ENHANCEMENT OF DRUG OXIDATION AND CONJUGATION BY CARCINOGENS IN DIFFERENT RAT TISSUES

A. AITIO, H. VAINIO and O. HÄNNINEN
Departments of Physiology, University of Turku, Turku 52
and *University of Kuopio, Kuopio 10, Finland*

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1. Introduction

The administration of carcinogenic polycyclic hydrocarbons has been shown to enhance hepatic drug oxidation [1, 2]. An increase takes place also in the skin [3–6], lungs and gastrointestinal tract [7–9], kidneys [8] and placenta [10]. The oxidation step in detoxification is followed *in vivo* by conjugation. In separate studies an enhancement of glucuronide synthesis has been reported in the liver [11–15] and skin [16]. The time courses of the responses have not, however, received much attention. The present study was performed to clarify the responses of arylhydrocarbon hydroxylase (EC 1.14.1.1) and UDP glucuronyltransferase (EC 2.4.1.17) in the intestinal mucosa, liver and kidneys after an administration of 3,4-benzpyrene and 3-methylcholanthrene to rats. These two enzymes have a close functional and topochemical relationship which suggests that their synthesis might have a common control mechanism.

The rise of the UDP glucuronyltransferase activity was preceded with a much more pronounced rise of the arylhydrocarbon hydroxylase activity in all tissues studied. The induction of the hepatic hydroxylase was significantly higher when the intraperitoneal administration route was used instead of the intragastric one.

2. Methods

Male Wistar rats (about 3 months old, total number 120) fed on commercial pellets *ad libitum* were used. A single dose of 3,4-benzpyrene (Sigma Chemical Co., St. Louis, Mo.) or 3-methylcholanthrene (Koch-Light Laboratories Ltd, Colnbrook, England) was administered either intragastrically or intraperitoneally (100 mg/kg of body weight) as a suspension in olive oil (20 mg/ml). The control rats received an equal amount of olive oil. The animals were decapitated at 8 a.m. to eliminate the diurnal variation in drug metabolism [17]. The small intestine, liver and kidneys were excised and cooled in 0.25 M sucrose (0°). Ten cm long segments from duodenum, low jejunum and aboral ileum were halved longitudinally and the mucosa was scraped with an ampoule file. Half of the mucosal samples and one kidney were homogenized in 1% aqueous digitonin for UDP glucuronyltransferase determinations [18, 19] and the rest of mucosal samples together with the other samples in 0.25 M sucrose using a Potter Elvehjem homogenizer. From the 9000 *g* supernatant fraction of the liver homogenate a microsomal preparation was sedimented by centrifuging at 105 000 *g* for 60 min. The activity of arylhydrocarbon hydroxylase was determined from the sucrose homogenates of the mucosa and kidney and the liver microsomal preparation by using 3,4-benzpyrene as substrate as described by Wattenberg et al. [7]. The determination of UDP glucuronyltransferase activity was carried out on the digitonin homogenates of the mucosa and kidney and the liver microsomal preparation in the presence of digitonin, with *p*-nitrophenol as the aglycone and 4.7 mmole/l concentration of UDP glucuronic acid [18, 19] at pH 7.0.

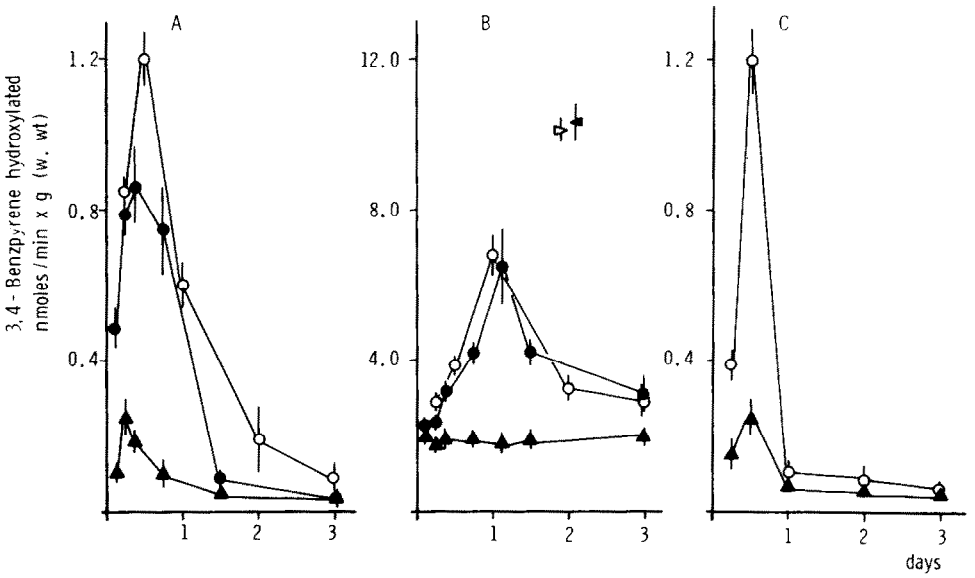

Fig. 1. The effect of intragastric (○, ●) and intraperitoneal (▷, ◀) administration of 3-methylcholanthrene (open symbols) and 3,4-benzpyrene (closed symbols) on the arylhydrocarbon hydroxylase activity of the rat in the duodenal mucosal homogenate (A), liver microsomes (B) and kidney homogenate (C). The triangles (▲) represent the controls receiving olive oil intragastrically. The standard errors of the means are indicated.

3. Results

The intragastric administration of a single dose of 3,4-benzpyrene or 3-methylcholanthrene caused an increase of both arylhydrocarbon hydroxylase and UDP glucuronyltransferase in the three tissues studied. The fastest response was observed in the hydroxylase activity of the intestinal mucosa (fig. 1A). As early as 3 hr after the administration of 3,4-benzpyrene a significant increase had taken place. In the duodenum the maximal hydroxylase activity, 9- and 12-fold increase after 3,4-benzpyrene and 3-methylcholanthrene administration, respectively, was observed in 9–12 hr. Also the decrease of enzyme activity to the control level was rapid. The UDP glucuronyltransferase activity increased in duodenal mucosa less and at considerably slower rate (fig. 2A). The maximal activity (about 2 times higher than in controls) was observed 24–27 hr after the administration of either drug.

The response of both arylhydrocarbon hydroxylase and UDP glucuronyltransferase activity was like that of the duodenum in the low jejunum and aboral ileum (not shown in the figure). The relative increase in hydroxylase activity was, however, much higher due to lower initial levels in the aboral gut. The vehicle, olive oil, used in the administration of carcinogens also caused a transient increase in the activity of both enzyme systems studied in the intestinal mucosa.

In the liver both carcinogens, after an intragastric administration, caused about a 3.5-fold increase in the microsomal arylhydrocarbon hydroxylase activity (fig. 1B). The maximum was reached in 24 hr. The enzyme activity increased and also diminished at a slower rate than in the intestinal mucosa. It was still significantly elevated 3 days after treatment. Little change could be observed in hepatic UDP glucuronyltransferase activity during the first 24 hr after the exposure to 3,4-benzpyrene, but during the second day a maximum, 2 times the control level, was observed. 3-Methylcholanthrene administration caused a somewhat faster, and also a greater increase in hepatic UDP glucuronyltransferase activity than did 3,4-benzpyrene (fig. 2B). Intragastric administration of olive oil had a negligible effect on hepatic drug metabolism. When the carcinogens were administered intraperitoneally, a much

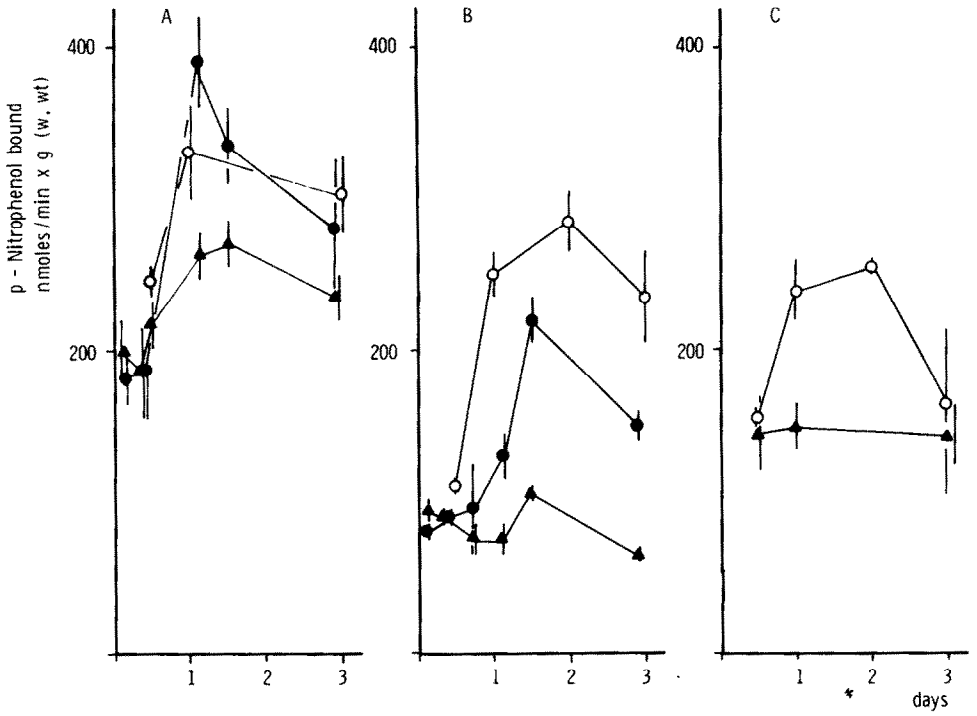

Fig. 2. The effect of intragastric 3-methylcholanthrene (100 mg/kg in olive oil (○) and 3,4-benzpyrene (100 mg/kg in olive oil (●) and olive oil (▲) on the UDP glucuronyltransferase activity of rat duodenal mucosal homogenate (A), liver microsomes (B) and kidney homogenate (C). The standard errors of the means are indicated.

higher response in hepatic arylhydrocarbon hydroxylase activity was observed. Two days after the injection, the enzyme activity was 3 times higher than after administration of a similar amount intragastrically. In the kidney the time course of the changes in the arylhydrocarbon hydroxylase and UDP glucuronyltransferase activity after intragastric administration of 3-methylcholanthrene was intermediate between that in the duodenum and liver (fig. 1C and 2C). The administration of 3,4-benzpyrene caused an accumulation of fluorescent metabolites in the kidney, which hampered the determination of arylhydrocarbon hydroxylase by using 3,4-benzpyrene itself as substrate.

4. Discussion

The enhancement of arylhydrocarbon hydroxylase preceeded the smaller and slower increase in the UDP glucuronyltransferase activity in the three tissues studied after the administration of either 3,4-benzpyrene or 3-methylcholanthrene. The hydroxylase activity was already decreasing when the UDP glucuronyltransferase activity was still increasing. This suggests different control mechanisms in the regulation of the levels of these two functionally and topochemically related enzymes. Since the products of arylhydrocarbon hydroxylase are substrates of UDP glucuronyltransferase, the possibility of a substrate induction of the last mentioned enzyme cannot be excluded.

The rapid response in the levels of the drug metabolizing enzymes in the intestinal mucosa after an exposure to carcinogens resembles the reaction in lungs, where a measurable increase of arylhydrocarbon hydroxylase can be detected as early as two hours after an exposure to cigarette smoke [9]. The capacity of intestinal mucosa to metabolize drugs reflects the composition of the diet [22]. Also, olive

oil probably contains compounds which are potent inducers of drug metabolizing enzymes. Corn oil has been shown to lack such compounds [20]. The induced state of mucosal drug metabolism was rapidly normalized. This may at least partly be explained by the rapid cell renewal [23]. In two days both mucosal cells are replaced by new ones and the hydroxylase activity is normalized.

The mucosal metabolism of carcinogens is probably of considerable importance, since an intragastric administration of either 3,4-benzpyrene or 3-methylcholanthrene caused only a 3.5-fold increase of microsomal arylhydrocarbon hydroxylase in the liver in contrast to the much higher increases described in the literature [8, 24] or obtained in the present study after an intraperitoneal injection of equivalent amount of carcinogen. In the liver the induced state persists over the follow up period. Five days after an administration of carcinogens a significant enhancement of glucuronide synthesis can be detected in liver slices [12].

Acknowledgements

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References

[1] A.H. Conney, E.C. Miller and J.A. Miller, J. Biol. Chem. 228 (1957) 753.
[2] A.H. Conney, Pharmacol. Rev. 19 (1967) 317.
[3] H.V. Gelboin, F. Wiebel and L. Diamond, Science 170 (1970) 169.
[4] E. Schlede and A.H. Conney, Life Sci. II 9 (1970) 1295.
[5] L.W. Wattenberg and J.L. Leong, Cancer Res. 30 (1970) 1922.
[6] W. Levin and A.H. Conney, Science 176 (1972) 419.
[7] L.W. Wattenberg, J.L. Leong and P.J. Strand, Cancer Res. 22 (1962) 1120.
[8] H.V. Gelboin and N.R. Blackburn, Cancer Res. 24 (1964) 356.
[9] R.M. Welch, A. Loh and A.H. Conney, Life Sci. I 10 (1971) 215.
[10] R.M. Welch, Y.E. Harrison, A.H. Conney, P.H. Poppers and M. Finster, Science 160 (1968) 541.
[11] J.K. Inscoe and J. Axelrod, J. Pharmacol. Exp. Ther. 129 (1960) 128.
[12] O. Hänninen and A. Aitio, Biochem. Pharmacol. 17 (1968) 2307.
[13] J.A. Goldstein and A. Taurog, Biochem. Pharmacol. 17 (1968) 1049.
[14] R.D. Howland and A. Burkhalter, Biochem. Pharmacol. 20 (1971) 1463.
[15] R.F. Potrepka and J.L. Spratt, Biochem. Pharmacol. 20 (1971) 861.
[16] G.J. Dutton and I.H. Stevenson, Biochem. Biophys. Acta 58 (1962) 633.
[17] A. Jori, E. DiSalle and V. Santini, Biochem. Pharmacol. 20 (1971) 2965.
[18] O. Hänninen, Ann. Acad. Sci. Fennicae Ser. A II (1968) No. 142.
[19] A. Winsnes, Biochem. Biophys. Acta 191 (1969) 279.
[20] L.W. Wattenberg and J.L. Leong, Proc. Am. Assoc. Cancer Res. 12 (1971) 17.
[21] P. Sims and P.L. Grover, Nature 216 (1967) 77.
[22] L.W. Wattenberg, Cancer 28 (1971) 99.
[23] F.O. Bertalanffy, Acta Anat. 40 (1960) 130.
[24] E. Schlede and H.-J. Merker, Naunyn-Schmiedebergs Arch. Exp. Path. Pharmak. 272 (1972) 89.