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STUDIES WITH TRYPTOPHAN METABOLITES *IN VITRO*—V

EFFECT OF THE METHANESULPHONATE DERIVATIVE OF HYCANTHONE (ETRENOL) AND LEAD ACETATE ON KYNURENINASE AND KYNURENINE TRANSAMINASE OF NORMAL MOUSE LIVER

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Abstract—The effect of the new antischistosomal drug methanesulphonate derivative of hycanthone(1-{[2-(diethylamino)ethyl]amino}-4-(hydroxymethyl)-thioxanthen-9-one)-(etrenol) on the metabolism of kynurenine was compared with that of lead acetate. Etrenol produced inhibition of both kynureninase and kynurenine transaminase enzymes whereas lead acetate inhibited the former enzyme only. It has been suggested that the inhibitory effects of etrenol were brought about by inactivation of the sulfhydryl groups of these enzymes since these inhibitions were not reversed by the supplementation of exogenous pyridoxal phosphate and that lead acetate protected both enzymes from the inhibitory effects of etrenol.

THE ASSOCIATION between vesical schistosomiasis and bladder cancer is generally accepted, but the exact nature of this association is still not clear.[1]

In a previous work from this laboratory[2] it was observed that infestation with *Schistosoma mansoni* created a deficiency in the phosphorylated pyridoxal (Plp) of the infested liver. This was accomplished by concentrating the cofactor and simultaneously inhibiting the phosphorylation of pyridoxal. Since active pyridoxal is essential for the two main reactions utilizing kynurenine, kynureninase, and kynurenine transaminase, the observed lack of Plp in the infested liver resulted in modified levels of the two metabolites of kynurenine. The observation that several kynurenine metabolites are known bladder carcinogens[3–5] raises the possibility that the increased concentration of these metabolites may be implicated in the high incidence of bladder tumours observed in bilharzial patients.[6–9]

Since potassium antimonyl tartrate (tartar emetic) and some other antimony-containing drugs have long been used in the treatment of bilharziasis both in the United Arab Republic and abroad, with obvious toxic effects, it seems important to refer to our previous studies on their effects on kynurenine metabolism *in vitro*. Three antimony containing antischistosomal drugs, tartar emetic, Stibophen, and lithium antimonyl thiomalate, produced inhibition of both kynureninase and kynurenine transaminase in normal mouse liver[10,11] and kidney homogenates.[12] It does not seem that the sulphur contained in lithium antimonyl thiomalate plays any significant role in its inhibitory effects on kynureninase or kynurenine transaminase.[11] The inhibition

was directly related to the antimonial contents of these drugs.[11] The antischistosomal drug 5-(nitro-thiazolyl)-2-oxo-tetrahydro-imidazole, which does not contain antimony, failed to inhibit either enzyme.[11] The possibility of the formation of an inactive chelate between the antimony and pyridoxal phosphate, which is a needed cofactor in both enzyme reactions, was offered as a mechanism for the observed inhibitions.[10,11]

In the present study, the effect of the methanesulphonate derivative of hycanthone, i.e. 1-{[2-(diethylamino)ethyl] amino}-4-(hydroxymethyl)-thioxanthen-9-one (Etrenol, Winthrop Products Inc., New York, U.S.A.) was compared with the effect of lead acetate. Etrenol is a new thioxanthone compound with chemotherapeutic activity against *Schistosoma haematobium* and *S. mansoni.*

MATERIALS AND METHODS

Animals. Adult albino mice weighing from 15 to 20 g fed *ad lib.* on a specially prepared diet containing all the necessary factors were used.

Materials. Kynurenine sulfate was purchased from Schuchardt Co. (Munchen, Germany). Kynurenic acid was supplied by Sigma Chemical Co. (St. Louis, Mo. U.S.A.). α-Ketoglutarate was supplied by L. Light & Co., Ltd. (Colnbrook, England). Anthranilic acid (AR) was purchased from Merck AG (Darmstadt, Germany). Pyridoxal phosphate was prepared by using the method described by Beiler and Martin.[13] Etrenol was supplied by Winthrop Products Inc. (New York, U.S.A.). Lead acetate (AR) was purchased from the General Chemical Co., Ltd. (England). Redistilled water from all-glass still was used to make solutions.

Preparation of the homogenates. The mice were killed by exsanguination after stunning by a blow on the head. The fresh livers were quickly removed and placed in ice-cold 0·25 M sucrose solution. Tissue homogenates (10% based on the wet weight of the tissue) were prepared in the cold isotonic sucrose solution by using a Potter–Elvehjem homogenizer.

Incubations. Reaction mixtures (final volume, 4 ml) were incubated in 20 ml test tubes shaken by the rotating drum of Gallenkamp anhydric incubator at 37° with air as the gas phase. At the end of the incubation, 1 ml of 16% trichloroacetic acid (TCA) was added to each test tube and the mixture transferred to centrifuge tubes with 1 ml of bidistilled water. The precipitate was removed by centrifugation and the supernatants were analyzed. The experimental test tubes were run in duplicate and a zero-time test tube was included in each set of experiments. The concentration of the different materials, when present in the incubation medium, unless otherwise stated, were: DL-kynurenine sulfate, 5·0 μmoles; potassium phosphate buffer (pH 7·4), 0·05 M; α-ketoglutarate, 30·0 μmoles; calcium chloride, 0·005 M; magnesium sulfate, 0·001 M; pyridoxal phosphate, 40·0 μg; 10% whole liver homogenate, 2·0 ml.

Quantitative estimation of metabolites. Kynurenine and kynurenic acid were determined by the method of Miller *et al.*[14] Anthranilic acid was determined by the method of Mason and Berg.[15]

The concentration of kynurenine was not rate limiting for the enzymatic reactions studied.[2] An incubation period of 3 hr was found adequate for optimal utilization of kynurenine and the formation of both metabolites, kynurenic acid and anthranilic acid.

RESULTS

The u.v. spectrum of aqueous etrenol solution (0·1 μmole/6 ml), in the presence and in the absence of kynurenine, was studied at pH 1, i.e. the pH of the supernatants after precipitation of proteins in the experimental tubes (Fig. 1). There were two maximum absorption peaks at 255 and 325 nm. Additive absorption readings were obtained when kynurenine sulfate was added to the etrenol solution. The other constituents in the incubation medium, except liver homogenates, did not interfere with the absorption of etrenol and of kynurenine sulfate at 365 nm. However, the maximum absorption peaks at 255 and 365 nm for kynurenine sulfate and at 255 and 325 nm for etrenol, have completely disappeared when the 10% whole liver homogenate (2·0 ml) were present in a solution (6 ml) containing more than 3×10^{-4} M of etrenol and 0·05 μmole of kynurenine sulfate at pH 1. In the latter experiment the u.v. spectrum was measured after precipitation of the added liver homogenate by TCA as above mentioned. Therefore, the kynurenine utilized and the kynurenic acid produced could not be calculated in some experiments, e.g. expts. 3 and 4 (Table 1a) and in the presence of more than 3×10^{-4} M of etrenol in Table 2a.

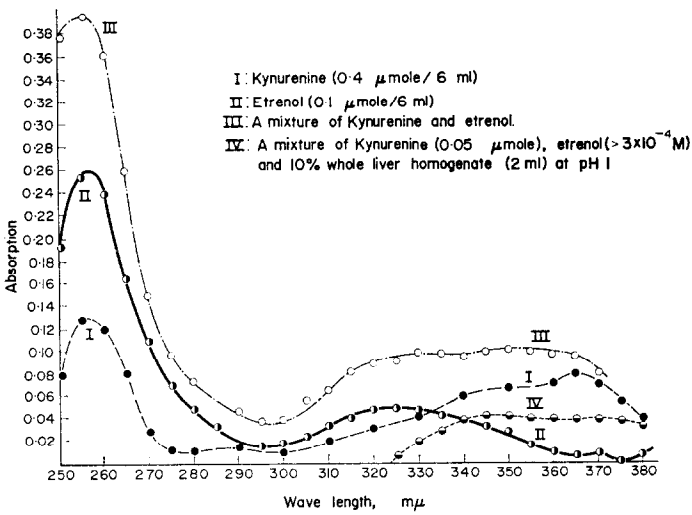

FIG. 1. Ultra-violet absorption spectrum of kynurenine (0·4 μmole/6 ml), etrenol (0·1 μmole/6 ml), a mixture of kynurenine and etrenol, and a mixture of kynurenine (0·05 μmole), etrenol ($> 3 \times 10^{-4}$ M) and 10% whole liver homogenate (2 ml) at pH 1.

The effect of increasing the concentration of etrenol and Pb^{2+} on kynurenine transaminase and kynureninase, as indicated by the amounts of kynurenic acid and anthranilic acid (in μmole/g liver) produced, respectively, are shown in Table 1. The relative inhibition of both enzyme systems is best illustrated by (a) the ratio of the amount of anthranilic acid to the amount of kynurenic acid produced, and (b) the percentage inhibition. The percentage inhibition was taken as the percentage difference between the amount of the metabolite produced in the control experiment and that produced at varying concentrations of either etrenol or Pb^{2+} with reference to the control value.

Table 1. Effect of increasing the concentration of etrenol and lead (Pb^{2+}) on the metabolism of kynurenine by normal mouse liver homogenates*

Expt. No.	Concentration (M)	Metabolites determined (μmole/g liver)† Kynurenine utilized‡	Kynurenic acid (KA)	Anthranilic acid (AA)	Ratio AA/KA	Inibition (%) KA	AA
			(a) *Etrenol*				
1	0	5·25	4·32	0·43	0·99	00	00
2	3×10^{-4}	3·60	2·52	0·22	0·87	42	49
3	3×10^{-3}	0·00§	2·52	0·00	0·00	42	100
4	$7{\cdot}8 \times 10^{-3}$	0·00§	1·08	0·00‖	0·00	75	100
			(b) *Lead* (Pb^{2+})				
1	0	9·00	2·05	0·85	0·41	00	00
2	3×10^{-4}	7·88	2·55	0·30	0·12	00	65
3	3×10^{-3}	6·00	3·75	0·23	0·06	00	73
4	$7{\cdot}8 \times 10^{-3}$	7·12	2·70	0·23	0·09	00	73

* The incubation medium (4 ml) contained 5 μmoles DL-kynurenine sulfate, 30 μmoles α-ketoglutarate, 40 μg pyridoxal phosphate, 0·005 M calcium chloride, 0·001 M magnesium sulfate and 10% whole liver homogenate (2 ml) in 0·05 M potassium phosphate buffer, pH 7·4. Incubations were carried out for 3 hr at 37°.

† Average values of four experiments.

‡ These values represent the difference between the kynurenine recovered and that originally present in the medium.

§ The amount of kynurenine utilized could not be calculated since the absorption at 365 nm of the "zero time experiment" which contained 5 μM DL-kynurenine sulfate, was found to be less than the absorption of the "experimental tubes" which were incubated for 3 hr at 37°.

‖ The amount of anthranilic acid could not be calculated since the absorption at 560 nm of the "zero time experiment" which gives the initial concentration of anthranilic acid, was found to be more than the absorption of the "experimental tubes" which were incubated for 3 hr.

At a concentration of 3×10^{-4} M, etrenol caused a pronounced inhibition of kynureninase enzyme as evidenced by the reduced level of anthranilic acid; the per cent inhibition for anthranilic acid is higher than that for kynurenic acid (expt. 2), which clearly illustrates the greater inhibition encountered on the production of anthranilic acid as compared with that of kynurenic acid. Increasing the concentration of etrenol produced a more pronounced inhibition of kynureninase enzyme (expts. 3 and 4). At concentrations above 3×10^{-4} M, the kynureninase enzyme became progressively inhibited. At a concentration of $7{\cdot}8 \times 10^{-3}$ M, the kynureninase enzyme was completely inhibited whereas the kynurenine transaminase showed 75 per cent inhibition (expt. 4, Table 1a). On the other hand, increasing the concentration of Pb^{2+} produced a pronounced inhibition of the kynureninase enzyme leading to the synthesis of anthranilic acid, with no significant effect on the kynurenine transaminase leading to the synthesis of kynurenic acid. However, the amounts of kynurenic acid produced in the livers to which Pb^{2+} was added, were higher than the corresponding values obtained with Pb^{2+}-free livers. The latter effect might be due to the kynureninase blockage by Pb^{2+} with the accumulation of kynurenine and its degradation by the uninhibited kynurenine transaminase (Table 1b). The amounts of kynurenine utilized in the presence of etrenol reflected closely the amounts used in the synthesis of the two metabolites. However, more kynurenine was utilized than that converted to both metabolites in the presence of Pb^{2+} (Table 1b). The difference might reflect hydroxylation to 3-hydroxykynurenine or the further metabolism of either of the products.

The effect of increasing the concentration of pyridoxal phosphate on the inhibitory action of etrenol, and Pb^{2+} on both enzyme systems was also investigated and the results are shown in Table 2. The results indicate that in the absence of both inhibitors, pyridoxal phosphate stimulated the synthesis of both kynurenic and anthranilic acids. In the presence of etrenol, however, increasing concentrations of pyridoxal phosphate partially reversed the inhibition encountered on the transaminase enzyme system to an extent dependent upon the amount of etrenol present in the medium. Furthermore, the addition of pyridoxal phosphate in the presence of 3×10^{-4} M of etrenol failed to result in the production of equivalent amounts of kynurenic acid to that obtained in the absence of etrenol; the difference became more pronounced when higher concentrations of etrenol were present. However, the addition of pyridoxal phosphate failed to result in the production of any detectable amounts of anthranilic acid in the presence of higher than 3×10^{-4} M of etrenol (Table 2a).

In the presence of 3×10^{-5} and 3×10^{-4} M of Pb^{2+}, the addition of 50 μg/g liver of pyridoxal phosphate partially reversed the inhibition encountered on the kynureninase enzyme. However, in the presence of Pb^{2+}, a constant amount of anthranilic acid was produced irrespective of whether the amounts of pyridoxal phosphate added were 100, 150 or 200 μg/g liver (expts. 3, 4 and 5, Table 2 b). On the other hand, the addition of pyridoxal phosphate stimulated the production of kynurenic acid in amounts equivalent to or even more than that obtained in the absence of Pb^{2+}.

Although Plp is not a substrate for either kynureninase or kynurenine transaminase, a simulated Lineweaver–Burk double reciprocal plot was constructed for both enzymes in presence of different concentrations of etrenol and Pb^{2+} ions. The amount of product formed after incubation was taken as an estimate of the velocity of the

TABLE 2. EFFECT OF INCREASING CONCENTRATIONS OF PYRIDOXAL PHOSPHATE ON THE ACTION OF ETRENOL AND LEAD (Pb^{2+}) ON THE METABOLISM OF KYNURENINE BY THE NORMAL MOUSE LIVER HOMOGENATES*

(a) *Etrenol*

Expt. No.	Pyridoxal phosphate (μg/g liver)	Molar concentration of etrenol† 0 KYN‡§	0 KA‡§	0 AA‡§	3×10^{-4} KYN‡§	3×10^{-4} KA‡§	3×10^{-4} AA‡§	3×10^{-3} KYN‡§	3×10^{-3} KA‡§	3×10^{-3} AA‡§	$7{\cdot}8 \times 10^{-3}$ KYN‡§	$7{\cdot}8 \times 10^{-3}$ KA‡§	$7{\cdot}8 \times 10^{-3}$ AA‡§
1	0	0·75	0·36	0·04	1·50	0·00	0·00	0·00¶	0·00	0·00	0·00¶	0·00‖	0·00**
2	25	2·10	1·35	0·18	2·70	0·36	0·00	0·00¶	0·72	0·00	0·00¶	0·00‖	0·00**
3	50	3·30	2·16	0·32	2·70	0·72	0·07	0·00¶	1·08	0·00	0·00¶	0·00‖	0·00**
4	75	4·05	2·90	0·36	2·85	1·44	0·14	0·00¶	1·80	0·00	0·00¶	0·00‖	0·00**
5	100	4·80	3·00	0·36	3·30	1·80	0·18	0·00¶	2·16	0·00	0·00¶	0·00	0·00**
6	150	5·25	3·96	0·43	3·45	2·16	0·22	0·00¶	2·52	0·00	0·00¶	0·36	0·00**
7	200	5·25	4·32	0·43	3·60	2·52	0·22	0·00¶	2·52	0·00	0·00¶	1·08	0·00**

(b) *Lead* (Pb^{2+}) Molar concentration of lead (Pb^{2+})†

Expt. No.	Pyridoxal phosphate (μg/g liver)	0 KYN	0 KA	0 AA	3×10^{-5} KYN	3×10^{-5} KA	3×10^{-5} AA	3×10^{-4} KYN	3×10^{-4} KA	3×10^{-4} AA	3×10^{-3} KYN	3×10^{-3} KA	3×10^{-3} AA	$7{\cdot}8 \times 10^{-3}$ KYN	$7{\cdot}8 \times 10^{-3}$ KA	$7{\cdot}8 \times 10^{-3}$ AA
1	0	3·00	0·37	0·18	1·05	0·08	0·18	2·62	0·00	0·00	1·50	0·00	0·00	0·75	0·00	0·00
2	50	3·60	0·90	0·36	3·45	0·30	0·30	4·50	1·35	0·18	4·12	2·40	0·11	3·37	1·72	0·08
3	100	6·00	1·35	0·60	4·50	0·53	0·45	7·50	2·00	0·20	4·87	3·60	0·18	6·75	2·47	0·13
4	150	7·80	1·88	0·80	6·00	0·75	0·50	7·87	2·20	0·25	5·81	3·70	0·22	7·12	2·62	0·22
5	200	9·00	2·05	0·85	8·40	1·03	0·54	7·88	2·55	0·30	6·00	3·75	0·23	7·12	2·70	0·23

* The incubation medium (4 ml) contained 5 μmoles DL-kynurenine sulfate, 30 μmoles α-ketoglutarate, 0·005 M calcium chloride, 0·001 M magnesium sulfate, 10% whole liver homogenate (2 ml) in 0·05 M potassium phosphate buffer, pH 7·4. Incubations were carried out for 3 hr at 37°.

† The metabolites determined are expressed in μmole/g liver.

‡ Average value of four experiments.

§ The abbreviations KYN, KA and AA represent kynurenine utilized, kynurenic acid and anthranilic acid produced, respectively.

‖ The amount of kynurenic acid produced could not be calculated since the absorption at 330 mμ of the "zero time experiment" was found to be more than the absorption of the "experimental tubes", which were incubated for 3 hr at 37°.

¶ As in § Table 1.

** As in Table 1.

reaction and pyridoxal phosphate concentration was used in place of the substrate since Plp interacts with kynurenine and a polyvalent cation to form a complex. This complex is the intermediate involved in the action of B_6-dependent kynreninase and kynurenine transaminase enzymes.[16–18] The thus, constructed graphs are shown in Fig. 2 for kynureninase, Fig. 3 for kynurenine transaminase using etrenol in two different concentrations, and Fig. 4 for kynureninase enzyme using four different oncentrations of Pb^{2+}.

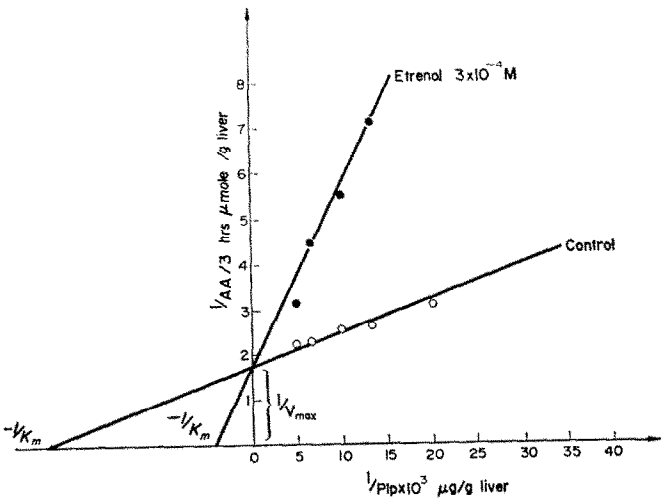

FIG. 2. Effect of 3×10^{-4} M of etrenol on the production of anthranilic acid (AA) from kynurenine by normal mouse liver homogenates in the presence of increasing concentrations of pyridoxal phosphate (Plp) in the medium. The incubation medium (4 ml) contained 5 µmoles DL-kynurenine sulfate, 30 µmoles α-ketoglutarate, 0·005 M calcium chloride, 0·001 M magnesium sulfate and 10% whole liver homogenate (2 ml) in 0·05 M potassium phosphate buffer, pH 7·4. Incubations were carried out for 3 hr at 37°. The ordinate represents the reciprocal of the amount of AA (µmole/g liver) produced in 3 hr. The abscissa represents the reciprocal of the µg concentrations of Plp/g liver.

It could be seen from Figs. 2 and 3 that the relationship of etrenol to Plp–substrate complex is not similar; in the former figure it is a competitive inhibition on the kynureninase enzyme, and in the latter figure it is a typical non-competitive inhibition on the kynurenine transaminase enzyme. It seems, therefore, that when both etrenol and the Plp–substrate complex are present, they compete for the same binding sites on the surface of the kynureninase enzyme, i.e. an enzyme–inhibitor complex is formed rather than an enzyme–substrate complex. The inability of the added Plp to produce any detectable amounts of anthranilic acid, in the presence of higher than 3×10^{-4} M of etrenol (Table 2a), may indicate that the degree of association of kynureninase enzyme with Plp was diminished. On the other hand, the non-competitive nature of the relationship between Plp–substrate complex and etrenol (Fig. 3), on the kynurenine transaminase enzyme, may indicate that etrenol binds to a different region on the kynurenine transaminase enzyme. The relationship of Pb^{2+} to Plp–substrate complex is similar to that of a non-competitive inhibitor on the Plp-dependent kynureninase enzyme (Fig. 4). The affinity between Plp–substrate complex and the kynureninase enzyme seems to be constant and not affected by the increasing con-

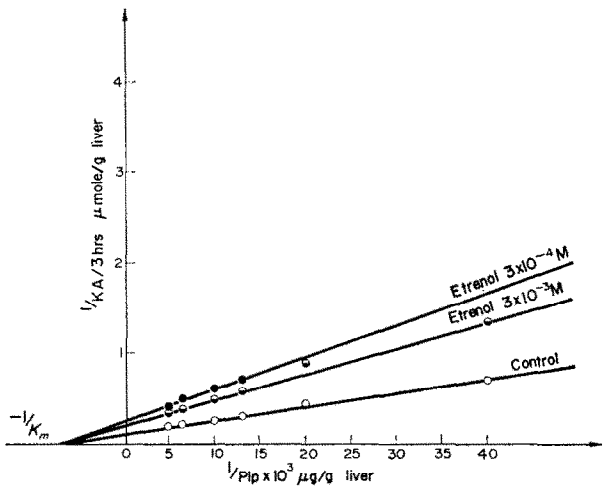

FIG. 3. Effect of two different concentrations of etrenol on the production of kynurenic acid (KA) from kynurenine by normal mouse liver homogenates in the presence of increasing concentrations of pyridoxal phosphate (Plp) in the medium. The incubation medium (4 ml) contained 5 µmoles DL-kynurenine sulfate, 30 µmoles α-ketoglutarate, 0·005 M calcium chloride, 0·001 M magnesium sulfate and 10% whole liver homogenate (2 ml) in 0·05 M potassium phosphate buffer, pH 7·4. Incubations were carried out for 3 hr at 37°. The ordinate represents the reciprocal of the amount of KA (µmole/g liver) in 3 hr. The abscissa represents the reciprocal of the µg concentrations of Plp/g liver.

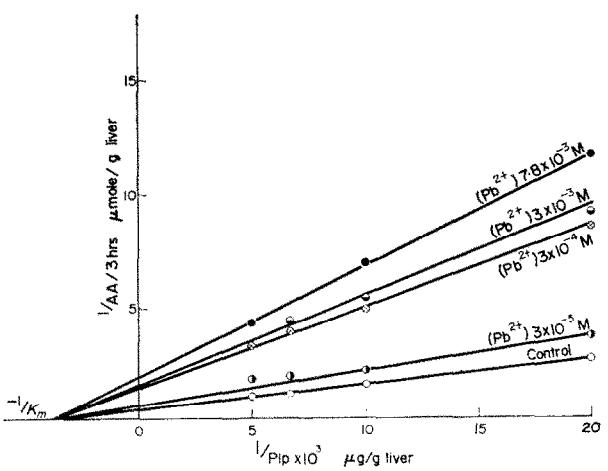

FIG. 4. Effect of four different concentrations of Pb^{2+} on the production of anthranilic acid (AA) from kynurenine by normal mouse liver homogenates in the presence of increasing concentrations of pyridoxal phosphate (Plp) in the medium. The incubation medium (4 ml) contained 5 µmoles DL-kynurenine sulfate, 30 µmoles α-ketoglutarate, 0·005 M calcium chloride, 0·001 M magnesium sulfate and 10% whole liver homogenate (2 ml) in 0·05 M potassium phosphate buffer, pH 7·4. Incubations were carried out for 3 hr at 37°. The ordinate represents the reciprocal of the amount of AA (µmole/g liver) in 3 hr. The abscissa represents the reciprocal of the µg concentrations of Plp/g liver.

TABLE 3 (a). EFFECT OF INCREASING CONCENTRATIONS OF ETRENOL IN THE PRESENCE OF CONSTANT CONCENTRATION OF Pb^{2+} (3×10^{-4} M) ON THE METABOLISM OF KYNURENINE BY THE NORMAL MOUSE LIVER HOMOGENATES*

Expt. No.	Concentration of etrenol (M)	Metabolites determined (μmole/g liver)† Kynurenine utilized‡	Kynurenic acid (KA)	Anthranilic acid (AA)	Ratio AA/KA	% Inhibition KA	% Inhibition AA
1	Control experiment	4·80	4·65	0·75	0·16	0	0
2	3×10^{-5}	4·45	4·86	0·57	0·12	0	24
3	$1{\cdot}5 \times 10^{-4}$	4·64	4·62	0·65	0·14	6	13
4	3×10^{-4}	4·75	4·60	0·68	0·15	11	9
5	$1{\cdot}5 \times 10^{-3}$	4·80	2·40	0·29	0·12	48	61
6	3×10^{-3}	4·35	2·85	0·08	0·03	39	89

TABLE 3 (b). EFFECT OF INCREASING CONCENTRATIONS OF Pb^{2+} IN THE PRESENCE OF CONSTANT CONCENTRATION OF ETRENOL (3×10^{-4} M) ON THE METABOLISM OF KYNURENINE BY THE NORMAL MOUSE LIVER HOMOGENATES*

Expt. No.	Concentration of Pb^{2+} (M)	Metabolites determined (μmole/g liver)† Kynurenine utilized‡	Kynurenic acid (KA)	Anthranilic acid (AA)	Ratio AA/KA	% Inhibition KA	% Inhibition AA
1	Control experiment	4·80	4·65	0·75	0·16	0	0
2	3×10^{-5}	3·30	4·20	0·65	0·15	10	13
3	$1{\cdot}5 \times 10^{-4}$	3·18	4·32	0·60	0·14	7	20
4	3×10^{-4}	4·38	4·32	0·65	0·15	7	13
5	$1{\cdot}5 \times 10^{-3}$	3·90	4·14	0·50	0·12	11	33
6	3×10^{-3}	3·35	4·02	0·48	0·12	14	36

* The incubation medium (4 ml) contained 5 μmoles DL-kynurenine sulfate, 30 μmoles α-ketoglutarate, 40 μg pyridoxal phosphate, 0·005 M calcium chloride, 0·001 M magnesium sulfate, 10% whole liver homogenate (2 ml) in 0·05 M potassium phosphate buffer pH 7·4. Incubations were carried out for 3 hr at 37°.

† Average values of four experiments.

‡ As in Table 1.

centrations of Pb^{2+} (Table 1 b). The non-competitive nature of the relationship between Plp–substrate complex and Pb^{2+} might be due to a reduced functional concentration of Plp–substrate complex at the enzyme level. In this case no competition occurs between Plp–substrate complex and Pb^{2+}, but Pb^{2+} may be assumed to bind to a different region on the enzyme. Therefore, the degree of association of the kynureninase enzyme with the added Plp, in the presence of Pb^{2+}, was depressed as evidenced by the finding that a constant amount of anthranilic acid was produced irrespective of whether the added amounts of Plp were 100, 150 or 200 μg/g liver (Table 2b).

Therefore, it may be suggested that the inhibitory effect of etrenol on the kynurenine transaminase and of Pb^{2+} on the kynureninase enzyme is through an irreversible non-competitive mechanism since increasing concentrations of Plp was unable to counteract the inhibitory effects of either etrenol and Pb^{2+} (Table 2). On the other hand, heavy metals, which reduce enzyme activity and bear no structural resemblance to the substrate, exhibit an irreversible non-competitive inhibition.[19] Inhibition patterns which are apparently non-competitive are frequently encountered with irreversible inhibitors since the decrease in V_{max} simply reflects the fact that some enzyme has been removed from the system.

A wide variety of enzyme "poisons" such as heavy metal ions, reduce enzyme activity. The sulfhydryl groups of enzyme proteins are known to have a natural chelating ability for arsenic, zinc, copper[20] and lead.[21,22] Therefore, it was of interest to study the possible mechanisms by which both etrenol and lead affect the kynureninase and kynurenine transaminase enzymes. This was done by increasing the concentration of one inhibitor in the presence of a constant amount of the other (Table 3). The per cent inhibition obtained in the presence of varying concentrations of either inhibitor was much less than that obtained in the presence of each individual inhibitor alone (see, for example, Table 1). However, minimal inhibition for the production of anthranilic acid was observed when equimolar concentrations of both inhibitors were present in the medium (expt. 4, Table 3 a and 3 b). It is noteworthy that the per cent inhibition induced by increasing etrenol concentrations, in the presence of a constant concentration of Pb^{2+} (3×10^{-4} M), was much higher than those obtained when increasing concentrations of Pb^{2+} were present (expts. 5 and 6, Table 3 a and 3 b).

DISCUSSION

It is evident from the present study that etrenol inhibits both the vitamin B_6-dependent kynureninase and kynurenine transaminase enzymes which are responsible for the conversion of kynurenine to anthranilic acid and kynurenic acid, respectively; the kynureninase enzyme is markedly inhibited than the kynurenine transaminase enzyme (Table 1a). It seems that pyridoxal phosphate is not the factor directly responsible for the observed inhibition since increasing concentrations of pyridoxal phosphate were unable to counteract the effects of etrenol (Table 2a).

In investigating the site of action of etrenol, it has been now suggested that this inhibition is brought about by reducing the affinity of the kynureninase enzyme for the pyridoxal phosphate–kynurenine complex. When both the substrate–complex and etrenol are present, they compete for the same binding sites on the enzyme surface (Fig. 2). However, no competition occurs between the substrate-complex and etrenol for the same binding sites on the kynurenine transaminase enzyme (Fig. 3) but it is

assumed that etrenol may bind to a different region on this enzyme. Thus the degree of association of the kynurenine transaminase enzyme with pyridoxal phosphate–substrate complex will be depressed. It is noteworthy that lead inhibited the kynureninase enzyme in a manner similar to that induced by etrenol on the kynurenine transaminase enzyme (Fig. 4). In either case, increasing concentrations of pyridoxal phosphate were unable to counteract this inhibitory effect (Table 2b). This finding coincides with the previously reported observation that lead intoxication in the rat does not induce modifications in tryptophan metabolism similar to those in pyridoxine deficiency.[23] Furthermore, the inhibitory effects of etrenol even in concentrations as high as 3×10^{-3} molar (expt. 6, Table 3a) were reduced in the presence of lead which is known as a sulfhydryl inhibitor.[20–22] Lead has, at least partially, protected the kynureninase and kynurenine transaminase enzymes from the inhibitory effects of etrenol (Table 3b). Therefore, the inhibition induced by etrenol and lead may be brought about by inactivation of the sulfhydryl groups of these enzymes. The reasons for the different mechanisms by which etrenol inhibits the sulfhydryl groups of both enzymes are still obscure. However, the competitive mechanism by which etrenol acts on the catalytic site of the kynureninase enzyme may be cautiously interpreted by considering etrenol as antimetabolite the chemical structure of which resembles that of the substrate required for this particular enzymic reaction. Moreover, this is not an extraordinary observation since a similar difference in the response of these two enzymes to pyridoxine antimetabolites was shown earlier.[17,24–27]

If etrenol and lead are sulfhydryl inhibitors, both of which act by a non-competitive mechanism, then one would expect that lead will induce an inhibitory effect on the kynurenine transaminase enzyme rather than on the kynureninase enzyme (Table 1b). However, this unexpected finding may be attributed to the inadequate sequence of the sulfhydryl groups on the kynurenine transaminase for lead action. This means that etrenol has a higher affinity than lead for the sulfhydryl groups on the kynureninase enzyme, since etrenol induced its inhibitory effect by a competitive mechanism (Fig. 2), whereas lead has acted by a non-competitive mechanism (Fig. 4).

It is noteworthy, however, that in contrast to etrenol 5-(nitro-thiazolyl)-2-oxo-tetrahydroimidazole (ambilhar), which does not contain antimony, failed to inhibit either enzyme.[11] This is not an extraordinary finding since the chemical structures of both drugs are different.

It has been suggested that the inhibitory effects of 3 antimony-containing antischistosomal drugs on the kynureninase and kynurenine transaminase enzymes of normal mouse liver homogenates, are brought about by the formation of an inactive chelate between the antimony and pyridoxal phosphate.[10,11] However, there is no evidence that this mechanism is involved in the inhibitory effects of etrenol observed in the present study.

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