Biochemical Pharmacology, Vol. 31, No. 9, pp. 1717–1721, 1982.
Printed in Great Britain.

0006-2952/82/091717-05 $03.00/0

THE EFFECT OF *TRANS*-STILBENE OXIDE AND OTHER STRUCTURALLY RELATED INDUCERS OF DRUG-METABOLIZING ENZYMES ON THE PENTOSE PHOSPHATE PATHWAY AND OTHER ENZYMES OF CARBOHYDRATE METABOLISM

JANERIC SEIDEGÅRD and JOSEPH W. DEPIERRE
Department of Biochemistry, Arrhenius Laboratory, University of Stockholm, S-106 91 Stockholm, Sweden

(*Received* 21 *September* 1981; *accepted* 10 *December* 1981)

Abstract—*trans*-Stilbene oxide has been found earlier to be a new type of inducer of drug-metabolizing systems. Here we demonstrate that treatment of rats with this xenobiotic results in an increase in the activity of the cytosolic glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase, the first and third enzymes in the pentose phosphate pathway, to 350% and 170% of the control values, respectively. At the same time microsomal glucose 6-phosphate dehydrogenase activity was unaffected by administration of *trans*-stilbene oxide or benzil. The time course and dose–response of the increases in glucose 6-phosphate and 6-phosphogluconate dehydrogenase activities have been characterized. The activities of ribulose 5-phosphate 3-epimerase and ribose 5-phosphate ketol isomerase, enzymes further along in the pentose phosphate pathway, were not significantly affected by *trans*-stilbene oxide or benzil. An investigation of the effect of treating rats with different metabolites of stilbene and with other structurally related compounds on hepatic cytosolic glucose 6-phosphate dehydrogenase activity revealed the structural features which are important for increasing this activity. Finally, it was found that administration of *trans*-stilbene oxide did not affect the activities of glucokinase and phosphoglucose isomerase, the two glycolytic enzymes which can produce glucose 6-phosphate, the link between glycolysis and the pentose phosphate shunt.

It has been established in our laboratory [1] and in others [2, 3] that *trans*-stilbene oxide induces drug-metabolizing enzymes in rat liver with a pattern unlike that seen after administration of phenobarbital or 3-methylcholanthrene. Treatment with *trans*-stilbene oxide increase the specific activity of microsomal epoxide hydrolase by 620%, the specific content of cytochrome P-450 by 120%, and the specific activities of cytosolic glutathione *S*-transferases by 200–300% [1]. Recently, we have also characterized the induction of drug-metabolizing enzymes and related systems by other metabolites and structural analogues of stilbene [4]. Our results suggest that so-called phase II enzymes (epoxide hydrolase and glutathione *S*-transferase(s) are induced to a much greater extent by such compounds than are the activities of the phase I cytochrome P-450 system.

It is also of interest to determine the effects of inducers of drug-metabolizing systems on enzyme pathways which are only indirectly involved in the metabolism of xenobiotics. The pentose phosphate shunt provides the cofactor NADPH required by the cytochrome P-450 system. We have measured the activities of glucose 6-phosphate dehydrogenase, the first enzyme in the shunt, together with 6-phosphogluconate dehydrogenase, the third enzyme, both of which provide NADPH, after administration of *trans*-stilbene oxide and other stilbene derivatives. The effect of these treatments on the subsequent enzymes 5-phosphate 3-epimerase and ribose 5-phosphate ketol isomerase has also been investigated.

In addition, since glucose 6-phosphate is the first substrate in both the pentose phosphate shunt and in the metabolic sequence which leads to the synthesis of UDP-glucuronic acid, it was of interest to determine whether the production of glucose 6-phosphate is affected by inducers of drug-metabolizing enzymes. Consequently, the effect of induction by *trans*-stilbene oxide and other stilbene derivatives on the three glycolytic enzymes which can give rise to glucose 6-phosphate, i.e. glucokinase (hexokinase IV), hexokinase and phosphoglucose isomerase, has also been studied here.

MATERIALS AND METHODS

Chemicals. Dibenzyl, 4,4′-dihydroxydiphenyl and 4,4′-dihydroxystilbene (ICN Pharmaceuticals Inc., Life Sciences Group, Plainview, NY); *cis*- and *trans*-stilbene oxide, desoxybenzoin, dibenzoylmethane, chalcone, 2,2′-pyridil, metyrapone, phenanthrene and diacetyl (EGA-Chemie, Steinheim/Albruch, West Germany); styrene oxide, benzoin and benzil (Fluka AG, Buchs S. G., Switzerland): $NADP^+$, ATP, ribose 5-phosphate ketol isomerase, glucose 6-phosphate dehydrogenase, fructose 6-phosphate, glucose 6-phosphate, ribose 5-phosphate, 6-phosphogluconate, D-glucose and phloridzin (Sigma Chemical Co., St. Louis, MO); biphenyl, diphenylmethane, *meso*-1,2-diphenylethane-1,2-diol and 2,3-epoxybutane (Merk-Schuchardt, Munich, West Germany); benzoic acid (Merck, Darmstadt, West Germany) and 3,3′-pyridil (Chem. Service,

West Chester, PA) were all purchased from commercial sources. *cis*-Stilbene oxide and *threo*-1,2-diphenylethane-1,2-diol were synthesized by the Synthesis Service, Chemical Center, Lund, Sweden. Chalcone epoxide was synthesized according to the method of Yang and Finnegan [5], recrystallized, and its structure confirmed by NMR and by its melting point. All other chemicals were of reagent grade and obtained from common commercial sources.

Animals. Male Sprague–Dawley rats weighing 180–200 g were starved overnight in order to reduce liver glycogen before decapitation. Since intraperitoneal injection of certain of the substances reduced the animal's appetite, treated animals were pair-fed with controls. Liver microsomes were prepared according to Ernster *et al.* [6] and the supernatant fraction taken as the cytosol.

Induction. For *trans*-stilbene oxide and benzil 2 mmoles/kg body wt was administered once daily for 5 days. In one experiment all derivates were administered at a dose of 1 mmole/kg body wt. Routinely, the substance was dissolved in 1 ml sunflower oil and injected i.p. into the rats. However, benzoin, *erythro*-1,2-diphenylethane-1,2-diol, and *threo*-1,2-diphenylethane-1,2-diol gave suspensions when mixed with sunflower oil. The inductions obtained with these suspensions were no different than those seen after injection of these three substances dissolved in 20% dimethylsulfoxide–30% polyethyleneglycol-A300–50% sunflower oil. In no case did the vehicle alone have any effect on the enzymes measured.

With most of the substances tested at these doses no toxic effects were seen. However, animals receiving *trans*-stilbene, benzoin, 4,4′-dihydroxystilbene and 4,4′-dihydroxydiphenyl showed a loss of appetite and in the case of *trans*-stilbene a listlessness as well.

Enzyme assays. Glucose 6-phosphate dehydrogenase was assayed in the cytosol according to Taketa and Watanabe [7] and in microsomes after treatment with 1% Triton X-100 according to Takahashi *et al.* [8]. 6-Phosphogluconate dehydrogenase was assayed according to Glock *et al.* [9]. Ribulose 5-phosphate 3-emiperase and ribose-5-phosphate ketol isomerase were assayed by monitoring the formation of ketopentose phosphate at 290 nm at 37° [10]. Glucokinase, hexokinase and phosphoglucose isomerase were measured using coupled reactions in which the reduction of $NADP^+$ was followed spectrophotometrically [11, 12].

Protein. Protein was measured using a modification of the Lowry procedure [13] with bovine serum albumin as standard.

Statistics. In all tables the mean ± standard deviation is given. Statistical differences between means of control and treated animals were determined using Student's *t*-test.

RESULTS

Induction of glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase

Figure 1 illustrates the time course of induction of glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase by *trans*-stilbene oxide. It can be seen that glucose 6-phosphate dehydrogenase rapidly increases to its maximal level of 3.7 times that of control after 3 days of treatment, while 6-phosphogluconate dehydrogenase activity also increases within the first 3 days to 70% above the control value. The time course of induction of the drug metabolizing enzyme glutathione *S*-transferase(s) by *trans*-stilbene oxide is similar to these time courses [1].

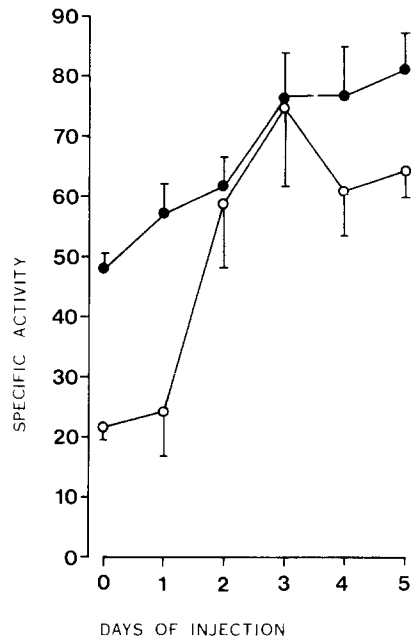

Fig. 1. Time course of the changes in glucose 6-phosphate dehydrogenase (○) and 6-phosphogluconate dehydrogenase (●) activities caused by treatment with *trans*-stilbene oxide (400 mg/kg). For details of the induction procedure and enzyme assays, see Materials and Methods. Specific activity = nmole NADPH produced/min/mg cytostolic protein. The means ± S.D. of 6 to 16 different animals are shown.

Figure 2 illustrates the return of glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase activities to control values after cessation of treatment with *trans*-stilbene oxide. Neither activity began to decrease until 2 days after the final injection, which was also the case after induction of cytosolic glutathione *S*-transferase(s), microsomal epoxide hydrolase and cytochrome P-450. This lag may be a depot effect, i.e. the large amount of sunflower oil used as a carrier for *trans*-stilbene oxide (1 ml per animal) during the course of several days. Both glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase returned essentially to control levels within 6 days after the final injection of *trans*-stilbene oxide.

Figure 3 illustrates the induction of glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase by different doses of *trans*-stilbene oxide. It appears that even higher levels of both activities might be obtained by administering more than 400 mg/kg. However, higher doses were not attempted for fear of toxic affects. This type of continually increasing dose–response curve was also seen for microsomal epoxide hydrolase and gluta-

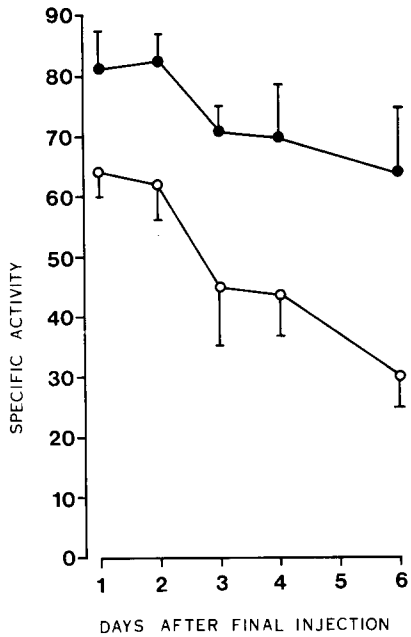

Fig. 2. Time course of return of glucose 6-phosphate dehydrogenase (○) and 6-phosphogluconate dehydrogenase (●) activities to control levels after cessation of treatment with *trans*-stilbene oxide. See legend to Fig. 1.

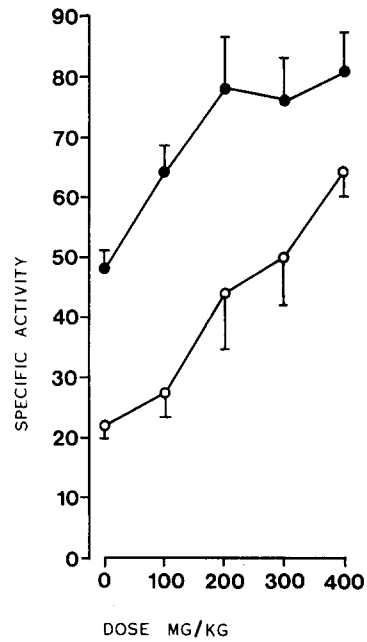

Fig. 3. Response of glucose 6-phosphate dehydrogenase (○) and 6-phosphogluconate dehydrogenase (●) activities to different doses of *trans*-stilbene oxide. See legend to Fig. 1. 400 mg *trans*-Stilbene oxide (mol. wt = 196) corresponds to 2 mmoles. The animals were injected once daily for 5 days.

thione *S*-transferase, but not for cytochrome P-450, which is induced maximally by 100 mg *trans*-stilbene oxide/kg × body wt [1].

It has been suggested that hexose 6-phosphate dehydrogenase or type III glucose 6-phosphate dehydrogenase in microsomes from rat liver might be involved in drug metabolism [8]. In Table 1 it can be seen that this microsomal activity is not affected to any great extent either by *trans*-stilbene oxide or benzil.

Induction of glucose 6-phosphate dehydrogenase by other metabolites and analogues of stilbene

In Table 2 are shown glucose 6-phosphate dehydrogenase activities after rats have been treated with various metabolites and structural analogues of stilbene. In this experiment the animals were treated with 1 mmole of substance/kg body wt in order to avoid the toxic effects seen for certain derivates at twice that dose. The first half of this table presents stilbene metabolites and it can be seen that benzoin and benzil both enhance glucose 6-phosphate dehydrogenase to the same level as *trans*-stilbene oxide, while both diols and *cis*-stilbene oxide increase this activity half as much.

Among the other stilbene derivatives, only dibenzoylmethane enhances glucose 6-phosphate dehydrogenase activity to the same level as does *trans*-stilbene oxide. Half as much increase occurs with desoxybenzoin, 2,2′-pyridil and metyrapone. The pattern seen in this table for glucose 6-phosphate dehydrogenase is not unlike that seen for the phase II enzymes, microsomal epoxide hydrolase and cytosolic glutathione *S*-transferase, although these two enzymes are induced to a greater extent [1].

Table 1. Comparison of microsomal and cytoplasmic glucose 6-phosphate dehydrogenase activities*

Glucose 6-phosphate dehydrogenase	None	Inducer *trans*-Stilbene oxide	Benzil
Microsomal†	11.4 ± 0.3	10.9 ± 0.3 (96)	13.5 + 0.5 (118)
Cytoplasmic	21.6 ± 1.7	64.4 ± 4.4 (298)	38.0 ± 5.1 (176)

* Two mmoles/kg body wt of each compound was injected i.p. into rats once daily for 5 days. Values given are the specific activity (nmoles NADPH formed/min per mg of cytoplasmic or microsomal protein) and are the means ± S.D. for 3 different animals. Values in parentheses are % of control.

† The microsomal enzyme has been designated as hexose 6-phosphate dehydrogenase type III glucose 6-phosphate dehydrogenase.

Table 2. The effect of administering stilbene; compounds related to stilbene and its metabolites on glucose 6-phosphate dehydrogenase activity*

Metabolite	G6PDH† % of control	Derivative	G6PDH† % of control
trans-Stilbene	119 ± 20 (6)	Dibenzyl	94 ± 15 (6)
trans-Stilbene oxide	188 ± 35 (12)‖	4,4'-Dihydroxystilbene	125 ± 25 (3)
erythro-1,2-Diphenylethane-1,2-diol	161 ± 17 (5)‖	Desoxybenzoin	154 ± 24 (3)§
cis-Stilbene	122 ± 17 (6)	2,2'-Pyridil	152 ± 6 (3)§
cis-Stilbene oxide	158 ± 18 (6)‖	3,3'-Pyridil	110 ± 9 (3)
threo-1,2-Diphenylethane-1,2-diol	153 ± 16 (6)‖	Metyrapone	144 ± 26 (3)§
Benzoin	218 ± 31 (6)‖	Styrene oxide	86 ± 7 (3)
Benzil	214 ± 42 (6)‖	Epoxy butane	105 ± 8 (4)
Benzoic acid	79 ± 10 (3)	Diacethyl	109 ± 12 (4)
		Biphenyl	79 ± 11 (3)
		4,4'-Dihydroxydiphenyl	114 ± 14 (3)
		Diphenylmethane	99 ± 10 (3)
		Chalcone	105 ± 17 (3)
		Chalcone epoxide	136 ± 12 (3)‡
		Dibenzoylmethane	226 ± 35 (3)‖
		Phloridzin	115 ± 16 (3)
		Phenanthrene	139 ± 20 (3)‡

* One mmole/kg body wt of each compound was injected i.p. into rats once daily for 5 days. Values given are the means ± S.D. of the number of animals shown in parentheses.

† Glucose 6-phosphate dehydrogenase. The control activity was 21.6 ± 1.7 (34) nmoles $NADP^-$ reduced/min per mg cytoplasmic protein.

‡ $P < 0.05$; § $P < 0.01$; ‖ $P < 0.001$.

In order to control whether the substances injected might be present in the supernatant and directly activate glucose 6-phosphate dehydrogenase, the derivatives were added to control cytosol at concentrations of 0.1–2 mM, which is calculated to be the concentration range which would be reached in the cytoplasm of hepatocytes *in vivo* if no metabolism occurred. In general, no direct effect of any of these compounds on glucose 6-phosphate dehydrogenase activity could be seen.

Effect on other enzymes of carbohydrate metabolism

The effect of induction with *trans*-stilbene oxide and benzil on other enzymes of the pentose phosphate shunt and of other pathways of carbohydrate metabolism are shown in Table 3. As can be seen, glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase, the first and third enzymes of the shunt and those enzymes which produce NADPH are induced to a much greater extent than the other enzymes. Ribulose 5-phosphate 3-epimerase and ribose 5-phosphate ketol isomerase are largely unaffected; a slight decrease to 80% of the control value could be seen for the later enzyme. Hexokinase (not shown) and phosphoglucose isomerase, glycolytic enzymes which can produce glucose 6-phosphate, are also largely unaffected by induction with *trans*-stilbene oxide or benzil. Glucokinase (hexokinase IV) activity may be somewhat increased when *trans*-stilbene oxide is administered to the animal.

Table 3. The effect of administering *trans*-stilbene oxide or benzil on enzymes in the pentose phosphate pathway or glucolysis*

Enzyme†	Reaction‡	None	Inducer *trans*-Stilbene oxide	Benzil
Glucose 6-phosphate§ dehydrogenase	G-6-P → 6-PGl	21.6 ± 1.7	64.4 ± 4.4 (298)	38.0 ± 5.1 (176)
6-Phosphogluconate§ dehydrogenase	6-PG → Ru-5-P	48.1 ± 2.1	81.3 ± 6.1 (169)	68.8 ± 5.6 (143)
Ribulose-5-phosphate§ 3-epimerase	Ru-5-P → Xu-5-P	272 ± 11	278 ± 21 (102)	246 ± 38 (90)
Ribose-5-phosphate§ ketol isomerase	R-5-P → Ru-5-P	52.6 ± 3.7	44.4 ± 2.6 (84)	40.2 ± 1.8 (76)
Glucokinase	G → G-6-P	9.9 ± 1.0	12.2 ± 0.7 (123)	10.8 ± 0.4 (109)
Phosphoglucose isomerase	F-6-P → G-6-P	1018 ± 22	890 ± 74 (87)	832 ± 74 (82)

* Two mmoles/kg body wt of each compound was injected i.p. into rats once daily for 5 days. Values given are the specific activity (nmoles product formed/min per mg of cytoplasmic protein) and are the mean ± S.D. for three different animals. Values in parentheses are % of control.

† For enzyme assays see Materials and Methods.

‡ Abbreviations: G-6-P, glucose 6-phosphate; 6-PGl, 6-phosphogluconolactone; 6-PG, 6-phosphogluconate; Ru-5-P, ribulose 5-phosphate; Xu-5-P, xylulose 5-phosphate; R-5-P, ribose 5-phosphate; G, glucose; F-6-P, fructose 6-phosphate.

§ Enzymes in pentose phosphate shunt.

DISCUSSION

trans-Stilbene oxide as an inducer for drug-metabolizing systems has now been investigated by a number of different laboratories. This xenobiotic has been shown to induce microsomal epoxide hydrolase, cytosolic glutathione *S*-transferase(s), cytochrome P-450 [1–3], UDP-glucose dehydrogenase [14], UDP-glucuronyl transferase [15], DT-diaphorase [16] and also the first and the third enzymes of the pentose phosphate shunt. This increase in activity is probably important in providing cytosolic NADPH, the cofactor required by the cytochrome P-450 system.

The present study also shows that glucose 6-phosphate dehydrogenase is induced to a higher extent than the third enzyme 6-phosphogluconate dehydrogenase. Subsequent enzymes in the shunt, i.e. ribose 5-phosphate ketol isomerase and ribulose 5-phosphate epimerase, are largely unaffected. This suggests that it is the first enzyme in the shunt which is the primary regulatory point.

It is, of course, possible that the increase in glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase activities observed here do not reflect a true inducer (i.e. increased amounts of enzyme protein), but rather some other mechanism of activation. However, in those cases which have been carefully investigated—viz., cytochrome P-450, epoxide hydrolase [2,4], glutathione *S*-transferases A, B and C [17] and DT-diaphorase [16]—the increases in enzyme activity caused by administering *trans*-stilbene oxide were found to reflect increases in the amount of enzyme protein. It seems therefore likely that the process described here is also one of induction.

Acknowledgements—This study was supported by grants from the Swedish Cancer Society and the National Cancer Institute (Grant 1 RO ICA 26261-01), Bethesda, MD, U.S.A. J. Seidegård was supported by a fellowship from the Ekströms foundation.

REFERENCES

1. J. Seidegård, R. Morgenstern, J. W. DePierre and L. Ernster, *Biochim. biophys. Acta* **586**, 10 (1979).
2. H. Schmassmann and F. Oesch, *Molec. Pharmac.* **14**, 834 (1978).
3. H. Mukhtar, T. H. Elmamlouk and J. R. Bend, *Chem. Biol. Interact.* **22**, 125 (1978).
4. J. Seidegård, J. W. DePierre, R. Morgenstern, Å. Pilotti and L. Ernster, *Biochim. biophys Acta* **672**, 65 (1981).
5. N. C. Yang and R. A. Finnegan, *J. Am. chem. Soc.* **80**, 5845 (1958).
6. L. Ernster, P. Siekevitz and G. E. Palade, *J. Cell Biol.* **15**, 541 (1962).
7. K. Taketa and A. Watanabe, *Biochim. biophys. Acta.* **235**, 19 (1971).
8. T. Takahashi, K. Tanahashi, T. Kodama and S. H. Hori, *J. Fac. Sci., Ser. VII, Zool.* **22**, 12 (1979).
9. G. E. Glock and P. McLean, *Biochem. J.* **55**, 400 (1953).
10. T. Wood, *Analyt. Biochem.* **33**, 297 (1970).
11. C. Sharma, R. Manjeshwar and S. Weinhouse, *J. biol. Chem.* **238**, 3840 (1963).
12. E. A. Noltham, in *Methods in Enzymology*, (Eds. S. P. Colowick and N. O. Kaplan), Vol. 9, p. 557. Academic Press, New York (1966).
13. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
14. J. Seidegård, J. W. DePierre and L. Ernster, *Acta Chem. Scand.* **B34**, 382 (1980).
15. T. H. Elmamlouk and H. Mukhtar, *Biochem. Pharmac.* **28**, 539 (1979).
16. C. Lind, B. Höjeberg, J. Seidegård, J. W. DePierre and L. Ernster, *FEBS Lett.* **116**, 289 (1980).
17. C. Guthenberg, R. Morgenstern, J. W. DePierre and B. Mannervik, *Biochim. biophys. Acta* **631**, 1 (1980).