GENETIC AND PHYSIOLOGICAL CONTROL OF ESTERASES IN EXPERIMENTAL SMALL ANIMALS III. HORMONAL REGULATION IN THE ACTIVITIES OF PSEUDO-CHOLINESTERASE ISOZYMES

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III. HORMONAL REGULATION IN THE ACTIVITIES OF PSEUDO-CHOLINESTERASE ISOZYMES

By

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Introduction

Different molecular forms of enzymes which act on the same substrates have been reported to occur within a single tissue. The various types of the enzymes have been called "isozymes", "hybrid" enzymes, "atypical" form and variant form, respectively (1, 2, 3, 4, 5). Some of the enzymes in animal sera and tissues having different electrophoretic mobilities have been demonstrated to be genetically controlled by two separate genes or autosomal allelic genes (2, 3, 5, 6). The various activity-levels of the enzymes in animal sera and tissues also have been reported to be genetically determined by multiple allelic genes (7, 8). However, the activity-levels of enzymes are not only genetically controlled by allelic genes, but also physiologically regulated by various hormones.

Differences of the activity-levels of some serum enzymes have been observed according to sex, or stage of pregnancy or lactation in mice, rats and rabbits (9, 10, 11). It has been demonstrated that the castration of males and females of mice and rats resulted in marked alterations of the intensities of the various enzymes, i.e., succinic dehydrogenase, β-glucuronidase, alkaline phosphatase and esterase etc., in the epididymis and seminal vesicle of male and the uterus and vagina of female, respectively, and the treatment with sex hormones to the castrated animals restored the enzyme activities to normal levels (12, 13, 14, 15, 16, 17, 18).

Although there have been extensive clinical investigations of human and animal serum cholinesterases, little is known about the physiological function and the regulative mechanisms of the cholinesterase isozymes. The approach that will make the function of the enzyme clear is to determine the source-organs and the hormonal regulative mechanisms in the synthesis of the enzymes. In the previous reports (19, 20, 21), several zones of cholinesterase activity were separated by starch-gel electrophoresis and showed remarkable individual variations in horse sera.

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In this connection, the present paper deals with the differences of the activity-levels of pseudo-cholinesterase isozymes among males, non-pregnant, pregnant and lactating females and the effects of castrations and the sex hormone treatments on the enzyme activities in the sera and organs of mice and rats.

Materials and Methods

Sera and tissues from mice of DD/Sd strain and C_3H strain and rats of Donryu strain were used to separate the esterases by vertical starch-gel electrophoresis (22) using the following discontinuous buffer system. The Tris-citrate buffer used for preparing the gel contained: 4.6 g of Tris (0.038 M) and 0.525 g of citric acid (0.0025 M) dissolved in 1L of distilled water. The buffer used in the electrode vessels contained: 18.5g of boric acid (0.3 M) and 2.4g of NaOH (0.06M) dissolved in 1L of distilled water.

Organs for the examination were weighed by torsion balance and homogenized with deionized water in a glass homogenizer. The supernatant fluid separated from the homogenate of one part organs in three parts water was used for the analysis.

The electrophoretic separation was carried out for 16～18 hours at room temperature with 135V applied over the gel. The esterases on the starch-gels were stained histochemically with α-naphthyl acetate or β-carbonaphthoxycholine iodide and naphthanil diazo blue B in 0.2M phosphate buffer, pH 6.8.

Categories of the experimental samples included: (a) male sera, non-pregnant, pregnant and lactating female sera of mice, (b) male or female sera from mice and rats which were castrated and treated with testosterone propionate or estradiol benzoate, respectively, (c) uterus and other organs from mice and rats which were ovariectomized and treated with estradiol benzoate or progesterone. Testosterone propionate was injected subcutaneously as sesame oil solution into castrated male mouse, 0.5 mg daily for 7 days and into castrated male rat, 1mg daily for 10 days. Estradiol benzoate was injected subcutaneously as water suspension into castrated female mouse, 1 μg daily for 7 days and into castrated female rat, 10 μg daily for 10 days. Progesterone was injected as sesame oil solution into castrated female mouse, 50 μg daily for 7 days.

Results

1. Variations of the the activity-levels of serum cholinesterase isozymes in male, non-pregnant, pregnant and lactating female mice.

In the course of the genetic study on the serum esterase zone D, which was reported in the previous paper (8), wide variation of the activity-levels of serum esterase zone C_3, which had slightly greater mobility than that of the zone D, was found in the mice of DD/Sd strain and C_3H strain as shown in Fig. 1 and Table 1. The activity-levels of zone C_3 in the sera of adult female mice were always higher

Table 1. Variations of the activity-levels of serum cholinesterase isozymes in male, non-pregnant, pregant and lactating female mice.

Sex	Physiological conditions	Numbers of cases	Activity-levels of serum cholinesterase				
			−	+	++	+++	++++
Male	Normal	50	3	47	0	0	0
Female	Normal	38	0	3	35	0	0
Female	Pregnant	20	0	0	1	9	10
Female	Lactating	8	0	0	3	5	0
Total numbers		116	3	50	39	14	10

than those in the sera of adult male and immature mice (Fig. 1a, b and c). The activity of zone C_3 increased markedly as the pregnancy developed in all cases and reached a maximum activity-level at the end of the pregnant period (Fig. 1d and Table 1). After parturition, the activity decreased progressively to the normal level at the end of the lactation period (Fig. 1e).

Since this zone C_3 and the other zone C_4 hydrolyzed β-carbonaphthoxycholine

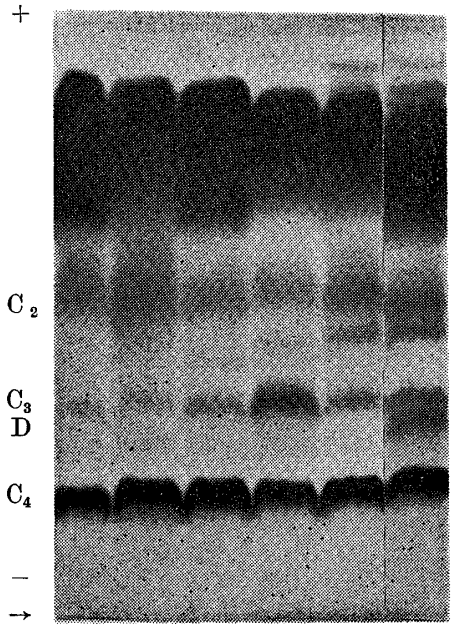

Fig. 1.

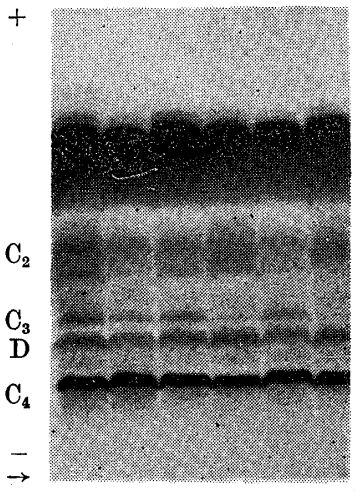

Fig. 2.

Fig. 1. Zymograms of serum cholinesterase isozymes in mice under various physiological conditions following starch-gel electrophoresis.
(a) Immature female, (b) normal adult male, (c) normal adult female, (d) pregnant female, (e) lactating female mice of DD/Sd strain, and (f) lactating female mouse of C_3H strain.

Fig. 2. Zymograms showing effects of castrations and treatments with sex hormones on the activity-levels of serum chlolinesterase isozymes in mice of C_3H strain.
(a) Normal female, (b) ovariectomized female, (c) ovariectomized female administered estradiol benzoate 1 μg daily for 7 days, (d) normal male, (e) castrated male, (f) castrated male administered testosterone propionate 0.5 mg daily for 7 days.

iodide histochemically or benzoylcholine spectrophotometrically and their activities were inhibited by eserine, 5×10^{-5}M, and TEPP, 10^{-5}M, they were considered to be multiple molecular forms, i.e., isozymes of pseudocholinesterase. Zone C_2 also hydrolyzed the β-carbonaphthoxycholine iodide, but the zone appeared to be mixture of two kinds of esterases, one being of a cholinesterase activity. Zone C_4 always had the greatest activity among the three esterase C zones. In the contrast to the activity of zone C_3, the activity of zone C_4 did not increase, but rather slightly decreased during pregnancy.

2. Effects of castrations and treatments with sex hormones on the activity-levels of serum cholinesterase isozymes in mice and rats.

Mouse serum cholinesterases: The activity-levels of serum cholinesterase isozymes on the starch-gels were compared among normal, castrated, and sex hormone treated castrated mice in both sexes as shown in Fig. 2. Castration of female mice resulted in a decline of the serum cholinesterase zone C_3(Fig. 2b). Estradiol benzoate administration to castrated female mice was followed by elevation of the enzyme activity to normal level (Fig. 2c). Progesterone administration had no effect on the enzyme activity of the castrated females. The activity-level of zone C_3 of normal male mice was apparently lower than those of normal female mice (Fig. 2d). Castration of males, on the other hand, was followed by a rise of the enzyme activity toward an intermediate level somewhat below those in normal females (Fig. 2e). Testosterone propionate administration to castrated male mice depressed the enzyme activity to a level as low as those in normal males (Fig. 2f).

Rat serum cholinesterases: The activity of serum cholinesterase zone C_4 was much greater in mature female rats than in male rats and increased during pregnancy. In contrast to the cholinesterase zone C_3 in mouse sera, the activity-level of zone C_3 in rat sera was very low in both sexes. Figure 3 illustrates the effects of castration and estrogen treatment on the activity-levels of serum cholinesterases in adult female rats. Castration of the female rats resulted in a marked decrease of the activity of the serum cholinesterase zone C_4 to levels as low as those in normal male rats (Fig. 3c and d). Estradiol benzoate administration to castrated female rats was followed by an elevation of the enzyme activities to levels as high as those in normal female rats (Fig. 3 e, f, g and h). Zone C_5, which was frequently found in the various activity-levels in the male and female rats of Donryu strain, indicated also the characteristic of pseudocholinesterase (Fig. 3 f). Some of the rat sera contained zone C_5, but not zone C_4. The activity of this zone also increased by the administration of estradiol benzoate to the castrated females. The activity of zone C_3 was low, but the enzyme indicated the similar changes to those of zone C_4 by the castration and the estrogen treatment. On the other hand, castration of male rats was followed by a rise of the cholinesterase zone C_4 toward an intermediate

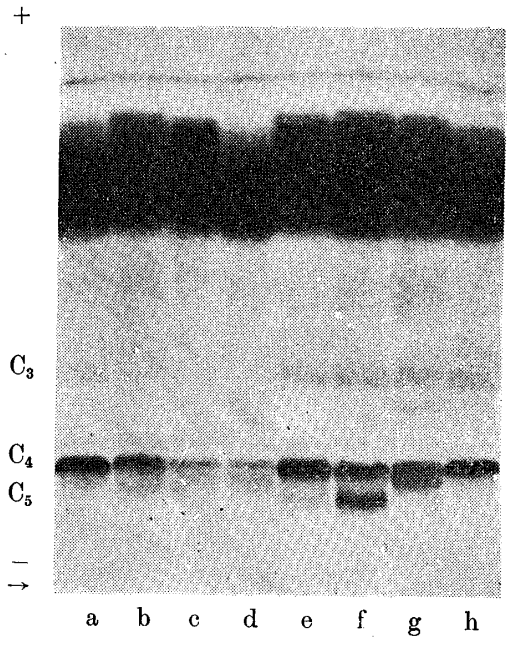

Fig. 3.

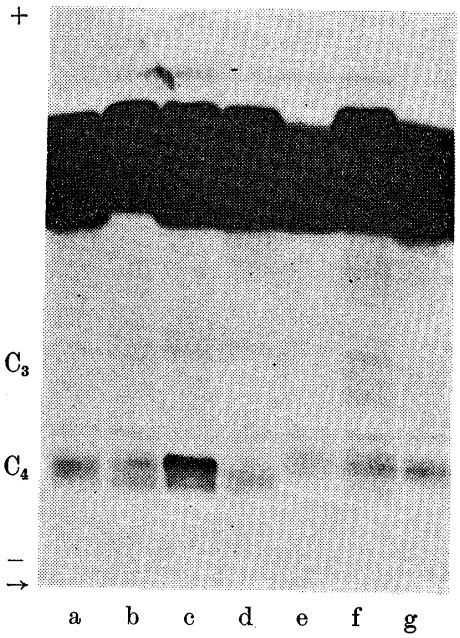

Fig. 4.

Fig. 3. Effects of ovariectomy and estradiol treatment on the activity-levels of serum cholinesterases in female rats of Donryu strain.
(a) and (b) Normal females, (c) and (d) ovariectomized females, (e), (f), (g) and (h) ovariectomized females administered estradiol benzoate 10 μg daily for 10 days, respectively.

Fig. 4. Effects of castration and testosterone tratment on the activity-levels of serum cholinesterases in male rats of Donryu strain.
(a) and (b) Normal males, (c) castrated male, (d), (e), (f) and (g) castrated males administered testosterone propionate 1 mg daily for 10 days, respectively.

level somewhat below those in normal females as shown in Fig. 4. Testosterone propionate treatment depressed the serum enzyme activity of castrated rats to levels as low as those in normal males (Fig. 4 d, e, f and g).

3. Effects of castration, estrogen treatment, and pregnancy on the activity-levels of pseudo-cholinesterase isozymes in the uterus and other organs in mice and rats.

Mouse uterus and placenta cholinesterases: Zymograms of mouse uterus and placenta esterases produced with α-naphthyl acetate following the starch-gel electrophoresis showed about 9 zones with esterase activity as shown in Fig. 5. Two zones, C_1 and C_4, of these esterase zones hydrolyzed the β-carbonaphthoxycholine iodide and were inhibited by eserine, 5×10^{-5}M, indicating the characteristic of pseudo-cholinesterase. In contrast with the zymograms of the serum cholinesterase isozymes, the activity-levels of zone C_1 in the normal and pregnant uteruses hydrolyzing the choline ester were greater than those of zone C_4 in the same tissues. While,zone C_3, which were apparently demonstrated in the sera of normal and pregnant mice, was not found in the normal and pregnant

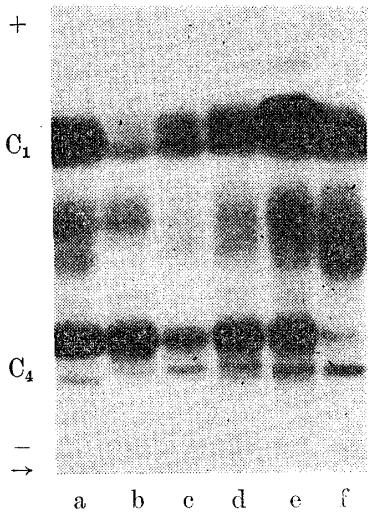

Fig. 5.

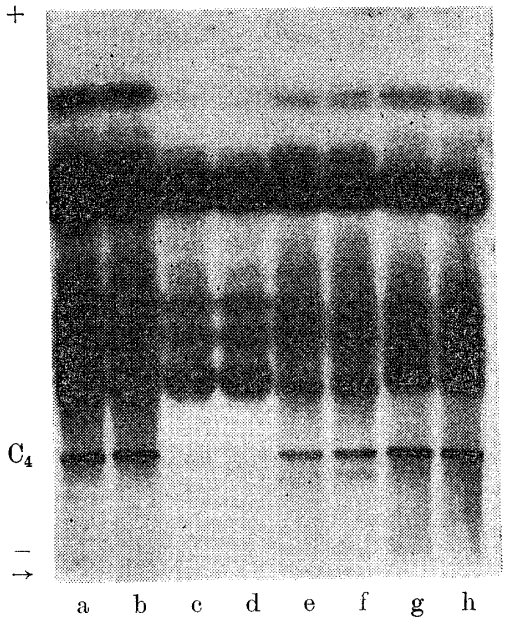

Fig. 6.

Fig. 5. Effects of ovariectomy, estradiol treatment and pregnancy on the activity- levels of pseudo-cholinesterase isozymes in uterus and placenta of mice.
(a) Normal female uterus, (b) ovariectomized female uterus, (c) and (d) ovariectomized female uteruses treated with estradiol benzoate 1 μg daily for 7 days. (e) pregnant uterus, (f) pregnant placenta.

Fig. 6. Effects of ovariectomy and estradiol treatment on the activity-levels of pseudo-cholinesterase in uterus of rats.
(a) and (b) Normal female, (c) and (d) ovariectomized female, (e), (f), (g) and (h) ovariectomized females treated with estradiol benzoate 10 μg daily for 10 days, respectively.

uteruses as well as the other organs, placenta, mammary glands, liver, spleen and kidney. Ovariectomy of female mice depressed the activity-levels of the cholinesterase zones C_1 and C_4 in the uterus samples to those of normal female mice (Fig. 5a and b). Estradiol benzoate treatment to the castrated mice elevated the activity of zone C_4 to the normal female levels and the activity of zone C_1 to intermediate levels somewhat below those in normal females (Fig. 5c and d). The activity-levels of the two zones, C_1 and C_4, in the uterus and placenta of the pregnant mice were similar to those found in normal mouse uterus (Fig. 5 e and f).

Rat uterus cholinesterases: Zymograms of rat uterus esterases separated by the electrophoresis showed about 9 distinct zones as shown in Fig. 6. Of these esterase zones, only one zone, C_4, indicated the characteristic of pseudo-cholinesterase. Ovariectomy abolished the activity of zone C_4 in uterus (Fig. 6c and d). Estradiol benzoate administration to the ovariectomized rats restored the enzyme activities to the levels of those found in normal females (Fig. 6 e, f, g and h).

Discussion

It has been reported that sex hormones regulate the activity-levels of several enzymes and the synthesis of proteins in male and female animals (23, 24, 25, 26). However, the hormonal regulation of isozymes in organs have not been apparent except for lactic dehydrogenase isozymes (27). In the present paper, it was found that the activity-levels of serum cholinesterase isozyme, zone C_3, in female mice were always higher than those in male mice and increased markedly as pregnancy developed. The activity-levels of the main cholinesterase, zone C_4, in the females were also higher than those in the males, but the enzyme activity did not increase but rather slightly decreased as pregnancy developed. On the other hand, in the electrophoretic analysis of several organs of the normal and pregnant female mice, the uterus and placenta apparently contained the pseudo-cholinesterase isozymes, zones C_1 and C_4. While, the liver had only very low activities of the enzymes. In rat sera, the activity-level of the cholinesterase zone C_4 in females was considerably greater than that in males and still more increased during pregnancy. Zone C_4 was also contained in the rat uterus. The results suggest that the main source-organs in the synthesis of the serum cholinesterases in females were not liver, but uterus and placenta which had an affinity to the physiological effects of the sex hormone and increased the weights during pregnancy. This consideration will offer the explanation for the sex differences of serum cholinesterase isozymes and the elevations of the activity-levels of the enzymes during pregnancy. However, the cholinesterase isozyme zone C_3, which increased markedly in pregnant mouse sera, was not found in the all organs examined, uterus, placenta, mammary glands, liver, spleen and kidney. Reiner et al. (28) suggested that serum cholinesterase is an aggregate of polymerized sub-units and the enzyme may have different molecular weights according to the degree of aggregation.

However, in the course of the experiments of the previous reports (19, 20, 21), the cholinesterase isozyme zone C_2 in horse sera, corresponding to zone C_3 in mouse sera, showed immunochemically partially different characteristic from the main cholinesterase zone C_4. In the experimental results (21), cholinesterase isozymes, zone C_2 and zone C_4, in horse serum were purified and concentrated respectively by the combination of Strelitz's method (29) and starch-gel electrophoresis. The purified and concentrated zone C_4 was injected with Freund's adjuvant into rabbits to produce the specific antibody. The activity of the eluate of main cholinesterase zone C_4 was inhibited 78 and 93% by the rabbit anti-C_4,. While, the activity of zone C_2 which was equal to that of zone C_4 was only inhibited 50 and 75% by the anti-C_4. The results suggested that the immunological and chemical structures of zone C_2 were partially different from those of zone C_4. On the other hand, it has been demonstrated that lactic dehydrogenase from animal tissues was electrophoretically separated into five isozymes each of which was a tetramer (1, 2). Two of the isozymes, which were called "parental" types, were chemically and

immunologically different. Three other isozymes, which were called "hybrid" enzymes, were intermediate types consisting of the two kinds of components, respectively (2, 3). From the electrophoretic and immunological characteristics, it is conceivable that some of the serum cholinesterase isozymes also consist of two kinds of components, one deriving from the main cholinesterase zone C_4.

Castrations of females depressed the activities of zone C_4 in rat sera and uterus, and also the activities of zone C_3 in mouse sera as well as zones C_1 and C_4 in mouse uterus. Estradiol benzoate treatments to the castrated rats and mice restored the activities of all the enzymes to the normal levels. On the other hand, castration of males resulted in elevation of the activities of zone C_4 in rat sera and zone C_3 in mouse sera. Testosterone propionate administrations to the castrated animals depressed the activities of the enzymes. It was concluded that the activity-levels of the pseudo-cholinesterase isozymes were regulated by both sex hormones.

The biological role of pseudo-cholinesterase has not been apparent. However, it has been proposed that the enzyme behaves principally similar to the specific cholinesterase and participates only in relatively slow nerve conductive processes (30). Clitherow, Mitchard and Harper (31) suggested that the principal biological function of serum cholinesterase might be to hydrolyze butyrylcholine preferentially which had a powerful nicotinic action. From the present results, it seems likely that as one of the physiological functions, pseudo-cholinesterase isozymes may be concerned with the metabolism of choline ester and the relaxation of uterus.

Summary

Zymograms of pseudo-cholinesterase isozymes in sera and tissues of mice and rats were examined under the various physiological conditions by starch-gel electrophoresis.

The activity-level of serum cholinesterase isozyme, zone C_3, in female mice was greater than that in male mice and increased markedly as pregnancy developed. In female rats, the activity-level of serum cholinesterase, zone C_4, was much greater than that in males and elevated during pregnancy.

The activity-levels of zone C_3 in female mice and zone C_4 in female rats decreased by ovariectomy and elevated to the normal level by estradiol benzoate treatment to the ovariectomized animals. While, the activities of zone C_3 in male mouse sera and zone C_4 in male rat sera increased by castration and were depressed to normal levels by testosterone propionate treatment, respectively.

The activity-levels of the pseudo-cholinesterase isozymes, zones C_1 and C_4 in mouse uterus and zone C_4 in rat uterus, were also depressed by ovariectomy and elevated to normal levels by estradiol benzoate administration to the ovariectomized animals.

It was considered that the activity-levels of pseudo-cholinesterase isozymes in mice and rats were regulated by both sex hormones, estradiol and testosterone, and

that the main source-organs on the synthesis of the cholinesterase isozymes in the female animals were uterus and placenta having an affinity to the physiological effects of estradiol. The mechanism of occurrence of the serum cholinesterase isozymes was also discussed.

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