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DIFFERENTIAL RESPONSES OF RAT HEPATIC MICROSOMAL CARBOXYLESTERASE ISOZYMES TO GLUCOCORTICOIDS AND PREGNENOLONE 16α-CARBONITRILE

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Abstract—Differences in the responses to glucocorticoids and pregnenolone 16α-carbonitrile (PCN) of three isozymes of hepatic microsomal carboxylesterase, namely RL1, RL2 and RH1, in male rats were studied. The administration of dexamethasone dose-dependently increased isocarboxazid hydrolase activity, whereas *p*-nitrophenyl acetate-hydrolyzing activity was decreased dose-dependently. Betamethasone, methylprednisolone and PCN also markedly increased isocarboxazid hydrolase activity. A radial immunodiffusion assay indicated that carboxylesterase reactive with antibodies was induced by these steroids. Carboxylesterase isozyme RL2 was strongly induced by dexamethasone, methylprednisolone and PCN. In contrast, RL1 and RH1 were decreased by dexamethasone, but not by the other steroids. Estradiol benzoate had a synergic effect on the PCN-induced increase of isocarboxazid hydrolase, but the actions of the glucocorticoids were not affected. It is concluded that hepatic microsomal carboxylesterase isozymes in rats differ considerably from each other in their response to various steroids. These data are also indicative of the importance of glucocorticoids in hepatic xenobiotic metabolism.

Hepatic microsomal carboxylesterase (EC 3.1.1.1) catalyzes the hydrolysis of a wide variety of endogenous and exogenous compounds, such as carboxylester, thioester and aromatic amide [1], and plays an important role in drug and lipid metabolism. This enzyme serves as a means of detoxification, but, in contrast, is also responsible for metabolic activation of some carcinogens [2]. Recently, we showed that changes in hepatic carboxylesterase occur during hepato-carcinogenesis in rats [3]. Hepatic microsomes contain multiple forms of carboxylesterase isozymes [4–6]. We and other workers have reported that hepatic microsomal carboxylesterases are induced by many exogenous compounds, such as phenobarbital [5, 7, 8], polycyclic aromatic hydrocarbons [9], polychlorinated biphenyl [10], aminopyrine [10], and clofibrate [10–12].

We have also shown that several xenobiotics can induce hepatic carboxylesterase isozymes [5, 10]. We further reported that administration of testosterone propionate to castrated male rats induces carboxylesterase activity towards various substrates and causes changes in isozyme levels [5, 13]. However, little is known about the effects of other steroids on the hydrolytic capability of liver microsomes and the quantity of each carboxylesterase isozyme. The purpose of the present study was to clarify the effects of synthetic steroids, such as dexamethasone, betamethasone, triamcinolone acetonide, fluocinolone acetonide, methylprednisolone and pregnenolone 16α-carbonitrile (PCN)‡, on the amounts of three carboxylesterase isozymes, RL1, RL2 and RH1, and on various hydrolytic enzyme activities in hepatic microsomes of rats.

MATERIALS AND METHODS

Throughout the present study, adult male rats of the Sprague–Dawley strain were used. Animals were fed laboratory animal chow and water *ad lib.* and were housed in wire-bottomed cages at constant temperature (22–24°) and humidity (50–60%) under a 12-hr light:12-hr dark cycle (7:00 a.m. to 7:00 p.m.). All chemicals were dissolved in corn oil. Daily intraperitoneal injections were given of dexamethasone (10 mg/kg), betamethasone (10 mg/kg), triamcinoline acetonide (10 mg/kg), fluocinolone acetonide (10 mg/kg), methylprednisolone (10 mg/kg) or PCN (50 mg/kg) each for 5 days. Estradiol benzoate (0.5 mg/kg) was given s.c., daily for 5 consecutive days. Rats were decapitated, and livers were removed, weighed and perfused with 1.15% KCl solution. Microsomes were isolated by differential centrifugation as previously described [5, 10]. All subsequent procedures were performed at 0–4°.

Carboxylesterase activities towards three substrates, i.e. *p*-nitrophenyl acetate, isocarboxazid and butanilicaine, were determined essentially

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‡ Abbreviations: PCN, pregnenolone 16α-carbonitrile; RL1, rat hepatic carboxylesterase RL1; RL2, rat hepatic carboxylesterase RL2; RH1, rat hepatic carboxylesterase RH1; butanilicaine, *N*-butylaminoacetyl-2-chloro-6-methylanilide; and ACTH, adrenocorticotropic hormone.

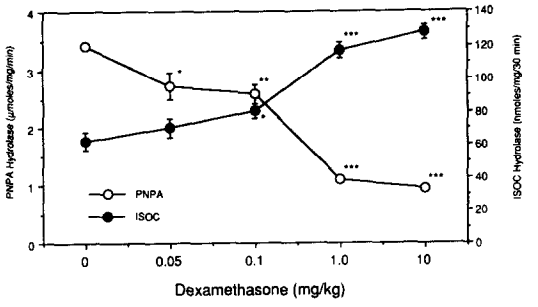

Fig. 1. Dose- and substrate-dependent effects of dexamethasone on hepatic microsomal carboxylesterase activities in male rats. Male rats at 6 weeks of age were treated by intraperitoneal injection with dexamethasone (in corn oil, 0.05 to 10 mg/kg) for 5 consecutive days. Control (0 mg/kg) animals were treated by intraperitoneal injection with corn oil. Each value is the mean ± SEM for five to six animals. Abbreviations: PNPA, *p*-nitrophenyl acetate; and ISOC, isocarboxazid. Key: (*) $P < 0.05$ vs control; (**) $P < 0.01$ vs control; and (***) $P < 0.0001$ vs control.

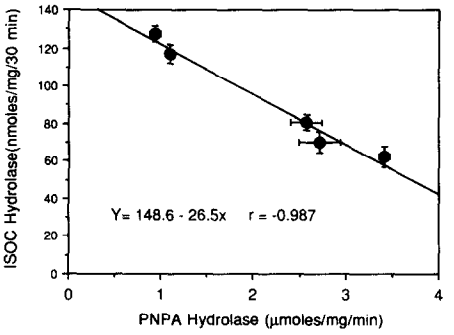

Fig. 2. Correlation between *p*-nitrophenyl acetate hydrolase and isocarboxazid hydrolase activities in dexamethasone-treated rats. Male rats at 6 weeks of age were treated by intraperitoneal injection with dexamethasone (in corn oil, 0.05 to 10 mg/kg) for 5 consecutive days. Control (0 mg/kg) animals were given the vehicle alone. Vertical and horizontal lines indicate SEM from five animals for isocarboxazid and *p*-nitrophenyl acetate hydrolase activities, respectively. The correlation coefficient and regression equation are $r = -0.987$ and $y = 148.6 - 26.5x$, respectively. Abbreviations: PNPA, *p*-nitrophenyl acetate; and ISOC, isocarboxazid.

according to the methods described previously [5, 6]. Carboxylesterases RL1, RL2 and RH1 were purified from liver microsomes of untreated male rats as described previously [5]. Antibodies to these three isozymes were prepared as reported previously [5]. Immunochemical quantitation of the forms of carboxylesterase isozymes in hepatic microsomes was conducted by the method of Thomas *et al.* [14] with minor modifications. Protein was determined by the method of Lowry *et al.* [15]. All data in this study were statistically analyzed by Student's *t*-test.

Chemicals. Dexamethasone, betamethasone, triamcinolone acetonide and fluocinolone acetonide were purchased from the Sigma Chemical Co., St. Louis, MO, U.S.A. Butanilicaine (*N*-butylaminoacetyl-2-chloro-6-methylanilide) was donated by Hoechst AG, Frankfurt, Germany. Isocarboxazid was donated by the Nippon Roche Research Center, Kamakura, Japan. Methylprednisolone and PCN were obtained from Upjohn Pharmaceutical Limited. Other chemicals were of reagent grade and were purchased from commercial sources.

RESULTS

Dose- and substrate-dependent effects of dexamethasone on hepatic microsomal carboxylesterase activities in adult male rats. Figure 1 shows the effects of various doses of dexamethasone, administered for 5 days, on carboxylesterase activities toward *p*-nitrophenyl acetate and isocarboxazid. Dexamethasone induced a dose-dependent increase of carboxylesterase activity towards isocarboxazid. The induction was maximal at a dose of 10.0 mg/kg body weight. Conversely, dexamethasone caused a dose-dependent decrease of carboxylesterase activity towards *p*-nitrophenyl acetate, producing the maximal effect at a dose of 10.0 mg/kg. In previous reports, we investigated the induction of carboxylesterase isozymes in liver microsomes of male and female rats by various xenobiotics [5, 10]. However, most of these xenobiotic compounds induced the activity towards both substrates, unlike dexamethasone. Figure 2 shows that there was an excellent inverse correlation (correlation coefficient, −0.987) between the changes of isocarboxazid hydrolase and *p*-nitrophenyl acetate hydrolase activities in liver microsomes of dexamethasone-treated rats.

Substrate-dependent effects of various glucocorticoids and PCN on hepatic microsomal carboxylesterase activities in adult male rats. Table 1 shows the effects of various glucocorticoids and PCN on hepatic microsomal carboxylesterase activities towards three substrates that are rather specific for the three isozymes, i.e. *p*-nitrophenyl acetate for RL1, isocarboxazid for RL2, and butanilicaine for RH1 [5, 10]. Carboxylesterase activity towards isocarboxazid was induced by dexamethasone, betamethasone, methylprednisolone and PCN. Conversely, carboxylesterase activities towards *p*-nitrophenyl acetate and butanilicaine were decreased greatly by dexamethasone, betamethasone, triamcinolone acetonide and fluocinolone acetonide, and were decreased slightly by methylprednisolone and PCN. Triamcinolone acetonide and fluocinolone acetonide also caused decreases of isocarboxazid hydrolase activity. On the other hand, we have already reported that the adrenocorticotropic hormone (ACTH) has no effect on carboxylesterase activities [16]. Also, when natural glucocorticoids, such as hydrocortisone or corticosterone, were administered, there were no significant effects on isocarboxazid hydrolase activity (107.8 and 106.5% of control activity, respectively).

Radial immunodiffusion assay to determine the amounts of carboxylesterases RH1, RL1 and RL2 in liver microsomes from male rats given glucocorticoids and PCN. In two previous papers [5, 10], we reported

Table 1. Substrate-dependent effects of various glucocorticoids and PCN on hepatic microsomal carboxylesterase activities in adult male rats

	Specific activity		
	p-Nitrophenyl acetate (μmol/mg/min)	Isocarboxazid (nmol/mg/30 min)	Butanilicaine (nmol/mg/min)
Control	3.03 ± 4.5 (1.00)	67.6 ± 4.5 (1.00)	132.3 ± 4.5 (1.00)
Dexamethasone	0.57 ± 0.07* (0.19)	122.6 ± 4.1* (1.81)	24.2 ± 5.4* (0.18)
Betamethasone	0.50 ± 0.06* (0.17)	101.9 ± 0.04† (1.51)	27.8 ± 3.0† (0.21)
Triamcinolone acetonide	0.39 ± 0.03* (0.13)	47.8 ± 3.5† (0.71)	33.1 ± 4.4† (0.25)
Fluocinolone acetonide	0.25 ± 0.04* (0.08)	40.9 ± 2.0* (0.61)	19.1 ± 1.2† (0.14)
Methylprednisolone	1.79 ± 0.16† (0.59)	102.5 ± 10‡ (1.52)	82.3 ± 1.1‡ (0.62)
PCN	2.78 ± 0.11 (0.92)	126.4 ± 7.1* (1.87)	95.5 ± 18 (0.72)

Each value is the mean ± SEM from five animals. Male rats at 6 weeks of age were treated intraperitoneally with dexamethasone (10 mg/kg), betamethasone (10 mg/kg), triamcinolone acetonide (10 mg/kg), fluocinolone acetonide (10 mg/kg), methylprednisolone (10 mg/kg) or PCN (50 mg/kg) daily for 5 consecutive days. All chemicals were dissolved in corn oil. Numbers in parentheses are the relative ratio with respect to the control value.

* $P < 0.001$ vs control.

† $P < 0.01$ vs control.

‡ $P < 0.05$ vs control.

Table 2. Immunochemical quantitation by radial immunodiffusion assay of carboxylesterase isozymes in the livers of male rats treated with glucocorticoids and PCN

	Carboxylesterase isozyme contents (μg/mg protein)		
	RL1	RL2	RH1
Control	16.1 ± 1.2 (1.00)	34.4 ± 1.0 (1.00)	28.6 ± 3.9 (1.00)
Dexamethasone	5.5 ± 0.9* (0.34)	59.3 ± 7.4† (1.72)	16.7 ± 3.7‡ (0.58)
Methylprednisolone	16.1 ± 1.2 (1.00)	50.7 ± 6.6‡ (1.47)	25.4 ± 2.4 (0.89)
PCN	15.9 ± 2.8 (0.99)	65.9 ± 4.8* (1.92)	39.9 ± 3.3 (1.40)

Each value (μg/mg protein) is the mean ± SEM from four to five preparations. Male rats at 6 weeks of age were treated intraperitoneally with dexamethasone (10 mg/kg), methylprednisolone (10 mg/kg) or PCN (50 mg/kg) daily for 5 consecutive days. All chemicals were dissolved in corn oil. Numbers in parentheses are the relative ratio with respect to the control value. For other experimental conditions, see the text.

* $P < 0.001$ vs control.

† $P < 0.01$ vs control.

‡ $P < 0.05$ vs control.

the purification of three forms of carboxylesterase, the preparation of specific antibodies to each of the isozymes, and the quantititation of carboxylesterase isozymes in liver microsomes by using a radial immunodiffusion assay. To the best of our knowledge the above papers were the first report of the quantitation of each carboxylesterase isozyme in rat liver microsomes. In the present study, we also determined the amounts of each isozyme by using a radial immunodiffusion assay. The results of the radial immunodiffusion assay for carboxylesterase reactive with antibodies are shown in Table 2. Carboxylesterase RL2 was significantly induced by dexamethasone, methylprednisolone, and PCN. The

Table 3. Correlation between isozyme contents and hydrolase activities in rat liver microsomes after treatment with glucocorticoids and PCN

Isozyme	Substrate	Linear regression y	r^2
RL1	vs PNPA	−0.45 + 0.19x	0.766
RL1	vs BUTA	−17.5 + 7.56x	0.788
RL1	vs ISOC	136.2 − 2.34x	0.209
RL2	vs PNPA	3.70 − 0.03x	0.148
RL2	vs BUTA	190.1 − 2.02x	0.377
RL2	vs ISOC	1.93 + 1.96x	0.978
RH1	vs PNPA	−0.63 + 0.10x	0.687
RH1	vs BUTA	−0.71 + 3.05x	0.427
RH1	vs ISOC	101.1 + 0.13x	0.002

Abbreviations: PNPA, *p*-nitrophenyl acetate hydrolase; ISOC, isocarboxazid hydrolase; and BUTA, butanilicaine hydrolase. Enzyme activity and isozyme contents are given in Tables 1 and 2, respectively.

result of this experiment suggests that the increase in isocarboxazid hydrolase activity caused by glucocorticoids and PCN was a reflection of an increase in carboxylesterase RL2 contents. Conversely, RL1 and RH1 were not induced by these steroids. Indeed, when dexamethasone was administered to male rats, RL1 and RH1 were decreased. These results are similar to those obtained by measuring the activities towards the rather specific substrates, i.e. RL1 for *p*-nitrophenyl acetate hydrolase activity and RH1 for butanilicaine hydrolase activity.

Correlation between isozyme contents and hydrolase activities in rat liver microsomes after treatment with glucocorticoids and PCN. Table 3 shows the correlations between isozyme contents and several hydrolase activities. Our previous results [5, 10] suggested that the three substrates were rather specific, i.e. *p*-nitrophenyl acetate for RL1, isocarboxazid for RL2, and butanilicaine for RH1, and so could be used to measure and discriminate among the hepatic carboxylesterases. The result of this experiment suggests that the increase in isocarboxazid hydrolase activity caused by treatment with glucocorticoids and PCN was reflective of an increase in carboxylesterase RL2 contents. There was a good correlation between isocarboxazid hydrolase activity and RL2 content ($r^2 = 0.978$), but the correlation coefficient between RL2 and *p*-nitrophenyl acetate ($r^2 = 0.148$) or between RL2 and butanilicaine ($r^2 = 0.377$) was very low. On the other hand, the correlation coefficient between RL1 content and *p*-nitrophenyl acetate ($r^2 = 0.766$) or butanilicaine ($r^2 = 0.788$) hydrolase activity was significant. The correlation coefficient between RL1 content and isocarboxazid hydrolase activity was very low ($r^2 = 0.209$). The correlation between RH1 contents and *p*-nitrophenyl acetate hydrolase activity ($r^2 = 0.687$) was slightly higher, but the relationship between RH1 contents and butanilicaine ($r^2 = 0.427$) or isocarboxazid ($r^2 = 0.002$) hydrolase activity was low or independent of treatment with glucocorticoid and PCN.

Effects of estradiol on glucocorticoid- and PCN-induced changes in hepatic microsomal carboxylesterases in rats. We have already reported that carboxylesterase RL2 contents and isocarboxazid hydrolase activities are induced slightly by estradiol in castrated male and female rats [5, 13]. Table 4 shows the effects of estradiol on glucocorticoid- and PCN-induced changes in hepatic microsomal carboxylesterases in rats. Estradiol enhanced the increase of isocarboxazid hydrolase activity induced by PCN. Similar inductive responses have been reported for glutathione *S*-transferase [17]; one subunit of glutathione *S*-transferase (Ya) is synergistically induced by dexamethasone in the presence of the primary inducing agent 1,2-benzanthracene. Interestingly, the effects of other synthetic glucocorticoids, such as dexamethasone and methylprednisolone, were not altered significantly by estradiol. Also *p*-nitrophenyl acetate and butanilicaine hydrolase activities were not changed significantly by glucocorticoids and PCN in the presence of estradiol.

DISCUSSION

In a previous paper, we described the induction of carboxylesterases RL2 and RH1 in hepatic microsomes of male and female rats by phenobarbital treatment, but RL1 was not affected [5]. It was the first report, to our knowledge, of the quantitation of each carboxylesterase isozyme in rat liver microsomes. Later, we reported the induction of carboxylesterase isozymes by several xenobiotics [10]. The administration of aminopyrine markedly increased the amounts of RL1 and RL2, but not that of RH1. On the other hand, the administration of *trans*-stilbene oxide and Aroclor 1254 to male rats only increased the amount of carboxylesterase RL2 [10]. The administration of clofibrate to rats markedly increased the amounts of all three carboxylesterase isozymes. We also showed that administration of gonadal steroids to castrated male rats induced carboxylesterase activity and caused changes in isozyme level [5, 13]. However, little is known about the inducibility of each carboxylesterase isozyme in rat hepatic microsomes caused by another steroid, such as glucocorticoid, and PCN.

Glucocorticoids, which influence a great number of physiological processes, induce several drug-metabolizing enzymes, such as cytochrome P450 [18–21], γ-glutamyltranspeptidase [22], NADPH-cytochrome P450 oxidoreductase [18], and tyrosine aminotransferase [23]. Some isozymes of glutathione *S*-transferases are synergistically induced by dexamethasone in the presence of 1,2-benzanthracene [18]. Some members of the P450 family are also induced by PCN [17, 19–21]. In the case of the glucocorticoids in this study, we found that administration of dexamethasone strongly induced isocarboxazid hydrolase activity and the amount of carboxylesterase RL2 protein (Fig. 1 and Table 2). Conversely, it markedly decreased *p*-nitrophenyl acetate hydrolase and butanilicaine hydrolase activities and the amounts of carboxylesterase RL1

Table 4. Effects of estradiol (E2) on glucocorticoid- and PCN-induced changes in hepatic microsomal carboxylesterases in rats

	Specific activity		
	p-Nitrophenyl acetate (μmol/mg/min)	Isocarboxazid (nmol/mg/30 min)	Butanilicaine (nmol/mg/min)
Control	2.81 ± 0.2 (1.00)	65.5 ± 7.9 (1.00)	147.5 ± 30.5 (1.00)
Estradiol (E2)	1.89 ± 0.05* (0.67)	66.4 ± 9.3 (1.01)	108.2 ± 22.0 (0.73)
Dexamethasone	0.94 ± 0.06† (0.33)	127.6 ± 4.2† (1.95)	42.4 ± 6.22* (0.29)
Dexamethasone + E2	1.03 ± 0.07† (0.37)	137.4 ± 5.8† (2.10)	54.0 ± 6.7* (0.37)
Methylprednisolone	2.65 ± 0.26 (0.94)	102.9 ± 4.5* (1.57)	88.3 ± 18.8* (0.60)
Methylprednisolone + E2	2.07 ± 0.19 (0.73)	98.2 ± 4.2* (1.50)	64.6 ± 9.6 (0.44)
PCN	2.88 ± 0.12 (1.02)	120.4 ± 7.1† (1.84)	95.0 ± 18.0 (0.64)
PCN + E2	2.73 ± 0.13 (0.97)	166.8 ± 16.9†‡ (2.54)	62.9 ± 8.4 (0.43)

Each value is the mean ± SEM from five animals. Male rats at 6 weeks of age were treated intraperitoneally with dexamethasone (10 mg/kg), methylprednisolone (10 mg/kg) or PCN (50 mg/kg) daily for 5 consecutive days or subcutaneously with estradiol benzoate (E2, 0.5 mg/kg) for 5 consecutive days. All chemicals were dissolved in corn oil. Numbers in parentheses are the relative ratio with respect to the control value.

* $P < 0.05$ vs control.

† $P < 0.01$ vs control.

‡ $P < 0.05$ PCN-treated animals.

and RH1 protein (Fig. 1 and Tables 1 and 2). Figure 2 shows that there was an excellent inverse correlation ($r^2 = 0.987$) between the change of isocarboxazid hydrolase and *p*-nitrophenyl acetate hydrolase activities in liver microsomes of dexamethasone-treated rats. Table 1 shows the substrate-dependent effects of various glucocorticoids and PCN on hepatic microsomal carboxylesterase activities in male rats. Isocarboxazid hydrolase activity was induced by dexamethasone, betamethasone, methylprednisolone and PCN. However, this activity was reduced by fluocinolone acetonide and triamcinolone acetonide. Detailed studies of the tyrosine aminotransferase gene have indicated the presence of multiple glucocorticoid-responsive elements that are located upstream from the initiation site of transcription [22]. The regulation mechanism of carboxylesterase isozymes is unclear as of yet. It may be regulated by a multiple glucocorticoid-responsive element or by another mechanism.

When PCN was administered to rats, isocarboxazid hydrolase activity and carboxylesterase RL2 contents were induced (Tables 1 and 2). Conversely, *p*-nitrophenyl acetate hydrolase and RL1 content were not changed (Tables 1 and 2). On the other hand, dexamethasone produced a significant decline (66%) in carboxylesterase RL1 but PCN had little or no effect (Table 2). Similar responses have been reported for epoxide hydrolase in rat liver microsomes [17]. Dexamethasone has different effects on the regulatory mechanisms of carboxylesterase isozymes, both inducible and deductive. However, PCN has only an inducible response. These results suggested that dexamethasone and PCN have similar regulatory responses on hepatic microsomal carboxylesterase isozymes at least in part, but dexamethasone may be another regulator of carboxylesterase RL1 and RH1.

Table 3 shows the correlations between isozyme contents and several hydrolase activities. Our previous results [5, 10] suggested that the three substrates were rather specific for each carboxylesterase isozyme, i.e. *p*-nitrophenyl acetate for RL1, isocarboxazid for RL2, and butanilicaine for RH1, and so could be used to measure and discriminate among the hepatic carboxylesterases. There was a good correlation between isocarboxazid hydrolase activity and RL2 content ($r^2 = 0.978$), but the other correlations were low. The result of this experiment suggests that the increase in isocarboxazid hydrolase activity caused by treatment with glucocorticoids and PCN was reflective of an increase in carboxylesterase RL2 contents in liver microsomes. However, the correlation coefficient between RL1 content and *p*-nitrophenyl acetate ($r^2 = 0.766$) or butanilicaine ($r^2 = 0.788$) hydrolase activity was significant. The correlation between RH1 content and *p*-nitrophenyl acetate hydrolase activity ($r^2 = 0.687$) was slightly high, but that between RH1 content and butanilicaine ($r^2 = 0.427$) or isocarboxazid ($r^2 = 0.002$) hydrolase activity was low or there was no relationship when rats were treated with glucocorticoid and PCN. The obvious discrepancy between substrate specificity and isozyme

contents, especially in the case of RH1, may be dependent on enzyme amounts, because carboxylesterase RL2 content is much higher than the contents of the other two isozymes.

Table 4 shows the effects of estradiol on glucocorticoid- and PCN-induced changes in carboxylesterases. We have already reported that carboxylesterase RL2 contents and isocarboxazid hydrolase activity are slightly induced by estradiol in castrated male and female rats [5, 13]. However, when estradiol benzoate was administered to sham-operated male and female rats, carboxylesterase RL2 contents and isocarboxazid hydrolase activity were not induced [5, 13]. In the present study, isocarboxazid hydrolase activity was synergistically induced by PCN in the presence of estradiol benzoate (Table 4). This result led to a 2.5- or 1.4-fold increase in isocarboxazid hydrolase activity, compared with estradiol benzoate alone or PCN alone, respectively. Similar inductive responses have been reported for glutathione *S*-transferase [17]; one subunit of glutathione *S*-transferase (Ya) is synergistically induced by dexamethasone in the presence of the primary inducing agent 1,2-benzanthracene. However, the effects of other synthetic glucocorticoids, such as dexamethasone and methylprednisolone, were not synergistically altered by estradiol. These results suggest that glucocorticoids and PCN affect the inducibility of carboxylesterases in rat liver by different mechanisms. Further work is needed to determine the regulatory mechanisms of carboxylesterase by these steroid derivatives. In conclusion, from the experiments described above, we believe that hepatic microsomal carboxylesterase isozymes in rats differ considerably from each other in their response to these synthetic steroids. These data are also indicative of the importance of glucocorticoids in hepatic xenobiotic metabolism.

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