Br. J. Pharmac. Chemother. (1967), **30**, 224–228.

THE EFFECT OF ANAPHYLACTIC SHOCK AND PREDNISOLONE ADMINISTRATION ON THE HISTAMINOLYTIC ACTIVITY OF GUINEA-PIG LIVER *

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(*Received September* 1, 1966)

Rose & Leger (1952) reported the appearance of a histamine destroying factor in the blood of rabbits during anaphylaxis. Code, Cody, Hurn, Kennedy & Strickland (1961) demonstrated a similar phenomenon in rats. Logan (1961) confirmed this in guinea-pigs. Logan (1962) produced evidence that the release of the histamine destroying factor occurred from the small intestines of rats. In rats the small intestine has been reported to be the organ richest in histaminase (Waton, 1956).

In guinea-pigs, liver has been shown by Lindell & Westling (1953) to contain considerable histaminolytic activity. The effect of anaphylactic shock on the histaminolytic activity of a homogenate of guinea-pig liver has therefore been studied in the hope that any change might indicate the site of release of histaminase, and help to define the importance of histaminase in anaphylactic shock.

In another series the effect of prednisolone administration on histaminolytic activity of the guinea-pig liver, in control and shocked animals, has been studied. Since corticosteroid administration to adrenolectomized animals restores depleted histaminase activity (Kahlson, 1956) it was hoped that the basal level of histaminolytic activity might be raised by prednisolone and that any difference due to shock would be easily detected. These expectations were interestingly belied.

METHODS

Four groups, each of six guinea-pigs weighing between 400–700 g, were used. Group 1 was used as control (see Table 1).

Sensitization and anaphylactic shock

Groups 2 and 4 were sensitized with 0.1 ml. of a freshly prepared 5% solution of crystalline ovalbumin in 0.9% saline, injected intramuscularly on four consecutive days. They were challenged with an intraperitoneal injection of 0.1 ml. of 5% ovalbumin in saline, 3–13 weeks after sensitization.

Animals in Group 3 were given an intramuscular injection of 5 mg/kg prednisolone acetate daily for 14 days before death. Group 4 also received the same treatment with prednisolone before being challenged with the antigen.

* The investigation was supported by a grant from the Gujarat University, Ahmedabad.

Preparation of liver homogenate

Animals either died of anaphylactic shock or were killed by a blow on the head and severance of neck vessels. The abdomen was quickly opened and the liver collected in ice-cold Tyrode solution. It was dried between filter papers and weighed to the nearest 0.1 g. It was then cut into slices and transferred to a chilled Waring blender containing 5 ml. Tyrode solution/g of tissue. The blender was run for 2 min and the homogenate centrifuged for 30 min at 900 *g*.

Incubation

To two conical flasks of 250 ml. capacity, each containing 5 ml. and 10 ml. homogenate and 94.5 ml. and 89.5 ml. Tyrode solution respectively, 0.5 ml. histamine acid phosphate (1 mg/ml.) solution was added, and the flasks were kept in a thermostatically controlled water-bath at 37° C and shaken occasionally.

A 5 ml. sample was collected immediately after mixing and further 5 ml. samples were collected at 20, 40, 60 and 100 min. The samples were immediately dipped in boiling water to destroy enzymatic activity. The volume of the boiled samples was adjusted and the samples were stored at 4° C until biological assay for histamine content was carried out.

Histamine assay

Histamine was assayed on guinea-pig ileum in atropinized Tyrode solution by the method of Code & McIntire (1956).

Histaminolytic activity

The histaminolytic activity of the sample was calculated by the method described by Spencer (1963). After determining the histamine concentrations in the samples, the incubation time t was plotted against $\log_{10} X_o/X_t$, where X_o is the histamine content of the first sample and X_t is the histamine content of the sample after incubation for t min. An approximately linear relationship was obtainable over the incubation range. The times in which 20% and 50% histamine was inactivated (DT 20 and DT 50 values) were obtained from these plots by reading the abscissae (t) for the ordinates ($\log_{10} X_o/X_t$) of 0.1 and 0.3 respectively.

The incubation mixture containing 10 ml. homogenate was included to confirm the validity of experiments and to calculate the DT 50 values of samples of low histaminase activity (Morrison, 1964).

RESULTS

The mean DT 50 value of the six control animals was 40 ± 18 (S.D.) min. The mean DT 50 value of the sensitized animals given anaphylactic shock was 64.66 ± 19 min. The histaminolytic activity of the liver in the shocked group was reduced by 38%, the difference being statistically significant ($P<0.02$) (Table 1).

Administration of 5 mg/kg prednisolone acetate intramuscularly daily for 14 days to group 3 reduced the histaminolytic activity of the liver by 63.3% of that of the control. The mean DT 50 value of this group was 109 ± 40 min ($P<0.01$).

Animals in group 4 were sensitized to ovalbumin. For 14 days before challenge they received prednisolone in the same dose as in group 3. The mean DT 50 value of this group was found to be 124 ± 32 min. Thus a further reduction of the histaminolytic activity of the liver was observed, though the difference between the values of group 3 and 4 was not statistically significant ($P<0.5$). As compared with the control, group 1, the mean histaminolytic activity was reduced by 68% ($P<0.01$).

TABLE 1

THE EFFECT OF ANAPHYLACTIC SHOCK ON THE HISTAMINOLYTIC ACTIVITY OF THE LIVER HOMOGENATES OF NORMAL AND PREDNISOLONE TREATED GUINEA-PIGS

Note the significant reduction in histaminolytic activity of liver by prednisolone and acute anaphylactic shock. The DT-50 value in the sixth animal in fourth group was unobtainable, the extrapolation of curves gave the calculated value of 413 min. This observation has not been included in computing the mean.

Group	Treatment	Guinea-pig weight (g)	Sex	Liver weight (g)	DT-50 with 1 g liver (min)	Mean DT-50±S.D.	Histaminolytic activity (as % of control)	In comparison with control group 1	
								Student's *t* test t	*P*
1	Control	560	F	27	21	40±18	100	—	—
		553	M	20	29				
		409	M	11	30				
		430	M	10	35				
		450	F	14	63				
		541	F	13·5	62				
2	Anaphylactic shock (i.p. injection of challenging antigen to ovalbumin sensitized animals)	450	M	18	36	64·6±19·4	61·9	2·278	<0·02
		525	M	18	50				
		516	F	18·5	60				
		450	F	12	86				
		515	F	17·5	80				
		537	F	17·8	76				
3	Prednisolone acetate (5 mg/kg i.m. for 14 days)	650	M	21·3	73	109±40·2	36·7	3·387	<0·01
		700	M	24·7	87				
		700	M	24	150				
		418	M	13·5	167				
		385	M	10	74				
		685	M	18·2	103				
4	Anaphylactic shock in prednisolone treated animals	605	M	23	110	124±32·6	32·3	5·141	<0·01
		650	M	21	78				
		650	M	22·2	140				
		700	M	25·4	127				
		500	M	21·7	165				
		505	M	15	—				

DISCUSSION

Code *et al.* (1961) showed the appearance of histaminolytic activity in the blood of white rats during anaphylactic shock. Rose & Leger (1952) reported similar findings in rabbits during anaphylactic shock.

Logan (1961) produced evidence for the appearance of histaminolytic activity (histaminase) during anaphylactic shock in guinea-pigs. He showed that blood histamine was reduced by 50 to 92% if the blood of guinea-pigs in acute anaphylactic shock was incubated at 37° C for 1½ hr before extraction. Aminoguanidine could protect histamine in blood from this destruction if added before incubation. In pre-shock samples there was no destruction of histamine. Histaminolytic activity appeared within 3 min of the challenging injection. In rats, Logan (1962) showed that the histaminolytic activity in blood appeared 6 to 13 min after the challenge. In this species he tried to locate the site of "histaminase" release. He ligated intestinal and renal vessels in rats before challenging and found that no destruction of blood histamine occurred on incubation in this group. The destruction of histamine was seen only if the renal vessels were ligated or in sham operated animals, but it was significantly less or almost none in the animals in which intestinal vessels were ligated. It was concluded from these findings that most of the histaminolytic activity was released from the small intestine. Waton (1956) had shown that the intestines were the richest source of histaminase in rats.

The source of release of histaminolytic activity in guinea-pigs undergoing anaphylactic shock has not been determined. Lindell & Westling (1953) have demonstrated that in the guinea-pig the kidney and the intestinal tract contain only small amounts of histaminase whereas the liver is rich in the enzyme. The changes in the histaminolytic activity of guinea-pig liver during anaphylaxis have therefore been studied to provide a clue to the site of its release, and to assess it quantitatively.

Spencer's (1963) method was used and was found suitable for studying the histaminolytic activity of guinea-pig liver. After acute anaphylactic shock in group 2, sensitized to ovalbumin and challenged, the histaminolytic activity of liver was reduced to about 62% of the control activity, 38% of its activity presumably being "released" during anaphylactic shock.

It is not possible to say from this study which particular cell of the liver releases the histaminolytic activity. Logan (1961) has suggested that mast cells may be involved in the release of both histamine and histaminase.

Prednisolone administration for 14 days has been tried to study the changes induced in the histamine metabolizing capacity of liver under the influence of corticosteroids. It was found that the capacity of liver to metabolize histamine was diminished considerably after prednisolone administration.

Decrease in the liver histaminase activity after prednisolone administration may be due either to diminished synthesis of the enzyme by the liver or to increased release of the enzyme from the liver into the circulation.

Prednisolone administration did not protect animals from acute anaphylactic shock. The mean histaminolytic activity was further reduced as compared with that of animals receiving prednisolone alone but the difference was not significant.

The reduction of the histaminolytic activity of the liver during anaphylactic shock indicates that in guinea-pigs the liver is the source of histaminase appearing in the circulation. Beall (1966) has shown that the lung tissue of shocked guinea-pigs failed to inactivate histamine for several hours. The possibility that repeated subclinical anaphylactic episodes may result in the exhaustion of the tissue stores of histaminase is therefore worth studying, since exhaustion might be an important factor in the causation and maintenance of some chronic allergic conditions. Since release of histaminolytic activity has been demonstrated in several species, it might well occur also in man.

SUMMARY

1. After acute anaphylactic shock in ovalbumin-sensitized guinea-pigs the histaminolytic activity of the liver was reduced by 38% as compared to the control.

2. Prednisolone acetate (5 mg/kg) injected intramuscularly for 14 consecutive days reduced the histaminolytic activity of the liver by 63% as compared to the control.

3. Prednisolone acetate did not protect guinea-pigs against acute anaphylactic shock.

4. Both anaphylaxis and prednisolone administration reduced the histaminolytic activity of liver significantly.

We are grateful to Dr. S. M. Sen, Dean of the M.P. Shah Medical College, Jamnagar, for permission to publish this article, to Hoechst Pharmaceuticals, Bombay, for liberal supplies of prednisolone acetate, to Shri D. J. Joshi and Shri A. R. Trivedi for technical assistance, and to Dr. Ranita Aiman, Professor of Pharmacology, Christian Medical College, Vellore, for helpful suggestions. We are grateful to the Gujarat University for a research grant.

REFERENCES

BEALL, G. N. (1966). Histamine in guinea pig tissues. *Archs int. Pharmacodyn. Ther.*, **159**, 484–495.

CODE, C. F., CODY, D. T., HURN, M., KENNEDY, J. C. & STRICKLAND, M. J. (1961). The simultaneous release of histamine and a histamine-destroying factor during anaphylaxis in rats. *J. Physiol.*, **156**, 207–216.

CODE, C. F. & MCINTIRE, F. C. (1956). Quantitative determination of histamine. *Meth. biochem. Analysis*, **3**, 49–95.

KAHLSON, G. (1956). The significance of histaminase in the body. In *Histamine, Ciba Foundation Symposium*, edited by WOLSTENHOLME, G. E. W. & O'CONNOR, C. M., pp. 248–257. Churchill, London.

LINDELL, S. E. & WESTLING, H. (1953). The histaminase activity of different organs in the guinea pig. *Acta physiol. scand.*, **30**, suppl. No. 111, 202–206.

LOGAN, G. B. (1961). Release of histamine-destroying factor during anaphylactic shock in guinea-pigs. *Proc. Soc. exp. Biol. Med.*, **107**, 466–469.

LOGAN, G. B. (1962). Source and time of release of histamine-destroying factor (histaminase) during anaphylactic shock in rats. *Proc. Soc. exp. Biol. Med.*, **111**, 171–174.

MORRISON, G. A. (1964). Kinetics of histaminase. *J. Pharm. Pharmac.*, **16**, 285–286.

ROSE, B. & LEGER, J. (1952). Serum histaminase during rabbit anaphylaxis. *Proc. Soc. exp. Biol. Med.*, **79**, 379–381.

SPENCER, P. S. J. (1963). A new method for the estimation of histaminase activity. *J. Pharm. Pharmac.*, **15**, 225–232.

WATON, N. G. (1956). Studies on mammalian histidine decarboxylase. *Br. J. Pharmac. Chemother.*, **11**, 119–127.