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NADH Dependent Aminopyrine N-demethylation and Lipid Peroxidation during Baygon Intoxication in Hepatic and Extrahepatic Guinea Pig Tissues

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Many foreign compounds are metabolized by the mixed function oxidase system in the presence of NADPH and molecular oxygen (BRODIE et al 1958, GILLETTE 1966, MANNERING 1971). PAWAR and MAKHIJA (1975 a,b) have earlier reported the effect of tw_0 injections of Baygon (10 mg/kg) on mixed function oxidase system in rats. KRISCH and STAUDINGER (1961) and STAUDINGER et al. (1961) reported that cyanide enhanced (62%) the microsomal hydroxylation of acetanilide with NADH over that with NADH alone. In the presence of cyanide and NADH the rate of microsomal acetanilide hydroxylation was enhanced an additional 20% by ascorbic. Mason et al. (1965) reported that 10 mM cyanide increased the NADPH induced microsomal hydroxylation of acetanilide by 33%. Recent reports of SHIGAMATSU et al.(1976) have indicated the existence of NADH dependent deethylation of p-nitrophenetole with rabbit liver microsomes. The present studies report the involvement of NADH in N-demethylation of aminopyrine and lipid peroxidation in guinea pig hepatic and extrahepatic tissues and the effect of Baygon intoxication in this system.

MATERIALS AND METHODS

Male guinea pigs initially weighing 600-650 gms were obtained from Hindustan Antibiotics, Poona. They were fed on a standard diet and water ad libitum for about two weeks prior to the initiation of the experiments so as to acclimatize, The animals were then divided into two groups - control and Baygon treated.

Baygon (10 mg/kg in corn oil) was injected intraperitoneally daily in the morning before feeding for 4 successive days. The control animals received an equivalent amount of corn oil and pair fed during the entire experimental period. The animals were sacrificed 24 hours after the last injection by cervical dislocation. Various organs as livers, kidney, intestine, testis, heart, lungs, spleen and adrenals were immediately removed, placed in ice-cold 0.9% saline and weighed.

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The livers, kidneys and intestine were homogenized(1:4 w/v) in Tris HCl buffer pH 7.4 containing 1.15% KCl. The homogenates were centrifuged and microsomes isolated as reported by us earlier (1975 a). The aminopyrine N-demethylase activity, electron transport components and lipid peroxidation were measured as reported recently (1975 b). The microsomal protein content was estimated according to the method of Gornall et al(1957) using crystalline bovine serum albumin as the standard.

RESULTS

Effect of Baygon intoxication on organ weights-

It was observed that during Baygon intoxication the body weights were increased by only 6.6%. However, a significant increase in the weight of heart, lungs and adrenals was noted and the increase in liver, kidney, intestine and testis ranged from 7.6 to 17.3%. A significant decrease of the order of 31.5% in the weight of spleen was noticed (TABLE 1).

TABLE 1

Effect of Baygon intoxication on organ weights in male guinea pigs.

Organs wt.(gms)	Control group	Baygon treated group	% difference
Body wt. gms	673.0±10.0	717.50±12.5	+ 6.61
Liver	23.0±2.0	27.00±3.0	+ 17.39
Kidney	5.11±0.5	5.50±0.8	+ 7.63
Intestine	14.03±0.9	16.20±0.6	+ 15.46
Testis	4.94±0.1	5.54±0.2	+ 12.03
Heart	1.83±0.4	2.94±0.5	+ 60.75
Lungs	4.00±0.3	5.72±0.1	+ 42.96
Spleen	1.41±0.1	0.97±0.1	- 31.54
Adrenals	0.43±0.1	0.52±0.1	+ 20.22

Values represent the average mean of 5-6 animals in each group.

Effect of Baygon intoxication on hepatic and extrahepatic microsomal protein content (TABLE 2)

A significant increase in the hepatic and intestinal microsomal protein content was observed during

TABLE 2

Effect of Baygon intoxication on microsomal protein content in male guinea pigs-

Microsomal protein (mg/gm tissue)	Control	Baygon treated
Hepatic	42.10±0.9	54.68±0.5
Renal	18.90±0.5	20.00±0.2
Intestinal	14.72±0.6	22.00±0.4

Values represent the average mean of 5-6 animals in each group.

Baygon intoxication. The magnitude of increase in hepatic microsomal protein was 29.8% and that in intestine was 49.4%. Baygon administration did not register any significant increase in the renal protein contemt.

Effect of Baygon intoxication on aminopyrine N-demethylase activity-

Baygon intoxication resulted in a decrease and a significant increase in aminopyrine N-demethylase activity using NADPH and NADH as electron donors respectively. However, when NADH and NADPH were used in combination a marginal increase of 6.25 per cent was noticed.

In vitro addition of 1 mM ascorbate to the incubation media of control microsomes caused a 5 fold increase in NADH linked aminopyrine N-demethylase activity, whereas, addition of 1 mM KCN resulted into 16.7 per cent increase in the enzyme activity.Combination of ascorbate and KCN in the NADH mediated system resulted in 3.5 fold elevation of the enzyme activity, KCN did not significantly alter the N-demethylation of aminopyrine during Baygon intoxication. Ascorbate addition to Baygon intoxicated microsomes caused the decrease of 74.2% in the NADH mediated system and when ascorbate and KCN were used in combination the percentage of decrease was 64.9% (TABLE 3).

Baygon intoxication caused a marginal and significant increase in renal aminopyrine N-demethylase activity using NADPH and NADH as electron donors respectively. However, when both NADH and NADPH were used in combination, 28.1% decrease in the enzyme activity

was noted. Baygon intoxication reduced the NADH mediated enzyme activity by 14.4% in presence of KCN. In the presence of ascorbate 82.1% decrease was noticed in Baygon treated animals. However, when ascorbate and KCN were used in combination the percentage of decrease was 66.0.

The intestinal NADH and NADPH mediated aminopyrine N-demethylase activity was increased with Baygon intoxication. The magnitude of increase was higher with NADH linked than with NADPH linked. When NADH and NADPH were added in combination 21.6% decrease in the enzyme activity was noted. However, addition of 1 mM ascorbate to the NADH system manifested 78.5% decrease in the enzyme activity. Addition of KCN to the NADH system or KCN and ascorbate in combination caused 53 per cent decrease in aminopyrine N-demethylase activity.

Effect of Baygon intoxication on microsomal electron transport components-

Baygon intoxication resulted in a marginal increase in cytochrome b5 and cytochrome P 450 content. The per cent increase in the total heme content was 33.3 (FIG 1), however, a concomitant decrease was noticed in cytochrome c reductase.

Effect of Baygon intoxication on lipid peroxidation-

Baygon administration caused 55.5% increase in NADPH linked hepatic lipid peroxidation, whereas, it decreased the ascorbate induced and non enzymatic lipid peroxidation by 21.6 and 28.9% respectively.

The pattern of renal lipid peroxidation was similar to that found in the liver (TABLE 4). The magnitude of increase in renal NADPH linked lipid peroxidation was 20.8%. However, 15.1 and 20.0 per cent decrease in ascorbate induced and non enzymatic lipid peroxidation was noted due to Baygon intoxication.

The behaviour of intestinal lipid peroxidation was quite different from that observed in hepatic and renal. A significant increase in NADPH and ascorbate promoted lipid peroxidation was observed due to Baygon intoxication. The magnitude of increase was 68.1 and 35.5 per cent respectively.

TABLE 4

Effect of Baygon intoxication on lipid peroxidation in male guinea pigs-

Organ	Group	NADPH linked	Ascorbate induced	Non-enzymatic
Liver	Control	8.1±0.2	13.3±0.1	7.6±0.1
	Baygon	12.6±0.3	10.4±0.2	5.4±0.1
Kidney	Control	12.0±0.1	13.2±0.2	3.5±0.1
	Baygon	14.5±0.2	11.2±0.2	2.8±0.1
Intestine	Control	6.9±0.3	11.2±0.2	3.1±0.1
	Baygon	11.6±0.2	15.4±0.1	3.0±0.1

Values represent the average mean of 5-6 animals in each group.
All activities expressed as n moles malonaldehyde produced/min/mg protein.

DISCUSSION

Our studies indicate the presence of NADH linked aminopyrine N-demethylase activity in guinea pigs and its increase during Baygon intoxication. CONNEY et al.(1957) originally noticed only a marginal activity with NADH for demethylation of 4-dimethyl-aminoazobenzene. However, the activity was significantly enhanced when a small amount of NADH was added to the NADPH system. Late on, HILDEBRANDT and ESTABROOK (1971) reported the synergistic effect of NADH on the NADPH linked ethylmorphine N-demethylation and suggested that the second electron which was used for activation of the oxygen molecule might be transferred from NADH through cytochrome b5. Furthermore, STAUDT et al.(1974) suggested that active oxygen which is not used for monoxygenation is reduced to water by NADH cytochrome b5 system and that this sparing effect could be the main mechanism of the well known synergistic action of NADH on microsomal hydroxylation reported by CORREIA and MANNERING (1973). More recently, SHIGAMATSU et al.(1976) reported NADH linked dealky-

lation with rabbit liver microsomes. Although it is believed that NADH mediated metabolism of drugs is slow as compared to the NADPH mediated metabolism , the observed enhanced effect of KCN alone and in presence of ascorbate in the NADH system of aminopyrine N-demethylation is supported by similar increase in acetanilide hydroxylation noted by STAUDINGER et al. (1961). This indicates the existence of a NADH dependent oxygenase system via cytochrome b5 different from that of cytochrome P 450. Studies also indicate that NADPH linked enzyme system was decreased due to Baygon intoxication which can be explained on the basis of decreased levels of cytochrome c reductase. It may also be due to the change in the conformation of cytochrome P 450 or active site of the enzyme by the compound administered. Though the addition of cyanide did not inhibit the NADH linked system in the liver during Baygon intoxication it had a significant inhibitory effect in kidney and intestine thereby indicating an organ variation phenomenon. The in vitro effect of ascorbate on the NADH mediated reactions indicate the involvement of ascorbate dependent NADH oxidase system which was slightly inhibited by KCN. The significant decrease in NADH mediated aminopyrine N-demethylation in presence of ascorbate during Baygon intoxication indicates the inhibition of NADH oxidase which was observed in all three tissues.

The significant increase in the NADPH linked lipid peroxidation system of the liver and kidney due to Baygon treatment in guinea pigs is in accordance with those reported by us in rats(1975 a,b). The decrease in nonenzymatic lipid peroxidation indicates the effect of Baygon in the in vitro system. However, increase in intestinal NADPH linked and ascorbate promoted lipid peroxidation and no change in nonenzymatic may be due to mucosal absorption of lipids and/or phospholipids during Baygon intoxication. However, further studies in guinea pigs regarding this system are in progress.

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