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Inhibition of Rat Liver Mitochondrial Monoamine Oxidase by Chloramphenicol and 2-Amino-1-*p*-nitrophenylpropane-1,3-diol

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Abstract □ Inhibition of rat liver mitochondrial monoamine oxidase by chloramphenicol and its hydrolytic product, 2-amino-1-*p*-nitrophenylpropane-1,3-diol, was studied. The enzyme activity and its inhibition by these two compounds were optimum at pH 7.0 after preincubation for 60 min, the time needed for maximum enzyme–inhibitor complex formation. Enzyme activity could be restored after prolonged dialysis. Monoamine oxidase inhibition by chloramphenicol and its hydrolytic product was noncompetitive and reversible. Deamination of various monoamines was not to the same degree by these compounds. Of the different antimicrobials studied, only chloramphenicol and its hydrolytic product had a strong inhibitory effect on monoamine oxidase.

Keyphrases □ Chloramphenicol—effect on *in vitro* activity of rat liver mitochondrial monoamine oxidase □ Monoamine oxidase, rat liver mitochondrial—activity *in vitro*, effect of chloramphenicol and 2-amino-1-*p*-nitrophenylpropane-1,3-diol □ Enzyme activity—rat liver mitochondrial monoamine oxidase, effect of chloramphenicol and 2-amino-1-*p*-nitrophenylpropane-1,3-diol *in vitro* □ Antimicrobials—chloramphenicol and others, effect on *in vitro* activity of rat liver mitochondrial monoamine oxidase

Chloramphenicol and its hydrolytic product, 2-amino-1-*p*-nitrophenylpropane-1,3-diol, when administered intravenously to cats, produced vasodepression followed by an overshooting rise in blood pressure (1). Monoamine oxidase inhibitor drugs also produce hypotension followed by hypertension (2, 3). Therefore, it is possible that chloramphenicol and 2-amino-1-*p*-nitrophenylpropane-1,3-diol act as monoamine oxidase inhibitors, the subject of the present investigation.

Table I—Effect of Different Antimicrobials on Rat Liver Mitochondrial Monoamine Oxidase [a]

System	Monoamine Oxidase Activity[b]	Inhibition, %
Control	46	—
Plus streptomycin sulfate	46	Nil
Plus penicillin G sodium	46	Nil
Plus tetracycline hydrochloride	36	21.7
Plus chloramphenicol	16	65
Plus 2-amino-1-*p*-nitrophenylpropane-1,3-diol	17	63

[a] Results are means of five observations. Variations in individual observations were minimal. [b] Activity was defined as aldehyde formed, micromoles per 2.5 mg of protein per 30 min of incubation at 37°.

EXPERIMENTAL

Preparation of Monoamine Oxidase—Adult male albino rats, 200 g (average weight), were killed by stunning and decapitation after an overnight fast. The livers were pooled, homogenized in ice-cold 0.25 *M* sucrose to obtain a 10% (w/v) homogenate (4), washed with 0.25 *M* sucrose at 27,000×*g* for 20 min, and suspended in 0.001 *M* phosphate buffer (pH 7.0). A 1-ml aliquot of this suspension was equivalent to 1 g of tissue, and it was used as the source of monoamine oxidase.

Assay of Monoamine Oxidase Activity—The reaction mixture for the monoamine oxidase assay contained 0.025 *M* phosphate buffer (pH 7.0), 0.0125 *M* semicarbazide hydrochloride adjusted to pH 7.0, 0.01 *M* tyramine, and 0.1 ml of mitochondrial suspension as the enzyme source in a final volume of 2 ml. The optimum concentration of tyramine for measuring monoamine oxidase activity is 0.01 *M* (5–7). A higher concentration inhibits monoamine oxidase activity (8). Monoamine oxidase activity was measured at 420 nm (9).

Table II—Variation of Preincubation Time on Rat Liver Mitochondrial Monoamine Oxidase Inhibition by Chloramphenicol and 2-Amino-1-*p*-nitrophenylpropane-1,3-diol [a]

System	Time of Preincubation, min 30	60	90
Control	32	46	28
Plus chloramphenicol, 12 mg	22 (31)	13 (72)	10 (62.5)
Plus 2-amino-1-*p*-nitrophenylpropane-1,3-diol, 12 mg	15 (40)	6 (86)	14 (50)

[a] The results are given as monoamine oxidase activity (aldehyde formed, micromoles per 2.5 mg of protein per 30 min of incubation at 37°) and are means of five observations. Variations in observations in each group were minimal. Figures in parentheses indicate percent inhibition.

Table III—Reversibility of Rat Liver Mitochondrial Monoamine Oxidase Inhibition by Chloramphenicol and 2-Amino-1-*p*-nitrophenylpropane-1,3-diol [a]

System	Monoamine Oxidase Activity[b] Before Dialysis	After Dialysis for 24 hr
Control	40	40
Plus chloramphenicol, 12 mg	11 (72.5)	38
Plus 2-amino-1-*p*-nitrophenylpropane-1,3-diol, 12 mg	5 (87.5)	41

[a] Results are means of five observations. Variations in observations in each group were minimal. An aliquot of the enzyme preparation was mixed with the inhibitor and dialyzed with 0.001 *M* cold phosphate buffer at 4–5°. In the control, the enzyme was dialyzed without any inhibitor. The time of preincubation was 60 min at 37°. Figures in parenthesis indicate percent inhibition. [b] Activity was defined as aldehyde formed, micromoles per 2.5 mg of protein per 30 min of incubation at 37°.

The *p*-hydroxyphenylacetaldehyde semicarbazone formed in the assay was converted to the corresponding 2,4-dinitrophenylhydrazone, the value of which was calculated from a calibration curve obtained with 2,4-dinitrophenylhydrazone derivative crystallized three times (10). When amines other than tyramine, *e.g.*, 5-hydroxytryptamine, norepinephrine, or epinephrine, were used, actual absorbance values also were recorded since the extinction of various 2,4-dinitrophenylhydrazone derivatives differs from that of *p*-hydroxyphenylacetaldehyde dinitrophenylhydrazone.

All incubations prior to the addition of the substrate were carried out at 37° for 1 hr. Later incubations were carried out at 37° for 30 min (maximum linearity) with air as the gas phase. Protein of the mitochondrial enzyme preparations was determined by the biuret method (11).

Effect of Different Antimicrobials on Rat Liver Mitochondrial Monoamine Oxidase—The effects of the addition of streptomycin sulfate, penicillin G sodium, tetracycline hydrochloride, chloramphenicol, and 2-amino-1-*p*-nitrophenylpropane-1,3-diol on liver monoamine oxidase activity were studied. All compounds were dissolved in water. Chloramphenicol and 2-amino-1-*p*-nitrophenylpropane-1,3-diol were dissolved in boiling water, an aliquot of which, when added to the incubation medium, did not show any precipitation. The same quantity of boiling water was added to the control system. The final concentration of each compound in the experimental system was 13×10^{-3} *M*.

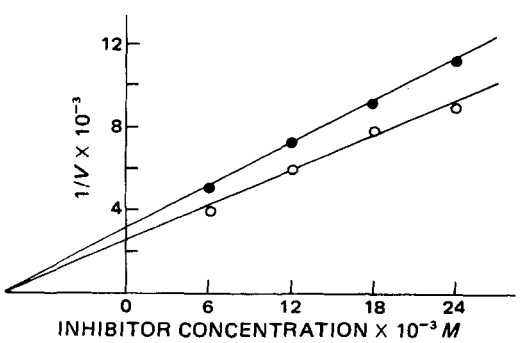

Figure 1—*Inhibition of rat liver mitochondrial monoamine oxidase by chloramphenicol. The enzyme preparation and the drug were mixed and added to the medium. After 60 min of preincubation, the substrate was added; the enzyme activity was determined after 30 min of incubation at 37°. Key:* ●, *0.005* M *tyramine; and* ○, *0.01* M *tyramine.*

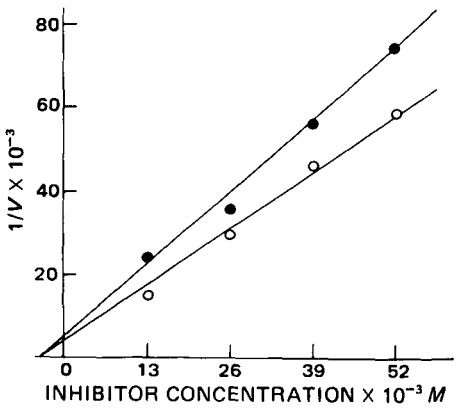

Figure 2—*Inhibition of rat liver mitochondrial monoamine oxidase by 2-amino-1-*p*-nitrophenylpropane-1,3-diol. Conditions of experiments were the same as described in Fig. 1. Key:* ●, *0.005* M *tyramine; and* ○, *0.01* M *tyramine.*

Reversibility of Rat Liver Monoamine Oxidase Inhibition by Chloramphenicol and 2-Amino-1-*p*-nitrophenylpropane-1,3-diol—To study the reversibility of monoamine oxidase inhibition, the enzyme and compound mixture was preincubated for 60 min at 37° for the formation of enzyme–inhibitor complex. It was then dialyzed in a dialysis sac with 0.001 *M* cold phosphate buffer (pH 7.0) for 24 hr. A portion of the same enzyme preparation was also dialyzed without the compounds in a similar manner. After 24 hr, the enzyme inside the sac was incubated with the substrate to determine monoamine oxidase activity.

Effect of Treatment of Rats with Chloramphenicol and 2-Amino-1-*p*-nitrophenylpropane-1,3-diol on Monoamine Oxidase Activities of Liver and Cardiac Muscle—Each of eight rats was given two daily intramuscular injections of 10 mg of chloramphenicol or 2-amino-1-*p*-nitrophenylpropane-1,3-diol dissolved in 0.5 ml of isotonic saline for 8 days. Control rats received only injections of isotonic saline. On the 9th day, after an overnight fast, the animals were sacrificed. The liver and heart were removed, the mitochondrial enzymes were isolated, and monoamine oxidase activity was determined.

RESULTS AND DISCUSSION

Inhibition of liver mitochondrial monoamine oxidase activity was maximum with chloramphenicol and 2-amino-1-*p*-nitrophenylpropane-1,3-diol, minimal with tetracycline, and absent with streptomycin and penicillin G (Table I). Therefore, liver monoamine oxidase inhibition was not a generalized effect of antimicrobials.

Enzyme–inhibitor complex formation started at 30 min, reached a maximum at 60 min, and diminished at 90 min (Table II). During the preincubation period of 60 min, concentrations of chloramphenicol assayed microbiologically (12) and of 2-amino-1-*p*-nitrophenylpropane-

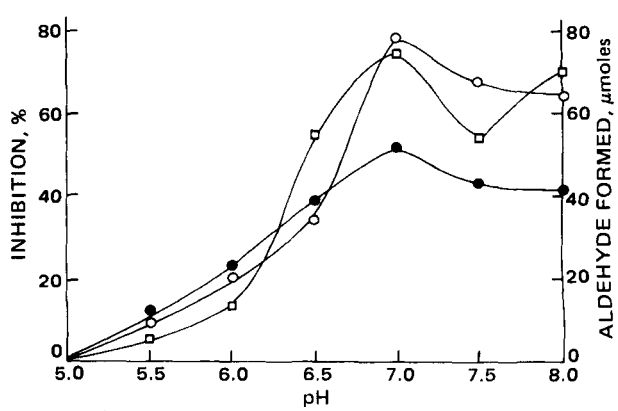

Figure 3—*Influence of pH on rat liver mitochondrial monoamine oxidase inhibition by chloramphenicol (○) or 2-amino-1-*p*-nitrophenylpropane-1,3-diol (□). The system without (●) or with the compounds (1.3×10^{-2} M) was first incubated for 60 min at 37° followed by the addition of tyramine (0.01* M*). The activity was determined after incubation for another 30 min. Phosphate buffer was used.*

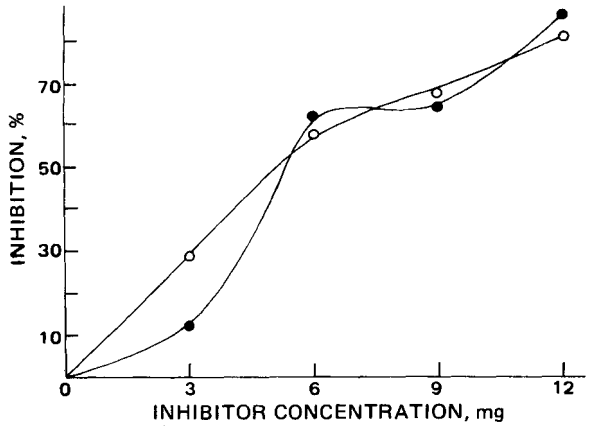

Figure 4—*Effect of different concentrations of chloramphenicol (○) or 2-amino-1-p-nitrophenylpropane-1,3-diol (●) on rat liver mitochondrial monoamine oxidase activity. Conditions of experiments were the same as described in Fig. 3.*

1,3-diol determined spectrophotometrically (13) in the incubation medium did not change. Therefore, the inhibition of monoamine oxidase activity by these compounds was the effect of these compounds *per se* and not a result of their degradation in the incubation medium. The diminution of monoamine oxidase inhibition after preincubation for 90 min might be due to the dissociation of the enzyme–inhibitor complex by the backward reaction.

The inhibition of rat liver monoamine oxidase by chloramphenicol and 2-amino-1-*p*-nitrophenylpropane-1,3-diol was reversible since the enzyme activity could be restored after dialysis for 24 hr (Table III). The inhibition was noncompetitive, with K_i values of 9×10^{-3} *M* for chloramphenicol (Fig. 1) and 3.9×10^{-3} *M* for 2-amino-1-*p*-nitrophenylpropane-1,3-diol (Fig. 2). Reversible noncompetitive inhibition with these compounds, however, is rarely seen.

Liver monoamine oxidase activity and its inhibition by chloramphenicol were optimum at pH 7.0, while the inhibition by 2-amino-1-*p*-nitrophenylpropane-1,3-diol exhibited a peak at pH 7.0 followed by a second peak at pH 8.0 (Fig. 3). With the addition of 12 mg of these compounds in the experimental system, inhibition of monoamine oxidase activity was 78% with chloramphenicol and 86% with 2-amino-1-*p*-nitrophenylpropane-1,3-diol (Fig. 4). These two compounds did not affect

Table IV—Effect of Chloramphenicol and 2-Amino-1-*p*-nitrophenylpropane-1,3-diol on the Oxidative Deamination of Different Monoamines by Rat Liver Mitochondrial Monoamine Oxidase [a]

	Monoamine Oxidase Activity[b]		
Monoamine	Control	With Chloramphenicol	With 2-Amino-1-*p*-nitrophenylpropane-1,3-diol
Tyramine	46 (0.28)	15 (0.09)	6 (0.04)
5-Hydroxytryptamine	29 (0.17)	14 (0.08)	19 (0.12)
Norepinephrine	5 (0.03)	4 (0.02)	3 (0.018)
Epinephrine	5 (0.03)	1 (0.006)	3 (0.018)

[a] Results are means of five observations. Variations in observations in each group were minimal. Figures in parentheses denote absorbance. The time of preincubation was 60 min. [b] Activity was defined as aldehyde formed, micromoles per 2.5 mg of protein per 30 min of incubation at 37°.

Table V—Tissue Mitochondrial Monoamine Oxidase Activities of Rats Injected with Chloramphenicol or 2-Amino-1-*p*-nitrophenylpropane-1,3-diol, 20 mg/Animal/Day for 8 Days [a]

	Monoamine Oxidase Activity[b]	
	Liver (per 2.5 mg of protein)	Cardiac Muscle (per 15 mg of protein)
Control	37 ± 0.05	25 ± 0.25
Chloramphenicol	25 ± 0.03	3 ± 0.14
2-Amino-1-*p*-nitrophenylpropane-1,3-diol	10 ± 0.008	3 ± 0.15

[a] Values are means of eight observations ± *SE*. [b] Activity was defined as aldehyde formed, micromoles per 30 min of incubation.

deamination of various monoamines to the same degree (Table IV), perhaps because of the unequal affinity of the substrate for the enzyme to form an enzyme–inhibitor complex. Tranylcypromine, a potent monoamine oxidase inhibitor, also showed similar variations with different biogenic monoamines (5). The monoamine oxidase inhibition by these compounds could be observed *in vivo*. Mitochondrial monoamine oxidase activities of liver and cardiac muscle of rats injected with these compounds were diminished (Table V).

Unlike tranylcypromine, pheniprazine, or iproniazid, which are potent monoamine oxidase inhibitors, chloramphenicol and 2-amino-1-*p*-nitrophenylpropane-1,3-diol were weak monoamine oxidase inhibitors; fairly high concentrations of these compounds had to be used.

When hydrolyzed, chloramphenicol loses dichloroacetic acid and is converted to 2-amino-1-*p*-nitrophenylpropane-1,3-diol, which has no antibacterial properties (14). Since both compounds inhibit liver mitochondrial monoamine oxidase activity, the inhibitory effect of chloramphenicol has no relation to its antimicrobial activity.

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