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Involvement of Molybdenum Hydroxylases in Reductive Metabolism of Nitro Polycyclic Aromatic Hydrocarbons in Mammalian Skin

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d) Abbreviation: PAHs, polycyclic aromatic hydrocarbons; NF, 2-nitrofluorene; NP, 1-nitropyrene; NB, 4-nitrobiphenyl; HPLC, high-performance liquid chromatography.

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ABSTRACT:

Molybdenum hydroxylases, aldehyde oxidase and xanthine oxidoreductase, were shown to be involved in the nitroreduction of 2-nitrofluorene (NF), 1-nitropyrene and 4-nitrobiphenyl, environmental pollutants, in the skin of various mammalian species. NF was reduced to 2-aminofluorene by hamster skin cytosol in the presence of 2-hydroxypyrimidine, 4-hydroxypyrimidine, N^1-methylnicotinamide or benzaldehyde, but not hypoxanthine or xanthine. Inhibitors of aldehyde oxidase markedly inhibited these nitroreductase activities, but oxypurinol, an inhibitor of xanthine oxidoreductase, had little effect. In DEAE column chromatography of hamster skin cytosol, the major fraction exhibiting nitroreductase activity also showed aldehyde oxidase activity. 2-Hydroxypyrimidine-linked nitroreductase activities of skin cytosol from rabbits and guinea pigs were also inhibited by an inhibitor of aldehyde oxidase. In contrast, nitroreductase activities of skin cytosols of rats and mice were markedly inhibited by oxypurinol. When aldehyde oxidase activity was estimated in skin cytosol of various mammals using benzaldehyde oxidase activity as a marker, considerable variability of the activity was found. The highest activity was observed with hamsters, and the lowest activity with rats. On the other hand, the highest xanthine oxidoreductase activity was observed with rats, and the lowest activity with rabbits. These skin cytosols of various mammals also exhibited significant 2-hydroxypyrimidine-linked nitroreductase activities toward 1-nitropyrene and 4-nitrobiphenyl catalyzed by aldehyde oxidase and xanthine oxidoreductase. Thus, NF was mainly reduced by aldehyde oxidase and xanthine oxidoreductase in skins of animals. However, the contributions of these two molybdenum hydroxylases were considerably different among animal species.

Introduction

Nitrated polycyclic aromatic hydrocarbons (nitro-PAHs) are widely distributed in the environment, chiefly as the result of incomplete combustion processes, and have various biological activities including mutagenicity and carcinogenicity through skin contact (El-Bayoumy et al., 1982; Beije and Möller, 1988; Purohit and Basu, 2000). 2-Nitrofluorene (NF) is classified as a nitro-PAH, and requires metabolic activation, being converted into electrophilic metabolites, which ultimately bind to cellular macromolecules. Reduction of the nitro group is thought to be a key step in the metabolic activation (Fu, 1990). In addition, nitroreduction is considered the major reaction in the metabolism of NF *in vivo* (Möller et al., 1987; Ueda et al., 2001). Therefore, the nitroreduction of these compounds in animal livers has been investigated extensively, and it was reported that the reductive metabolism of nitro-PAHs is catalyzed by cytochrome P450, aldehyde oxidase and/or xanthine oxidoreductase in mammalian liver (Saito et al., 1984; Tatsumi et al., 1986; Bauer and Howard, 1990).

Recently, we examined reductive metabolism of NF in rat skin preparations and showed that nitroreduction in skin was mainly catalyzed by cytosolic xanthine oxidoreductase, but not by microsomal cytochrome P450, cytosolic aldehyde oxidase or DT-diaphorase (Ueda et al., 2003). However, an earlier study using rabbit liver had shown that aldehyde oxidase played a major role in the reduction of NF and other nitro-PAHs (Tatsumi et al., 1986). In sea bream liver, nitroreduction of NF was catalyzed by aldehyde oxidase (Ueda et al., 2002). Furthermore, our preliminary study using rat liver found that aldehyde oxidase was mainly involved in reduction of NF, with xanthine oxidoreductase playing a lesser role. These facts suggested that both of the molybdenum hydroxylases contribute to the nitroreduction of nitro-PAHs in mammalian tissues, especially in skin, which is one of target organs for environmental contaminants.

Moreover, there are marked species differences and strain differences (in rats and mice) of

liver aldehyde oxidase (Krenitsky et al., 1974; Rashidi et al., 1997; Kitamura et al., 1999a; Sugihara et al., 1995). For example, in liver, high aldehyde oxidase activity is observed in rabbits and hamsters. Therefore, the enzyme primarily responsible for nitroreduction of nitro-PAHs in skin may well be species-dependent.

In the present study, we demonstrated the involvement of both aldehyde oxidase and xanthine oxidoreductase in nitroreduction of nitro-PAHs in skin cytosols of various mammals. Furthermore, the relative contributions of these molybdenum hydroxylases to the nitroreduction in skin cytosols of various mammals were determined.

Materials and Methods

Chemicals.

NF, 1-nitropyrene (NP), 4-nitrobiphenyl (NB), 2-aminofluorene, 1-aminopyrene, 4-aminobiphenyl, 2-hydroxypyrimidine hydrochloride, 4-hydroxypyrimidine, xanthine, 1-methylxanthine and benzaldehyde were purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). N^1-Methylnicotinamide, menadione, chlorpromazine, quercetin dihydrate, isovanillin, oxypurinol, hypoxanthine and phenylmethylsulfonyl fluoride were from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Other chemicals used were of the highest grade commercially available.

Animals.

Male Syrian golden hamsters (65-80 g), Japanese albino rabbits (2.0-2.5 kg), Hartley guinea pigs (150-190 g) and ddY mice (23-30 g) were purchased from Japan SLC Inc. (Shizuoka, Japan). Sea/Sprague Dawley rats (200-240 g) were purchased from Seiwa Experimental Animals, Ltd. (Fukuoka, Japan).

Preparation of skin cytosol.

Skin cytosol and microsomes were prepared by employing reported method (Ueda *et al.*, 2003). Briefly, the skin was excised and subcutaneous tissues were scraped off with scissors. Scraped sheets of skin were homogenized with three volumes of 0.1 M K,Na-phosphate buffer (pH 7.4) containing 0.1 mM dithiothreitol, 0.1 mM phenylmethylsulfonyl fluoride, 0.1 mM EDTA and 0.1 mM ethylene glycol bis(β-aminoethyl ether)-*N,N,N',N'*-tetraacetic acid (buffer A). Microsomes and cytosol were obtained from the homogenate by successive centrifugation

at 9000 g for 20 min and at 105,000 g for 90 min.

DEAE-cellulose column chromatography.

The skin cytosolic fraction was subjected to ammonium sulfate fractionation, and proteins that precipitated between 30% and 60% ammonium sulfate saturation were collected. The precipitate was dissolved in buffer A, and dialyzed against 200 volumes of 10-fold-diluted buffer A for 12 h. The dialyzed solution was centrifuged at 9000 g for 20 min, and the supernatant was adsorbed on a column (1.5 x 12 cm) of DE-52, which was equilibrated with buffer A. The column was washed with 50 ml of buffer A and eluted with a 100-ml linear gradient of 0 to 0.3 M sodium chloride in buffer A. The fractions were assayed for aldehyde oxidase activity, xanthine oxidase activity and nitroreductase activity towards NF in the presence of 2-hydroxypyrimidine, and the active fractions were pooled and stored at –80ºC.

Assays of nitroreductase activity.

The incubation mixture consisted of 0.1 µmol of NF, NP or NB, 0.5 µmol of an electron donor and a skin preparation in a final volume of 1 ml of 0.1 M K,Na-phosphate buffer (pH 7.4). The incubation was performed at 37ºC for 30 min under an atmosphere of nitrogen using a Thunberg tube. The protein concentration used in assays was 2 mg/ml. In some cases, incubation was also performed in the presence of 10 µM menadione, 100 µM isovanillin, quercetin or chlorpromazine, which are potent inhibitors of aldehyde oxidase, or 100 µM oxypurinol, an inhibitor of xanthine oxidoreductase. After incubation, 50 µg of phenacetin was added to the mixture as an internal standard, and then the mixture was extracted with 7 ml of ethyl acetate. The extract was evaporated to dryness in vacuo and the residue was subjected to high-performance liquid chromatography (HPLC). Nitroreductase activity was determined by

measuring amino derivatives formed from NF, NP and NB.

Assay of aldehyde oxidase activity.

Aldehyde oxidase activity was measured in terms of the oxidation of benzaldehyde to benzoic acid. Each incubation mixture consisted of 0.1 μmol of benzaldehyde and skin cytosol in a final volume of 0.5 ml of 0.1 M K,Na-phosphate buffer (pH 7.4). The incubation was performed at 37ºC for 15 min. The protein concentration used in assays was 1 mg/ml. After incubation, the reaction was stopped by addition of 500 μl of methanol and 50 μg of estriol was added to the mixture as an internal standard. After mixing, the solution was centrifuged at 10,000 rpm for 5 min. An aliquot of the supernatant was subjected to HPLC.

Assays of xanthine oxidoreductase (xanthine oxidase and xanthine dehydrogenase) activities.

Xanthine oxidase activity in skin cytosol was measured in terms of the oxidation of 1-methylxanthine to 1-methyluric acid in the absence of NAD. In the case of xanthine dehydrogenase activity, 0.27 μmol of NAD was added to the incubation mixture. Each incubation mixture consisted of 0.1 μmol of 1-methylxanthine and skin cytosol in a final volume of 0.5 ml of 0.1 M K,Na-phosphate buffer (pH 7.4). The incubation was performed at 37ºC for 20 min. The protein concentration used in assays was 2 mg/ml. After incubation, the reaction was stopped by addition of 500 μl of methanol and 50 μg of acetaminophen was added to the mixture as an internal standard. After mixing, the solution was centrifuged at 10,000 rpm for 5 min, and an aliquot of the supernatant was subjected to HPLC. In this study, xanthine oxidoreductase activity was expressed as the total of xanthine oxidase and xanthine dehydrogenase activities.

HPLC.

HPLC was performed in an LC-10ADvp (Shimadzu Co., Ltd., Kyoto, Japan) chromatograph fitted with a 250 x 4.6 mm column of CAPCELL PAK C18 UG120 S-5 μm (Shiseido Co., Ltd., Tokyo, Japan). For the determination of 2-aminofluorene, 1-aminopyrene or 4-aminobiphenyl, the column was operated at a flow rate of 0.7 ml/min of acetonitrile : water (6 : 4) at 40ºC, using phenacetin as an internal standard, with the detector set at 280 nm. Retention times of authentic phenacetin (an internal standard), 4-aminobiphenyl, 2-aminofluorene, 1-aminopyrene, NB, NF and NP were 5.1, 8.5, 8.8, 13.6, 17.0, 18.9 and 30.7 min, respectively. For the determination of benzoic acid, the column was operated at a flow rate of 0.5 ml/min of 0.5% acetic acid : acetonitrile (1 : 1) at 40ºC, using estriol as an internal standard, with the detector set at 272 nm. Retention times of authentic estriol (an internal standard), benzoic acid and benzaldehyde were 6.5, 7.6 and 10.0 min, respectively. For the determination of 1-methyluric acid, the column was operated at a flow rate of 0.5 ml/min of 0.5% acetic acid : methanol (9 : 1) at 40ºC, using acetaminophen as an internal standard, with the detector set at 280 nm. Retention times of authentic acetaminophen (an internal standard), 1-methyluric acid and 1-methylxanthine were 11.3, 14.0 and 17.1 min, respectively.

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Results

Nitroreduction of NF in hamster skin preparations.

Nitroreductase activity toward NF in hamster skin microsomes and cytosol in the presence of various electron donors was examined. The skin cytosol exhibited significant nitroreductase activity with 2-hydroxypyrimidine, 4-hydroxypyrimidine (electron donors of aldehyde oxidase and xanthine oxidoreductase), N^1-methylnicotinamide or benzaldehyde (electron donors of aldehyde oxidase). However, NADPH, NADH (electron donors of DT-diaphorase), hypoxanthine, xanthine and 1-methylxanthine (electron donors of xanthine oxidoreductase) had no effect on the activity (figure 1A). The 2-hydroxypyrimidine- or 4-hydroxypyrimidine-linked nitroreductase activity was markedly inhibited by menadione, isovanillin, chlorpromazine and quercetin (inhibitors of aldehyde oxidase), but oxypurinol, an inhibitor of xanthine oxidoreductase, had little effect. Moreover, N^1-methylnicotinamide- or benzaldehyde-linked activity was completely inhibited by menadione, but not by oxypurinol (figure 1B). In contrast, microsomal nitroreductase activity was enhanced with NADPH, but the full activity (3.5 pmol/min/mg protein) was much lower than that of skin cytosol. These facts suggest that the nitroreduction by hamster skin cytosol is mainly due to aldehyde oxidase.

[Insert figure 1 about here]

DEAE column chromatography of hamster skin cytosol.

The hamster skin cytosol was subjected to DEAE-cellulose column chromatography, and the fractions were assayed for nitroreductase activity in the presence of 2-hydroxypyrimidine, as well as for aldehyde oxidase and xanthine oxidase activities. The fractions that exhibited

2-hydroxypyrimidine-linked nitroreductase activity were separated into two active fractions. The minor active fractions (fraction I; fraction 26-32) were coeluted with xanthine oxidoreductase, and the major active fractions (fraction II; fraction 37-46) were coeluted with aldehyde oxidase (figure 2).

[Insert figure 2 about here]

Fraction I exhibited nitroreductase activity in the presence of 2-hydroxypyrimidine or hypoxanthine. The 2-hydroxypyrimidine-linked nitroreductase activity of fraction I was inhibited by oxypurinol, but not by menadione. On the other hand, the nitroreductase activity of fraction II was enhanced by addition of 2-hydroxypyrimidine, N^1-methylnicotinamide and benzaldehyde, but not by hypoxanthine. The 2-hydroxypyrimidine-linked nitroreductase activity was inhibited by menadione, but not by oxypurinol (figure 3). These results confirmed that the nitroreductase activities in fractions I and II are due to xanthine oxidoreductase and aldehyde oxidase, respectively. Fraction II accounted for the majority of the nitroreductase activity in hamster skin cytosol.

[Insert figure 3 about here]

Nitroreduction of NF in skin cytosols of various mammals.

2-Hydroxypyrimidine-linked nitroreductase activity toward NF in skin cytosol of various mammals was examined. The skin cytosols of rabbits, guinea pigs, mice and rats all exhibited significant nitroreductase activity. The effects of various chemicals were then examined. In rabbits, similar to hamster, the nitroreductase activity in skin cytosol was markedly inhibited by

menadione, but not by oxypurinol. In skin of guinea pigs, the nitroreductase activity was partly inhibited by both oxypurinol and menadione. Moreover, the activities in skin cytosols of mice and rats were significantly inhibited by oxypurinol, but little affected by menadione (figure 4). These results suggest that aldehyde oxidase participates predominantly in nitroreduction of NF in skins of hamsters and rabbits, whereas xanthine oxidoreductase participates predominantly in mice and rats, and both flavoenzymes participate in guinea pigs.

[Insert figure 4 about here]

Aldehyde oxidase and xanthine oxidoreductase activities in skin cytosol.

When aldehyde oxidase activity in skin cytosol was measured in terms of the oxidation of benzaldehyde, the highest activity was observed with hamsters, followed by rabbits and guinea pigs. In mice and rats, the activities were slight. The difference in the activity between hamsters and rats was about 6-fold. When xanthine oxidase activity was assayed in the absence of NAD, marked differences were found in skin cytosols from various mammals (table 1). The activities were completely inhibited by oxypurinol, but not menadione (data not shown). The highest activity was observed with rats, followed by mice, guinea pigs and hamsters. However, very low activity was observed in rabbits. The difference in activity between rats and rabbits was 32-fold. Species difference was also observed in xanthine dehydrogenase activity, which was assayed in the presence of NAD. In most species, xanthine dehydrogenase activity of skin cytosol was higher than xanthine oxidase activity.

[Insert table 1 about here]

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Nitroreduction of nitro-PAHs in skin cytosols of various mammals.

The 2-hydroxypyrimidine-linked nitroreductase activity toward NP and NB, as well as NF, was examined in skin cytosols of various mammals. These compounds were significantly reduced to the corresponding amines by skin cytosols of hamsters, rabbits, guinea pigs, rats and mice. Relatively high activity was observed in mice and rats, while rabbits showed the lowest activity among other mammals (table 2). This may be due to very low xanthine oxidoreductase activity. The activity observed with rats was inhibited by oxypurinol, and that in hamsters was inhibited by menadione (data not shown). NP and NB, as well as NF, were also reduced by aldehyde oxidase and xanthine oxidoreductase in skin cytosols of various mammals, but the contributions of the two flavoenzymes were different among species.

[Insert table 2 about here]

Discussion

Aldehyde oxidase, a cytosolic enzyme, contains FAD, molybdenum and iron-sulfur center, and is closely related to xanthine oxidoreductase (Turner et al., 1995; Hille, 2005). Both enzymes have similar primary and secondary structures, but their substrate specificities differ (Krenitsky et al., 1972; Beedham, 1985). Aldehyde oxidase can catalyze the oxidation of various aldehydes and nitrogenous heterocyclic compounds (Clarke et al., 1995; Jordan et al., 1999; Kitamura et al., 1999a), whereas xanthine oxidoreductase participates purine metabolism such as caffeine, hypoxanthine and 6-deoxyacyclovir (Kreninsky, 1984; Jones et al., 1987; Relling et al., 1992). The both enzymes in the presence of an electron donor can also mediate the reduction of a variety of compounds, such as sulfoxide, *N*-oxide, epoxide and aromatic nitro compounds (Tatsumi et al., 1986; Kitamura and Tatsumi, 1984; Hirao et al., 1994; Sugihara et al., 1996). In a previous study, we demonstrated that NF was mainly reduced by xanthine oxidoreductase in the presence of 2-hydroxypyrimidine or 4-hydroxypyrimidine as an electron donor in skin of Sea:Sprague-Dawley strain rats which exhibited high liver aldehyde oxidase activity (Ueda et al., 2003). However, in this study using hamster skin, 2-hydroxypyrimidine- and 4-hydroxypyrimidine-linked nitroreductase activities were slightly inhibited by an inhibitor of xanthine oxidoreductase, and markedly inhibited by inhibitors of aldehyde oxidase. Moreover, electron donors to aldehyde oxidase enhanced the nitroreductase activity, showing that aldehyde oxidase plays an important role in the nitroreduction in hamster skin. In contrast, the activity in skin of guinea pigs was partially inhibited by oxypurinol and menadione. These results suggested that xanthine oxidoreductase mainly contributed to nitroreduction in skin of mice and rats, while both molybdenum hydroxylases contributed in skin of guinea pigs. Therefore, it appears that the patterns of participation of these molybdenum hydroxylases in

nitroreduction in mammalian skin can be classified into three groups, i.e., i) participation of mainly aldehyde oxidase (hamsters and rabbits), ii) participation of mainly xanthine oxidoreductase (mice and rats); and iii) participation of both molybdenum hydroxylases (guinea pigs) (figure 5).

[Insert figure 5 about here]

Marked species difference exists in the liver aldehyde oxidase activity. We determined aldehyde oxidase activity using benzaldehyde as a substrate in skin cytosols from hamsters, rabbits, guinea pigs, mice and rats, and clear species differences in the levels of the activity were observed. The aldehyde oxidase and xanthine oxidoreductase activities in skin of various species appeared to be complementary, i.e., when one was high, the other was low. In addition, the pattern of species difference observed in these experiments using skin cytosol was similar to that in liver, except that rats (Sea:Sprague-Dawley strain), which have high liver aldehyde oxidase activity, exhibited relatively high xanthine oxidoreductase and low aldehyde oxidase activity in skin cytosol. In humans, it was reported that methotrexate, benzaldehyde, N^1-methylnicotinamide and 6-methylpurine were metabolized by liver aldehyde oxidase (Rodrigues, 1994; Kitamura et al., 1999b; Sugihara et al., 1997), and famciclovir was metabolized by liver xanthine oxidoreductase (Fowles et al., 1994). Since human aldehyde oxidase and xanthine oxidoreductase both play important roles in the metabolism of exogenous compounds, they may both be involved in metabolism of nitro compounds, such as some hair dyes and various topically applied therapeutic antiviral agents and anticancer agents, in human skin (Van Duuren, 1980; Rashidi et al., 1997).

In this study, skin cytosols from various mammals also exhibited significant nitroreductase

activity towards NP and NB. The highest activity toward NF was observed with hamsters, which also exhibited high aldehyde oxidase activity. On the other hand, the highest activities toward NP and NB were observed with rats, which exhibited high xanthine oxidoreductase activity. This substrate specificity of the nitroreduction observed in skin cytosol from various mammals presumably reflects the specificity of the two flavoenzymes i.e., aldehyde oxidase in the reduction of NF and xanthine oxidoreductase in the reduction of NP and NB.

The tissue distributions and activities of molybdenum hydroxylases have been investigated extensively. Krenitsky et al. (1974) found no aldehyde oxidase activity in skin of Sprague-Dawley rats and rhesus monkeys. On the other hand, Kooij et al. (1992) reported that xanthine oxidoreductase was present in rat skin. Immunohistochemical analysis confirmed that xanthine oxidoreductase was distributed in epidermis, sebaceous glands and hair follicle epithelium, while aldehyde oxidase was not detected in epidermis of Wistar rats (Moriwaki et al., 1996). Moreover, in mice, mRNA of aldehyde oxidase was detected in esophagus, lung, liver and testis, but not skin (Kurosaki et al., 1999). In contrast, Tereo et al. (2001) cloned cDNA for AOH1 and AOH2, novel molybdenum hydroxylases, and demonstrated that mRNA of AOH2 was present in the basal layer of the epidermis and hair follicle in mice. We detected aldehyde oxidase and xanthine oxidoreductase activities in skins of various mammals. However, the physiological roles of these enzymes are still unknown. Possible roles include homeostatic control of vitamins such as retinoids and other biologically active molecules, and elimination of biogenic wastes or breakdown products generated by ultraviolet radiation and other environmental stresses (Kishore and Boutwell, 1980; Siegenthaler et al., 1990; Beedham et al., 1995; Garattini et al., 2003).

The present study has demonstrated that nitroreductase activities toward some nitro-PAHs in skin of various mammalian species are due to both aldehyde oxidase and xanthine

oxidoreductase, and the species differences reflect differences of relative aldehyde oxidase and xanthine oxidoreductase activities. Although the physiological roles of aldehyde oxidase and xanthine oxidoreductase in skin are unknown, it is clear that both molybdenum hydroxylases play important roles in the metabolism of xenobiotics in skin.

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Footnotes

a) Unnumbered footnote

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b) Reprint request

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Legends to Figures

Figure 1. Reduction of 2-nitrofluorene to 2-aminofluorene by hamster skin cytosol.

Each bar represents the mean ± SD of four hamsters. A, requirement of electron donor. B, the effects of inhibitors on 2-hydroxypyrimidine-, 4-hydroxypyrimidine-, N^1-methylnicotinamide- or benzaldehyde-linked nitroreductase activity. Incubation was performed at 37ºC for 30 min with 2.0 mg/ml of skin cytosol with various electron donors in the presence or absence of an inhibitor under anaerobic conditions. Open column, in the absence of an inhibitor; hatched column, in the presence of an inhibitor. Menadione was added at $1x10^{-5}$ M, and isovanillin, chlorpromazine, quercetin and oxypurinol were added at $1x10^{-4}$ M. The amount of 2-aminofluorene formed was determined by HPLC. Other details are described in *Materials and Methods.* **, $p<0.05$ compared with control. ND, not detected.

Figure 2. DEAE-cellulose column chromatography of 2-hydroxypyrimidine-linked nitroreductase (○), xanthine oxidase (▲) and aldehyde oxidase (●) in ammonium sulfate precipitate (30%-60%) from hamster skin cytosol.

In the assay of 2-nitrofluorene nitroreductase, the incubation mixture consisted of 0.1 μmol of 2-nitrofluorene, 0.5 μmol of 2-hydroxypyrimidine, and 0.2 ml of each fraction in a final volume of 1 ml of 0.1 M K,Na-phosphate buffer (pH 7.4). The incubation was performed at 37ºC for 30 min under anaerobic conditions. Other details are described in *Materials and Methods*.

Figure 3. Reduction of 2-nitrofluorene to 2-aminofluorene by Fraction I and Fraction II separated by DEAE-cellulose column chromatography.

Each bar represents the mean ± SD of three experiments. The incubation was performed at 37ºC for 30 min with 0.1 ml of fraction I or fraction II and various electron donors under anaerobic conditions. Menadione was added at $1x10^{-5}$ M, and oxypurinol was added at $1x10^{-4}$ M. The amount of 2-aminofluorene formed was determined by HPLC. Other details are described in *Materials and Methods*. **, $p<0.05$ compared with control.

Figure 4. Reduction of 2-nitrofluorene to 2-aminofluorene by skin cytosol from various mammals.

Each value represents the mean ± SD of three or four animals. Incubation was performed at 37ºC for 30 min with 0.1-0.3 ml of skin cytosol (2.0 mg/ml) with 2-hydroxypyrimidine in the presence or absence of an inhibitor under anaerobic conditions. Menadione was added at $1x10^{-5}$ M, and oxypurinol was added at $1x10^{-4}$ M. The amount of 2-aminofluorene formed was determined by HPLC. Other details are described in *Materials and Methods*. **, $p<0.05$ compared with control.

Figure 5. Aldehyde oxidase and xanthine oxidoreductase-catalyzed reduction of nitro-PAHs in skin cytosols from various mammals.

Table 1 Aldehyde oxidase and xanthine oxidase/xanthine dehydrogenase activities in skin

Species	Aldehyde Oxidase[a)]	Xanthine Oxidoreductase	
		Xanthine Oxidase[b)]	Xanthine Dehydrogenase[c)]
	(nmol/min/mg protein)		
Hamster	1.43 ± 0.26	0.15 ± 0.01	0.66 ± 0.14
Rabbit	0.91 ± 0.07	0.05 ± 0.01	0.24 ± 0.16
Guinea pig	0.47 ± 0.17	0.36 ± 0.12	1.81 ± 0.21
Mouse	0.26 ± 0.08	0.83 ± 0.14	1.95 ± 0.78
Rat	0.24 ± 0.11	1.23 ± 0.15	1.97 ± 0.39

Each value represents the mean±SD of four animals.

[a] The mixture containing 0.1 µmol of benzaldehyde and skin cytosol in 0.1 M K,Na-phosphate buffer (pH 7.4) was incubated at 37ºC for 15 min. Benzoic acid formed was determined using HPLC.

[b] The mixture containing 0.1 µmol of 1-methylxanthine and skin cytosol in 0.1 M K,Na-phosphate buffer (pH 7.4) was incubated at 37ºC for 20 min. 1-Methyluric acid formed was determined using HPLC.

[c] The mixture containing 0.1 µmol of 1-methylxanthine, 0.27 µmol of NAD and skin cytosol in 0.1 M K,Na-phosphate buffer (pH 7.4) was incubated at 37ºC for 20 min. 1-Methyluric acid formed was determined using HPLC.

Other details are described in *Materials and Methods.*

Table 2 Nitroreductase activity toward nitro-PAHs in skin cytosols of various mammals

Species	Nitroreductase activity (pmol/min/mg protein)	
	1-Nitropyrene	4-Nitrobiphenyl
Hamster	15.0 ± 6.2	132.7 ± 30.1
Rabbit	5.5 ± 3.3	± 9.4
Guinea pig	16.4 ± 3.0	121.4 ± 19.3
Mouse	23.4 ± 3.8	175.7 ± 15.5
Rat	35.1 ± 6.7	173.7 ± 10.5

Each value represents the mean±SD of three animals.
The mixture containing 0.1 µmol of 1-nitropyrene or 4-nitrobiphenyl, 0.5 µmol of 2-hydroxypyrimidine and skin cytosol in 0.1 M K,Na-phosphate buffer (pH 7.4) was incubated at 37°C for 30 min under an anaerobic condition.
The amounts of 1-aminopyrene and 4-aminobiphenyl formed were determined by HPLC.
Other details are described in *Materials and Methods.*

Fig.1

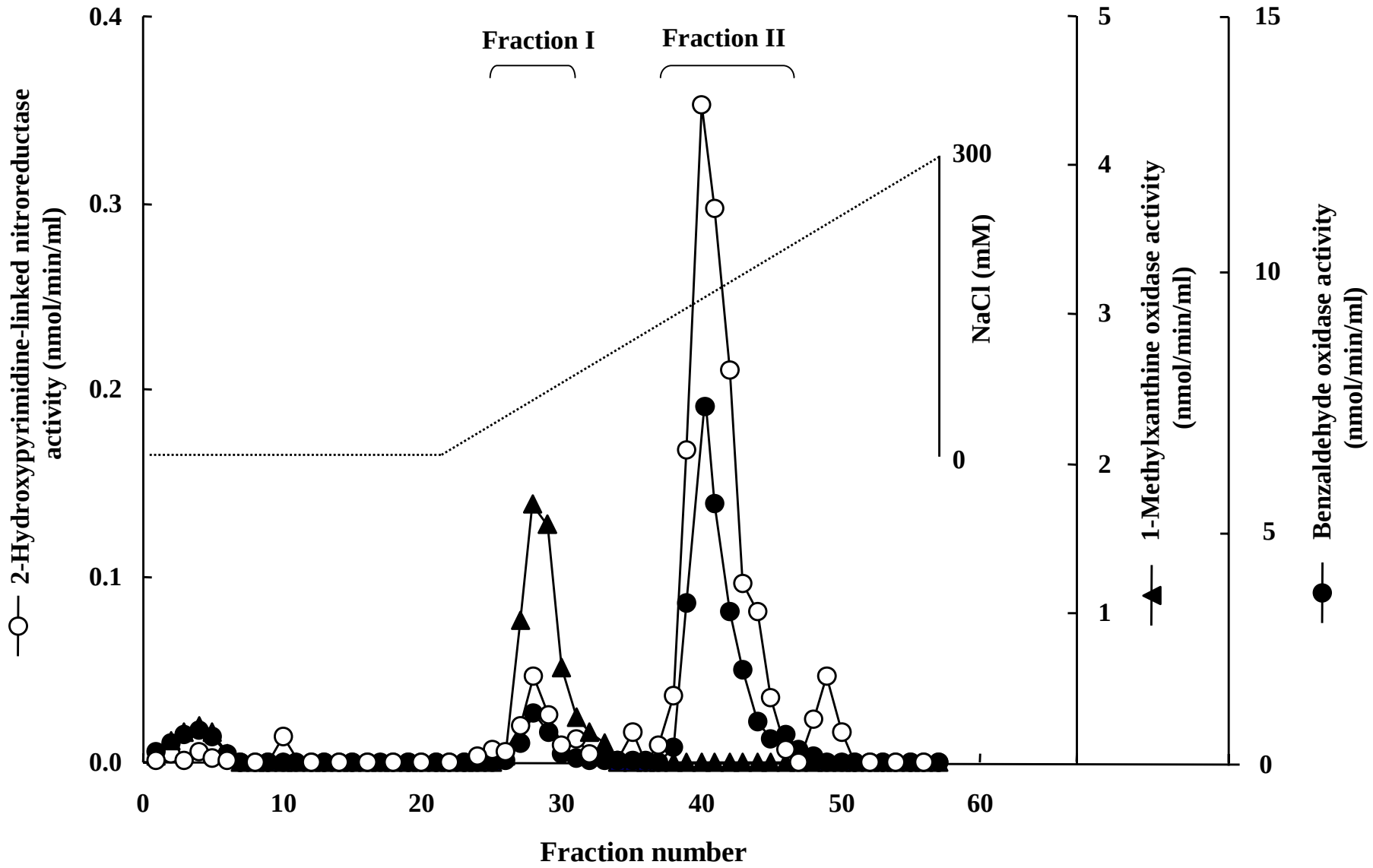

Fig.2

Fig.3

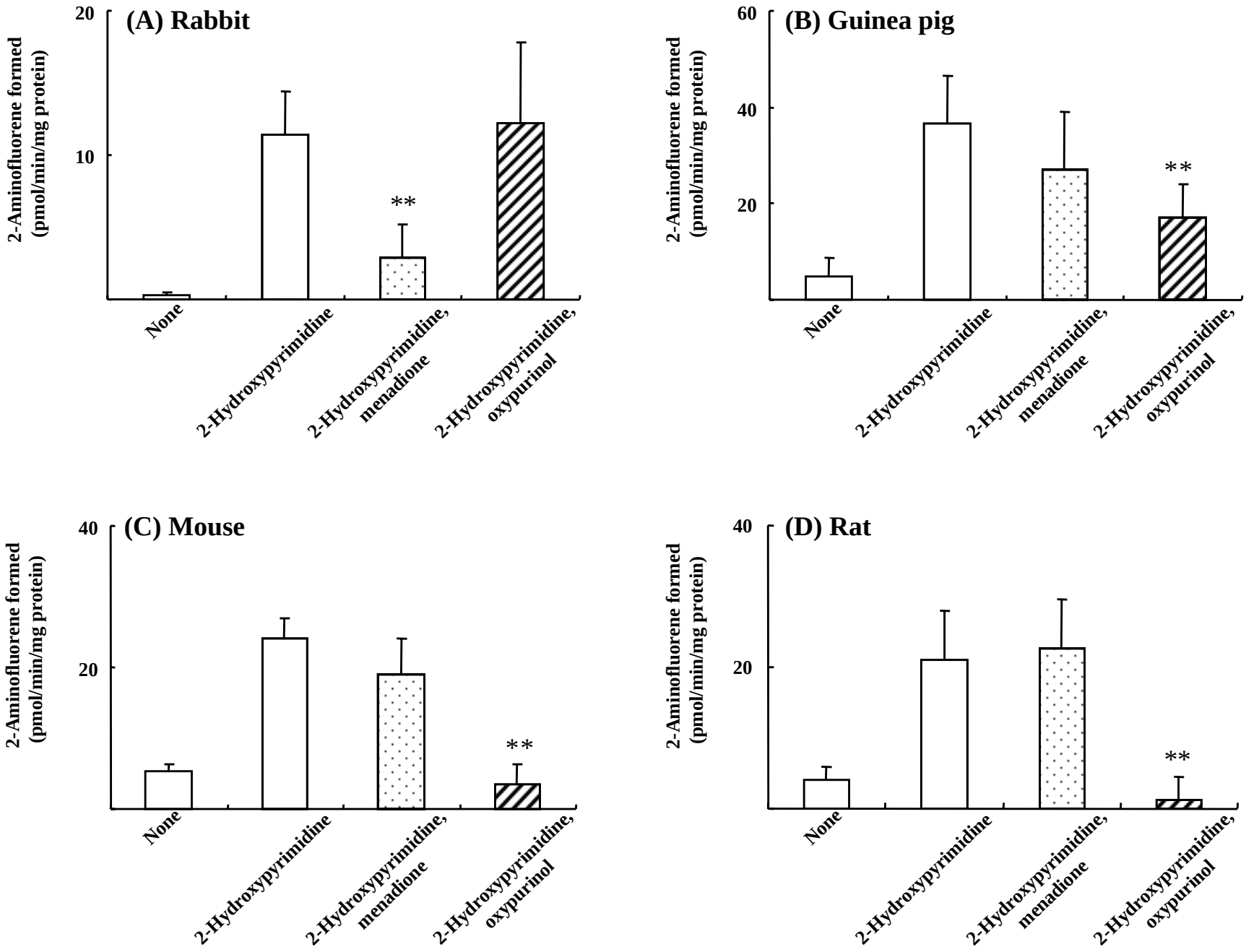

Fig.4

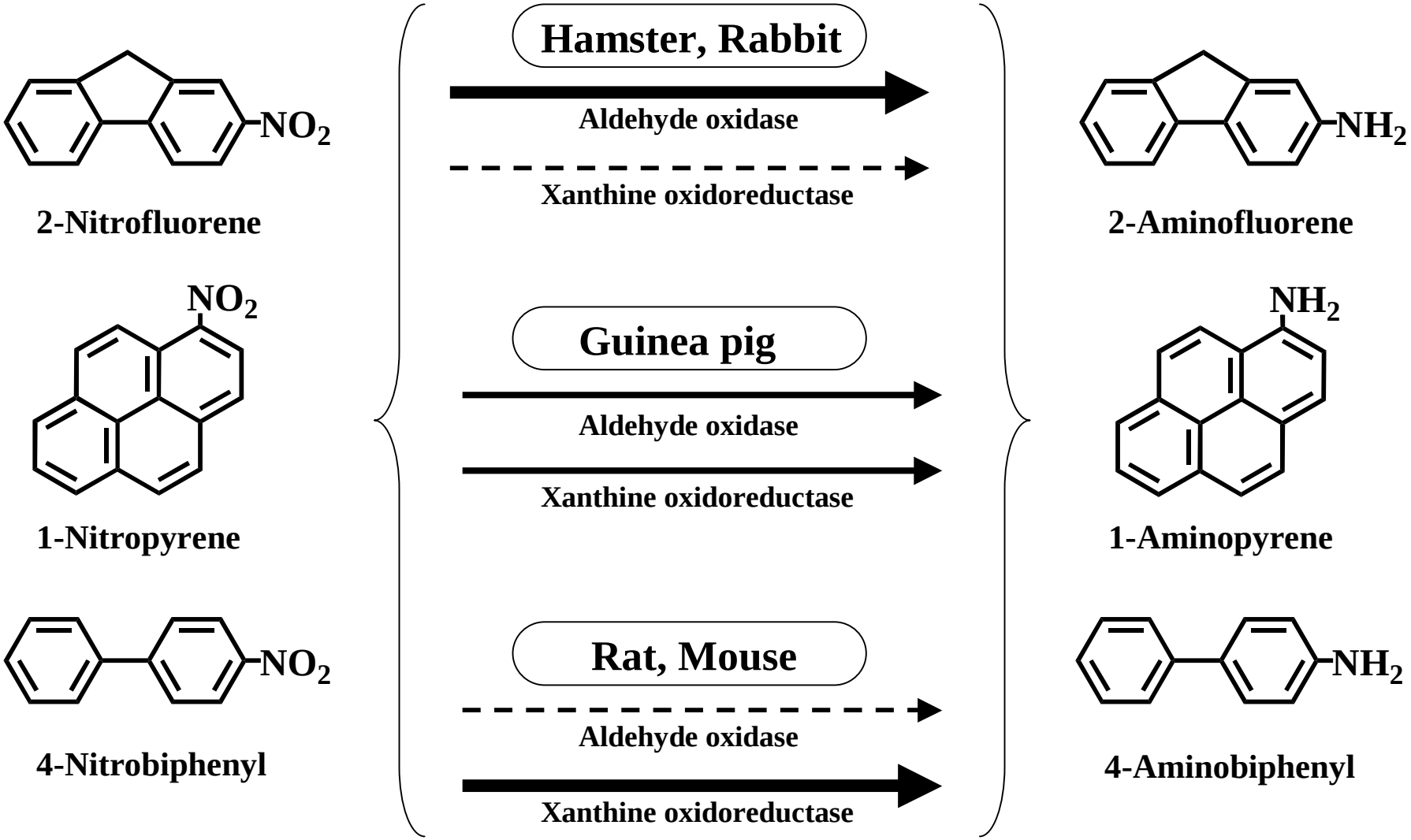

Fig. 5.