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EFFECTS OF HYPOPHYSECTOMY ON ACETYLCHOLINESTERASE AND BUTYRYLCHOLINESTERASE IN THE RAT

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Abstract—Acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) activities were examined in several tissues of normal and hypophysectomized male and female rats. Significant sex differences in the mean AChE activities of normal rats were observed in the superior cervical ganglion (three times more activity in males) and in serum (50% more activity in females). Sex differences in the BuChE activity of serum and liver were even larger (ten times more activity in females), but the activity of other tissues was similar in both sexes. Hypophysectomy had little effect on the mean activity of AChE but did alter BuChE activity in certain tissues. Most of the effects of hypophysectomy on mean BuChE activity were opposite in direction in the two sexes. For example, in males hypophysectomy caused increases in the BuChE activity of serum (300%) and liver (43%), while in females it caused decreases in both tissues (25 and 30% respectively). In rats of a given group, the AChE activity of each tissue appeared to be regulated independently of the activity in other tissues. By contrast, BuChE activity showed statistically significant correlations in more than half of the tissue-pairs examined in control rats of either sex. These correlations can be considered to reflect a tendency toward body-wide regulation. In female rats, the cross-tissue correlations were largely eliminated by hypophysectomy. This finding indicates that the regulation of BuChE may be strongly affected by hormones under the control of the pituitary gland. However, in male rats, only the correlations involving atria were altered by hypophysectomy. Therefore, the effects of hormones on BuChE are probably both sex and tissue dependent.

Acetylcholinesterase (AChE, EC 3.1.1.7) and butyrylcholinesterase (BuChE, EC 3.1.1.8) are widely distributed enzymes whose roles outside the cholinergic synapse are not yet completely defined [1]. Recent work shows that both AChE and BuChE are capable of degrading substance P [2, 3]. Furthermore, BuChE is known to be involved in the metabolism of many drugs that act on nerve and muscle cells, including muscle relaxants (e.g. succinylcholine [4]), local anesthetics (e.g. procaine [5]), and narcotic analgesics (e.g. diacetylmorphine [6]). For these reasons, it is of pharmacological interest to elucidate the factors responsible for regulating the cholinesterases.

In an approach to this problem, we previously examined the individual variation of cholinesterase activities in different tissues of male rats of various strains [7]. As expected, genetic factors were found to be important determinants of cholinesterase activity. However, in outbred rats of the Sprague–Dawley strain, we noted a striking divergence between the pattern of variation of AChE activity and that of BuChE. On the one hand, the relative AChE activity of each tissue was independent of that in other tissues of the same rat; on the other hand, the relative BuChE activity of most tissues varied in parallel from rat to rat.

One factor that could cause parallel variation of enzyme activity in different tissues would be an endocrine hormone. Both AChE and BuChE are known to respond to endocrine influences. For example, castration lowers the BuChE activity of serum in female rats while raising it in males [8]. The cholinesterases have also been found to be affected by adrenalectomy [9] and thyroidectomy [10].

Recent work suggests that the effects of sex steroids on serum cholinesterases of the rat are mediated through the hypothalmic-hypophyseal axis [11]. To test the possibility that this neuro-endocrine system is responsible for the tendency towards body-wide regulation of BuChE activity, we have investigated the effects of hypophysectomy on the cholinesterases in several tissues of male and female rats.

MATERIALS AND METHODS

Animals. Male and female Sprague–Dawley rats (4 months old) were obtained from Hormone Assay Laboratories, Chicago, IL. Hypophysectomy was performed 3 weeks before shipment; rats were held for 1 month after arrival to allow for recovery from the stress of surgery and transport. Sham-operated and unoperated animals were used as controls. Since no differences between the cholinesterase activities of these groups were observed, data from the appropriate sex were pooled.

The rats had free access to Purina rat chow and water. The diet was supplemented daily with fresh oranges, which significantly improved the survival rate after hypophysectomy. Animals were housed from birth in wire mesh cages. Soap, organic detergents, and insecticides were never introduced into the animal quarters, and there were no identifiable

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chemical influences in the environment that would have been expected to affect the cholinesterases.

Tissues. Rats were anesthetized with ether. A 1-ml sample of blood was then removed by cardiac puncture, allowed to clot for 1 hr at 4°, and centrifuged at 1500 *g* for 10 min at 4°. The isolated serum was diluted 1:10 with ice-cold antiprotease buffer of the following composition: 50 mM sodium phosphate, pH 7.4; 4% (w/v) sucrose; 1% (v/v) Triton X-100; 1 mg/ml bacitracin; 2 mM benzamidine hydrochloride; 20 μg/ml pepstatin; and 5 mM *N*-ethylmaleimide. Diluted serum samples were stored at 4° and assayed for cholinesterase activity within 24 hr.

Immediately after the blood sample was taken, a catheter was introduced into the left ventricle, and the rats were exsanguinated by perfusion with 150–200 ml of 0.9% NaCl (room temperature). Organs were then removed, including the left hemidiaphragm, the two atria (sampled in only a few females), the two superior cervical ganglia, the brain, and the liver (not assayed for AChE in all of the males). These organs were cleaned of connective tissue, washed in cold antiprotease buffer, blotted and weighed on a Mettler HL32 electronic balance (Mettler Instrument Corp., Hightstown, NJ) or a Cahn Gram Electrobalance (Cahn Instrument Co., Paramount, CA). For further work, pieces of about 200 mg were cut from the larger organs (e.g. from the left front lobe of the brain and from the median lobe of the liver). In some cases, the water content of the tissues was determined. For this purpose, the samples were weighed fresh and then dried at 80° in a convection oven for 4 days or until a constant weight was reached. Dry weight was then calculated as a percentage of the wet weight, and the difference from 100% was taken to represent tissue water (recognizing that it might include a small proportion of other volatile components).

Tissue samples to be used for the enzyme assay were frozen in antiprotease buffer immediately after removal. They were stored at −20° until assay, usually within 1 week. On the day of the assay, the tissues were thawed and homogenized. The paired superior cervical ganglia were homogenized in all-glass homogenizers containing 1.5 ml of cold antiprotease buffer. Other tissues were homogenized in 10 vol. of fresh buffer in a Polytron homogenizer (Brinkmann Instruments, Westbury, NY) at setting No. 5 for 30 sec. Homogenates were centrifuged at 16,000 *g* for 10 min at 4°. On the basis of previous work [7] it was anticipated that about 80% of the total cholinesterase activity in each tissue would be recovered in the supernatant fractions.

Assays. AChE was assayed by a modification of the method of Potter [12] using [^{14}C]acetate-labeled acetylcholine as a substrate (1 mM) as previously described [13]. Duplicate 50-μl samples were assayed at 37° in the presence of 10^{-4} M ethopropazine hydrochloride (Warner Lambert Pharmaceuticals, Morris Plains, NJ) to inhibit BuChE by over 99%. Blank samples also contained the specific AChE inhibitor BW284C51 [1,5-bis(4-allydimethylammoniumphenyl)pentan-3-onedibromide, Burroughs Wellcome Co., Research Triangle Park, NC] in a concentration of 10^{-5} M. Activity was calculated in units of μmoles substrate hydrolyzed per hr.

BuChE was measured in duplicate samples by an essentially similar radiometric method using [^{14}C]butyrate-labeled butyrylcholine as a substrate (0.5 mM) as previously described [7]. Reaction mixtures contained BW284C51 (10^{-5} M) to inactivate AChE. Ethopropazine (10^{-4} M) was added to the blank samples. Activity was calculated in units of μmoles substrate hydrolyzed per hr.

Statistical analysis. The data were analyzed by standard statistical methods, using a Hewlett–Packard model 85 computer. Since the cholinesterase activities of most tissues do not seem to be normally distributed [7], non-parametric statistics were employed. Differences between sample means were tested for statistical significance by the rank-sum technique of Wilcoxon as described by Snedecor and Cochran [14]. Spearman's rank correlation coefficient, r_s, was calculated as an index of the mutual relationship between two variables.

The experimental design required multiple comparisons of mean values and multiple calculations of correlation coefficients, which raised the possibility of obtaining apparently significant results by chance alone (i.e. type I error). To reduce the overall likelihood of this sort of error to less than 5%, individual P values were accepted as statistically significant only when smaller than 0.05/N, where N is the number of measurements in a given "family". For this purpose, a family was defined as (1) comparisons of the mean activity of AChE *and* BuChE in corresponding tissues of any two groups of rats or (2) correlations of the activity of either enzyme within all tissue pairs of a given group of rats.

RESULTS

Weights and water content. We anticipated that the total body weight and the wet weight of certain tissues would differ among the experimental groups. Such differences raise questions about the proper means of normalizing enzyme activities for intergroup comparisons. In previous work [7], we observed that individual variation of cholinesterase activity was not reduced by expressing the data in terms of protein content. Therefore, we expressed data in units of enzyme activity per g wet weight of tissue, except for ganglia (units/ganglion) and serum (units/ml). An experiment was performed to determine if variations in water content between groups would be large enough to affect the normalized activities. As Table 1 shows, there was a 2-fold range of mean total body weight from group to group. Some organs (e.g. liver) showed even larger group differences in mean wet weight, while others (e.g. brain) showed differences of less than 10%. Each tissue showed a characteristic water content, ranging from a high of 92.6% in serum to a low of 73% in liver. However, there were *no* statistically significant differences in the water content of corresponding tissues from different groups (Table 1). From these results we concluded that cholinesterase activity per g wet weight provided a realistic index of the concentration of enzyme in each sample.

Mean enzyme activities. AChE and BuChE activities, expressed per g wet weight of sample, showed

Table 1. Weights and water content of total carcass and selected tissues of unoperated (control) and hypophysectomized (hypox) male and female Sprague–Dawley rats*

	Total	Brain†	Superior cervical ganglion‡	Diaphragm†	Atria†	Liver†	Serum§
			Weights				
Male control	457 ± 19	2.1 ± 0.03	0.54 ± 0.05	0.36 ± 0.01	0.13 ± 0.008	17.0 ± 0.7	1.00 ± 0.00
Male hypox	327 ± 9.4	2.1 ± 0.03	0.38 ± 0.02	0.32 ± 0.01	0.09 ± 0.009	9.5 ± 0.6	1.01 ± 0.01
Female control	290 ± 9.3	1.9 ± 0.02	0.40 ± 0.02	0.26 ± 0.01	0.11 ± 0.003	10.5 ± 0.6	0.99 ± 0.01
Female hypox	233 ± 9.2	1.8 ± 0.03	0.35 ± 0.02	0.23 ± 0.01	0.10 ± 0.007	7.1 ± 0.3	1.00 ± 0.01
				Water content¶			
Male control		81.9 ± 0.7		80.5 ± 0.3	84.3 ± 0.4	73.0 ± 0.3	92.6 ± 0.1
Male hypox		80.9 ± 0.7		81.8 ± 0.2	86.9 ± 0.5	73.5 ± 0.4	92.6 ± 0.1
Female control		82.3 ± 0.3		81.7 ± 0.9	84.9 ± 0.5	73.5 ± 0.6	92.4 ± 0.1
Female hypox		80.9 ± 0.5		81.4 ± 0.5	85.8 ± 0.4	74.3 ± 0.8	92.5 ± 0.2

* Means ± S.E.M. of six observations are given.
† Wet weight, in grams.
‡ Dry weight, in milligrams (wet weights were unreliable because of rapid water loss after dissection).
§ Grams per milliliter.
¶ Calculated from the ratio of dry weight to wet weight.

considerable variation from tissue to tissue. There were also significant sex differences (Table 2). Thus, AChE activity was 50% higher in the sera of female rats. Conversely, the AChE activity in superior cervical ganglia of female rats was only one-third as great as in the ganglia of males, even though the dry weights of these tissues were quite similar (Table 1). Sex differences in BuChE activity, although confined to serum and liver, were dramatic. On the average, female sera and livers had about ten times higher concentrations of BuChE activity than did the corresponding male tissues (Table 2).

Because some cholinesterases (e.g. serum BuChE) are known to be sensitive to endocrine influences, hypophysectomized rats were studied as an extreme model of altered hormonal control. The results (see Table 2) showed that hypophysectomy had little effect on the *AChE* of most tissues. An apparent exception was the superior cervical ganglion, where the total AChE activity was reduced significantly in comparison with that of the normal controls. This effect may have been at least partly due to reduction in tissue mass (see Table 1). Hypophysectomy had greater effects on the concentration of *BuChE* (Table 2). The most striking of the effects were increases in the BuChE activity of male serum (300%) and liver (43%), which contrasted with *decreases* in the BuChE activity of female serum (24%) and liver (30%). The net result of all these changes was to reduce but not eliminate the normal sex differences in cholinesterase activity.

Correlation of enzyme activities. The data were anlayzed by non-parametric techniques to determine if there were any consistent patterns in the variation of cholinesterase activities from tissue to tissue and from rat to rat. With regard to AChE, the general finding was a lack of relation between the enzyme activities of any one tissue and that of any other: the

Table 2. Effects of hypophysectomy on mean tissue cholinesterase activities*

	Brain	Superior cervical ganglion	Diaphragm	Atria	Liver	Serum
			AChE activities			
Male control	580 ± 39	3.8 ± 0.4	26 ± 1.3	28 ± 1.4	15 ± 1.2†	10 ± 0.6
Male hypox	590 ± 28	1.8 ± 0.25‡	23 ± 1.0	26 ± 1.8	15 ± 0.5†	8.9 ± 0.5
Female control	560 ± 17	1.3 ± 0.06‡	26 ± 1.5		16 ± 0.5	15 ± 0.7‡
Female hypox	560 ± 26	0.9 ± 0.05§	26 ± 0.8		16 ± 0.8	14 ± 0.6
			BuChE activities			
Male control	8.0 ± 0.7	0.69 ± 0.06	2.5 ± 0.23	66 ± 6.9	4.1 ± 0.4	3.1 ± 0.4
Male hypox	8.9 ± 0.9	0.27 ± 0.04‡	2.2 ± 0.22	56 ± 4.6	5.8 ± 0.5	12.5 ± 1.4‡
Female control	6.6 ± 0.4	0.53 ± 0.03	2.8 ± 0.20		42 ± 3.2‡	36 ± 3.4‡
Female hypox	6.6 ± 0.2	0.32 ± 0.02§	1.9 ± 0.10§		29 ± 1.2§	27.6 ± 1.8

* Enzyme activities (in general, means ± S.E.M. of eighteen to twenty-five observations) are given in units (μmoles/hr) per g of tissue wet weight (most tissues) per ganglion or per ml serum.
† Based on a subset of twelve observations.
‡ Significantly different from control males ($P < 0.0001$).
§ Significantly different from control females ($P < 0.001$).

Table 3. Correlation* of AChE activity among tissues of male rats†

	AChE activity			
	Superior cervical ganglion	Diaphragm	Atria	Serum
(A) Controls				
Brain	0.18	0.37	0.27	0.16
Superior cervical ganglion		0.14	0.09	−0.14
Diaphragm			0.00	0.29
Atria				0.13
(B) Hypophysectomized				
Brain	−0.12	0.01	0.26	0.22
Superior cervical ganglion		−0.30	−0.40	−0.32
Diaphragm			−0.26	0.01
Atria				0.26

* Spearman rank correlation coefficient, r_s.
† Eighteen to twenty-four rats per group.

correlation coefficients between tissues averaged 0.20, and none reached statistical significance. This pattern of independent variation in the AChE activity of different tissues was noted in both males (Table 3) and females (Table 4) and was not affected by hypophysectomy.

By contrast, BuChE activity tended to vary in parallel among the tissues of control rats: the cross-tissue correlation coefficients averaged 0.6, some were as high as 0.9, and the majority reached statistical significance (Tables 5A and 6A). Hypophysectomy affected this pattern of variation, but the effects were different in the two sexes. In females, hypophysectomy more than halved the magnitude of the average cross-tissue correlation coefficient, leaving no statistically significant tendency for parallel variation of BuChE activity (Table 6B). A scatter diagram of BuChE activity in brain and diaphragm illustrates this effect (Fig. 1). In male rats, the effects of hypophysectomy on the variation of BuChE activity were more selective. The correlations involving atria behaved like those in females. Thus, in the five comparisons with control male atria, four of the cross-tissue correlations of BuChE activity were statistically significant (average magnitude, 0.78), but none of them was significant in the hypophysectomized males (average magnitude, 0.35). A scatter diagram of BuChE activity in brain and atria illustrates this effect (Fig. 2). The other cross-tissue correlations of BuChE activity in male rats were not changed by hypophysectomy (Table 5B and Fig. 3).

DISCUSSION

The present results are consistent with a large body of information indicating that the regulation of the cholinesterases is influenced by endocrine hormones. In fact, gonadal hormones appear to be

Table 4. Correlation* of AChE activity among tissues of female rats†

	AChE activity			
	Superior cervical ganglion	Diaphragm	Liver	Serum
(A) Controls				
Brain	0.02	0.02	0.29	0.21
Superior cervical ganglion		−0.47	0.24	0.13
Diaphragm			0.22	−0.05
Liver				0.36
(B) Hypophysectomized				
Brain	0.21	0.02	−0.19	0.15
Superior cervical ganglion		−0.19	0.23	0.39
Diaphragm			−0.03	0.18
Liver				0.08

* Spearman rank correlation coefficient, r_s.
† Twenty-two to twenty-five rats per group.

Table 5. Correlation* of BuChE activity among tissues of male rats†

	BuChE activity				
	Superior cervical ganglion	Diaphragm	Atria	Serum	Liver
(A) Controls					
Brain	0.60	0.73‡	0.90‡	0.58	0.76‡
Superior cervical ganglion		0.43	0.58	0.27	0.40
Diaphragm			0.74‡	0.63	0.75‡
Atria				0.79‡	0.87‡
Serum					0.83‡
(B) Hypophysectomized					
Brain	0.58	0.78‡	0.20	0.66‡	0.70‡
Superior cervical ganglion		0.43	0.33	0.16	0.17
Diaphragm			0.43	0.70‡	0.67‡
Atria				0.52	0.29
Serum					0.79‡

* Spearman rank correlation coefficient, r_s.
† Eighteen to twenty-four rats per group.
‡ Statistically significant ($P < 0.0005$).

major determinants of cholinesterase activity. This view is supported by the original findings of Everett and Sawyer [8] and Sawyer and Everett [15] demonstrating sex differences in serum cholinesterase activity (BuChE) that were abolished by castration. The studies of Leeuwin and co-workers [16–18] provided further evidence that gonadal hormones affect the regulation of serum BuChE.

Recently the mechanisms of the endocrine regulation of serum BuChE in the rat have been analyzed more carefully. From the work of Illsey and Lamartinierre [11] it appears that the pituitary gland is involved, since hypophysectomy has major effects on the activity of serum BuChE. However, these effects are not reversed by the implantation of an ectopic pituitary, even when appropriate gonadal steroids are supplied in addition [11]. Therefore, a role for the hypothalamus in the endocrine control of serum BuChE must also be postulated.

Our observations show that sex differences, which are presumably hormonally mediated, involve AChE and BuChE, as do the effects of hypophysectomy. Furthermore, these endocrine influences are not confined to the serum enzymes. The biochemical bases for such effects remain to be defined, although they are unlikely to represent direct actions of steroids on the cholinesterase molecules [11]. More probably, hormonal mechanisms serve to alter the relative balance between synthesis and degradation of the cholinesterases. In any case, the present findings provide a basis for evaluating the possibility that the different patterns of individual variation of AChE

Table 6. Correlation* of BuChE activity among tissues of female rats†

	BuChE activity			
	Superior cervical ganglion	Diaphragm	Liver	Serum
(A) Controls				
Brain	0.51	0.67‡	0.68‡	0.64‡
Superior cervical ganglion		0.71‡	0.64‡	0.40
Diaphragm			0.56	0.27
Liver				0.72‡
(B) Hypophysectomized				
Brain	0.35	0.10	0.24	0.30
Superior cervical ganglion		0.19	−0.02	−0.29
Diaphragm			0.39	0.16
Liver				0.53

* Spearman rank correlation coefficient, r_s.
† Twenty-two to twenty-five rats per group.
‡ Statistically significant ($P < 0.003$).

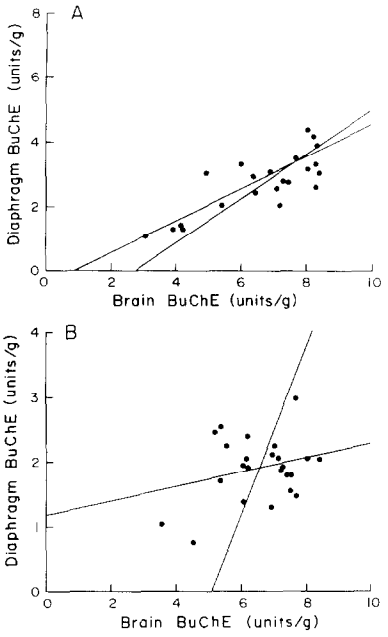

Fig. 1. Scatter diagram comparing the BuChE activities in brain and diaphragm of female Sprague–Dawley rats. (A) Normal females. (B) Hypophysectomized females. Each point represents data from a single animal. Enzyme units are μmoles of BuChE hydrolyzed per hr of incubation. The regression lines were fitted by the method of least squares. Note that when the correlation coefficient is high (A) the regression of y on x has essentially the same slope as that of x on y, but when the correlation coefficient is low (B) these slopes diverge considerably.

and BuChE activities in normal rats reflect different degrees of endocrine control. The result that bears most directly on this possibility is the effect of hypophysectomy on the relationship among the enzyme activities of different tissues.

In normal animals of both sexes, none of the

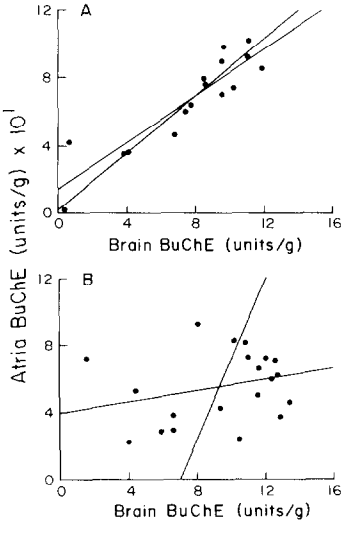

Fig. 2. Scatter diagram comparing the BuChE activities in brain and atria of male Sprague–Dawley rats. (A) Normal males. (B) Hypophysectomized males.

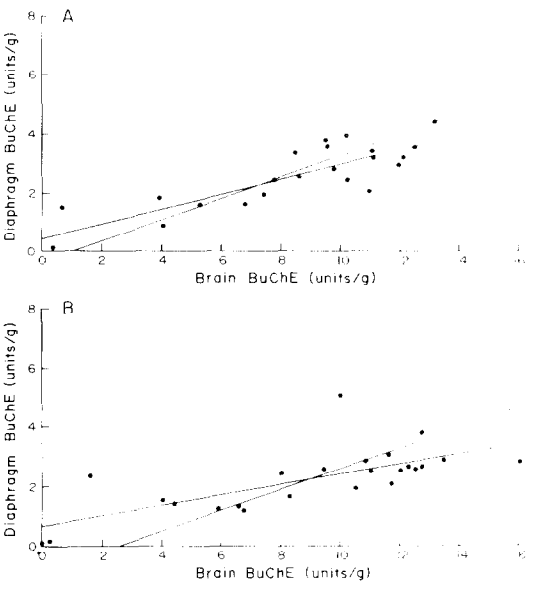

Fig. 3. Scatter diagram comparing the BuChE activities in brain and diaphragm of male Sprague–Dawley rats. (A) Normal males. (B) Hypophysectomized males.

tissue-pairs yielded correlations of AChE activity that were statistically significant, whereas over half of the "cross-tissue" correlations of BuChE activity were highly significant. This result is qualitatively similar to one we obtained earlier in a study of the cholinesterases of male Sprague–Dawley rats [7]. It may be concluded that, in the Sprague–Dawley strain, there is a general tendency for the BuChE activity of different tissues to vary in parallel, but the tendency for parallel variation of AChE activity is far weaker. Wide individual and even day-to-day variations in the levels of circulating gonadal hormones are known to occur in rats [19]. Therefore, to the extent that these hormones affect the regulation of BuChE and are under the control of the pituitary gland, one would expect hypophysectomy to disrupt the tendency for body-wide, parallel variations of BuChE activity. This expectation was confirmed in the present experiments with regard to the BuChE activities of female rats. However, because hypophysectomy of male rats affected only those cross-tissue correlations that involved atria, one must use caution in generalizing about the role of the pituitary-endocrine system in "synchronizing" the cholinesterase activities of different tissues. Meanwhile, it is worth noting that tissue- and sex-specific effects of endocrine hormones on the regulation of enzymes are by no means unprecedented (for a recent example, see the report of Woodson *et al.* [20] concerning the effects of testosterone on the levels of thiopurine methyltransferase).

Further experiments are required to define the nature of the tissue-specific mechanisms that appear to dominate the regulation of AChE in rats. Elucidating the hormonal mechanisms that affect the regulation of BuChE is another challenge for the future. At present it is safe to conclude that the two families of cholinesterases are regulated in different ways and probably serve distinct physiological roles.

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