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SHORT COMMUNICATIONS

Induction of acute renal porphyria in Japanese quail by Aroclor 1254

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One of the manifestations of polychlorinated biphenyl (PCB) intoxication in mammals and birds is porphyria [1, 2]. Porphyria is produced in rats or mice within weeks or months following exposure to PCBs [3–5]. In chicks, gross accumulation of porphyrins in liver occurs after feeding hexachlorobiphenyl isomers for 21 days [6] or PCB mixtures for 60 days [7]. In Japanese quail, daily oral administration of Aroclor 1260, 500 or 1000 mg/kg, for 1 week produces an increase in hepatic δ-aminolevulinic acid (ALA) synthetase and in tissue fluorescence indicative of porphyrin accumulation [8]. Daily consumption of feed containing 2000 ppm of the PCB mixture, Phenoclor DP6, by Japanese quail also causes increased fluorescence in liver, kidney, small intestine, and muscle characteristic of porphyria in 5–13 days [7]. In contrast, rats fed the same diet for 3 weeks show no tissue fluorescence under u.v. light [7].

Studies on the effects of a single dose of PCBs on heme metabolism are limited. Goldstein *et al.* [3] has reported that the administration of a large oral dose of Aroclor 1254 (1000 mg/kg) to rats increases hepatic ALA synthetase activity 5-fold in 24 hr but does not increase urinary porphyrins, ALA, or porphobilinogen (PBG) 2 or 3 days or 4 months after treatment. Data on tissue porphyrins were not presented. In another rat study [9], a single oral dose of 2.12 mmoles/kg of the PCB mixtures (Aroclors 1232, 1248, or 1260) caused a slight increase in hepatic ALA synthetase activity as well as in liver porphyrins in 24 hr. A small but significant increase in liver porphyrins has been found to persist for 28 days when Aroclor 1248 is given i.p. at a dose of 1.7 mmoles/kg [9].

The present work was undertaken to examine the mechanism of Aroclor 1254-induced porphyria in Japanese quail. Aside from the hepatic effects, the effects on the kidney were also determined since this tissue was primarily affected following acute exposure to other porphyrogenic agents such as hexachlorobenzene (HCB) and octachlorostyrene [10]. The studies of Vos and Koeman [7] also showed that the kidney is one of the tissues of PCB-treated quail that accumulate porphyrins.

Adult male Japanese quail (100–110 g), after an overnight fast, were dosed orally with Aroclor 1254, 500 mg/kg, in corn oil. Control birds received corn oil alone. The birds were transferred to metabolism cages to allow collection of feces (plus urine) for 24 hr prior to sacrifice. The birds were killed by CO_2 inhalation 48 hr after dosing. Liver and kidneys were removed and homogenized in 1.15% KCl containing 0.01 M phosphate buffer, pH 7.4.

Porphyrins were extracted from liver, kidney, bile, and feces by a two-step procedure using an ethyl acetate–acetic acid (4:1) mixture and 3 N HCl. To remove interfering pigments, the HCl extracts were neutralized with 2 M acetate buffer, pH 4.6, and passed through an anion exchange column [11]. Total porphyrins were measured fluorometrically by the method of Poulos and Lockwood [12], and porphyrin profiles were determined by high performance liquid chromatography (HPLC) [13]. ALA content of feces was determined by the method of Carlson [11]. ALA synthetase activity was assayed by the method of Sassa *et al.* [14]. Uroporphyrinogen-I synthetase (URO-I-S) activity was measured by detecting the formation of porphyrins following a 1-hr incubation period using porphobilinogen as substrate [15]. The procedure described by Kardish and Woods [16], as modified by Woods *et al.* [13], was used for the measurement of uroporphyrinogen decarboxylase (URO-D) activity. URO-D activity was expressed as pmoles coproporphyrin formed/mg protein/hr. Values obtained from Aroclor 1254-treated quail were compared to values from corresponding controls by Student's *t*-test at $P < 0.05$.

Table 1 shows that a single oral dose of Aroclor 1254 produced a marked increase in fecal ALA and fecal porphyrins after 48 hr. The birds were killed after 48 hr because preliminary studies showed peak values in porphyrin contents at this time. These increases were associated with an 11-fold increase in renal ALA synthetase activity and a 16-fold increase in hepatic ALA synthetase activity (Table 2). In the rat, no increases in ALA or porphyrin content of feces after a single dose of Aroclor 1254 were obtained [3], suggesting a difference in species.

In the kidney, administration of Aroclor 1254 caused a tremendous (1700-fold) increase in porphyrin content, whereas in liver porphyrin levels increased only 76-fold (Table 1). Acute poisoning of quail with other compounds such as HCB also produces greater accumulation of porphyrins in kidney than in liver [10]. The quail kidney appears to be a major target of porphyrogenic compounds administered acutely in large doses.

Uroporphyrins and 7-carboxyporphyrins were the major porphyrins found in kidney of Aroclor-treated birds (Fig. 1A). Increases in uroporphyrin and other multicarboxylated porphyrin levels may result either from enhanced synthesis and/or inhibition of decarboxylation of uroporphyrinogen *in vivo*. To determine the mechanism for the observed response, the activities of URO-I-S and URO-D were measured in quail tissues. Aroclor administration was

Table 1. Effect of Aroclor 1254 on fecal δ-aminolevulinic acid (ALA) and on porphyrin content of kidney, liver, and feces of quail

Treatment	Fecal ALA (μmoles/g dry)	Porphyrin (μg/g)		
		Kidney	Liver	Feces (dry)
Control	0.16 ± 0.01	0.124 ± 0.003	0.149 ± 0.01	6.88 ± 2.1
Aroclor 1254	2.87 ± 0.66*	212 ± 26.8*	11.4 ± 6.4*	250 ± 56*

Values are mean ± S.E. for four birds.
* Significantly different from control value at $P < 0.05$.

Table 2. Effect of Aroclor 1254 on δ-aminolevulinic acid (ALA) synthetase, uroporphyrinogen I synthetase (URO-I-S), and uroporphyrinogen (URO) decarboxylase activities in kidney and liver of quail

	ALA synthetase (nmoles/g tissue/hr)		URO-I-S (nmoles/g tissue/hr)		URO decarboxylase (pmoles coproporphyrin formed/mg protein/hr)	
Treatment	Kidney	Liver	Kidney	Liver	Kidney	Liver
Control	4.21 ± 0.28	7.05 ± 0.77	9.37 ± 1.14	12.4 ± 0.4	89.5 ± 2.7	117 ± 5.5
Aroclor 1254	46.1 ± 6.2*	110 + 23*	31.5 ± 5.4*	22.7 ± 5.2*	43.8 ± 6.5*	98 ± 8.1

Values are mean ± S.E. for four birds.
* Significantly different from control value at $P < 0.05$.

found to produce a 51% reduction of renal URO-D activity (as measured by coproporphyrinogen formation) and a 3-fold increase in renal URO-I-S activity (Table 2). In the *in vivo* situation it is possible that uroporphyrin accumulation in kidney may precede the inhibition of URO-D by Aroclor 1254. Since uroporphyrins may inhibit URO-D activity *in vitro* [17], increased levels of uroporphyrins in quail kidney as a result of enhanced uroporphyrin synthesis may inhibit URO-D activity *in vivo*, thereby aggravating the porphyrin accumulation in that organ. In addition, some of the porphyrins may be synthesized in the liver and transported to the kidneys.

Feces of Aroclor-treated birds contained mainly coproporphyrins (Fig. 1B). Since the feces of quail represents the products of both the urogenital and digestive systems, the lack of significant amounts of 8- or 7-carboxyporphyrins in feces may indicate a defective mechanism of eliminating these porphyrins through the kidneys. In contrast, urinary elimination of these porphyrins occurs in the rat as shown by the high concentration of these porphyrins in the rat urine following chronic exposure to PCBs [3]. The fecal coproporphyrins likely originated from both kidney and liver. Evidence for the latter source was provided by the coproporphyrins found in bile (Fig. 1C).

No significant reduction in uroporphyrinogen decarboxylase activity was observed in liver. However, hepatic URO-I-S activity was increased, but this increase (83%) was low compared to the 3-fold increase in renal URO-I-S activity. These observations may explain the lower accumulation of porphyrins in liver as compared to kidney. In addition, the liver has an effective mechanism of eliminating porphyrins through the bile or through the circulation. Bile of Aroclor 1254-treated birds contained large amounts of porphyrins consisting of 8-, 7-, 6-, 5-, and 4-carboxyporphyrins, the latter being the most abundant as isomer I (Fig. 1C). In control bile, only coproporphyrin I was detected by HPLC (unpublished data).

Tissue-dependent response of URO-D to xenobiotics has been demonstrated recently by Woods *et al.* [13]. They found that renal URO-D activity in rats acutely dosed with mercury (1.5 mg/kg) is inhibited to 39% of controls. In contrast, hepatic uroporphyrinogen decarboxylase activity is unchanged. Although our present results are consistent with these findings, further work on this aspect is required since endogenous porphyrins which might also inhibit URO-D were not removed from either liver or kidney prior to our URO-D assay. Actual tissue differences in URO-D activity in quail may perhaps be explained on the basis of

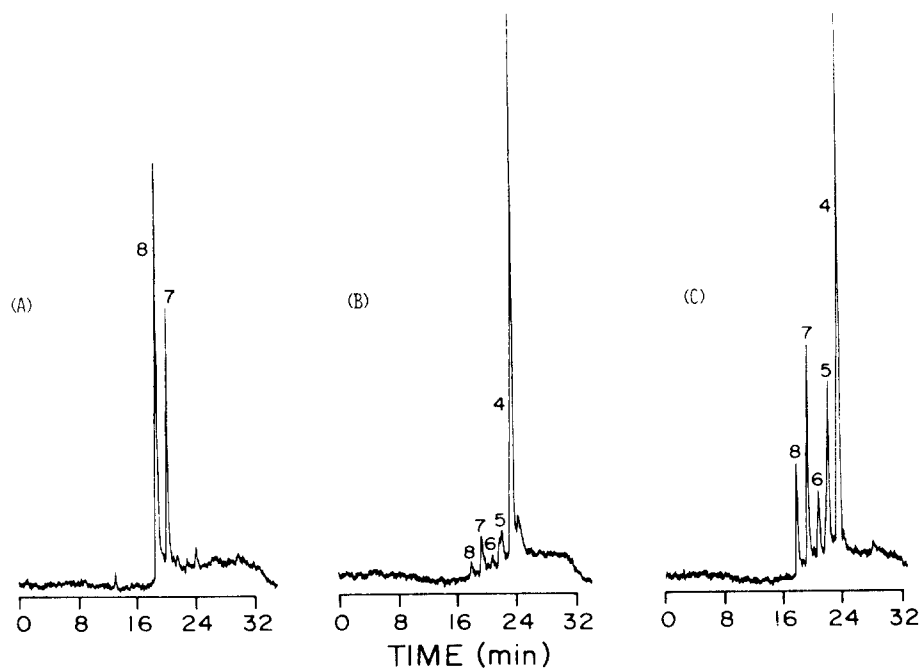

Fig. 1. HPLC separation of prophyrins in kidney (A), feces (B) and bile (C) of Aroclor-treated quail. Peaks 8, 7, 6, 5, and 4 co-eluted with 8-, 7-, 6-, 5-, and 4-carboxyporphyrin standards respectively.

differential tissue distribution of Aroclor 1254 but such data were not obtained in our study.

Another question left unanswered is whether PCBs present in Aroclor 1254 or contaminants such as chlorinated dibenzofurans [18] were responsible for the observed alterations in porphyrin metabolism. However, we failed to detect dibenzofurans in our Aroclor preparation by GC-mass spectrometric analysis. The effect of purified dibenzofurans at levels found in Aroclor 1254 [18] on enzymes of the heme pathway requires further investigation.

In summary, single oral administration of Aroclor 1254 produced porphyria in male Japanese quail characterized by excessive excretion of ALA and coproporphyrin I in feces, increased activity of ALA synthetase in liver and kidney, and a dramatic accumulation of uroporphyrins and 7-carboxyporphyrins in kidney. ALA-S activity was increased more in liver than in kidney, whereas URO-I-S activity was increased more in kidney than in liver. The activity of URO-D was decreased in kidney but not in liver. Further work is required to explain these tissue-dependent changes in porphyrin metabolism.

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Effects of phorbol esters and pertussis toxin on agonist-stimulated cyclic AMP production in rat osteosarcoma cells

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Tumor-promoting phorbol esters are thought to act through activation of protein kinase C and subsequent phosphorylation of specific substrates [1]. Treatment of intact cells with phorbol esters can alter cAMP production, but the effects are complex. Both decreased [2–5] and increased [6–10] cAMP production after phorbol ester treatment have been reported. The basis for the opposite effects of phorbol esters on cAMP production and the site(s) of action have not been defined.

We studied the effects of phorbol esters on cAMP accumulation in a well-characterized rat osteosarcoma cell line [11]. Parathyroid hormone (PTH*) and beta-adrenergic agonists stimulate cAMP production in these cells through distinct receptors interacting with the stimulatory guanine nucleotide regulatory protein, G_s [12]. These cells also possess a functional inhibitory regulatory protein, G_i [12]. We now report that phorbol esters can enhance agonist-stimulated cAMP accumulation in rat osteosarcoma cells, but that the effect is very rapid and transient. The effects of phorbol esters, moreover, are additive with those of pertussis toxin which inactivates G_i [13].

* Abbreviations: PTH, parathyroid hormone; TPA, 12-*O*-tetradecanoyl phorbol-13-acetate; 4-alpha-; PDD, 4-alpha-phorbol 12,13-didecanoate; ROS, rat osteosarcoma; G_s and G_i, the guanine nucleotide regulatory proteins associated with stimulation and inhibition, respectively, of adenylate cyclase.

Materials and methods

The 17/2.8 subclone of rat osteosarcoma (ROS) cells was originally obtained from Dr. Gideon Rodan. Phorbol esters and l-isoproterenol hydrochloride were from Sigma. Pertussis toxin was the gift of Dr. Ron Sekura. PTH (human 1-34 fragment) was from Bachem. The sources of other materials and the methods for culture of ROS cells and for cAMP measurement were as previously described [12, 14].