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ANTI-INFLAMMATORY ACTIVITY OF IMIPRAMINE AND CONGENERS*

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Abstract—Imipramine was found to possess potent anti-inflammatory property similar to hydrocortisone on formalin-induced arthritis in albino rats. Desmethyl imipramine and amitriptyline, the two congeners of imipramine, were devoid of such an activity.

Imipramine was shown to give significant protection against 5-HT and histamine aerosol-induced bronchospasm in guinea pigs, although it was a weaker antagonist of 5-HT and histamine than BOL-148 and mepyramine, respectively.

Imipramine, like hydrocortisone, prevented the elevation of S-GOT and S-GPT levels during inflammation. It reduced the S-GPT but not the S-GOT level in normal rats. ATPase activity was significantly increased by imipramine in liver and brain homogenates during the inflammatory state although inflammation did not alter the ATPase activity in tissue homogenates.

INTRODUCTION

IMIPRAMINE (Tofranil; Geigy) and its congeners, desmethyl imipramine (Pertofran; Geigy) and amitriptyline (Elavil; Merck Sharp and Dohme) are powerful anti-depressive agents.[1–3] Amitriptyline has been shown to stimulate the pituitary adrenal system in normal and stressed animals.[4] As adrenocorticotrophin and adrenal corticoids are potent anti-inflammatory agents, imipramine and its congeners may also possess such an activity. The anti-inflammatory property of imipramine and its congeners was studied using Brownlee's[13] arthritis method in rats. Also, anti-serotonin (5-hydroxytryptamine) and the antihistaminic activities of imipramine plus its effect on serum transaminases and tissues ATPase were studied in order to elucidate its mechanism of anti-inflammatory activity since anti-inflammatory agents are known to antagonize histamine[5, 6] and 5-hydroxytryptamine,[7] inhibit transamination[8–10] and uncouple oxidative phosphorylation.[11, 12]

METHODS

Anti-inflammatory studies

The anti-inflammatory activity of the drugs were studied in albino rats having formaldehyde-induced arthritis, employing the method of Brownlee.[13] Rats weighing 100–110 g were divided into groups of six. The antero-posterior diameters of the ankle joints were measured daily for 10 days and 0·1 ml of 2% (v/v) formaldehyde solution was injected subcutaneously under the planter aponeurosis in each foot on the first

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and third days. One group of animals served as control, and one group each was treated with intraperitoneal injections of hydrocortisone (0·5 mg/100g), phenylbutazone (2mg/100g), imipramine (0·8mg/100g), desmethyl imipramine and amitriptyline (2 and 8mg/100g each). The 10-day average diameter for each group was calculated and statistically analysed.

5-Hydroxytryptamine and histamine aerosol studies

The anti-5-hydroxytryptamine and antihistaminic activities of imipramine were investigated on the 5-hydroxytryptamine (5-HT) and histamine aerosol induced bronchospasm in the guinea pigs produced by the method of Herxheimer[14] and compared with that of 2-bromolysergic acid diethylamide (BOL-148) and mepyramine, respectively. The animals were divided into groups of six and exposed to 5-HT or histamine aerosol in a chamber. The aerosol was produced by passing compressed air at a pressure of 200 mm Hg through 0·5% aqueous solution of histamine acid-phosphate (pH 7·0) and 5-hydroxytryptamine creatinine sulphate (pH 6·0) as base kept in a nebulizer (Asmovan-Burrows Wellcome). The preconvulsion time for each animal was determined both before and after drug treatment. Graded doses of the drugs were injected intraperitoneally in different groups. The percentage protection afforded by these agents against 5-HT and histamine aerosol induced bronchospasm was calculated by the formula:

$\%$ protection $= (1\text{-}C/T) \times 100$ where C and T are mean preconvulsion times before and after drug treatment respectively.

The regression lines were plotted and PD_{50} of imipramine was compared with that of BOL-148 and mepyramine to determine its anti 5-hydroxytryptamine and antihistaminic potencies.

Biochemical studies

The enzyme estimations were carried out in normal and arthritic albino rats with or without treatment. Serum was obtained from the blood collected after decapitation of the rats. The liver and brain tissues were obtained immediately and pooled separately.

Serum glutamic oxaloacetic transaminase (S-GOT) and serum glutamic pyruvic transaminase (S-GPT) activities were estimated by the method of Reitman and Frankel.[15] One unit of enzyme activity was the change in the optical density of 0·001/min/ml of serum. Optical density was measured by a Bausch and Lomb spectronic "20" colorimeter.

ATPase activity was assayed in 10 per cent (w/v) homogenates of pooled tissues (liver and brain) prepared in 0·25 M sucrose by Potter Elvehjem homogenizer. The reaction mixture consisted of 0·05 M Tris, pH 8·0, 1mM ATP and 0·1 ml of 10 per cent tissue homogenates in a final volume of 2 ml. Release of P_i (inorganic phosphorus) from ATP was measured according to Fiske and SubbaRow[16]. 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

Effect on formaldehyde-induced arthritis

The effects of imipramine, desmethyl imipramine and amitriptyline were studied in albino rats having formaldehyde-induced arthritis and compared with that of standard

anti-inflammatory agents, hydrocortisone and phenyl butazone. The results are shown in Table 1. Imipramine proved to have potent anti-inflammatory properties ($P = 0{\cdot}05$–$0{\cdot}02$) similar to hydrocortisone ($P = <0{\cdot}001$). On the other hand, in another series of experiments desmethyl imipramine and amitriptyline were found to be devoid of such reactivity even in larger doses (2 mg/100 g) although phenylbutazone showed

TABLE 1. EFFECT OF IMIPRAMINE DESMETHYL IMIPRAMINE AND AMITRIPTYLINE ON FORMALIN-INDUCED ARTHRITIS IN RATS

	Dose mg/100 g (i.p.)	Initial ankle diameter (mm)	Average diameter of ankle during 10-day period (mm)	P*
Control	—	6·1 ± 0·07	7·53 ± 0·04	—
Hydrocortisone	0·5	6·04 ± 0·09	7·1 ± 0·09	∠0·001
Imipramine	0·8	5·98 ± 0·09	7·34 ± 0·05	0·05–0·02
Control	—	5·57 ± 0·07	7·14 ± 0·11	—
Phenylbutazone	2·0	5·56 ± 0·15	6·65 ± 0·17	0·05–0·02
Amitripyline	2·0	5·57 ± 0·15	7·08 ± 0·11	0·7
Desmethyl imipramine	2·0	5·51 ± 0·1	6·96 ± 0·15	0·4

* Compared with the control average 10-day diameter.

significant anti-inflammatory property ($P = 0{\cdot}05$–$0{\cdot}02$) in the same dose. Further increase in the dose of desmethyl imipramine and amitriptyline (8 mg/100 g) resulted in 100 per cent mortality. Therefore, it can be deduced that the anti-inflammatory activity of imipramine is not related to its degradation in the body forming the desmethyl congener as is suggested for its anti-depressive activity[1,3] since desmethyl imipramine was devoid of anti-inflammatory properties.

Effect on 5-hydroxytryptamine (5-HT) and histamine aerosol-induced bronchospasm

The effect of imipramine on guinea pigs having 5-HT and histamine aerosol-induced bronchospasm was investigated and compared quantitatively with the specific blockers of 5-HT and histamine i.e. 2-bromolysergic acid diethylamide (BOL-148) and mepyramine. Table 2 shows the percent protection afforded by graded doses of imipramine, BOL-148 and mepyramine against 5-HT, and histamine aerosol-induced bronchospasm. It is evident that imipramine protected against 5-HT and histamine aerosol-induced bronchospasm in a way similar to BOL-148 and mepyramine and the degree of protection varied directly with the dose.

The regression lines of BOL-148 and imipramine for the protection against 5-HT aerosol induced bronchospasm, and mepyramine and imipramine for protection against histamine aerosol-induced bronchospasm, are shown in Fig. 1. Imipramine was found to be a weaker antagonist of 5-HT than BOL-148, the ratio of PD_{50} of these agents being 1:11. Similarly, it was a weaker antagonist of histamine than mepyramine, the PD_{50} ratio being 1:4. Further more, the nature of antagonism of imipramine against 5-HT and histamine was similar to BOL-148 and mepyramine since the respective regression lines were parallel.

TABLE 2. EFFECT OF BROMOLYSERGIC ACID DIETHYLAMIDE (BOL-148), MEPYRAMINE AND IMIPRAMINE ON 5-HYDROXYTRYPTAMINE (5-HT) AND HISTAMINE AEROSOL-INDUCED BRONCHOSPASM IN GUINEA PIGS

	Drug	Dose (mg/kg)	Per cent protection
5-HT induced bronchospasm	BOL-148	0·2 (6)*	29
		0·8 (12)	48
		1·6 (6)	68
		3·2 (6)	78
	Imipramine	3·125 (4)	26
		6·25 (11)	45
		12·5 (5)	51
		25·0 (5)	84
Histamine induced bronchospasm	Mepyramine	0·5 (5)	56
		1·0 (6)	63
		2·0 (10)	90
	Imipramine	0·8 (6)	29
		1·6 (6)	45
		3·2 (6)	68
		6·4 (4)	84

* The number in parenthesis indicates the number of animals studied.

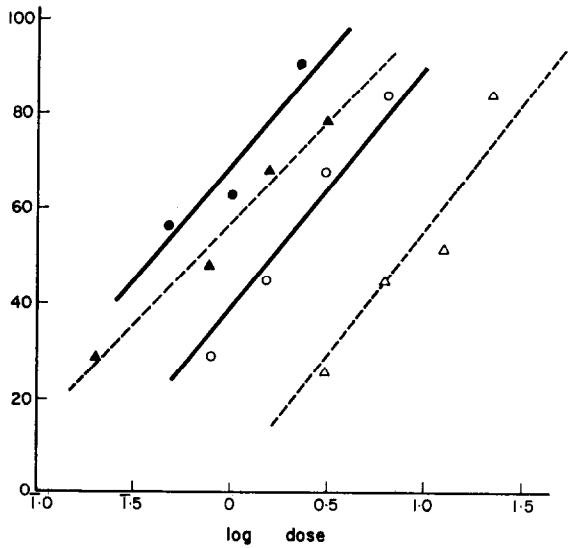

FIG. 1. Regression lines of mepyramine, BOL-148 and imipramine for protection against bronchospasm in guinea pigs induced by aerosolized histamine (———) and 5-hydroxytryptamine (- - - - -).

●—mepyramine
▲—Bol-148
○—imipramine
△—imipramine

The anti-5-hydroxytryptamine and anti-histaminic properties of imipramine may be related to its anti-inflammatory activity since histamine and 5-HT have been shown to act as mediators of certain inflammatory reactions.[17, 18] Salicylates and corticoids, the well known anti-inflammatory agents, have been shown to inhibit the increase in capillary permeability to intradermal injection of histamine[5, 6] inhibit histamine and 5-HT induced asthma[20] and are also shown to be mild 5-HT antagonists.[7]

The effect of serum glutamic oxaloacetic transaminase (S-GOT) and serum glutamic pyruvic transaminase (S-GPT)

The effect of hydrocortisone and imipramine on serum transaminases in normal and arthritic rats are shown in Table 3. The S-GOT and S-GPT activities were sig-

TABLE 3. EFFECT OF HYDROCORTISONE AND IMIPRAMINE ON SERUM GLUTAMIC OXALOACETIC TRANSAMINASE (S-GOT) AND SERUM GLUTAMIC PYRUVIC TRANSAMINASE (S-GPT) IN NORMAL AND ARTHRITIC RATS

	Drug and No. of observations	Control (15)	Hydrocortisone† (11)	Imipramine (6)
S-GOT*	Normal	28·3 ± 1·9	28·1 ± 0·7 ($P = 0·9$)	26·8 ± 0·5 ($P = 0·5$–$0·4$)
	Arthritic	50·0 ± 1·1	28·6 ± 1·3 ($P = <0·001$)	38·8 ± 1·2 ($P = 0·001$)
	Per cent increase with inflammation	76·7	1·8	44·7
S-GPT*	Normal	32·1 ± 1·5	22·5 ± 1·3 ($P = <0·001$)	21·5 ± 0·9 ($P = <0·001$)
	Per cent decrease with drug	—	30	33
	Arthritic	39·8 ± 1·7	25·7 ± 1·6 ($P = <0·001$)	33·0 ± 1·8 ($P = 0·05$–$0·01$)
	Per cent decrease with drug	—	39·9	22·9

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

† Data reported by Tangri *et al.*[10]

nificantly increased during inflammation. Imipramine prevented the increase in the enzyme activities due to the inflammatory reaction similar to that exhibited by hydro cortisone. Further more, both these drugs significantly decreased the normal S-GPT activity, but failed to alter the normal S-GOT activity. The inhibition of the transaminase activity by the anti-inflammatory agents could be due to either an independent effect of these agents or it could possibly be related to their anti-inflammatory property. The effect of imipramine on S-GOT was connected with its anti-inflammatory activity since this drug did not effect the normal enzyme activity whilst it checked S-GOT activity due to the inflammation. On the other hand, no such relation was observed between the anti-inflammatory property of imipramine and its effect on S-GPT activity, since imipramine also decreased the normal enzyme activity. Similar effects

on the transaminases were observed using glycyrrhetic acid and its methyl ester,[10] hydrocortisone[10] and with salicylates.[8, 20]

Effect on ATPase activity in pooled tissue homogenates

The effect of hydrocortisone and imipramine was studied on the ATPase activity of pooled liver and brain homogenates obtained from normal and arthritic rats. The result are shown in Table 4. The inflammatory reaction *per se* did not alter the

TABLE 4. EFFECT OF HYDROCORTISONE AND IMIPRAMINE ON THE ADENOSINE TRIPHOSPHATASE (ATPASE) ACTIVITY IN POOLED LIVER AND BRAIN HOMOGENATES OBTAINED FROM NORMAL AND ARTHRITIC RATS

		Control	Hydrocortisone†	Imipramine
Liver ATPase	Normal	11·6	17·0	15·3
	Per cent increase with drug	—	46·1	31·0
	Arthritic	11·6	17·0	14·4
	Per cent increase with drug	—	46·1	24·1
Brain ATPase*	Normal	8·9	8·9	8·9
	Per cent increase with drug	0	0	0
	Arthritic	8·9	13·4	13·4
	Per cent increase with drug	—	50·0	50·0

* Expressed in μ mols. of P_i split for 100 mg of tissue in 15 min at 37°.
† Data reported by Tangri *et al.*[10]

enzyme activity in both the liver and brain homogenates. Imipramine, like hydrocortisone, significantly increased the ATPase activity in liver homogenates obtained from both normal and arthritic animals, but concerning brain ATPase activity, imipramine did not have any affect on it although its activity was increased significantly in the arthritic animals by this drug. Hydrocortisone also showed similar effects. Salicylates, hydrocortisone, glycyrrhetic acid and methyl glycyrrhetic acid have been shown to uncouple oxidative phosphorylation and to stimulate ATPase activity.[10, 21] Glycyrrhetic acid and also its methyl ester, did not influence the normal brain ATPase activity although it was increased by these agents during the inflammatory state.[10] The results obtained in the present investigation with imipramine further confirm the contention that the effect of anti-inflammatory agents on ATPase activity in liver and brain homogenates may not be related to their anti-inflammatory property since ATPase activity itself was not influenced by the inflammatory reaction. This contention, however is based only upon our findings in liver and brain ATPase but it is presumed that the same may be true of the inflammatory tissue.

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