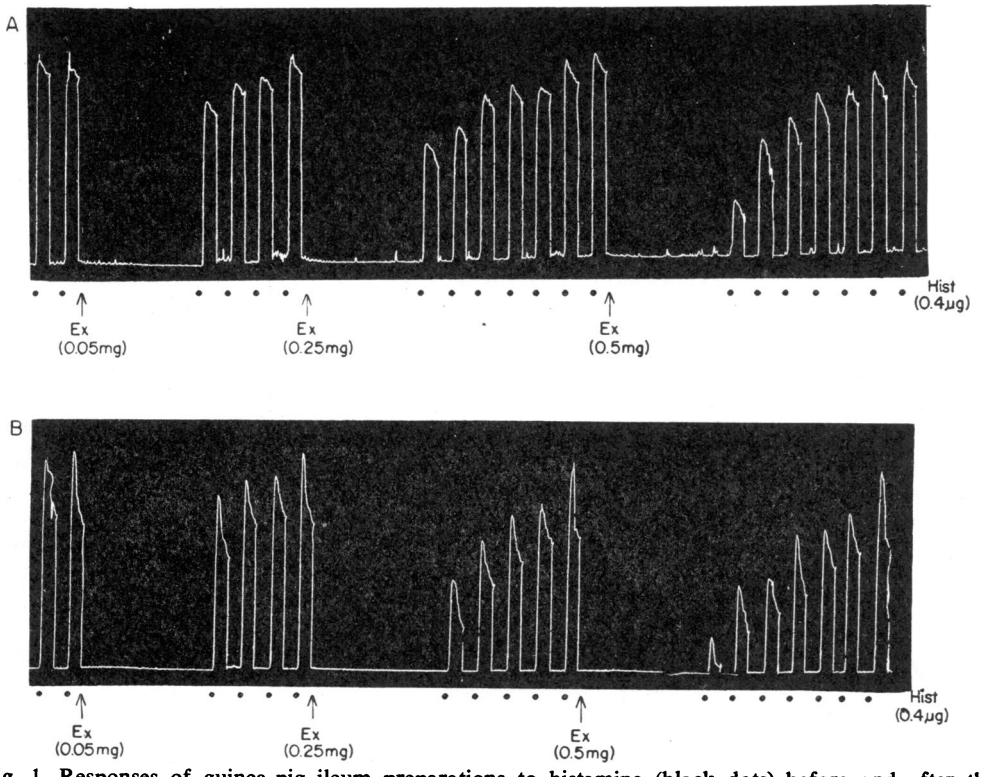

Fig. 1. Responses of guinea-pig ileum preparations to histamine (black dots) before and after the administration of either horse urine extract (Fig. A) or human urine extract (Fig. B) as marked at arrows (Ex) in increasing concentrations (0.05, 0.25 and 0.5 mg/50 ml. bath. Intervals of 3 min elapsed between administrations of histamine. In each instance the drum was temporarily stopped after washing and restarted 20 sec before the next test. After each administration of the extract the record was taken continuously for 3 min.

The administration of horse urine (Fig. 1,A) and human urine extract (Fig. 1,B) in concentrations of 1.10^{-6} g/ml. elicited about a 30% reduction in the contractions induced by 0.4 μg of histamine. The addition of the extract in a concentration of 5.10^{-6} g/ml. produced about a 50% inhibition, while the administration of the extract in a concentration of 1.10^{-5} g/ml. resulted in a nearly complete inhibition. After repeated washings, the gut regained its original sensitivity to the standard dose of histamine.

Inhibition of histamine-induced gastric hypersecretion

The result of the experiments obtained after the intraperitoneal administration of 1.5 g/kg of the urine extract is summarized in Fig. 2: the intraperitoneal injection of the vehicle or the extract did not induce any significant change in the basal gastric secretion during the 60-min observation period. Histamine (0.4 mg/kg) elicited a

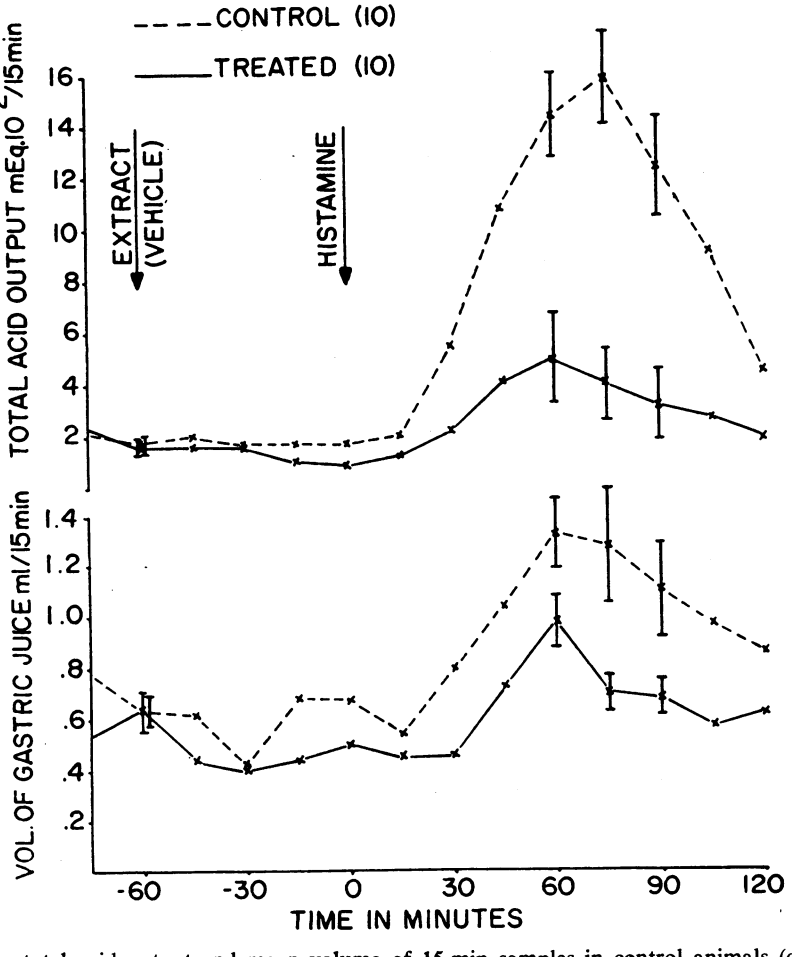

Fig. 2. Mean total acid output and mean volume of 15-min samples in control animals (dotted line) and in guinea-pigs treated with horse urine extracts (continuous line) before and after the administration of 0.4 mg/kg histamine. Acid output: $P<0.001$ (60, 75, 90 min); volume of gastric juice: Not significant (60 min), $P<0.05$ (75 and 90 min).

maximal secretory response in the controls, but, in the treated animals, it induced only a slight increase in gastric secretion. In three subsequent samples collected during peak secretion (60, 75, 90 min after histamine administration) both acid output and volume of gastric juice were found to be lower in animals treated with the extract.

Protection against ulcer formation

Shay method

Horse urine extracts: in the first series of experiments urine extract was administered in a dose of 2.0 and 3.0 g/kg orally into rats 6 hr prior to pylorus ligation. Though in the treated animals ulcer formation was strongly inhibited, these results could not be fully evaluated, since traces of the substance could be seen in the stomach of some animals during autopsy. Thus, the possibility of a local effect could not be excluded.

Table 1,A summarizes the results of those experiments in which the extract was administered orally 24 hr prior to pylorus ligation. It is shown that the administration of 2.0 g/kg extract produced a slight protection. However, when the dose was increased to 2.5 g/kg, the protection became evident. The administration of 3.0 g/kg of the extract resulted in a highly significant reduction of ulceration. The efficacy of the extract at this dose level was also clearly reflected in the analysis of gastric juice of 8 treated and 15 control animals (Table 2,A). In treated animals both the volume and free and total acid outputs were significantly reduced when compared with that of controls.

In order to exclude any possibility of a local effect in a series of experiments, the extract was given intraduodenally into rats immediately after pylorus ligation. Table 1,B summarizes the results obtained after the intraduodenal administration of 0.5 and 1.0 g/kg urine extract. It is shown that urine extract in a dose of 0.5 g/kg did not produce any significant reduction in ulcer formation, while the injection of 1.0 g/kg of the extract brought about a highly significant protection against ulceration. Similarly both the volume of gastric juice and acid output were significantly reduced (Table 2,B).

TABLE 1

THE EFFECT OF HORSE AND HUMAN URINE EXTRACT ON ULCER FORMATION IN SHAY RATS

The extract or the solvent was administered orally 24 hr prior to pylorus ligation (Section A), or injected intraduodenally immediately after pylorus ligation (Sections B and C). Animals were killed 17 hr after ligation. Determinations were made individually, results express mean values and standard errors. N.S.= Not significant.

	Treatment	Source of extract	No. of animals	Doses g/kg	Ulceration	P	Ulcer Index
A	SOLVENT	—	35	(1·5 ml.)	2·54± ·26		225·0
	EXTRACT	Horse	6	2·0	2·08± ·56	N.S.	208·0
	EXTRACT	Horse	5	2·5	1·00± ·49	<·01	60·0
	EXTRACT	Horse	20	3·0	0·85± ·30	<·001	34·0
B	SOLVENT	—	22	·6 ml.	3·0 ± ·24		286·2
	EXTRACT	Horse	6	0·5	2·0 ± ·78	N.S.	133·2
	EXTRACT	Horse	20	1·0	0·75± ·25	<·001	45·0
C	SOLVENT	—	12	·6 ml.	1·79± ·36		119·2
	EXTRACT	Human	3	1·0	2·17±1·19	N.S.	217·0
	EXTRACT	Human	6	1·75	0·42± ·16	<·01	28·0

TABLE 2

THE EFFECT OF HORSE URINE EXTRACT ON GASTRIC SECRETION IN SHAY RATS

The extract or the solvent was administered orally 24 hr prior to pylorus ligation (Section A), or injected intraduodenally immediately after pylorus ligation (Section B). Animals were killed 17 hr later. Determinations were made individually, results express mean values and standard errors. N.S.=Not significant.

	Treatment	No. of Animals	Doses g/kg	Vol. of Gastric juice	P	Free HCl mEq × 10^{-2}	P	Total acidity mEq × 10^{-2}	P
A	SOLVENT	15	1·5 ml.	7·3± ·65		46·6± 4·8		68·9± 8·5	
	EXTRACT	8	3·0	4·4± ·31	<·001	21·6± 3·0	<·001	39·4± 4·0	<·01
B	SOLVENT	14	0·6 ml.	8·6± ·9		62·1± 3·4		86·5± 3·4	
	EXTRACT	4	0·5	6·7±1·5	N.S.	49·9±14·1	N.S.	77·4±18·2	N.S.
	EXTRACT	16	1·0	4·8± ·2	<·001	42·0± 2·5	<·001	60·4± 3·2	<·001

Human urine extracts: extracts of male human urine when injected intraduodenally into rats exerted a protection against ulcer formation similar to that of pregnant mare urine. From human urine extracts, however, it was necessary to inject 1.75 g/kg intraduodenally to obtain a protection corresponding to that of 1.0 g/kg horse urine extract. Table 1,C summarizes the results obtained after the administration of 1.0 g/kg and 1.75 g/kg of the extract: 1.75 g/kg of the extract significantly reduced ulcer formation.

Restraint (stress) method

Table 3,A illustrates the protective effect of 0.75 g/kg and 1.0 g/kg horse urine extract administered intraperitoneally immediately prior to immobilization. The 24-hr immobilization period led to ulcer formation in 66.6% of the control rats. In animals which received 1.0 g/kg of the extract, ulceration was found in 21%. Table 3,B shows that the intramuscular injection of the extract in doses of 1.0 g/kg and 1.5 g/kg resulted in a protection similar to that obtained after the intraperitoneal administration of the extract; the results being different from the controls at the higher dose level.

Effect on blood pressure

Following the administration of urine extracts, neither the blood pressure nor the heart rate showed much change in either group during the 3-hr observation period.

TABLE 3

THE EFFECT OF HORSE URINE EXTRACT ON ULCER FORMATION IN IMMOBILIZED RATS

The extract or solvent was administered either intraperitoneally (Section A), or intramuscularly (Section B), immediately prior to immobilization. Animals were killed 24 hr later. N.S.=Not significant.

	Treatment	No. of Animals	Doses g/kg	% Showing ulceration	P
A	SOLVENT	15	(·3 ml.)	66·66	
	EXTRACT	13	·75	38·46	N.S.
	EXTRACT	14	1·0	21·43	<·05
B	SOLVENT	15	(·3 ml.)	80·00	
	EXTRACT	16	1·0	50·00	N.S.
	EXTRACT	14	1·5	37·71	<·05

DISCUSSION

The results of the present experiments not only confirm the presence of an antihistamine-like substance(s) in extracts of mammalian urine but they also show that these extracts exert a strong inhibitory effect on gastric secretion. The existence of a substance(s) with antihistamine-like effects in different cell, tissue or urine extracts seems to be well established (Kovacs, 1950; Kovacs & Juhasz, 1951; Karady, Kovacs, Kovacs, Szerdahelyi & Vajda, 1951; Francis & Melville, 1959; Feldberg & Kovacs, 1960; Archer, 1963; Kovacs & Melville, 1962, 1963; Kovacs, Pelletier & Rose, 1963, etc). These reports also indicate that the mechanism of action of the active principle(s) present in the extracts basically differs from that of the synthetic antihistamines. This assumption is further supported by the findings that the extracts are also capable of reducing the gastric acid secretion, at least under the experimental conditions described in this paper.

The results presented in this paper, however, were obtained with a purified but not with a chemically pure substance. Therefore we do not have any direct evidence which would indicate that the antihistaminic and antiulcerogenic effect is brought about by the same or a single substance. This possibility, however, seems to be supported at present by the fact that the purification procedures result in a similar increase of specific activity for both antihistaminic and antiulcerogenic effects.

It is well known that human and animal urines contain a substance called urogastrone (Gray, Culmer, Wells & Wieczorowski, 1941), which inhibits gastric secretion (Friedman, 1951). The properties of the substance and its extraction from urine have been studied extensively by Gray, Wieczorowski, Wells & Harris (1942) and Gregory (1955). On the basis of these facts one can safely conclude that the active principle present in our extracts is not urogastrone. For example, urogastrone is very well soluble in water but not in chloroform. Furthermore, urogastrone was found to be ineffective when administered orally. In contrast to these data the extracts used in our experiments were insoluble in water but readily soluble in chloroform and they were highly active when administered orally or intraduodenally.

One of the striking features of the antiulcerogenic action of urine extracts was its long duration. Administered in sufficient amounts the effect of a single oral dose could exert an inhibitory effect on the gastric secretion for at least 30 to 40 hr. On the other hand, when it was administered intraduodenally at the time of pylorus ligation, the same degree of protection could be obtained with correspondingly smaller doses of the extract.

From the experiments performed to date it is not possible to draw conclusions concerning the chemical nature of the active substance in urine extracts or about its mechanism of action. Nor is it possible to establish whether the active principle plays any important physiological role in the regulation of H-substances or gastric secretion in the human body.

SUMMARY

1. The action of horse and human urine extracts which exert antihistamine-like effects was investigated on gastric acid secretion.

2. The extract used in these experiments was found to be readily soluble in organic solvents (chloroform, ether, etc), but insoluble in water.

3. Urine extracts injected intraperitoneally into guinea-pigs inhibited the histamine-induced gastric hypersecretion.

4. The extract administered either orally 24 hr prior to pylorus ligation or intraduodenally immediately after ligation prevented or strongly reduced the development of ulcer formation in Shay rats.

5. The intraperitoneal or intramuscular administration of the extract into rats reduced ulcer development induced by the restraint (stress) method.

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