J. steroid Biochem. Vol. 35, Nos 3/4, pp. 473–479, 1990
Printed in Great Britain. All rights reserved

0022-4731/90 $3.00 + 0.00
Copyright © 1990 Pergamon Press plc

INTERACTION OF PHYTOESTROGENS AND OTHER ENVIRONMENTAL ESTROGENS WITH PROSTAGLANDIN SYNTHASE *IN VITRO*

GISELA H. DEGEN
Institute of Toxicology, SFB 172, University of Würzburg, Versbacher Str. 9, D-8700 Würzburg, F.R.G.

(*Received* 22 *May* 1989; *received for publication* 11 *October* 1989)

Summary—The phytoestrogens daidzein, genistein, equol and coumestrol were found to stimulate microsomal prostaglandin H synthase (PHS) *in vitro* in a concentration-dependent manner when PHS-activity was measured by arachidonic acid-dependent oxygen uptake. These compounds were co-oxidized by PHS and the conversion of parent compounds was measured by HPLC analysis. The stimulation of PHS-cyclooxygenase by these compounds was partially reversed at high concentrations probably due to their antioxidant properties causing inhibition. In contrast, the monomethyl ethers of daidzein and genistein, formononetin and biochanin A, had little or weakly inhibitory effect on PHS, and appear to be no or poor co-substrates for PHS. Compared to the equine estrogen equilin, its metabolite d-equilenin was poorly metabolized by PHS and inhibited rather than stimulated PHS-cyclooxygenase activity *in vitro*. The resorcylic acid lactones zearalenone and zeranol, on the other hand, were surprisingly good inhibitors of PHS-cyclooxygenase. Furthermore, zeranol inhibited both the arachidonic acid and the hydrogenperoxide-dependent oxidation of DES in contrast to indomethacin which inhibited only cyclooxygenase-dependent cooxidation of DES. The results of this *in vitro* study are discussed in the context of data on synthetic and steroidal estrogens and support the idea that PHS-activity may be modulated by interaction with certain estrogenic compounds.

INTRODUCTION

In addition to endogenously produced steroidal estrogens, humans and animals are exposed to environmental estrogens of plant, fungal and synthetic origin [1]. These structurally diverse substances are interesting from both endocrinological and toxicological points of view: phytoestrogens for example, although rather weak estrogens, can reach considerable concentrations in soy products [2] and have been associated with infertility in various animal species [3, 4]. On the other hand, the decreased breast cancer risk of vegetarian women has been related to their increased consumption of phytoestrogens and lignans [5]. It can be hypothesized that these rather weak estrogens compete with endogenous estrogens at the receptor level, but they may also interfere in estrogen-mediated processes at the non-genomic level, and affect estrogen metabolism or disposition [6].

Our interest in studying the effects of phytoestrogens and other compounds with estrogenic or anti-estrogenic properties on prostaglandin H synthase (PHS), a key enzyme in prostaglandin biosynthesis, has been provoked by several observations. PHS has both cyclooxygenase activity, catalyzing the incorporation of molecular oxygen into arachidonic acid (AA) to produce hydroperoxyendoperoxide PGG_2, as well as peroxidase activity which reduces PGG_2 to PGH_2, a reaction which is linked to the oxidation of endogenous cosubstrates or of xenobiotics and has been termed "co-oxidation" [7] (Fig. 1).

The carcinogenic estrogen, diethylstilbestrol (DES), and several of its structural analogs with phenolic groups are efficiently oxidized by PHS and metabolized to reactive intermediates [8, 9]. Co-oxidation of stilbene and of steroidal estrogens *in vitro* is accompanied by stimulation of PHS-activity with an increased, stoichiometric production of prostaglandin precursor [9–11]. Estrogen target tissues are known to contain PHS and the enzyme appears to be under hormonal control [12, 13]. PHS may play a dual role: (a) in the metabolic activation of estrogens to reactive intermediates [14, 15] and (b) in the production of prostaglandins which are thought to be involved in the progression of e.g. breast tumors [16]. Interestingly, antiestrogens of the triphenylethylene type have been found to inhibit PHS *in vitro* [17], and to prevent estrogen-induced increases in prostaglandin $F_{2\alpha}$ released by endometrial cells in culture [18].

In extension of our structure–activity studies with stilbene and steroidal estrogens [9, 10, 14, 19] we have studied *in vitro* effects of several naturally occurring estrogens on PHS. Our aim was to establish whether any of these compounds are co-substrates or inhibitors of PHS and whether the (stimulatory or

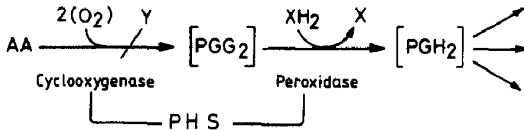

Fig. 1. Scheme of co-oxidation. Arachidonic acid (AA/20:4) is metabolized as described in the text with concomitant oxidation of a co-substrate (XH_2); PHS-cyclooxygenase is inhibited by indomethacin (Y).

inhibitory) effects can be rationalized in terms of the structures of the estrogens.

This paper summarizes our published and unpublished data on isoflavones, coumestrol, equol, resorcylicacid lactones, and on the ring B unsaturated estrogens, equilin and d-equilenin; their effects on PHS are compared with those of DES-indanyl derivatives and the classical inhibitor indomethacin. The *in vitro* approach taken here to assess direct effects of these compounds upon PHS and their PHS-mediated metabolism can also be applied to other compounds, for example antiestrogens, prior to elucidating the role of these interactions *in vivo*.

EXPERIMENTAL

Chemicals

Daidzein, genistein, formononetin, biochanin A, equilin, and d-equilenin were purchased from Fa. C. Roth (Karlsruhe and Sigma, Deisenhofen, F.R.G.). Professor D. N. Kirk (Department of Chemistry, Queen Mary College, London, England) kindly provided samples of equol and coumestrol; zeranol (P1496) and zearalenone (P1492) were obtained from ICM (Terre Hatue, Ind.). Indenestrol A and indenestrol B were a gift from Professor M. Metzler (Institute of Toxicology, University of Würzburg). All other chemicals and biochemicals were from the sources given previously [9, 10].

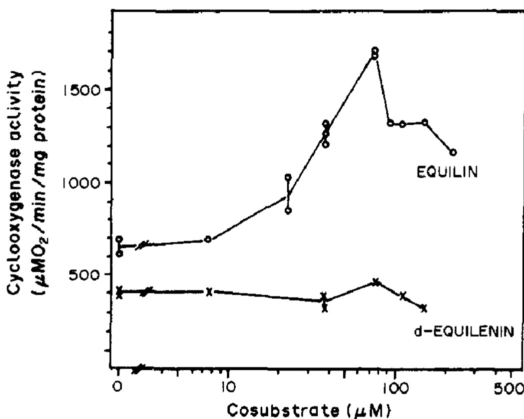

Fig. 2. PHS-cyclooxygenase activity measured in the presence of the indicated concentrations of equilin or d-equilenin as described in the Experimental section (basal assay). Data points show values of single or repeated measurements at a given concentration.

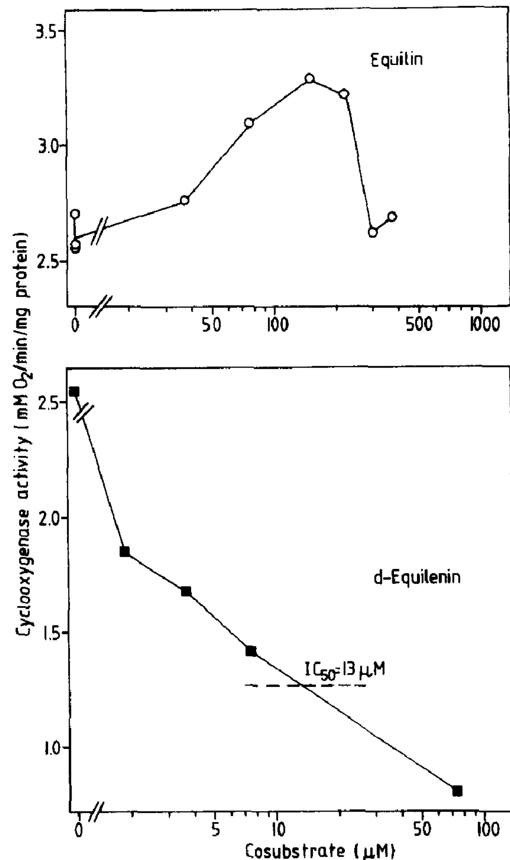

Fig. 3. Effect of increasing concentrations of equilin or d-equilenin on PHS-cyclooxygenase activity assayed in the presence of adrenalin (1 mM) as described in the Experimental section. One data point per concentration from a representative experiment is shown; variance between repeated measurements is 15%.

Effect of estrogenic compounds on PHS-activity

The effects on PHS were studied by measuring the arachidonic acid (AA)-dependent oxygen consumption (PHS-cyclooxygenase activity) by means of an oxygen-sensitive electrode in incubations with varying amounts of estrogenic compounds [9]. Concentration-dependent stimulation and/or inhibition of PHS was determined using enzyme activities derived from the initial tangential slope when plotted versus test compound concentration [20].

Moreover, inhibition of PHS was studied for some estrogenic compounds at varying concentrations of AA in the assay: PHS-cyclooxygenase-activity (in the presence of adrenalin) was measured with increasing concentrations of test compounds and yielded a series of curves from which the concentrations causing a 50% inhibition (IC_{50}-values) were determined [21]. Apparent IC_{50}-values plotted against AA concentration revealed essentially three types of dependency of IC_{50}-values on the AA concentration (see below, Fig. 5), an assay variation thus useful for detecting various types of PHS-inhibition [21, 22].

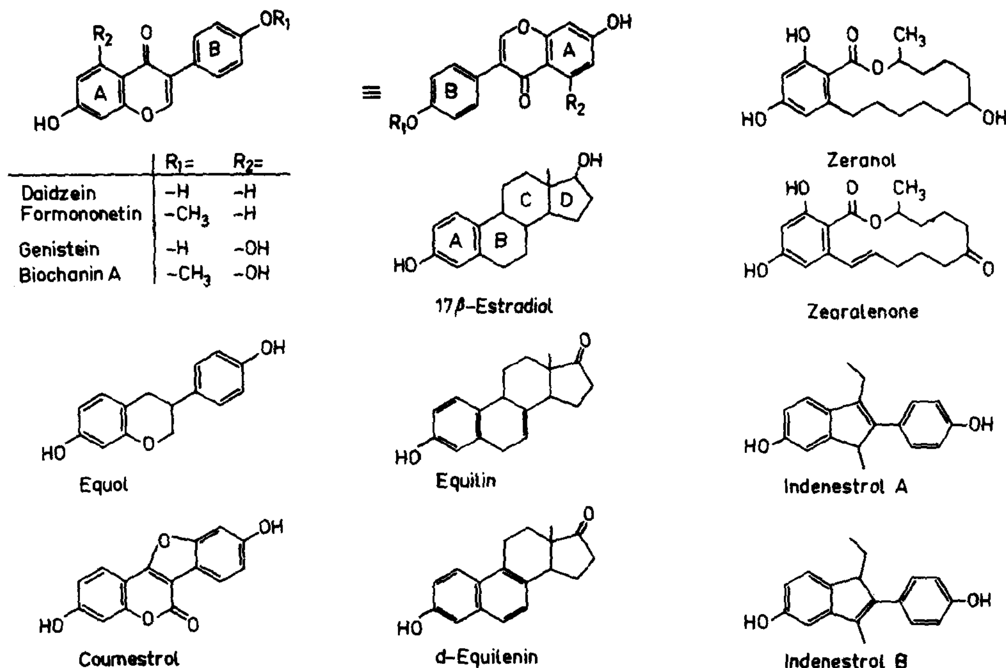

Fig. 4. Structures of selected environmental estrogens; see text for further details with respect to their interaction with PHS. The basic isoflavone structure is rotated to emphasize the similarity with estradiol.

Inhibition of PHS-catalyzed oxidation of DES

The effect of test compounds on the cooxidation of DES was studied essentially as described previously for naphthoflavones [21]. Incubations were started by addition of either AA or hydrogenperoxide as cofactors [20, 21], then extracted and the extracts were analyzed by HPLC for DES and its metabolites [8]. Thus compounds which inhibit both AA-dependent and peroxide-dependent metabolism of DES can be distinguished from inhibitors of

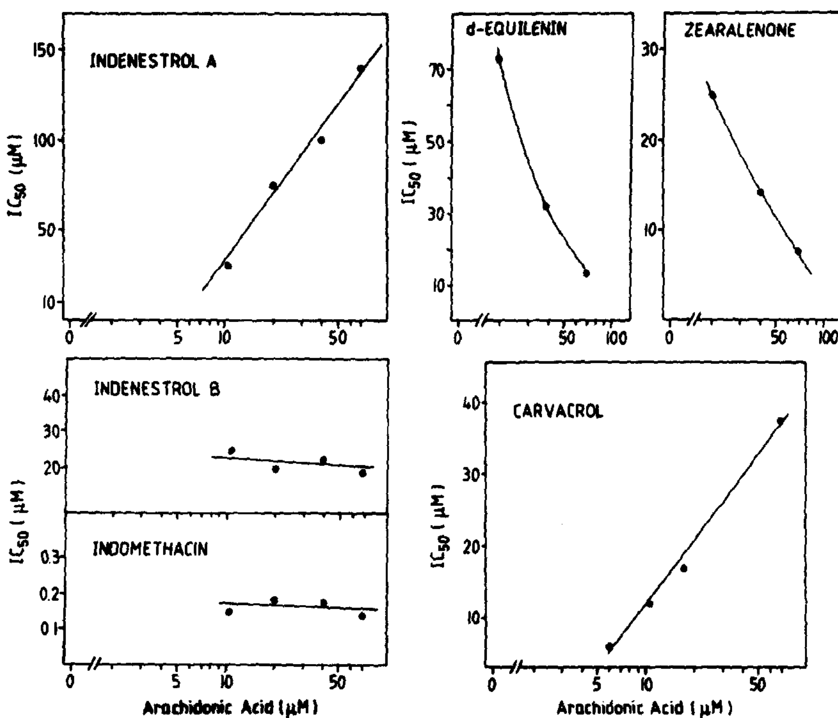

Fig. 5. Dependency of PHS-cyclooxygenase inhibition by various agents upon fatty acid assay (AA) concentration. IC_{50}-values for various inhibitors were derived from a series of dose–response curves measured as described in Fig. 3 and in the Experimental section.

Table 1. HPLC analysis of environmental estrogens

Compound	Detection (nm)	Retention time (min) with Gradient I	Gradient II	Gradient III
Daidzein	310	7.7	7.7	7.9
Equol	280	8.7	8.6	9.0
Genistein	262	10.2	10.1	10.7
Coumestrol	343	13.1	13.0	14.2
Formononetin	254	13.8	13.7	15.2
Biochanin A	254	17.7	17.5	20.1
d-Equilenin	280	15.0	—	16.6
Equilin	280	15.3	—	17.5
Zeranol	265	15.4	—	17.2
Zearalenone	236	17.3	—	19.8

Compounds were chromatographed on a RP_{18} column (Dupont Zorbax Sil ODS 4.6 × 250 mm) eluted at a flow rate of 1 ml/min at room temparature with either gradient I: 30 min linear 45–100/ B in A or with gradient II: same as I but solvents contain 0.1% acetic acid to improve peak shape; or separated with gradient III: 30 min linear 45–85% B in A; solvent A: 20% methanol in water; B: 100% methanol. Estrogens were detected at the given wavelength and quantitated in incubation extracts as described in the Experimental section and in Ref. [20].

PHS-cyclooxygenase which do not affect PHS-peroxidase mediated DES-oxidation.

PHS-catalyzed metabolism of estrogenic compounds

Cooxidation of estrogenic compounds was measured in incubations with PHS from ram seminal vesicle microsomes supplemented with AA as described previously [20]. Extracts were analyzed by HPLC (see below) and conversion was estimated by comparing the amount of recovered parent compound in incubations with PHS and AA (complete) to that found in the presence of inhibitor indomethacin, and in controls with heat-inactivated enzyme or in incubations lacking either AA or enzyme.

HPLC-analysis of environmental estrogens in incubation extracts

Compounds were analyzed on a HPLC-unit capable of gradient elution (KONTRON) using a reverse phase (RP_{18})-column (Dupont Zorbax Sil ODS; 4.6 × 250 mm) eluted with a linear methanol–water gradient and quantitation of the u.v.-signal by comparison with calibration curves of the different estrogens essentially as described for DES derivatives [9]. Details on separation and detection of the different environmental estrogens together with their retention times are provided in Table 1.

In essence, the HPLC analysis is used to determine the phytoestrogen decrease upon incubation with PHS and AA, and thus allows to measure conversion of the parent compound, also in the absence of specific metabolites (which could be non-extractable or escape u.v.-detection at a particular wavelength).

RESULTS AND DISCUSSION

Equine estrogens

Equilin is a component of conjugated estrogen preparations widely used in estrogen replacement therapy; it can be metabolized to d-equilenin in humans [23]. The effect of these two compounds on PHS-cyclooxygenase activity is shown in Fig. 1: a concentration-dependent stimulation of PHS was found with equilin which is partly reversed at higher concentrations. Similar dose–response curves have been observed with other phenolic estrogens [9] or with phenidone [24], compounds which are cooxidized by PHS. Stimulation of PHS by equilin was also found in the presence of other (potential endogenous) cosubstrates of PHS such as adrenalin (Fig. 2). In contrast, d-equilenin inhibited PHS under these conditions (Fig. 2: IC_{50} 13 μM) and hardly affected the basal enzyme activity (Fig. 1).

As expected, equilin was found to be efficiently metabolized by PHS *in vitro* (Table 2); its conversion was inhibited by indomethacin, and clearly depended upon both AA and microsomal native enzyme. By comparison, d-equilenin is poorly metabolized, its conversion being only partially inhibited by indomethacin and only slightly AA-dependent (Table 2). Apparently, equilin is co-oxidized in a manner similar to that of steroidal and stilbene estrogens [14, 9] whereas further desaturation of ring B in d-equilenin makes it an inhibitor rather than a reducing cosubstrate for PHS. That the PHS-catalyzed metabolic activation of these two compounds in target tissues has relevance for a carcinogenic effect *in vivo* is suggested by the observation that equilin, but not d-equilenin, was found to induce tumors in

Table 2. PHS-catalyzed conversion of equine estrogens

Incubation[a]	μg parent compound	% Recovery
Equilin complete	11.8 ± 1.1	37.0
with indo	22.0 ± 0.5	69.0
minus AA	29.2 ± 2.1	91.5
minus enzyme	31.9 ± 0.7	100.0
boiled enzyme	29.9 ± 0.9	93.7
d-Equilenin complete	22.0 ± 0.6	75.4
with indo	24.5 ± 0.3	84.2
minus AA	24.6 ± 0.5	84.4
minus enzyme	29.2 ± 1.4	100.0
boiled enzyme	28.7 ± 0.5	98.4

[a] Equilin or d-equilenin (60 μM) were incubated with RSVM (0.5 mg/ml) and AA (100 μM) (complete incubations) or in the presence of PHS-inhibitor indomethacin (70 μM) in phosphate buffer (0.05 M, pH 7.5) for 5 min at 37°C. These incubations ($n = 3$) and controls without AA or enzyme or with heat-inactivated enzyme were extracted and analyzed by HPLC as described in the Experimental section.

the hamster kidney [25]. Renal PHS-activity has been found to activate certain nephrocarcinogens in other species [26]; its role in mediating the carcinogenic effect of estrogens in hamster kidney is unclear at present, but clearly deserves further investigation.

Phytoestrogens

Genistein, daidzein, equol and coumestrol stimulated AA-dependent oxygen consumption (PHS-cyclooxygenase activity) in a dose-dependent manner [20] indicating that they may be co-substrates for PHS-peroxidase. The stimulation of PHS-cyclooxygenase activity was partially reversed at high concentrations, an effect also observed for other compounds cooxidized by PHS [9, 24]. This latter effect could be due to their radical scavenging properties decreasing the "peroxide tone" necessary for initiating the PHS reaction or to a direct effect on the enzyme [27].

Phytoestrogens which increased cyclooxygenase activity *in vitro* were metabolized by PHS via co-oxidation (measured independently): conversion of the compounds studied by HPLC-analysis was enzyme- and AA-dependent and was inhibited by indomethacin [20]. Thus, equol, daidzein, coumestrol, and genistein are apparently co-oxidized by PHS, and it should be of interest to study whether this results in the formation of reactive intermediates.

In contrast, the monomethyl ethers of daidzein and genistein, formononetin and biochanin A, had little or weakly inhibitory (IC_{50} 60 μM) effect on cyclooxygenase activity, and show little or no PHS-catalyzed conversion [20]. Thus, PHS appears to distinguish in the co-oxidation reaction between phenolic groups in the A- and B-rings of phytoestrogens with isoflavone structure: PHS apparently oxidizes preferentially the hydroxyl group of the isoflavone ring B which is thought to be equivalent to ring A of steroidal estrogens (see Fig. 4) and seems to have a lower redox potential [28]. The second hydroxyl group in ring A of genistein and biochanin A may form intramolecular hydrogen bonds to the adjacent keto group thereby decreasing the polarity of these compounds as observed in HPLC (elution order in Table 1; and [28]).

Resorcylic acid lactones

Zearalenone and zeranol, on the other hand, were surprisingly good inhibitors of PHS-cyclooxygenase (IC_{50} 3.5 μM) compared to indomethacin (IC_{50} 0.1 μM under identical assay conditions). Conversion of these compounds, if any, was minor and only partially inhibited by indomethacin or lack of AA as studied by HPLC-analysis [20]. Furthermore, zeranol inhibited both the AA- and the hydrogen peroxide-dependent oxidation of DES to Z, Z-DIES (IC_{50} 3.5 μM) by PHS [7] with a potency similar to that of indomethacin which, however, inhibited only the cyclooxygenase (AA)- dependent cooxidation of DES (IC_{50} 7 μM) whereas zeranol affected also the PHS-peroxidase. Moreover, a difference in the type of PHS-inhibition also becomes apparent when the dependency of IC_{50}-values on the fatty acid (AA)-concentration is studied: indomethacin displays no change, but zearalenone is less potent at lower AA-levels (Fig. 5). Whether these mycoestrogens may interfere with co-oxidation of DES or with PHS-catalyzed metabolism of endogenous estrogens *in vivo* remains presently a matter of speculation.

DES-Indanylderivatives

Indenestrol A (IA), a metabolite of DES found *in vivo*, and its synthetic isomer indenestrol B (IB) exerted opposite effects on PHS. IA is extensively metabolized by PHS as is evident from HPLC-analysis of incubation extracts, IA-cooxidation yields a reactive quinone and is accompanied by increased cyclooxygenase activity (half-maximal stimulation at 30 μM). At rather high concentrations of IA this effect is reversed, and inhibition of PHS (IC_{50} 190 μM) is observed [29]. With lower fatty acid concentrations in the assay the PHS-inhibition by IA is more pronounced; thus dependency of IC_{50}-values upon AA-levels is similar to that observed for antioxidant-type inhibitors of higher potency such as carvacrol (Fig. 5).

IB on the other hand inhibits PHS-cyclooxygenase under a range of test-conditions, but, unlike other oxidizable compounds, not by an antioxidant type of inhibition (Fig. 5). Like indomethacin, IB inhibits only the AA-dependent but not the peroxide-mediated oxidation of DES, indicating that it affects the cyclooxygenase but not the peroxidase activity. (Incomplete recovery of IB from incubations with native PHS is not AA-dependent nor inhibited by indomethacin and is probably due to affinity of IB for the enzyme rather than to metabolism [29].)

Conclusions

As we have illustrated with several examples, stimulation or inhibition of PHS or a dual role has been demonstrated for naturally occurring and synthetic estrogens when studying their *in vitro* interaction with the enzyme. A combination of assays and conditions had been found useful for a more differentiated picture of the effects which these environmental estrogens can exert on PHS *in vitro*.

Several points have to be considered when attempting to categorize these compounds as stimulators or inhibitors of PHS. Compounds which are co-substrates of PHS (such as) e.g. equilin, equol, and IA will increase enzyme activity while they are co-oxidized. As apparent from the dose–response curve for equilin for example (Figs 2 and 3A), this (stimulatory) effect can be extrapolated to low concentrations, and in analogy with DES or estradiol, co-oxidation of reducing co-substrate is expected to result in a stoichiometric production of prostaglandin precursor PGH_2 [11]. On the other hand, as described for phenol [27] stimulation of PHS becomes less

at successively higher concentrations of agent eventually giving way to inhibition. A similar characteristic probably applies to equol which has been previously reported to inhibit prostaglandin synthesis *in vitro* (cited in [2]) whereas we observed stimulation of PHS-activity by equol [20]. These observations are thus not necessarily discrepant, yet emphasize the need for a cautious classification of agents.

In general, effects observed at low concentrations of estrogen seem to be more pertinent for the *in vivo* situation although the bioavailability and binding to plasma proteins in different species can vary considerably for the investigated compounds [3, 6]. Moreover, an ingested compound and its metabolite may exert different effects upon PHS: at a concentration where biochanin A is inhibitory (IC_{50} 60 μM) genistein maximally stimulates the enzyme. Other examples of structurally related agents with opposite effects are the equine estrogens and indanyl derivatives of DES; they may provide useful tools for further studies on the interaction of estrogenic compounds with PHS.

Although a discussion on the mechanisms of PHS-inhibition is beyond the scope of this article, another aspect should be mentioned. Microsomal cyclooxygenase activity is commonly measured at AA-concentrations of 100 μM, yet cellular levels of free AA are 20 μM or lower [30], a factor clearly of relevance when judging the inhibitory potential of environmental estrogens (Fig. 5).

In summary, stimulation and inhibition of PHS by environmental estrogens has been studied *in vitro* together with structural requirements and factors pertinent for extrapolating the observed effects to the *in vivo* situation. In estrogen target tissues which contain PHS and in which estrogens can achieve fairly high concentrations these compounds may modulate prostaglandin biosynthesis by affecting PHS and other prostaglandin-synthesizing enzymes. This and the PHS-catalyzed formation of reactive intermediates from certain estrogenic compounds are two aspects which in our opinion deserve future investigation concerning their possible role for the (reproductive) toxicity of environmental estrogens.

Acknowledgements—The skilful technical assistance of S. Dekant, H. Raabe and K. Herberich-Ott is gratefully acknowledged. We thank Dr Ann Dunlop for editorial help and the Deutsche Forschungsgemeinschaft for financial support.

REFERENCES

1. McLachlan J. A., Korach K. S., Newbold R. R. and Degen G. H.: Diethylstilbestrol and other estrogens in the environment. *Fund. Appl. Toxic.* **4** (1984) 686–691.
2. Setchell K. D. R.: Naturally occurring non-steroidal estrogens of dietary origin. In *Estrogens in the Environment II* (Edited by J. A. McLachlan). Elsevier, New York (1985) pp. 69–85.
3. Stob M.: Naturally occurring food toxicants: estrogens. In *CRC Handbook of Naturally Occurring Food Toxicants* (Edited by M. Recheige). CRC Press, New York (1983) pp. 81–100.
4. McLachlan J. A. and Newbold R. R.: Estrogens and development. *Environm. Hlth Perspect.* **75** (1987) 25–27.
5. Adlercreutz, H.: Does fiber-rich food containing animal lignan precursors protect against both colon and breast cancer? An extension of the "fiber hypothesis". *Gastroenterology* **86** (1984) 761–766.
6. Adlercreutz H., Höckerstedt K., Bannwart C., Bloigo S., Hämäläinen E., Fotsis T. and Ollus A.: Effect of dietary components, including lignans and phytoestrogens, on enterohepatic circulation and liver metabolism of estrogens and on sex hormone binding globulin (SHBG). *J. Steroid Biochem.* **27** (1987) 1135–1144.
7. Marnett L. J. and Eling T. E.: Cooxidation during prostaglandin biosynthesis: a possible pathway for the metabolic activation of xenobiotics. *Rev. Biochem. Toxic.* **5** (1983) 135–172.
8. Degen G. H., Eling T. E. and McLachlan J. A.: Oxidative metabolism of diethylstilbestrol by prostaglandin synthetase. *Cancer Res.* **42** (1982) 919–923.
9. Degen G. H., Metzler M. and Sivarajah K.: Co-oxidation of diethylstilbestrol and structural analogs by prostaglandin synthase. *Carcinogenesis* **7** (1986) 137–142.
10. Degen G. H., McLachlan J. A., Eling T. E. and Sivarajah K.: Co-oxidation of steroidal and non-steroidal estrogens by purified prostaglandin synthase results in a stimulation of prostaglandin formation. *J. Steroid Biochem.* **26** (1987) 679–685.
11. Freyberger A. and Degen G. H.: Studies on the stoichiometry of estrogen oxidation catalyzed by purified prostaglandin-H-synthase holoenzyme. *J. Steroid Biochem.* **33** (1989) 473–481.
12. Ramwell P. W., Leovey E. M. K. and Sintetos A. L.: Regulation of the arachidonic acid cascade. *Biol. Reprod.* **16** (1977) 70–87.
13. Ham E., Cirillo V. J., Zanetti M. E. and Kuehl F. A.: Estrogen directed synthesis of specific prostaglandins in uterus. *Proc. Natn. Acad. Sci. U.S.A.* **72** (1975) 1420–1424.
14. Degen G. H.: Prostaglandin synthase catalyzed metabolism of synthetic and steroidal estrogens. In *Drug Metabolism: Molecular Approaches and Pharmacological Implications* (Edited by G. Siest). Pergamon Press, Oxford (1985) pp. 223–228.
15. Degen G. H., Freyberger A., Metzler M. and Schlatterbeck M.: Prostaglandin H synthase catalyzed metabolic activation of synthetic and natural estrogens. In *Primary Changes and Control Factors in Carcinogenesis* (Edited by T. Friedberg and F. Oesch). Deutscher Fachschriften-Verlag, Wiesbaden (1986) pp. 30–34.
16. Karmali R. A., Welt S., Thaler H. T. and Lefevre F.: Prostaglandins and breast cancer: relationship to disease stage and hormone status. *Br. J. Cancer* **48** (1983) 689–696.
17. Gilbert J., Michel J.-F., Précigoux G., Hospital M., Raynaud J.-P., Michel F. and Crastes de Paulet A.: inhibition of prostaglandin synthetase by di- and triphenylethylene derivatives: a structure-activity study. *J. Med. Chem.* **26** (1983) 693–699.
18. Schatz F., Markiewicz L., Barg P. and Gurpide E.: *In vitro* inhibition with antiestrogens of estradiol effects on $prostaglandin_{2\alpha}$, production by human endometrium and endometrial epithelial cells. *Endocrinology* **118** (1986) 408–412.
19. Degen G. H., Jellinck P. H. and Hershcopf R. J.: Prostaglandin H synthase catalyzes regiospecific release of tritium from labeled estradiol. *Steroids* **49** (1987) 561–580.
20. Degen G. H.: *In vitro* studies on the effect of different phytoestrogens on prostaglandin H synthase. *Proceedings of Eur. Food Tox. II* (Edited by Inst. Toxic., Swiss Fed. Inst. Food Technology) University of Zürich, Switzerland (1986) pp. 85–91.

21. Degen G. H.: Inhibition of prostaglandin H synthase catalyzed cooxidation of diethylstilbestrol by α-naphthoflavone and β-naphthoflavone. *J. Biochem. Toxic.* **3** (1988) 1–10.
22. Egan, R. W. and Gale, P. H.: Prostaglandin synthesis by intact and microsomal enzyme: control by antioxidants. *6th International Conference on Prostaglandins and Related Compounds*, Florence, Italy (1986) Abstr. p. 364.
23. Bhavnani B. R. and Woolever C. A.: The metabolism of equilin in normal men. *J. Steroid Biochem.* **17** (1982) 217–223.
24. Marnett L. J., Siedlik P. H. and Fung L. W. M.: Oxidation of phenidone and BW755C by prostaglandin endoperoxide synthetase. *J. Biol. Chem.* **257** (1982) 6957–6964.
25. Li J. J. and Li S. A.: Estrogen-induced tumorigenesis in hamsters: roles for hormonal and carcinogenic activities. *Arch. Toxic.* **55** (1984) 110–118.
26. Rice J. R., Zenser T. V. and Davis B. B.: Prostaglandin synthase-dependent cooxidation and aromatic amine carcinogenesis. In *Arachidonic Acid Metabolism and Tumor Initiation* (Edited by Marnett L. J.). Martinus Nijhoff, Boston (1985) pp. 125–169.
27. Egan R. W., Gale P. H., Beveridge G. C., Marnett L. J. and Kuehl F. A.: Direct and indirect involvement of radical scavengers during prostaglandin biosynthesis. In *Advances in Prostaglandin & Thromboxane Research* (Edited by Samuelsson B., Ramwell P. W. and Paoletti R.). Raven Press, New York, Vol. 6 (1980) pp. 153–155.
28. Setchell K. D. R., Welsh M. B. and Lim C. K.: High performance liquid chromatographic analysis of phytoestrogens in soy protein preparations with ultraviolet, electrochemical and thermospray mass spectrometric detection. *J. Chromat.* **386** (1987) 315–323.
29. Degen G. H. and Metzler M.: Indanyl analogs of diethylstilbestrol: differential interaction with prostaglandin H synthase. *Carcinogenesis* **10** (1989) 839–845.
30. Marshall P. J., Kulmacz R. J. and Lands W. E. M.: Constraints on prostaglandin biosynthesis in tissues. *J. Biol. Chem.* **262** (1987) 3510–3517.