Biochemical Pharmacology, 1965, Vol. 14, pp. 1277–1281. Pergamon Press Ltd., Printed in Great Britain.

BIOCHEMICAL STUDY OF ANTI-INFLAMMATORY AND ANTI-ARTHRITIC PROPERTIES OF GLYCYRRHETIC ACID*

K. K. TANGRI, P. K. SETH, SURENDRA S. PARMAR and K. P. BHARGAVA

Department of Pharmacology and Therapeutics, King George's Medical College, Lucknow University, Lucknow

(*Received* 31 *July* 1964; *accepted* 13 *October* 1964)

Abstract—The anti-inflammatory activity of glycyrrhetic acid, methyl glycyrrhetic acid and glycyrrhetic acid diacetate was found to be similar to hydrocortisone on the formalin-induced arthritis in albino rats. Methyl glycyrrhetic acid and glycyrrhetic acid diacetete were more potent anti-inflammatory agents when compared with glycyrrhetic acid.

Glycyrrhetic acid, methyl glycyrrhetic acid and hydrocortisone prevented the elevation of S-GOT and S-GPT during inflammation. These agents reduced the S-GPT level and not the S-GOT level in normal rats. ATPase activity in brain and liver homogenates remained unaltered during inflammation but was significantly elevated by these agents. The significance of these biochemical changes is discussed.

INTRODUCTION

THE EXACT mechanism of action of anti-inflammatory agents is not fully understood. Uncoupling of oxidative phosphorylation[1–4] and inhibition of certain enzyme systems such as transaminases[5–7] by these agents might be responsible for their anti-inflammatory properties. Glycyrrhetic acid, an aglycone obtained from Glycyrrhiza glabra has been shown to possess potent anti-inflammatory actions.[8, 9] Glycyrrhetic acid and glycyrrhizin have been reported to uncouple oxidative phosphorylation[10] and to inhibit biosynthesis of sulphated mucopolysaccharides.[11] In the present investigation, the anti-inflammatory activity of glycyrrhetic acid, methyl glycyrrhetic acid and glycyrrhetic acid diacetate was compared with that of hydrocortisone by the Brownlee arthritis method in rats.[12] In order to correlate the anti-inflammatory activity with the biochemical changes, the effects of glycyrrhetic acid, methyl glycyrrhetic acid and hydrocortisone on the transaminases and adenosine triphosphatase activity, were studied in normal and arthritic rats.

METHODS

Antiinflammatory studies

The drugs were assessed for their anti-inflammatory activity on the formaldehyde induced arthritis in albino rats, produced according to the method of Brownlee.[12] Rats weighing between 100–110 g were divided into five groups of six each. The antero-posterior diameter of the ankle joints were measured daily for ten days and 0·1 ml of 2% (v/v) formaldehyde was injected subcutaneously under the plantar aponeurosis in each foot on the first and third days. One group of animals served

* A preliminary report of this study was communicated to the Sixth International Congress of Biochemistry, New York **5**, 410 (1964).

as control. One group each was treated with intraperitoneal injections of hydrocortisone (0·5 mg/100 g), glycyrrhetic acid, methyl glycyrrhetic acid and glycyrrhetic acid diacetate (each 2 mg/100 g) daily for ten days. The ten-day average diameter for each group was calculated and statistically analysed.

Biochemical studies

Enzyme assays were carried out in normal and arthritic albino rats with or without drug treatment. Serum was obtained from the blood collected by decapitation of rats and liver and brain tissues removed immediately and pooled separately.

Serum glutamic oxaloacetic transaminase (S-GOT) and serum glutamic pyruvic transaminase (S-GPT) were assayed by the method of Reitman and Frankel.[13] One unit of enzyme activity was the change in the optical density of 0·001/min/ml of serum. Optical density was measured in a Bausch and Lomb Spectronic '20' colorimeter at 505 mμ.

Adenosine triphosphatase (ATPase) activity was assayed in 10% (w/v) homogenates of pooled tissues (liver or brain) prepared in 0·25 M sucrose by Potter–Elvehjem homogenizer. The reaction mixture consisted of 0·05 M Tris pH 8·0, 1 mM ATP and 0·1 ml of 10% tissue homogenate, in a final volume of 2 ml. Release of P_i (inorganic phosphorus) from ATP was measured according to Fiske and Subbarow.[14] The split of 1 μM of P_i/100 mg of tissue in 15 min at 37° was considered as one unit of the enzyme activity.

RESULTS AND DISCUSSION

A. *Effect of formaldehyde induced arthritis*

The effects of hydrocortisone, glycyrrhetic acid, methyl glycyrrhetic acid and glycyrrhetic acid diacetate were studied on formaldehyde induced arthritis in albino rats and the results are shown in Table 1. Glycyrrhetic acid, methyl glycyrrhetic acid and glycyrrhetic acid diacetate showed potent anti-arthritic activity similar to hydrocortisone. Quantitatively, methyl glycyrrhetic acid (32.2%) and glycyrrhetic acid diacetate (50%) had more potent anti-inflammatory activity when compared with glycyrrhetic acid (17·2%).

TABLE 1. EFFECT OF HYDROCORTISONE, GLYCYRRHETIC ACID, METHYL GLYCYRRHETIC ACID AND GLYCYRRHETIC ACID DIACETATE ON FORMALIN INDUCED ARTHRITIS IN RATS

	Dose mg/100 g (i.p.)	Average initial diameter (mm)	Average ten-day diameter (mm)*	% anti-inflammatory activity	P†
Control	—	6·1 ± 0·07	7·33 ± 0·04	0	—
Hydrocortisone	0·5	6·04 ± 0·09	7·1 ± 0·09	28·8	<0·001
Glycyrrhetic acid	2·0	6·08 ± 0·06	7·28 ± 0·06	17·2	0·01–0·001
Methyl glycyrrhetic acid	2·0	6·01 ± 0·08	7·04 ± 0·07	32·2	<0·001
Glycyrrhetic acid diacetate	2·0	6·19 ± 0·07	6·86 ± 0·08	50·0	<0·001

* Average of ten days measurements.
† Compared with the control average ten-day diameter.

B. *Effect on serum glutamic oxaloacetic transaminase (S-GOT) and serum pyruvic transaminase (S-GPT)*

The results of serum transaminase studies with hydrocortisone, glycyrrhetic acid

and methyl glycyrrhetic acid in the normal and arthritic rats, are summarized in Table 2.

The activities of the enzymes S-GOT and S-GPT were significantly increased during inflammation. The antiinflammatory drugs prevented the increase in the enzymatic activity due to the inflammatory reaction. These drugs decreased the S-GPT level in normal rats, but they did not significantly alter the normal S-GOT activity. The reduction in the transaminase activity by the anti-inflammatory agents may be related to

TABLE 2. EFFECT OF HYDROCORTISONE, GLYCYRRHETIC ACID AND METHYL GLYCYRRHETIC ACID ON SERUM GLUTAMIC OXALOACETIC TRANSAMINASE (S-GOT) AND SERUM GLUTAMIC PYRUVIC TRANSAMINASE (S-GPT) IN NORMAL AND ARTHRITIC RATS

		Control	Hydrocortisone	Glycyrrhetic acid	Methyl glycyrrhetic acid
	No. of observation	(15)	(11)	(10)	(6)
S-GOT*	Normal	28·27 ± 1·19	28·1 ± 0·68 (P = 0·9)	29·3 − 0·68 (P = 0·5 − 0·4)	34·76 − 0·67 (P = 0·2 − 0·1)
	Arthritic	44·5 ± 2·32	28·6 ± 1·31 (P = ∠0·001)	31·3 ± 0·47 (P = ∠0·001)	38·3 ± 0·65 (P = 0·05)
	Per cent increase in inflamation	57·4	1·77	6·82	10·4
S-GPT*	Normal	32·13 ± 1·5	22·5 ± 1·25 (P = ∠0·001)	24·0 ± 0·86 (P = ∠0·001)	18·7 ± 2·02 (P = ∠0·001)
	Per cent decrease with drug	—	30	25·4	41·8
	Arthritic	42·8 ± 2·25	25·7 ± 1·56 (P = ∠0·001)	26·8 ± 1·21 (P = ∠0·001)	28·5 ± 1·2 (P = ∠0·001)
	Per cent decrease with drug	—	39·9	37·4	37·4

* Enzyme activity in units. One unit = Change in optical density of 0·001 min/ml of serum.

their antiinflammatory action or may be an independent effect on the enzyme. Since these agents did not affect the S-GOT activity in the normal animals and significantly reduced the enzymatic activity in the arthritic rats, it may be concluded that the effect of these agents on S-GOT activity has a relationship to their anti-inflammatory action. However, such a relationship could not be established with S-GPT activity since these agents had an inhibitory action on the normal enzyme activity. Work with other anti-inflammatory agents is necessary to further establish a relationship between the anti-inflammatory activity and the enzyme inhibition. In a study of acute inflammation due to viral hepatitis, the serum transaminase activity was found to be increased and prednisone quickly reduced it.[15]

Huggins *et al.*[6, 7] reported that the inhibition of normal glutamic pyruvic transaminase was greater than glutamic oxaloacetic transminase studies *in vitro* with the anti-inflammatory agents like salicylates and resorcylic acid. Steggle *et al.*[5] have reported the inhibitory activity of salicylate congeners on the S-GPT in relation to

their chemical structures. However, Manso *et al.*[16] failed to observe the inhibitory action of salicylates on the serum transaminases. An inhibition of transamination in liver slices by butazolidine, chloroquin, cortisol and dexamethasone has also been reported.[17]

C. *Effect on adenosine triphosphatase (ATPase) activity in pooled tissue homogenates*

The results of the study of effects of hydrocortisone, glycyrrhetic acid and methyl glycyrrhetic acid on ATPase activity in pooled liver and brain homogenates obtained from normal and arthritic rats, are shown in Table 3. The ATPase activity both in the liver and brain homogenates was unaltered by the inflammatory reaction. The anti-inflammatory drugs, however, significantly raised the enzymic activity in the liver homogenates obtained from normal as well as arthritic animals. Though no change was seen in the brain ATPase activity with these agents in the normal animals, significant increase in the enzyme activity was observed in arthritic animals. The absence of the stimulant effect of these agents on brain ATPase activity in the normal animals could be due to their inability to cross the blood brain barrier. That the

TABLE 3. EFFECT OF HYDROCORTISONE, GLYCYRRHETIC ACID AND METHYL GLYCYRRHETIC ACID ON THE ADENOSINE TRIPHOSPHATASE (ATPASE) ACTIVITY* IN POOLED LIVER AND BRAIN HOMOGENATES OBTAINED FROM NORMAL AND ARTHRITIC RATS

		Control	Hydrocortisone	Glycyrrhetic acid	Methyl glycyrrhetic acid
Liver ATPase	Normal	11·62	16·98	14·31	14·31
	Per cent increase with drug	—	46·1	23·1	23·1
	Arthritic	11·62	16·99	16·99	16·99
	Per cent increase with drug	—	46·2	46·2	46·2
Brain ATPase	Normal	8·94	8·94	8·94	8·94
	Per cent increase with drug	—	0	0	0
	Arthritic	8·94	13·41	11·62	13·41
	Per cent increase with drug	—	50	24·99	50

* Expressed in μ M of P_i split per 100 mg of tissue in 15 min at 37°.

barrier was rendered more permable during inflammation, might account for the increased ATPase activity in the brain of arthritic rats.

Whitehouse and Haslam[10] observed that glycyrrhetic acid and several other anti-inflammatory agents uncoupled oxidative phosphorylation and increased ATPase activity in liver mitochondria. Also, the sulphation for the synthesis of mucopolysaccharides in the connective tissues was inhibited by these agents.[10, 11] Salicylates are known to uncouple oxidative phosphorylation by stimulating the ATPase activity.[18] However, the enzyme ATPase was unaffected by the inflammatory reaction in the present investigation and therefore the increased ATPase activity by these drugs may not be related to their anti-inflammatory action.

Acknowledgement—We gratefully acknowledge the financial support given by the Indian Council of Medical Research for the Drug Research Unit.

REFERENCES

1. T. M. Brody, *J. Pharmac.*, **117**, 39 (1956).
2. S. W. Teffery and M. J. H. Smith, *Biochem. J.*, **72**, 462 (1959).
3. M. W. Whitehouse, *J. Pharm. Pharmac.*, **15**, 556 (1963).
4. M. W. Whitehouse, *Biochem. Pharmac.*, **13**, 319 (1964).
5. R. A. Steggle, A. K. Huggins and M. J. H. Smith, *Biochem. Pharmac.*, **7**, 151 (1961).
6. A. K. Huggins, C. Bryant and M. J. H. Smith, *J. Pharm. Pharmac.*, **13**, 654 (1961).
7. A. K. Huggins, M. J. H. Smith and V. Moses, *Biochem. J.*, **79**, 271 (1961).
8. M. L. Gujral, K. N. Sareen, K. K. Tangri, A. K. Roy, G. P. Gupta and M. K. P. Amma, *Ind. J. Physiol. Pharmac.*, **3**, 39 (1959).
9. R. S. H. Finney and G. F. Somers, *J. Pharm. Pharmac.*, **10**, 613 (1958).
10. M. W. Whitehouse and J. M. Haslam, *Nature (Lond.)*, **196**, 1323 (1962).
11. H. Bastrom, K. Berusten and M. W. Whitehouse, *Biochem. Pharmac.*, **13**, 413 (1964).
12. G. Brownlee, *Lancet*, **1**, 157 (1950).
13. S. Reitman and S. Frankel, *Am. J. clin. Path.*, **28**, 56 (1957).
14. C. M. Fiske and V. Subbarow, *J. biol. Chem.*, **66**, 375 (1925).
15. E. Picchio and N. Benda, *Progr. Med. (Napoli)*, **14**, 68 (1958).
16. C. Manso, A. Taranta and I. Nydick, *Proc. Soc. exp. Biol., N.Y.* **93**, 84 (1956).
17. V. Moses and M. J. H. Smith, *Biochem, J.*, **61**, 424 (1961).
18. A. B. Falcone, *J. clin. Invest.*, **38**, 1002 (1959).