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RELATIONSHIP BETWEEN INDUCTIONS OF MONOOXYGENASE ACTIVITY AND γ-GLUTAMYLTRANSPEPTIDASE IN RAT HEPATOCYTE PRIMARY CULTURES

A. M. EDWARDS
Clinical Biochemistry, School of Medicine, Flinders University of South Australia, Bedford Park, S.A. 5042, Australia

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Abstract—The proposition that changes in activity of γ-glutamyltranspeptidase (GGT) in serum may provide a useful index of the extent of induction of liver drug-metabolizing enzymes by various drugs was examined by comparing control of GGT and monooxygenase activities in cultured hepatocytes. In rat hepatocyte monolayers maintained for up to 5 days the effects of xenobiotics and other factors on cellular GGT activity were compared with effects on a relatively broad measure of drug metabolism, the 7-ethoxycoumarin *O*-deethylase (ECD) activity of intact cells. A diverse group of drugs including phenobarbital and other barbiturates, diphenylhydantoin, glutethimide, aminopyrine and griseofulvin and the steroids dexamethasone and pregnenolone 16α-carbonitrile were shown to induce both GGT and ECD under comparable culture conditions. Inductions of both activities were potentiated by glucocorticoids and depressed (where tested) by dibutyryl cyclic AMP. Some other hormones or nutrients modulated the activities differently. The magnitude of GGT induction by different drugs did not correlate with relative ECD induction and for several drugs the concentration-dependence of the two effects was different. Interpretation is complicated by the possible contribution of multiple forms of cytochrome P-450 to ECD activity but it seems unlikely that drugs which induce both GGT and drug metabolism do so via a common regulatory mechanism. For such drugs changes in serum GGT could provide only a crude guide to likely changes in drug metabolism. Some compounds including polycyclic hydrocarbons and warfarin induced ECD but had no associated effect on GGT in hepatocytes.

Measurements of the enzyme γ-glutamyltranspeptidase (GGT)* in serum are used in assessing liver function and diagnosing hepatobiliary disease in man [1]. Patients found to have high serum GGT activity include those treated with phenobarbital (PB) [2, 3] or certain other drugs including other barbiturates [4], glutethimide [5], diphenylhydantoin [2], aminopyrine [6] and warfarin [4] which are all known to be inducers of the liver drug-metabolizing (monooxygenase) system [7, 8]. Some of these monooxygenase-inducers have been shown to increase GGT in the livers of experimental animals ([1, 9–11] and references therein) and in the case of PB, induction of GGT in hepatocytes has been demonstrated as a possible source of elevated GGT in serum [10, 11]. These observations led to the proposal that changes in serum GGT provide a convenient index of the extent of induction of liver drug-metabolizing enzymes in patients treated with inducing drugs [1, 12]. Such an index has potential value in adjusting drug dosages to likely rates of drug metabolism.

The first objective of this study was to determine whether a variety of monooxygenase inducers act, like PB [10, 11, 13], directly on hepatocytes to induce GGT. The phenomenon of enzyme induction in normal cells under investigation differs from the more complex process leading to high levels of GGT expression during liver carcinogenesis ([13] and references therein). The second objective was to clarify the extent to which regulation of GGT a plasma membrane enzyme [10], resembles that of the drug metabolizing system in the endoplasmic reticulum; in particular, to examine whether overlap in drug effects on GGT and monooxygenases is coincidental or reflects some common regulatory mechanism(s). Previous studies in this laboratory on regulation of monooxygenase [14] and GGT [13, 15] activities in rat hepatocyte primary culture provided the basis for comparing effects of xenobiotics and other factors on these two activities under fully-defined *in vitro* conditions.

Any attempt to compare GGT and monooxygenase regulation is complicated by the fact that multiple forms of cytochrome P-450, differing in substrate specificity, contribute to liver drug metabolism (reviewed in [7, 16]). Since these multiple gene products are known to be subject to varying controls [7, 16] it seems likely that any link with GGT regulation at a molecular level would be limited to a subset of cytochrome P-450 isozymes and inducers. Ethoxycoumarin deethylase (ECD) activity in intact cells, employed here as a measure of drug metabolism, is dependent on a number of P-450 isozymes and is increased by exposure to PB or other xenobiotics *in vivo* or *in vitro* ([14] and references

* Abbreviations used: dibutyryl cyclic AMP, N^6,O^2-dibutyryl adenosine-3′,5′-monophosphate; ECD, 7-ethoxycoumarin *O*-deethylase; GGT, γ-glutamyltranspeptidase (EC2.3.2.2.) PB, sodium phenobarbitone; PCN, pregnenolone 16α-carbonitrile.

therein). In comparing GGT and monooxygenase control, ECD has provided a convenient measure of broad changes in drug metabolism rather than of specific changes in individual components.

MATERIALS AND METHODS

Chemicals, enzymes and media. Glutethimide was a generous gift from Ciba-Geigy Australia Ltd. (Sydney, Australia); sodium phenobarbital was purchased from Prosana Laboratories (Sydney, Australia), amobarbital from Hamilton Laboratories (Adelaide, Australia); sodium barbital and barbituric acid from BDH chemicals (Poole, England) and all other biochemicals from Sigma Chemical Co. (St Louis, MO). Sources and formulations of media were as previously described [15]. Stock solutions of test chemicals in saline or dimethylsulphoxide were prepared fresh weekly. When added to cultures the final dimethylsulphoxide concentration was 0.1% or less.

Hepatocyte isolation and culture. Male Porton-strain Wistar rats (200–300 g) allowed free access to laboratory chow were used for all studies. Hepatocytes were isolated by a two-step collagenase perfusion procedure [17]. For studies on GGT about 7×10^6 freshly-isolated hepatocytes were added to collagen-coated 90 mm diameter culture dishes (Kayline, Adelaide, Australia) in modified Waymouth medium [15] with 3% fetal bovine serum (Flow Laboratories, North Ryde, Australia). For studies on ECD, 2.5×10^6 cells were plated onto 60 mm collagen-coated culture dishes (Disposable Products, Adelaide, Australia) in modified Waymouth medium plus 3% fetal bovine serum and 10 mM nicotinamide. After allowing 3 hr for cell attachment the medium was changed to a defined culture medium which unless otherwise specified, contained a modification of Waymouth medium MB 752/1 incorporating alanine, serine, asparagine, oleic and linoleic acids, insulin and antibiotics as defined in [15]. Thereafter cultures were maintained in most cases for 3 days (ECD experiments) or 5 days (GGT experiments) with daily medium changes. In most studies media were supplemented with dexamethasone which suppressed outgrowth of fibroblast-like cells [13]. In experiments where dexamethasone or other glucocorticoid was not present cultures were examined for fibroblast-like cells and discarded where significant outgrowth was observed.

Assays of GGT, ECD and protein. GGT was assayed colorimetrically in extracts of saline-washed hepatocytes as previously described [15]. A unit of GGT activity is defined as the amount catalysing formation of 1 μmole *p*-nitroaniline per min at 37°. ECD activity was measured by exposing monolayers of intact hepatocytes to ethoxycoumarin and measuring total hydroxycoumarin formation after enzymic hydrolysis of sulphate and glucuronide conjugates as previously described [14]. A unit of ECD activity is defined as the amount catalysing formation of 1 pmole hydroxycoumarin per min. Protein was assayed by the Lowry procedure [18].

The statistical significance of differences between sets of data was assessed using Student's *t*-test. In general the results shown in figures and tables were obtained with a single liver cell preparation. In every case, comparable results have been obtained in replicate experiments with independent cell preparations.

RESULTS

In the first part of this study the effects on GGT of the standard monooxygenase inducer, PB were examined in detail to allow comparison with previous findings on induction of ECD [14]. Figure 1 shows changes in GGT with time, in primary cultures of rat hepatocytes maintained in modified Waymouth medium supplemented with 30 nM dexamethasone. Under these conditions the initial low GGT activity of freshly isolated hepatocytes was preserved for 2–3 days but thereafter increased progressively with time. Maintenance of cultures with PB caused an earlier and more marked increase in GGT to levels 2- to 3-times those in control cultures after 5–6 days. If phenobarbital was omitted after 4 days GGT activity continued to increase for about 24 hr but not thereafter in contrast to cultures maintained with PB throughout (Fig. 1B). If cycloheximide was added to PB-treated cultures at a concentration sufficient to inhibit protein synthesis by 95% (A. M. Edwards, unpublished findings), further increase in GGT was largely prevented (Fig. 1B). These observations are consistent with the view that PB causes reversible enzyme induction requiring continuing protein synthesis. Since near-maximal induction by PB was observed after 5 days, this period of cell incubation was used for most studies on GGT below. The changes in ECD in hepatocyte culture in response to a variety of factors including induction by PB have previously been described [14]. For ECD studies below, the basic culture medium (with 30 nM dexamethasone and 10 mM nicotinamide) preserved ECD activity at or slightly above the activity of freshly-isolated cells and supported clear inductions

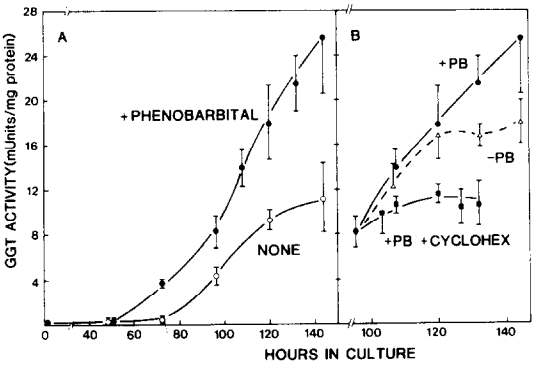

Fig. 1. Effect of PB on GGT activity in cultured hepatocytes. Panels A and B: hepatocytes were maintained in monolayer culture in modified Waymouth medium containing 30 nM dexamethasone with or without 2 mM PB. After various times, sets of dishes were harvested and assayed for GGT as in Materials and Methods. Panel B: from the group of dishes exposed to PB for 4 days, some dishes continued with PB (●); from some, PB was omitted (△); and some received PB plus cycloheximide, 20 μg/ml (■). Values and bars are means ± S.D. from 4 dishes.

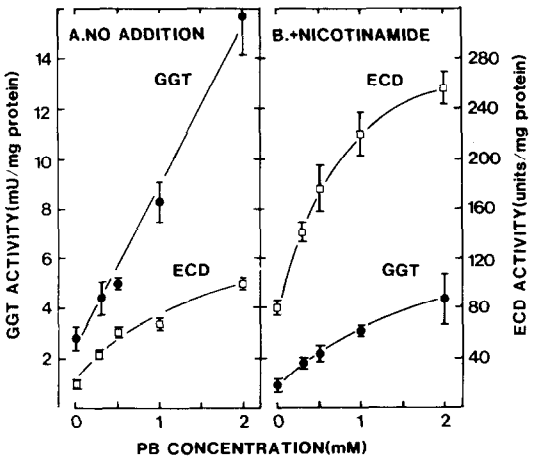

Fig. 2. Concentration-dependence of GGT and ECD inductions by PB in the presence and absence of nicotinamide. Hepatocytes were maintained in modified Waymouth medium with 30 nM dexamethasone and varying concentrations of PB without (panel A) or with 10 mM nicotinamide (panel B). ECD was assayed after 3 days in culture and GGT after 5 days. Values and bars are means ± S.D. from 3–4 dishes.

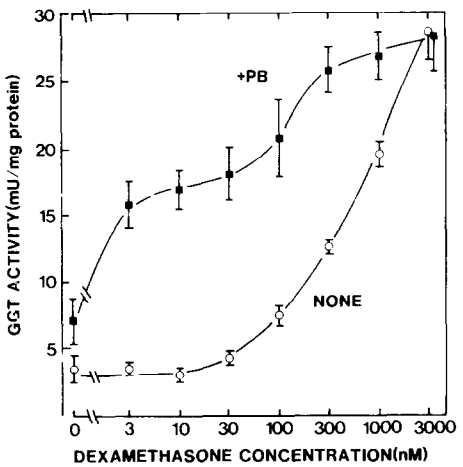

Fig. 3. Effect of dexamethasone concentration on GGT in the presence and absence of PB. Hepatocytes were maintained in modified Waymouth medium with or without 2 mM PB and with varying concentrations of dexamethasone. GGT was assayed after 5 days. Values and bars are means ± S.D. from 4 dishes.

by a variety of drugs [14]. Since drug effects were found to be near-maximal after 3 days [14] this period was used for most ECD studies below. In general, similar relative changes in ECD were observed if cells were assayed after 5 days rather than 3 days in culture.

Figure 2 shows the concentration-dependences of GGT and ECD inductions by PB and compares inductions by PB in the absence (panel A) and presence (panel B) of nicotinamide. Although nicotinamide had marked effects on the magnitude of inductions as discussed below, it had only minor effects on the shape of the concentration-dependence curves which were similar for ECD and GGT. For both parameters, inductions were maximal at 2 mM PB; higher concentrations gave lower activities (not shown).

Figure 3 shows the GGT activities of hepatocytes maintained for 5 days with or without PB and with varying concentrations of dexamethasone. Corresponding experiments for ECD were previously reported [14]. The steroid alone at levels up to 20–30 nM had little effect on GGT but at higher concentrations caused marked induction as previously reported [15]. Low, non-inducing dexamethasone concentrations (3–30 nM) markedly potentiated a small inducing effect of PB alone. At the optimal inducing concentration of dexamethase (3 μM) there was no additional effect of PB. As reported for ECD regulation [14], dexamethasone could be replaced by other glucocorticoids (hydrocortisone, corticosterone) but not by non-glucocorticoids (tetrahydrocortisol, fluoxymesterone) in potentiation of GGT induction (results not shown).

Figure 4 compares the effects of varying nicotinamide concentrations with or without PB, on GGT and ECD activities. Consistent with previous reports on ECD [14] and other monooxygenase activities [19, 20] addition of nicotinamide (up to 10 mM) to the culture medium favoured the preservation of ECD activity in control cultures and enhanced induction by PB. All nicotinamide concentrations tested caused a marked decline in GGT activities observed after 5 days in culture. In agreement with previous findings [21] it was shown that in effects on GGT, nicotinamide could be replaced by 3-aminobenzamide (10 mM) but not by the structurally-related 3-aminobenzoate (10 mM) which are respectively inhibitor and non-inhibitor of ADP-ribosylation in hepatocytes [21, 22]. However, neither compound

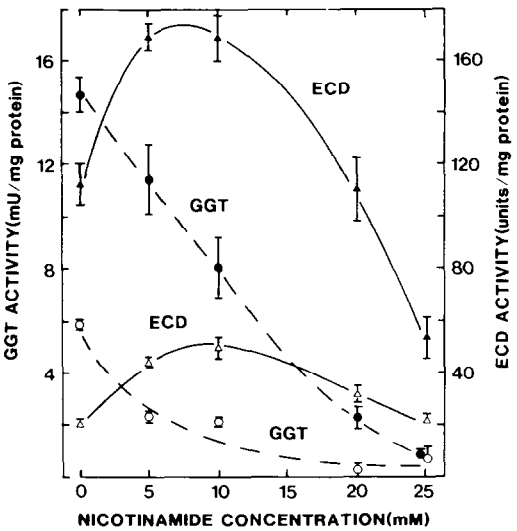

Fig. 4. Effect of nicotinamide concentration on GGT and ECD activities in hepatocytes. Cells were maintained in modified Waymouth medium with 30 nM dexamethasone, with or without PB and with varying concentrations of nicotinamide. ECD was assayed after 3 days: (△) no addition; (▲) plus 2 mM PB. GGT was assayed after 5 days: (○), no addition; (●), plus 2 mM PB. Values and bars are means ± S.D. from 4 dishes.

Table 1. Effects of dibutyryl cyclic AMP on inductions of GGT and ECD in cultured hepatocytes

Additions	GGT activity (mUnits/mg protein)		ECD activity (units/mg protein)	
	None	+dbcAMP	None	+dbcAMP
None	3.3 ± 0.3	2.3 ± 0.9	64 ± 5	21 ± 7
Dexamethasone, 3 μM	18.8 ± 0.6	5.9 ± 1.1	91 ± 6	21 ± 4
PB, 2 mM	10.7 ± 0.4	3.8 ± 0.5	185 ± 10	39 ± 9
PCN, 50 μM	17.3 ± 2.0	4.5 ± 0.4	78 ± 7	18 ± 3

After attachment, hepatocytes were maintained in modified Waymouth medium containing 30 nM dexamethasone with or without 50 μM dibutyrylcyclic AMP (dbcAMP) and other additions shown. Dishes for ECD measurement also contained 10 mM nicotinamide and were assayed after 3 days in culture. GGT was assayed after 5 days. Values are means ± S.D. from 4 dishes.

had significant effects on ECD activities when tested in place of nicotinamide (results not shown).

The effects of some nutritional, hormonal and other endogenous agents on both GGT and ECD were compared in parallel cultures with and without inducers. Table 1 shows that addition of dibutyryl cyclic AMP to culture media in general lowered both GGT and ECD activities markedly. The nucleotide had no significant effect on GGT activity in control cultures but reduced by 70–85% the extent of GGT inductions by high dexamethasone concentration, PB or the synthetic steroid, PCN; it caused a similar 70–85% reduction in control and induced ECD activities. The two activities differed, however, in sensitivity to some other agents which were added to media with and without PB. Fetal bovine serum and high glucose concentrations depressed GGT activity in cultured hepatocytes [15] but neither serum nor varying the glucose concentration of media from 2.5 to 50 mM had marked effects on ECD activity. Levels of ECD in cultures were found to be higher when insulin was omitted from the standard culture medium [14] but this had little effect on GGT activity (not shown).

As a further approach to comparing GGT and ECD regulation, compounds structurally-related to phenobarbital were used to compare structure–activity relationships and concentration-dependence of GGT and ECD inductions under comparable conditions in hepatocyte cultures. Figure 5 shows the relative effects of varying concentrations of the barbiturates sodium barbital and amobarbital and the structurally-related, glutethimide. All three compounds induced both GGT and ECD although in the case of sodium barbital only a small (though consistent) induction of GGT was observed. It is apparent that for all three compounds the concentration-dependences of ECD and GGT induction were different. For amobarbital and glutethimide higher drug concentrations were required for maximal induction of GGT and of ECD; for sodium barbital the converse was true. Additional related compounds tested as in Fig. 5 (results not shown) included barbituric acid which at concentrations up to 2 mM had little effect on either ECD or GGT activities; and diphenylhydantoin which increased both parameters with similar concentration-dependence, giving maximal inductions of 3-fold (GGT) and nearly 2-fold (ECD) at concentrations in the range 0.1–0.3 mM. For the phenobarbital-like compounds tested, the order of potency (at optimal concentrations) as inducers of GGT was glutethimide > amobarbital, diphenylhydantoin > PB > Na barbital > barbituric acid; while the order of potency as ECD inducers was amobarbital, PB > Na barbital, diphenylhydantoin > glutethimide > barbituric acid. These conclusions are based both on the results above and on a series of further experiments in which effects of multiple inducers were compared directly with the same liver cell preparation.

Figure 6 shows the effects of some monooxygenase inducers differing substantially in structure from PB and known to induce different monooxygenase components [16]. The polycyclic hydrocarbons, 3-methylcholanthrene and β-napthoflavone both induced ECD in the presence (Fig. 6) or absence (not shown) of nicotinamide. By contrast neither polycyclic hydrocarbon induced GGT (except for a minor effect at the highest concentrations of β-naphthoflavone) under any conditions tested. PCN is considered to be a monooxygenase inducer distinct from either the phenobarbital-type or polycyclic

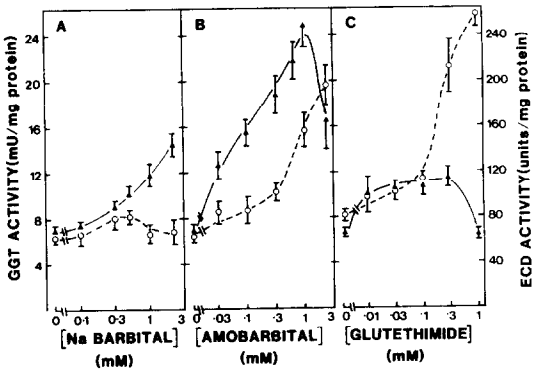

Fig. 5. Concentration dependences of GGT and ECD inductions by barbiturates and glutethimide. Cells were maintained in modified Waymouth medium with 30 nM dexamethasone and varying drug concentrations as shown. ECD was assayed after 3 days: the culture medium for these dishes contained 10 mM nicotinamide (▲). GGT (○) was assayed after 5 days. Values and bars are means ± S.D. from 4 dishes.

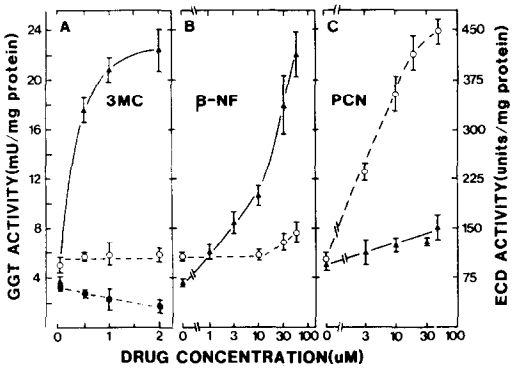

Fig. 6. Effects of polycyclic hydrocarbons and PCN on GGT and ECD activities in hepatocytes. Cells were maintained in modified Waymouth medium with 30 nM dexamethasone and varying concentrations of 3-methylcholanthrene (3MC), β-napthoflavone (β-NF) or PCN. ECD was assayed after 3 days: the culture medium contained 10 mM nicotinamide (▲). Dishes for GGT assay (after 5d) contained (○) no other additions; or (●) 10 mM nicotinamide. Values and bars are means ± S.D. from 3–4 dishes.

hydrocarbon-type [16, 23]. It induced both ECD and GGT with similar concentration-dependence. Although the increase in ECD was small, this may reflect a relatively low activity of PCN-induced form(s) of cytochrome P-450 towards ethoxycoumarin as a substrate.

Figure 7 shows the effects of three further compounds all of which have been reported to increase both monooxygenase activity in experimental animals and serum GGT in patients treated with these compounds [4, 6, 8]. Aminopyrine induced both parameters with similar concentration-dependence. Griseofulvin caused a small but clearly significant increase in ECD which was near-maximal at 3 μM and while griseofulvin was also a good inducer of GGT, higher concentrations were required, with a

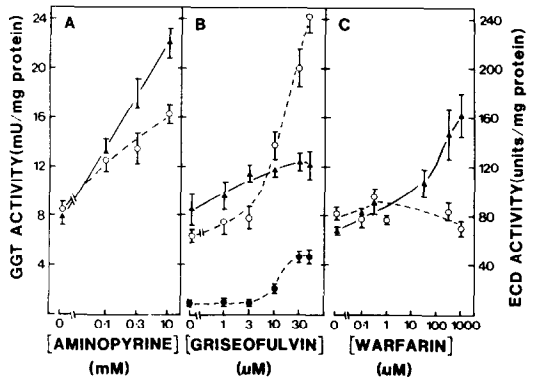

Fig. 7. Relative effects of further drugs on ECD and GGT activities in hepatocytes. Cells were maintained in modified Waymouth medium with 30 nM dexamethasone and varying concentrations of drugs as shown. ECD was assayed after 3 days: the culture medium contained 10 mM nicotinamide (▲). Dishes for GGT assay (after 5 days) contained (○) no other additions; or (●) 10 mM nicotinamide. Values and bars are means ± S.D. from 4 dishes.

maximal effect at about 50 μM. Since the one difference in culture media for examining responses of ECD and GGT was inclusion of nicotinamide in the ECD studies, this agent was included in some GGT dishes to determine whether its presence could account for the different concentration dependences of GGT and ECD inductions by griseofulvin. As shown in Fig. 7B nicotinamide lowered GGT activity in control cultures and decreased the magnitude of GGT induction but did not markedly alter the relative concentration-dependence of induction by griseofulvin.

Although warfarin treatment has been found to increase serum GGT activity in humans [4] it had little or no effect on GGT in hepatocytes under conditions where it caused clear induction of ECD (Fig. 7c).

DISCUSSION

Adaptive changes in a broad spectrum of liver drug metabolizing activities in reponse to various xenobiotics are known to occur predominantly in hepatocytes [24]. To establish that there is more than a chance association between induction of monooxygenase activities and elevation of GGT activity in serum a primary requirement is to show that at least some types of xenobiotic induce GGT and drug metabolism in the same cells. Increased GGT in hepatocytes is one possible source of serum enzyme but proliferation of GGT-containing bile duct epithelial cells, a frequent response to liver toxins ([25] and references therein) or a higher rate of enzyme leakage could be alternate causes of elevated GGT activity in serum. The use of a relatively pure population of hepatocytes in primary culture for this study eliminated the possible contribution of non-hepatocytes. The results show that a variety of compounds including barbiturates and the related drugs glutethimide and diphenylhydantoin, the steroids dexamethasone and PCN and the drugs aminopyrine and griseofulvin all induce GGT in hepatocytes under conditions where monooxygenase induction, measured as ECD activity of intact cells, is also observed. In a related study centred on the actions of known liver tumor promoters, a variety of further agents have been shown to induce both activities in experiments similar to those reported above: these agents include *p,p'*-dichlorodiphenyltrichloroethane, α- and γ-hexachlorocyclohexanes, butylhydroxytoluene, 17β-estradiol, testosterone and cyproterone acetate (A. M. Edwards and C. M. Lucas, "Induction of γ-glutamyltranspeptidase in primary cultures of normal rat hepatocytes by liver tumor promoters and related compounds" manuscript submitted and unpublished findings). Thus a substantial group of monooxygenase inducers with diverse structures also induce GGT in hepatocytes. This study, however, identified some monooxygenase inducers which had little effect on GGT under conditions where ECD was strongly induced. These included the polycyclic hydrocarbons 3-methylcholanthrene and β-naphthoflavone which are believed to increase a subset of P-450 isozymes, predominantly P-450$_c$ [16] by a mechanism involving the cytosolic *Ah* receptor [7]. It is not known whether warfarin, a further non-

inducer of GGT, also increases monooxygenase activity via the *Ah* receptor. The results indicate that GGT induction is not part of the pleiotrophic response mediated by the *Ah* locus.

For the substantial group of compounds which caused increases in hepatocyte ECD and GGT it seemed important to explore whether these dual effects are coincidental or reflect some common or closely-related control mechanisms. One approach was to compare inductions by PB of GGT and ECD and their modulation by other factors. PB-stimulated increases in both parameters apparently involved reversible enzyme induction dependent on continuing protein synthesis (Fig. 1 and ref. 14) and showed similar dependence on PB concentration (Fig. 3). Both effects were markedly potentiated by addition of low dexamethasone concentrations (Fig. 2 and ref. 14) or other glucocorticoids. Indeed for all compounds with dual effects on ECD and GGT so far tested glucocorticoids markedly potentiated both effects (results not shown). These observations and the effect of dibutyryl cyclic AMP in depressing both activities (Table 1) establish some similarities in GGT and monooxygenase controls but further factors (nicotinamide, serum, insulin, glucose) modulated the two activities differently. Dexamethasone alone induced both parameters but with quite different concentration-dependences (Fig. 2 and refs. 14, 15).

If there were indeed some common mechanism mediating inductions of GGT and monooxygenases by drugs (such as common receptor or common intracellular messenger) similar structure–activity relationships and concentration-dependences for induction of the two parameters might be expected, particularly where these were measured under quite similar conditions in culture. While for some compounds (PB, diphenylhydantoin, PCN, aminopyrine) the concentration-dependences were similar, for others (barbital, amobarbital, glutethimide, griseofulvin) optimal inductions of the two activities occurred at quite different drug concentrations. Within the group of compounds which induced both GGT and ECD (or within the subgroup structurally-related to PB) the compounds which were most effective in inducing GGT (e.g. glutethimide, PCN, griseofulvin) were not the best ECD inducers. Thus, there is no simple correlation between GGT and monooxygenase inductions as measured with one test substrate, 7-ethoxycoumarin. Interpretation of relative inductions is, however, complicated by the fact that multiple proteins, rather than a single gene product, may contribute to observed ECD activity. P-450 isozymes differ substantially in ECD activity [26, 27] and may be differentially-responsive to different inducers. The apparent concentration dependence of ECD induction may be the sum of overlapping inductions of multiple isozymes and more specific probes for individual P-450 isozymes would be required to clarify whether GGT induction is closely linked with that of any one or more specific isozymes. With this reservation it seems most likely that a range of hydrophobic compounds exert only loosely-related effects on GGT and drug metabolism.

Some caution is required in any detailed extrapolation of *in vitro* findings on cellular GGT of rat hepatocytes to diagnostic measurements of serum GGT in humans. A link between GGT induction and increased leakage from cells has been found previously [9] but leakage was not directly measured in this *in vitro* study. Also the intrahepatic distribution of GGT and hence the balance of factors contributing to the activity of GGT in serum differ between species including man and rat [1, 28]. The rat hepatocyte culture system, depsite its advantages for an ECD/GGT comparison under one fully-defined set of conditions, does not fully reproduce the conditions of the intact liver: expression of GGT appears to be somewhat facilitated in culture (see discussion in [15, 21]) whereas some P-450 isozymes are more poorly synthesized than *in vivo* (see discussion in [14, 24]). Nevertheless a number of general conclusions appear reasonable. Firstly there are some compounds such as polycyclic hydrocarbons which markedly increase components of the liver drug metabolizing system without an associated effect on GGT. For such compounds, measurements of serum GGT can provide no relevant information about the extent of monooxygenase induction. Also there may be some monooxygenase inducers (warfarin, for instance) which do increase serum GGT but by mechanisms unrelated to hepatic enzyme induction. Secondly for the substantial group of compounds which induced both GGT and ECD, it seems reasonable to conclude that higher levels of GGT in serum might signify relatively greater induction of some monooxygenase activities although for most drugs which elevate GGT the proportions of P.450 isozymes induced are still unknown. Since our findings suggest that there is unlikely to be any single mechanism at the molecular level by which different drugs induce GGT and a constant set of monooxygenase components, it would be inappropriate to use changes in serum GGT as more than a crude guide to likely broad changes in drug metabolism.

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