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INHIBITION OF DRUG METABOLIZING ENZYMES BY HEAVY METALS IN VITRO

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SUMMARY

Cadmium iodide was a potent inhibitor of benzo(a)pyrene hydroxylation, ethoxycoumarin deethylation, epoxide hydration, and glutathione conjugation, but had no effect on cytochrome c reduction. Mercuric acetate inhibited cytochrome c reduction, benzo(a)pyrene hydroxylation, ethoxycoumarin deethylation, and glutathione conjugation, but was without effect on epoxide hydration. Nickel, cobalt, chromium and zinc salts had no effect on epoxide hydration or glutathione conjugation. Lead salts did not affect either monooxygenation, cytochrome c reduction, epoxide hydration, or glutathione conjugation.

Administration of salts of lead (1-4), or cadmium (5-12), or organic mercurial compounds (13,14) have been shown to result in decreased activities of various mixed function oxidation reactions. Administration of inorganic mercuric acetate on the other hand caused an increase of p-nitroanisole-O-demethylation (15). The present investigation was performed in order to see whether these findings could be explained by the direct *in vitro* effect of the heavy metal salts on the enzymatic mechanisms concerned.

MATERIAL AND METHODS

Adult male Wistar (Af/Han/Mol) rats, fed *ad lib*, were used throughout this study. A washed microsomal fraction, and a postmicrosomal supernatant were prepared with the calcium aggregation method (16) as described (17). Benzo(a)pyrene hydroxylation was measured fluorimetrically (18) and radiochemically (19). The final concentration of benzo(a)pyrene in the incubation mixture was 80 µmol/l. Deethylation of ethoxycoumarin was determined with a modification (20,21) of the method of Ullrich and Weber (22). In the assays of monooxygenation, NADPH (0.2 mmol/l) was used instead of a NADPH regenerating system. The activity of NADPH cytochrome c reductase according to Phillips and Langdon (23). The activities of epoxide hydratase (24), and glutathione S-transferase (25), were determined with ^{3}H-styrene oxide as the substrate as described (26). The protein contents were measured with the biuret method (27) with bovine serum albumin as the reference. The metal salts were added to incubation mixtures as aqueous solutions to give final concentrations indicated in the Tables. By adding metal salts to a series of incubation mixtures also at the end of the incubation, it was ascertained that they had no effect on the procedure of the activity determination.

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RESULTS

Preliminary studies were carried out on mixed function oxidase activity using a regenerating system to provide the NADPH required. Results essentially similar to those presented in Table 1 (where NADPH was provided as such), were obtained. Cadmium iodide, up to concentrations of 0.5 mmol/l, did not affect cytochrome c reductase activity, whereas mercuric acetate at a low concentration, $2x10^{-6}$ mol/l, caused a 70 per cent inhibition. Mixed function oxidation, measured with ethoxycoumarin, or benzo(a)pyrene as the substrates, was inhibited by both cadmium and mercury. Cadmium was less effective, $2x10^{-4}$ mol/l was required for full inhibition, whereas less than one tenth of that was needed in case of mercury. Benzo(a)pyrene hydroxylation seemed to be more sensitive toward cadmium, whereas ethoxycoumarin deethylation was more sensitive toward mercury. The differences between these two substrates, as well as between different hydroxylation reactions of benzo(a)pyrene, as revealed by the two analytical methods, were however minor. The anions tested, iodide, borate, sulfate, acetate, nitrate, and chloride were without effect. Lead (acetate or nitrate) at concentrations of 0.8 and $2.0x10^{-4}$ mol/l, did not affect ethoxycoumarin deethylation or benzo(a)pyrene hydroxylation, respectively.

The effects of cadmium tetraborate, iodide, sulfate, potassium iodide, potassium sulfate, lead acetate, lead nitrate, mercuric acetate, chloride, natrium acetate, zinc acetate, nickelsulfate, cobalt chloride, and chromium-potassium sulfate, were studied on epoxide hydration and glutathione conjugation. Lead, nickel, cobalt, zinc, chromium salts, as well as all the anions, were without any effect at a concentration of $2.5x10^{-4}$ mol/l. The effects of cadmium iodide, and mercuric acetate have been summarized in the Table 2. Cadmium ($1.7x10^{-5}$ mol/l) did not affect epoxide hydrase, and $1x10^{-5}$ mol/l was without effect on glutathione conjugation. Higher concentrations of cadmium proved to be inhibitory to these reactions. Less than five percent of epoxide hydrase activity was left at $1.7x10^{-4}$ mol/l cadmium iodide

Table 1. Effect of cadmium iodide and mercury acetate on cytochrome c reductase and mixed function oxidase activities in vitro of rat hepatic microsomes. Means and standard errors from 7-11 experimental animals have been denoted.

Metal added, mol/l	Cytochrome c reduced nmol/min/mg protein	Benzo(a)pyrene[1] hydroxylated nmol/min/mg protein	Benzo(a)pyrene[2] hydroxylated pmol/min/mg protein	Ethoxycoumarin deethylated nmol/min/mg protein
None	106 ± 11	0.253 ± 0.043	14.9 ± 2.3	0.264 ± 0.032
Cadmium, 2×10^{-7}	ND[3]	ND	14.4 ± 2.5	0.252 ± 0.030
2×10^{-6}	ND	ND	13.3 ± 2.3	0.249 ± 0.031
2×10^{-5}	103 ± 11	0.033 ± 0.007	2.06 ± 0.41	0.091 ± 0.010
2×10^{-4}	82.6 ± 14.0	0.00	0.00	0.042 ± 0.005
5×10^{-4}	82.8 ± 11.0	0.00	ND	ND
Mercury, 2×10^{-7}	97.0 ± 12.0	ND	13.3 ± 2.3	0.253 ± 0.032
2×10^{-6}	32.4 ± 3.6	ND	8.6 ± 2.3	0.033 ± 0.006
2×10^{-5}	7.64 ± 3.8	0.118 ± 0.040	0.00	0.009 ± 0.002
2×10^{-4}	0.00	0.036 ± 0.014	0.00	0.00
5×10^{-4}	0.00	0.020 ± 0.010	ND	ND

1) Radiometric method (19), measuring total hydroxylation of benzo(a)pyrene (38)

2) Fluorometric method (18), measuring mainly formation of 3-OHbenzo(a)pyrene (38)

3) ND = Not determined

Table 2. Effect of cadmium iodide and mercury acetate on the activity of epoxide hydratase and glutathione S-transferase of rat liver toward styrene oxide. Means and standard errors from 6-7 experimental animals have been indicated.

Epoxide hydratase		Glutathione S-transferase	
Salt added, mol/l	Styrene oxide hydrated nmol/min/mg prot	Salt added, mol/l	Styrene oxide conjugated nmol/min/mg prot
None	4.39 ± 0.23	None	95.4 ± 7.5
Cadmium, iodide		Cadmium, iodide	
1.7×10^{-7}	4.33 ± 0.50	1.0×10^{-7}	94.0 ± 10.0
1.7×10^{-6}	4.50 ± 0.53	1.0×10^{-6}	86.6 ± 11.0
1.7×10^{-5}	4.20 ± 0.57	1.0×10^{-5}	83.3 ± 7.8
4.3×10^{-5}	2.49 ± 0.25		
8.7×10^{-5}	1.17 ± 0.27		
1.7×10^{-4}	0.18 ± 0.094	1.0×10^{-4}	23.0 ± 4.8
4.3×10^{-4}	0.00 ± 0.0		
Mercuric, acetate		Mercuric, acetate	
		1.0×10^{-7}	104 ± 11
		1.0×10^{-6}	88.9 ± 9.1
1.7×10^{-4}	4.21 ± 0.61	1.0×10^{-4}	54.4 ± 4.6
4.3×10^{-4}	3.18 ± 0.28		

concentration, whereas a slightly lower concentration inhibited glutathione conjugation by 75 per cent only. Mercuric acetate was considerably weaker inhibitor of both styrene oxide hydration and conjugation than cadmium. The highest concentration tested, 1.7×10^{-4} mol/l had no effect on styrene oxide hydration, and a similar concentration produced only a 50 per cent inhibition of glutathione conjugation.

DISCUSSION

Administration of lead salts to experimental animals leads to a decrease in the activity of mixed function oxidation reactions (1-4). However, no effect in vitro of lead on either ethoxycoumarin deethylation or benzo(a)-pyrene hydroxylation could be seen in the present study. Concentrations of lead high enough (0.1-1.0 mmol/l) to inhibit sulfhydryl enzymes (28) were shown to inhibit cytochrome c and cytochrome P-450 reduction (4); this

enzyme contains functional SH groups (29). In line with the present findings, lead concentrations in excess of 10^{-4} mol/l were required to inhibit mixed function oxidation (3,30,31). On the other hand, congeneers of lead, platinum and palladium were shown to inhibit aminopyrine demethylation effectively (32).

Cadmium has been shown to inhibit mixed function oxidation (6,12,33). Differences in the cadmium sensitivity have been observed between various substrates of monooxygenation (12). In the present study, the differences between different hydroxylation reactions of benzo(a)pyrene, as well as between benzo(a)pyrene and ethoxycoumarin, were minor. - Cadmium did not affect cytochrome c reduction.

Salts of methyl mercury, when administered to rats, decreased the activity of mixed function oxidase, whereas mercuric acetate administration was reported to result in an increase in p-nitroanisole demethylation (13-15). Mercury proved to be a potent inhibitor of mixed function oxidation, whereas the effect was less pronounced on cytochrome c reduction. Therefore, it is conceivable that mercury exerts its effect by a mechanism not dependent on SH groups. Mercury has been shown to attack a wide variety of different molecular structures (cf 28).

Rat liver glutathione S-transferases comprise a group of related enzymes, which share a common two subunit structure (34). Glutathione S-transferase A was shown to contain 3.5 SH groups per molecule (35). Iodoacetate was ineffective as an inhibitor of glutathione S-transferase A, whereas another sulfhydryl alkylating agent, o-iodobenzoate, proved to be an effective inhibitor (35). In the present study, inorganic mercury proved a weak inhibitor, 50 per cent inhibition was achieved at 0.1 mmol/l. This inhibition was not due to scavenging of glutathione or splitting the conjugate by mercuric acetate, because none of the metal salts studied affected the nonenzymatic conjugation reaction rate. Cadmium inhibited glutathione conjugation at a concentration of 10^{-4} mol/l. Organometallic compounds of germanium, lead,

and tin have been shown to inhibit glutathione S-transferases of rat liver supernatant fraction (36).

Iodoacetamide and p-hydroxymercuribenzoate were weak inhibitors of purified epoxide hydrase, 10 mmol/l concentrations inhibited the hydration by 15 per cent only (37). Similarly, inorganic mercuric acetate in the present study at a concentration of 0.2 mmol/l did not appreciably affect styrene hydration. On the other hand, cadmium, at a similar concentration, was able to inhibit epoxide hydrase activity by more than 95 per cent.

Calop and coworkers (31) have suggested that the carcinogenic effect of various metal salts might be due to an inhibition of epoxide detoxification (i.e., a cocarcinogenic effect). Cadmium inhibited both glutathione conjugation and hydration of an epoxide, styrene oxide. However, other carcinogenic metals, cobalt, nickel, chromium, had no similar effect. This hypothesis therefore, can not be generally applicable.

No straight forward conclusions about in vivo effects of heavy metal salts on drug metabolism can be drawn from in vitro studies.

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