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Multiplicity and Regulation of Hepatic Microsomal Carboxylesterases in Rats

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SUMMARY

Three isozymes of carboxylesterase were purified from rat liver microsomes by using Sephadex G-150 gel filtration, DE-52 ion exchange, and chromatofocusing column chromatographies. These isozymes each showed a single protein band on sodium dodecyl sulfate-polyacrylamide gel electrophoresis and were immunologically different from each other as determined by immunochemical blotting analysis and immunochemical inhibition of catalytic activity. The three isozymes were named RH1 (molecular weight 174,000, trimer, pI 6.0), RL1 (molecular weight 61,000, monomer, pI 6.5), and RL2 (molecular weight 61,000, monomer, pI 5.5). RL1 has the highest specific activities toward *p*-nitrophenylacetate and malathion. Acetanilide is a rather specific substrate for RL2, whereas RH1 has the highest specific activity for butanilicaine. RL1 has the highest specific activity for the hydrolysis of long-chain acyl-CoA. To investigate the hormonal regulation of carboxylesterase activities, we have quantitated RL1, RL2, and RH1 in liver microsomes from male and female rats using a radial immunodiffusion assay. The amount of RL1 in male rats was decreased by castration but recovered to almost the level in sham-operated rat liver microsomes after treatment of the castrated rats with testosterone. Conversely, in ovariectomized female rats, the amount of RL1 was increased as compared to that in sham-operated rats, and treatment of the ovariectomized rats with estradiol tended to decrease the quantity of RL1. In all cases of sex hormone treatment, the amount of RH1 remains unclear at present. However, the amount of RL2 may be, at least in part, regulated by estrogens. On the other hand, phenobarbital treatment of male and female rats caused a significant increase in the amounts of RH1 and RL2, whereas RL1 was not affected. It was concluded that the three isozymes differ considerably from each other in response to hormone treatment, inducibility, substrate specificity, and immunological properties.

Hepatic microsomal carboxylesterase (EC 3.1.1.1) catalyzes the hydrolysis of a wide range of xenobiotic carboxylesters and aromatic amides (1, 2), and is involved in a major route for the detoxification and activation of such compounds.

Recently, several workers have shown that there are multiple isozymes of carboxylesterase in rat liver microsomes (2, 3). Blomberg and Raftell (4) showed that the overall esterolytic activities are very similar in the livers of rats of both sexes, although there are quantitative differences in the carboxylesterase isozymes. Robbi and Beaufay (5) compared sex differences in the esterase activity pattern after isoelectrofocusing crude extracts from male and female rat liver microsomes. In a previous paper (6), we reported sex differences and substrate-dependent changes in carboxylesterase activity. Isozymes of hepatic microsomal carboxylesterase having a high pI and those having a low pI are dependent mainly upon androgen and estrogen, respectively (6).

Hepatic microsomal carboxylesterases have been reported to be induced by pretreatment of rats with drugs such as phenobarbital (7) and dichlorodiphenyltrichloroethane (8), but not by 3-methylcholanthrene (2).

Although several forms of purified carboxylesterases have been well characterized, there is little understanding of their regulatory mechanisms in tissues because of the lack of specific assay methods. In this paper, we describe the purification of three forms of carboxylesterase and the preparation of specific antibody to each isozyme. These antibodies were used as probes to examine the regulatory mechanisms of these isozymes in rat liver microsomes, as well as for quantitation.

Materials and Methods

Animals and surgical procedures. Throughout the present study, adult Sprague-Dawley rats of both sexes were used. Animals were fed a laboratory chow and water *ad libitum* and housed in plastic cages at constant temperature (22°–24°) and humidity (50–60%) under a 12-hr light/dark cycle (7 a.m.–7 p.m.). Rats were gonadectomized or sham operated at 5 weeks of age and subcutaneously injected daily with either

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ABBREVIATIONS: Bis-Tris, 2-2-bis(hydroxymethyl)-2,2′,2″-nitrilotriethanol; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis; CoA, coenzyme A; PBS, phosphate-buffered saline; Hepes, *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid.

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testosterone propionate (5 mg/kg) or estradiol benzoate (0.5 mg/kg) for 6 consecutive days before sacrifice. All animals were sacrificed at 8 weeks of age. Testosterone propionate and estradiol benzoate were dissolved in corn oil and given subcutaneously in a volume of 1 ml/kg body weight. Sham-operated, castrated, and ovariectomized rats received an equivalent volume of the vehicle.

Phenobarbital, dissolved in saline, was given intraperitoneally at a dose of 80 mg/kg for 7 days before sacrifice.

Preparation of microsomes. Rats were sacrificed by decapitation between 9 a.m. and 10 a.m. Livers were quickly removed, weighed, perfused with 1.15% KCl, then homogenized with 1.15% KCl in a Potter-Elvehjem homogenizer. The 20% homogenate was centrifuged at 9,000 × *g* for 20 min and the resulting supernatant was further centrifuged at 105,000 × *g* for 1 hr. The resulting microsomal pellet was suspended in 1.15% KCl and stored at −80° until required.

Purification of carboxylesterases. The purification procedures for carboxylesterases are summarized in Fig. 1. Carboxylesterase isozymes were isolated from male rat liver microsomes by a modification of the method of Mentlein *et al.* (9). All steps were carried out at 4°. Microsomes were solubilized in 100 mM Tris-HCl buffer, pH 8.5, containing 1% saponin, at a final protein concentration of 2.7 mg/ml. The solution was stirred for 60 min at 4° and then centrifuged at 105,000 × *g* for 60 min. The supernatant was fractionated by ammonium sulfate precipitation. The 30–70% precipitate was suspended in 10 mM Tris-HCl buffer, pH 8.0. The solution was gel filtrated on a Sephadex G-150 column (2.6 × 90 cm) equilibrated with 10 mM Tris-HCl buffer, pH 8.0. Two peak fractions (A and B) were applied to their columns (2.5 × 25 cm) of Whatman DE-52 which had been equilibrated with 10 mM Tris-HCl buffer, pH 8.0, independently. The column was washed with the same buffer and then eluted with a stepwise NaCl gradient in 10 mM Tris-HCl buffer, pH 8.0. One peak fraction (A-1) was collected by elution with 60 mM NaCl from fraction A, and two peak fractions (B-1 and B-2) were collected by elution with 30 mM and 150 mM NaCl, respectively, from fraction B. The three fractions were separately pooled and dialyzed for 24 hr against three changes of 2 liters of 25 mM Bis-Tris-HCl buffer, pH 7.4. The dialyzed fractions were applied to a column (1.0 × 30 cm) of chromatofocusing gel PBE 96 (Pharmacia Fine Chemicals), equilibrated with 25 mM Bis-Tris-HCl, pH 7.4. These enzymes were eluted with 270 ml of Polybuffer 74 (Pharmacia Fine Chemicals), diluted at a ratio of 1:8, and adjusted to pH 5.0 with HCl. The fractions showing a single protein band on SDS-PAGE were combined. The eluates from A-1, B-1, and B-2 fractions were termed RH1, RL1, and RL2, respectively. The three purified carboxylesterase fractions from chromatofocusing were dialyzed for 24 hr against two changes of 2 liters of 10 mM Tris-HCl buffer, pH 8.0. In order to remove Polybuffer, each preparation was then applied to a

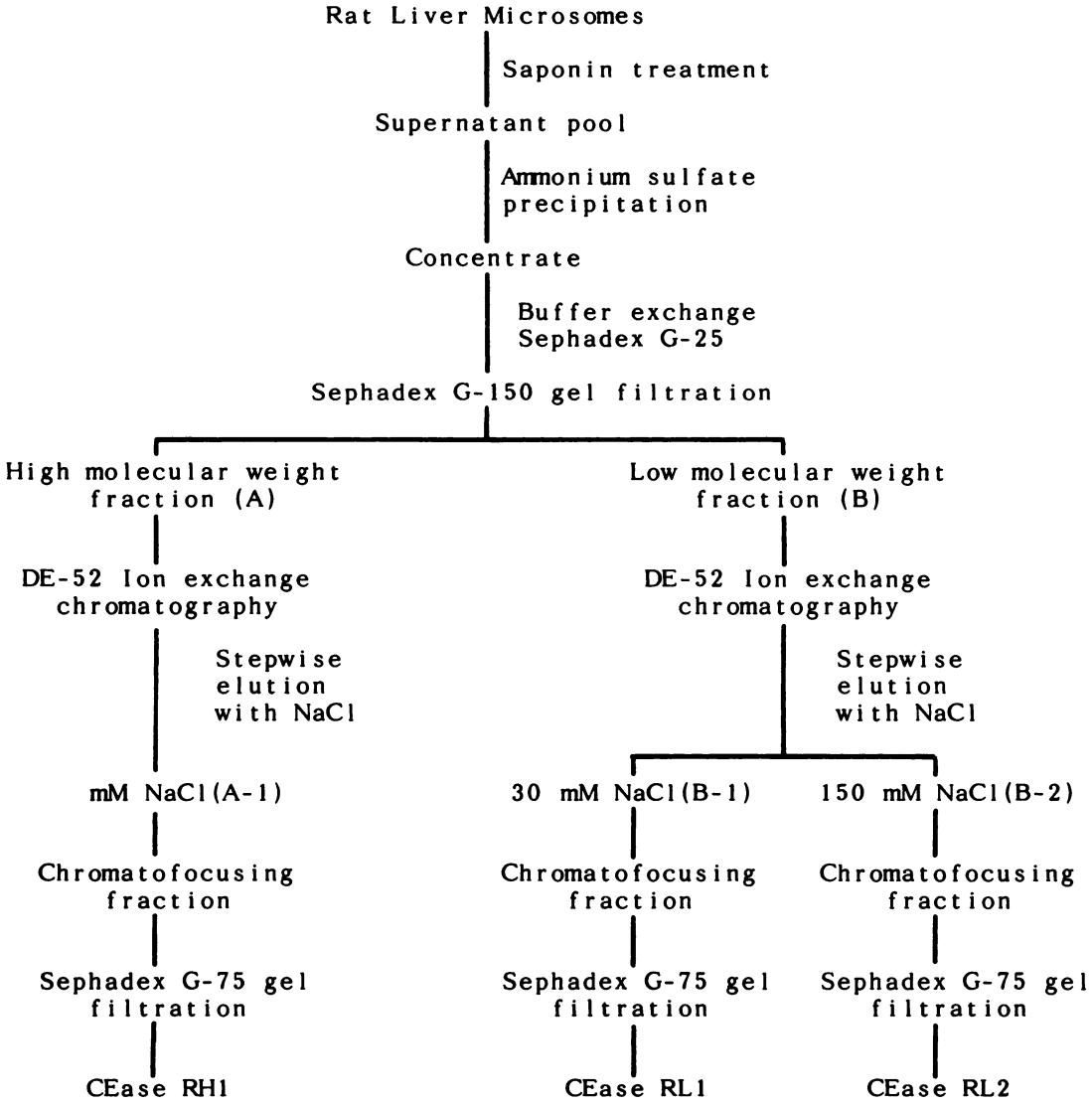

Fig. 1. Summary of the purification procedures for carboxylesterase (*CEase*) RH1, RL1, and RL2 from liver microsomes of male rats.

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column (1.5 × 70 cm) of Sephadex G-75 equilibrated with 10 mM Tris-HCl buffer, pH 8.0. The preparations could be stored at −80° for several months without loss of enzyme activity.

Preparation of antibodies. Antibodies against highly purified carboxylesterases RH1, RL1, and RL2 were raised in male Japanese White rabbits (2.5–3.0 kg). Purified preparations (300 μg) of the carboxylesterases were mixed with an equal volume of Freund's complete adjuvant (Difco Laboratories) and administered twice at each of five intramuscular sites and five sites of footpad. The rabbits were boosted intravenously via the ear vein with 150 μg of protein in 1 ml of 0.9% NaCl solution 3 weeks later. Collection of blood of the immunized animal from the marginal ear vein was conducted at 7 days after booster injection followed by once every 3 days. IgG was prepared by ammonium sulfate fractionation and a DE-52 column according to the method of Noshiro and Omura (10).

Enzyme assays. The hydrolysis of *p*-nitrophenylacetate, *p*-nitrophenylpropionate, and *p*-nitrophenylbutyrate were determined colorimetrically in 50 mM Tris-HCl buffer (pH 8.0/30°) by measuring the *p*-nitrophenol released according to the method of Krisch (11). The hydrolysis of malathion or clofibrate was assayed in 100 mM Tris-HCl buffer (pH 7.5/37°) by coupling the hydrolysis of substrate to the reduction of a tetrazolium dye according to the method of Talcott (12). The hydrolysis of acetanilide was assayed in 50 mM Tris-HCl buffer (pH 8.6/37°) by fluorometric determination of the released aniline (λ_{ex} = 282 nm, λ_{em} = 348 nm) according to the method of Heymann *et al.* (13). The hydrolysis of isocarboxazid was assayed in 100 mM Tris-HCl buffer (pH 8.0/37°) by colorimetric determination of the released benzylhydrazine according to the method of Satoh and Moroi (14). The hydrolysis of long-chain acyl-CoA was assayed in 30 mM Hepes buffer (pH 7.4/30°) by colorimetric determination (412 nm) of free thiol groups by reaction with 5,5′-dithiobis-(2-nitrobenzoic acid) according to the method of Berge (15). The hydrolysis of butanilicaine was assayed in 50 mM Tris-HCl buffer (pH 8.6/30°) by spectrophotometric determination according to the method of Heymann *et al.* (13).

When inhibition of enzyme activity by antibodies was studied, all assay tubes were brought to the same final concentration of IgG with control IgG. Microsomes and purified preparations were incubated with IgG for 30 min at 37° in 0.25 ml of 10 mM Tris-HCl buffer, pH 8.0. The mixtures were left overnight at 4° and then centrifuged at 10,000 × *g* for 5 min. The resulting supernatant was used for assaying carboxylesterase activity. The degree of inhibition of the activity by each antibody was expressed as a percentage of the control activity using control IgG. Protein was determined by the procedure of Lowry *et al.* (16) with bovine serum albumin as a standard.

Other methods. PAGE was performed in the presence of SDS as described by Laemmli (17) with 10% acrylamide gel. Proteins were silver stained as described by Wray *et al.* (18). The standard proteins used and their molecular weights were: phosphorylase *b* (94,000), bovine serum albumin (67,000), ovalbumin (43,000), and carbonic anhydrase (30,000). The molecular weights of the preparations were estimated by gel filtration on a Sephadex G-150 column (2.6 × 90 cm) equilibrated with 10 mM Tris-HCl buffer, pH 8.0. Ouchterlony double diffusion analysis was performed by the method of Noshiro and Omura (10). Radial immunodiffusion analysis of carboxylesterase isozymes of rat liver microsomes was performed by the method of Thomas *et al.* (19) using highly purified carboxylesterase isozymes.

Immunochemical staining was carried out as described by Towbin *et al.* (20) with the modification of Guengerich *et al.* (21). Briefly, SDS-PAGE was carried out essentially as described by Laemmli (17), with the exception that Pyronin Y was used as the tracking dye. Proteins were transferred from the polyacrylamide gels to nitrocellulose sheets using the general procedure of Towbin *et al.* (20). A current setting of 200 mamp was used for a period of 2 hr for electrophoretic transfers. Nitrocellulose sheets containing the transferred proteins were shaken gently at 37° in a minimal amount of PBS containing 3% (w/v) bovine serum albumin for 15 min to coat any reactive sites of the nitrocellulose. The sheets were washed six times with PBS. Each nitrocellulose sheet was then shaken with the appropriate antibody for 60 min at 37°. Antisera were used at 1:200 dilutions in PBS. Sheets were washed six times with PBS as before. Subsequently, each sheet was shaken with 2.5% (v/v) goat anti-rabbit IgG solution for 15 min at room temperature. After six washes with PBS, the sheets were shaken with a 1:100 dilution of peroxidase-antiperoxidase complex in PBS for 15 min at room temperature and then washed six times with PBS. To each tray containing a nitrocellulose sheet was added a fresh solution of 0.5 mM 3,3′-diaminobenzidine and 1.4 mM H_2O_2 in 50 mM Tris-HCl buffer (pH 7.6) to develop the stain. After 5 min of shaking at room temperature, each sheet was washed four times with water. The visualized sheets were dried under filter papers and stored.

Materials. Testosterone propionate, estradiol benzoate, palmitoyl-CoA, stearoyl-CoA, oleoyl-CoA, *p*-iodonitrotetrazolium violet, and 3-3′-diaminobenzidine hydrochloride were purchased from Sigma Chemical Co., St. Louis, MO. Saponin was obtained from Merck, Darmstadt, Germany. PBE 74, Polybuffer 94, Sephadex G-150, and protein standards were from Pharmacia, Uppsala, Sweden. *p*-Nitrophenylacetate, *p*-nitrophenylpropionate, and *p*-nitrophenylbutyrate were from Nakarai Chemicals, Kyoto, Japan. DE-52 was obtained from Whatman, Kent, England, and malathion and acetanilide were from Wako Pure Chemicals Inc., Ltd., Tokyo, Japan. Nitrocellulose sheets (0.45 μm) were obtained from Schleicher & Schuell, Dassel, West Germany. SDS, acrylamide, and other reagents for gel electrophoresis were purchased from Bio-Rad Laboratories, Richmond, CA. Goat antirabbit IgG was obtained from Cappel Laboratories, Cochranville, PA. Horseradish peroxidase-antiperoxidase complex was purchased from ICN Immunobiologicals, Lisie. Israel. Butanilicaine (*N*-butylaminoacetyl-2-chloro-6-methylanilide) and isocarboxazid were kindly donated by Hoechst AG, Frankfurt, Germany, and Nippon Roche Research Center, Kamakura, Japan, respectively. Other chemicals were of reagent grade and were purchased from commercial sources.

Results

Purification of carboxylesterase isozymes from rat liver. The procedures developed for the purification in this study are principally based on the methods of Mentlein *et al.* (9). Solubilization of carboxylesterase from microsomal membranes was carried out using 1% saponin according to the method of Mentlein *et al.* (9). Two peak fractions (A and B) were resolved by Sephadex G-150 gel filtration. Both fractions possessed *p*-nitrophenylacetate-hydrolyzing activity, whereas acetanilide-hydrolyzing activity was mainly found in the B fraction (low molecular weight). A single high molecular weight hydrolase peak (A-1) was eluted by 60 mM NaCl, and two low molecular weight hydrolase peaks (B-1 and B-2) were eluted by 30 mM and 150 mM NaCl, respectively. The 30 mM NaCl eluate fractions showed *p*-nitrophenylacetate hydrolase activity, whereas the 150 mM NaCl eluate fractions showed acetanilide and isocarboxazid hydrolase activities. These three fractions were then applied to a chromatofocusing column. The A-1, B-1, and B-2 fractions each gave a single hydrolase peak, pH 6.0, pH 6.5, and pH 5.5, respectively. Fig. 2 shows the result of SDS-PAGE of the elctrophoretically homogeneous carboxylesterases RH1, RL1, and RL2 isolated from male rats; these isozymes have different electrophoretic mobilities. A plot of the relative mobility versus the log of the molecular weight of the protein standards (data not shown) indicated a minimal molecular weight of 59,000 for RH1 and 61,000 for RL1 or RL2. Carboxylesterases RL1 and RL2 behave as monomers (M_r 60,000) on Sephadex G-150 gel chromatography, whereas RH1 behaves as a trimer (M_r 174,000) (data not shown).

Differences in the catalytic activities of the three carboxylesterase isozymes. Table 1 shows the catalytic activities of carboxylesterase isozymes RH1, RL1, and RL2 toward

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several substrates. RL1 has the highest activities for the hydrolysis of *p*-nitrophenylacetate, *p*-nitrophenylpropionate, and malathion. Acetanilide is a specific substrate of RL2, whereas RH1 has the highest specific activity for butanilicaine. RH1 and RL2 are capable of hydrolyzing isocarboxazid, and RH1 and RL1 hydrolyze clofibrate at similar rates. In contrast, RL1 efficiently catalyzes the hydrolysis of long-chain acyl-CoAs, which are poorly hydrolyzed by RH1.

Immunochemical differences in the three carboxylesterase isozymes. After proteins were transferred to nitrocellulose, staining using the immunochemical procedure indicated a high degree of selectivity as well as sensitivity of the antibodies: namely, purified isozymes, RH1, RL1, and RL2 were stained by only their respective antibodies (Fig. 3). In the case of microsomes, staining with antibodies showed only one band.

As shown in Fig. 4, antibodies toward purified isozymes affected the *p*-nitrophenylacetate hydrolase activity. Anti-RH1 IgG was capable of strongly inhibiting the activity of purified carboxylesterase RH1, whereas the other two preparations were not affected. In contrast, anti-RL1 IgG strongly inhibited only the activity of purified carboxylesterase RL1, and anti-RL2 IgG strongly inhibited only the activity of purified carboxylesterase RL2.

Radial immunodiffusion assay to determine the amounts of carboxylesterase RH1, RL1, and RL2 in liver microsomes from male and female rats. The results of radial immunodiffusion assay for carboxylesterase reactive with anti RL1-IgG are shown in Table 2. In our previous paper (6), we reported that castration of male rats resulted in a decrease in certain carboxylesterase activities, and that the decrease of activity could be restored by treatment of the castrated rats with testosterone propionate. In accordance with that conclusion, the amount of RL1 was decreased by castration

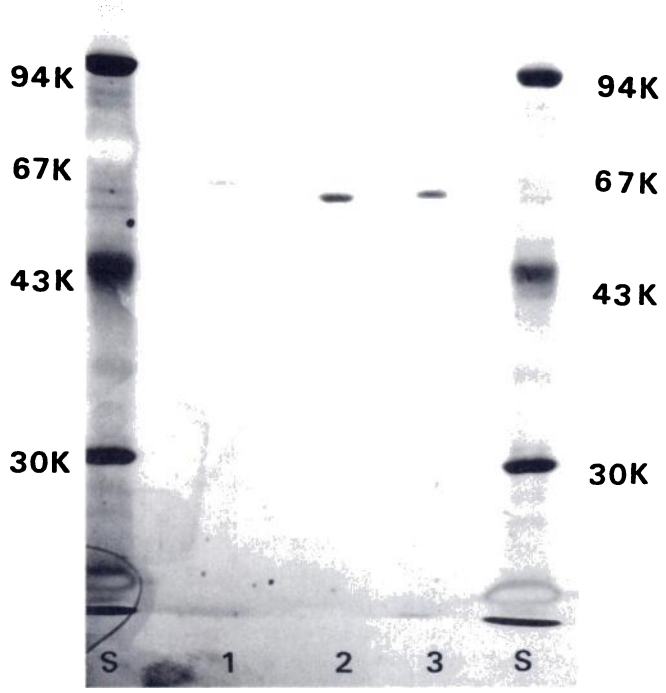

Fig. 2. SDS-PAGE of purified preparations of carboxylesterase RL1, RH1, and RL2. The gel was stained alkaline $AgNO_3$ as described elsewhere (18). *S*, standard proteins: phosphorylase *b* (*94K*), bovine serum albumin (*67K*), ovalbumin (*43K*), and carbonic anhydrase (*30K*). *1*, carboxylesterase RL1; *2*, carboxylesterase RH1; *3*, carboxylesterase RL2.

TABLE 1

Catalytic activity of carboxylesterase isozymes RH1, RL1, and RL2

Results are expressed as means of duplicate assays.

Substrate	Catalytic activities of isozymes		
	RH1	RL1	RL2
	μmol/mg protein/min		
p-Nitrophenylacetate	44.8	76.4	4.16
p-Nitrophenylpropionate	53.9	160.7	8.63
p-Nitrophenylbutyrate	20.5	20.7	3.43
Malathion	0.011	0.201	<0.002
Butanilicaine	3.44	<0.005	<0.005
Acetanilide	0.017	<0.002	0.116
Isocarboxazid	0.268	<0.001	0.263
Clofibrate	0.250	0.242	<0.002
Palmitoyl-CoA	0.009	0.686	<0.002
Stearoyl-CoA	0.009	0.343	<0.002
Oleoyl-CoA	0.009	0.196	<0.002

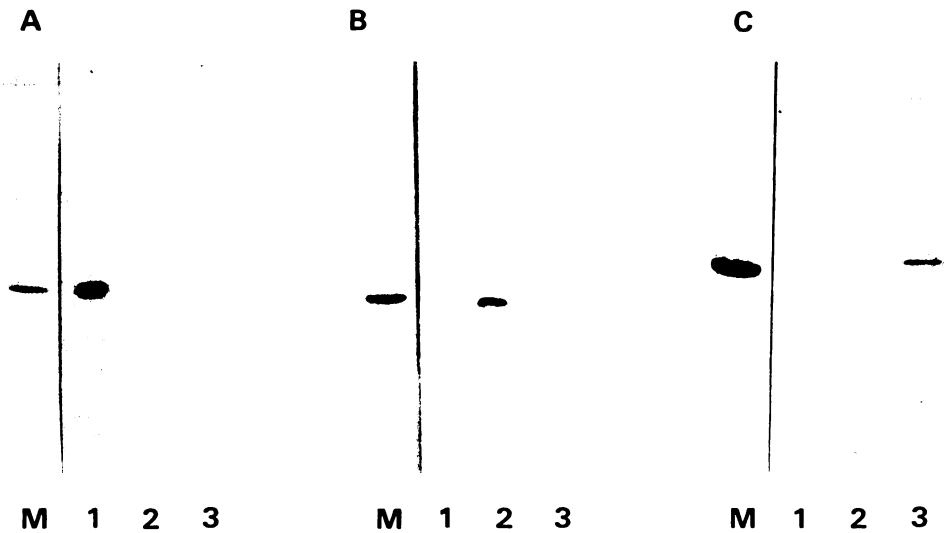

Fig. 3. Immunochemical staining of carboxylesterase RL1, RL2, and RH1 after transfer from SDS-polyacrylamide gel to nitrocellulose. Liver microsomes (10 μg) from male rat and the purified preparation of RL1 (0.25 μg), RL2 (0.25 μg), and RH1 (0.25 μg) were electrophoresed on SDS-polyacrylamide gels, transferred to a sheet of nitrocellulose, and stained immunochemically as described under Materials and Methods. Antibodies to carboxylesterase RL1 (A), RH1 (B), and RL2 (C) were used. *M*, rat liver microsomes; *1*, carboxylesterase RL1; *2*, carboxylesterase RH1; *3*, carboxylesterase RL2.

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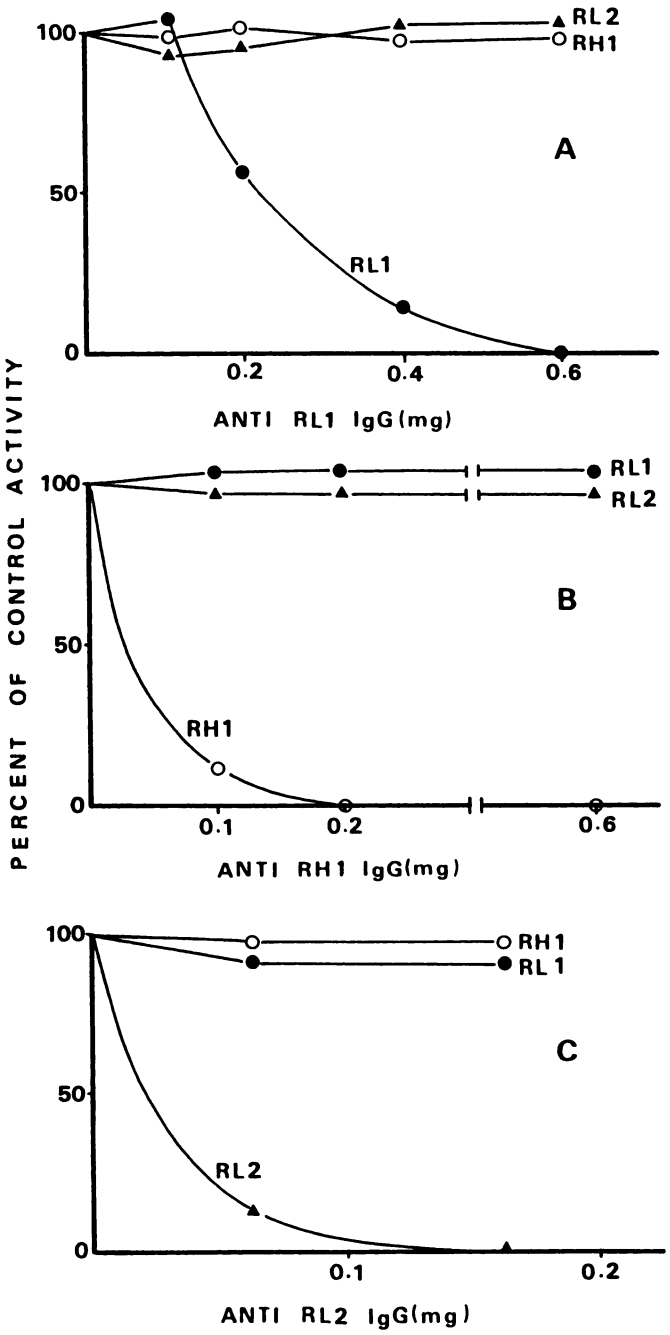

Fig. 4. Effect of antibodies on the *p*-nitrophenylacetate hydrolase activities of the three purified carboxylesterase isozymes. The specific activities of carboxylesterase RH1, RL1, and RL2 used in this experiment were 0.45, 0.40, and 0.41 μmol/mg of protein/min, respectively. A, anti-RL1 IgG; B, anti-RH1 IgG; C, anti-RL2 IgG.

and recovered after testosterone treatment. Conversely, in female rats, the amount of RL1 was increased by castration but was decreased by estradiol benzoate treatment. The amount of RH1 in male rats was decreased by castration and did not recover after testosterone treatment. Conversely, the amount of RL2 in female rats was decreased by castration and recovered after estradiol treatment. The results of analysis of carboxylesterase isozymes by radial immunodiffusion assay in the livers of male and female rats treated with phenobarbital are shown in Table 3. The amount of RL1 was not affected by phenobarbital treatment, whereas the amounts of RH1 and RL2 were greatly increased by phenobarbital treatment in male and female rats.

Discussion

In recent years, isozymes of carboxylesterases have been purified from mammalian liver microsomes (1, 2), and the molecular weight, catalytic activity, and subunit structure have been reported. However, few reports have appeared on sex-related differences in the regulation of the isozymes.

The results of SDS-PAGE and immunochemical blotting analysis, as well as the selective inhibition of catalytic activity by the homologous antibody, support the concept that antibodies prepared against each of the carboxylesterase isozymes have high specificity. Consequently, these antibodies can be used to quantitate each individual form of carboxylesterase in microsomes. The immunoquantitation of carboxylesterases in rat liver microsomes and the effects of sex hormones and xenobiotic treatment on the levels of these three carboxylesterase isozymes were therefore investigated.

In 1980, Mentlein *et al.* (9) reported the purification of five isozymes from rat liver microsomes; these isozymes showed different catalytic activities for the hydrolysis of various substrates. Some of these isozymes had similar substrate specificity and molecular weights similar to those of the isozymes which we purified: RL1, RH1, and RL2 resemble hydrolase pI 6.2/6.4, hydrolase pI 6.0, and hydrolase pI 5.6, respectively, although careful comparison, possibly involving exchange of the proteins between laboratories, will be required to establish whether any are identical.

Table 1 shows the substrate specificities of carboxylesterase isozymes toward several substrates. RL1 showed the highest catalytic activity toward the hydrolysis of *p*-nitrophenylesters, malathion, and long-chain acyl-CoAs. Hydrolysis of butanilicaine was catalyzed most effectively by RH1. Acetanilide is a rather specific substrate for RL2. RH1 and RL2 are capable of hydrolyzing isocarboxazid, and RH1 and RL1 hydrolyze clofibrate at similar rates. According to the substrate specificity data, these three isozymes play different roles in drug and lipid metabolism.

In the previous paper (6), we investigated the sex difference and changes in carboxylesterase activity toward various substrates, and it was suggested that hepatic microsomal carboxylesterases may be regulated by gonadal hormones which exert different effects on the several isozymes of carboxylesterases. In accordance with that conclusion, we found that the amount of carboxylesterase RL1 was exclusively regulated by gonadal hormones, whereas the amount of RH1 remains unclear at present. However, the amount of RL2 may be, at least in part, regulated by estrogens.

On the other hand, phenobarbital treatment increases the amounts of RH1 and RL2, but not that of RL1. Thus, low pI carboxylesterase (RL2) was induced by phenobarbital. Our data agree with the results of Robbi and Beaufay (5).

It is concluded that these three isozymes differ considerably in response to hormone treatment, inducibility, substrate specificity, and immunological properties.

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TABLE 2

Immunochemical quantitation by radial immunodiffusion assay of carboxylesterase isozymes in the livers of male and female rats: effects of castration and treatment with gonadal hormones

Each value (μg/mg of protein) is the mean ± SE from five preparations. For other experiments, see the text.

Isozyme	Sex	Sham operated	Castrated	Castrated + TSP[a]	Castrated + EDB[a]
RL1	male	11.61 ± 0.82	3.06 ± 0.08[b]	10.81 ± 0.88[c]	1.69 ± 0.53[b]
	female	4.16 ± 1.12	9.38 ± 1.33[d]	11.43 ± 1.90[d]	1.16 ± 0.23[e]
RH1	male	31.66 ± 0.99	15.53 ± 1.60	18.52 ± 2.27	7.46 ± 0.40[b,c]
	female	16.04 ± 1.79	21.17 ± 3.27	13.01 ± 1.73	7.82 ± 1.75[f,g]
RL2	male	35.99 ± 2.03	37.76 ± 4.41	31.79 ± 4.41	41.22 ± 4.06
	female	63.26 ± 7.28	49.95 ± 10.16	32.93 ± 1.32[d]	51.56 ± 5.94

[a] TSP, testosterone propionate; EDB, estradiol benzoate.
[b] $p < 0.001$ vs. sham operated.
[c] $p < 0.001$ vs. castrated.
[d] $p < 0.05$ vs. sham operated.
[e] $p < 0.01$ vs. castrated.
[f] $p < 0.01$ vs. sham operated.
[g] $p < 0.05$ vs. castrated.

TABLE 3

Immunochemical quantitation by radial immunodiffusion assay of carboxylesterase isozymes in the livers of male and female rats treated with phenobarbital (PB)

Each value is the mean ± SE from five preparations. For other experiments, see the text.

Isozyme	Sex	Concentration	
		Control	PB
		μg/mg protein	
RL1	male	14.15 ± 0.65	16.97 ± 0.72
	female	7.31 ± 0.15	10.11 ± 1.91
RH1	male	16.66 ± 2.73	40.33 ± 5.11[a]
	female	13.51 ± 2.42	23.53 ± 1.01[b]
RL2	male	31.27 ± 3.62	78.47 ± 4.07[c]
	female	86.26 ± 3.84	160.22 ± 20.45[b]

[a] $p < 0.01$ vs. control.
[b] $p < 0.05$ vs. control.
[c] $p < 0.001$ vs. control.

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