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STRESS-INDUCED ALTERATIONS IN ARYLESTERASE ACTIVITY IN THE RAT

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Abstract—Exposure of intact rats to emotional stress resulted in an increase of plasma arylesterase activity, more profound in males than in females. The intra class correlation of this effect between full-sibs suggests considerable maternal influence and/or additive genetic effects and dominance deviation, the additive part being minimal in females. Most animals subjected to muscle work showed a decrease in arylesterase activity. Adrenalin given intramuscularly to rats caused an increase in arylesterase activity; this effect was neutralized when adrenalin was administered together with ACTH.

Arylesterase is briefly discussed as an enzyme being under hormonal control.

ARYLESTERASE is the dominant esterase activity in mammalian blood plasma, but the physiological function of the enzyme is still unknown. The experimental observations so far, which may throw some light on a possible general function of arylesterases, can be summarized as follows: (1) great dependency of the esterase activity on the manner of handling the animals;[1] (2) increased content of free fatty acids (FFA) in the blood as a result of emotional stress[2, 3] and starvation;[4–6] (3) the control of FFA alteration by the pituitary-adrenal axis[7–9] and the involvement of cyclic 3′,5′-AMP in this control;[10, 11] (4) the control of arylesterase activity level by similar or related hormone systems;[12, 13] (5) the transportation of FFA associated with blood plasma albumin[14, 15] containing about 5 per cent of tyrosine, the *O*-acetylated derivative of which might be a natural substrate for arylesterases;[16–18] and (6) the possible role of arylesterases in determining the transesterification reactions between FFA and acetic acid.

The suggestion that these observations are related gave rise to the present investigations. It was felt also that an examination of an esterase activity under conditions of stress and suggestively being involved in the metabolism of ester drugs would be of interest.

In a previous experiment[19] causes of individual variation in arylesterase activity were analysed. Within the limits of the material it was concluded that additive genetic effects formed a minor part of the regulation of the enzyme activity especially in females. In the present experiment the reaction to emotional stress was analysed from a genetic point of view.

METHODS

Animals

Adult rats of a Wistar strain and weighing 175–200 g at an age of about 75 days

were used in all experiments. Except where otherwise stated the animals were allowed food and tap water *ad libitum*, and were housed separately in a relatively stress-free environment for at least 7 days.

The genetic aspects of the stress reaction were tested in a group of 81 males and 86 females subjected to 15 hr of emotional stress. These animals were produced by 20 females and 4 males unrelated to each other.

Treatments

Emotional stress. Each animal was wrapped into a fine wire-netting which prevented practically all movements.

Muscle work. A treadmill consisting of a continuous rubber mat with a running surface of about 20 × 50 cm was used for the physical work. The mat was run at a speed of about 20 cm/sec. The animals were trained before use in the experiments.

Drug treatment. Adrenalin was given as *adrenalini bitartras* 0·01 mg per indiv. in 0·5 ml *aq. steril.* ACTH was given as *corticotropinphloretin. phosh. polymer. respond. corticotropin.* (Reacthin, Leo) 1 I.E. per indiv. in 0·5 ml *aq. steril.*, and cyclic 3′,5′-AMP as adenosine-3′,5′-cyclic-phosphoric acid (Sigma) 5 mg per indiv. in 1 ml *aq. steril.*

All substances were injected intramuscularly.

Blood plasma

Heparinized blood samples were collected by cardiac puncture following intraperitoneal injection of a barbiturate (Mebumal). The plasma was centrifuged free of all cells and used for arylesterase determination.

Esterase determination

The arylesterase activity was measured by the Warburg technique at 25° in a sodium bicarbonate–CO_2 buffer of pH 7·4. Phenyl acetate in a final concentration of 10 mM was used as substrate. Corrections were made for spontaneous hydrolysis of substrate. The plasma was diluted 1:200 with the buffer solution, and 0·4 ml of the diluted plasma was mixed with 1·6 ml of the substrate solution. The esterase activity was expressed in μmoles of substrate hydrolysed/min/ml plasma.

RESULTS

Effects of emotional stress

The data in Fig. 1 and Table 1 demonstrate that exposure of intact rats to emotional stress results in an increase of plasma arylesterase activity. The most profound increase was seen in males. The maximal increase in activity occurred after about 15 hr of stress and was about 30 per cent above the control value. In females the increase was less profound and was about 10 per cent above control when the activity had reached its maximum.

The stress reaction was inversely correlated to the activity level. At 15 hr of immobilization stress 81 males showed a correlation coefficient of 0·39 ($P=0·001$) between activity level and percental stress reaction. The regression coefficient was −0·36. For 86 females the correlation coefficient was 0·49 ($P < 0·001$) and the regression coefficient −0·35.

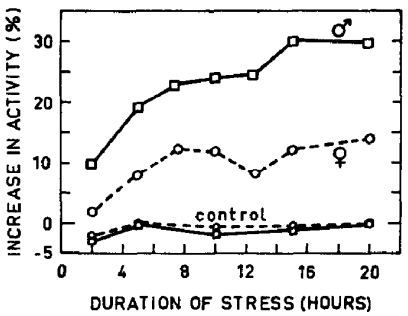

FIG. 1. Effect of stress on the arylesterase activity of rat plasma. The values indicate the means, based on 8 animals at each point from two series of experiments, of per cent alteration in arylesterase activity compared with control values taken 4 days before the animals were subjected to stress. At each point, male reactions to stress are significantly ($P < 0.05$) higher than female reactions. Cf. Table 1 for absolute values obtained after 10 hr of stress.

TABLE 1. EFFECT OF STRESS ON THE ARYLESTERASE ACTIVITY OF RAT PLASMA

Animals (no.)	Treatment	Arylesterase activity Mean	S.D.	Range
8 males	Before treatment	139·5	15	123–161
	After 10 hr stress	172	14·5	154–194
8 females	Before treatment	175·5	19·5	144–202
	After 10 hr stress	195	13·5	176–215

The animals were subjected to 10 hr of stress. Blood samples were collected 6 days before (control) and immediately after treatment. Activity values expressed in μmoles of substrate (phenyl acetate) split/min/ml plasma.

Genetic aspects

Before analysing the influence on the stress reaction of parent combinations the activity level of the animals was tested according to the same procedure as in a previous experiment.[19] With this new group of animals, almost exactly the same estimates for paternal and maternal effects were obtained. This confirms the conclusion that additive genes to a minimal extent govern the activity level especially in female offspring.

The magnitude of the emotional stress reaction is correlated to the activity level (see above). After correction of the percental stress reaction to an average activity

TABLE 2. FULL-SIB AND HALF-SIB ANALYSES OF PERCENTAL EMOTIONAL STRESS REACTION IN MALE OFFSPRING OF 4 SIRES AND 20 DAMS

Source of variation	D.f.	M.sq.	Component of variance	%
Sires	3	319·49	7·05	6·2
Dams within sires	16	176·42	22·93	20·2
Offspring within dams	61	83·54	83·54	73·6

$$\text{Quot.: } \frac{176{\cdot}42}{83{\cdot}54} = 2{\cdot}11 \quad \frac{319{\cdot}49}{176{\cdot}42} = 1{\cdot}81$$

$$0{\cdot}01 < P < 0{\cdot}05 \qquad P > 0{\cdot}05.$$

level, the intra class correlation between full-sibs and half-sibs was analysed. The results are given in Tables 2 and 3. The intra class correlation between full-sibs is clearly predominated suggesting considerable maternal effects and/or additive genetic effects and dominance deviations. The additive genetic effect as measured by the paternal intra half-sib correlation in 4 families can be estimated in males but not in females. Heritability in the males probably reaches a level of 20–25 per cent. The limited material makes more detailed interpretation impossible.

TABLE 3. FULL-SIB AND HALF-SIB ANALYSES OF PERCENTAL EMOTIONAL STRESS REACTION IN FEMALE OFFSPRING OF 4 SIRES AND 20 DAMS

Source of variation	D.f.	M.sq.	Component of variance	%
Sires	3	40·99		
Dams within sires	16	182·79	26·07	26·9
Offspring within dams	66	70·70	70·70	73·1

$$\text{Quot.}: \frac{182{\cdot}79}{70{\cdot}70} = 2{\cdot}59$$

$$0{\cdot}001 < P < 0{\cdot}01.$$

Effects of muscle work

Table 4 demonstrates some of the results obtained with these animals subjected to work for various periods of time. Most animals showed a decrease in esterase activity, but great individual variation in the response was observed and found to be independent of sex. The greatest response was observed with a female rat after 30 min of work (−40·6 per cent), and two male rats did not respond at all. These preliminary experiments have to be repeated with animals better trained for this work, because an emotional stress effect cannot be excluded. The results obtained show, however, that muscle work and emotional stress give rise to opposite effects as far as the alteration in arylesterase activity is concerned.

TABLE 4. EFFECT OF MUSCLE WORK ON THE ARYLESTERASE ACTIVITY OF RAT PLASMA

Animals tested (number)	Sex	Time in work (min)	Change in ArE activity (per cent) Mean	S.D.
10	♂	15	−17·4	5·6
7	♀	15	−12·2	7·6
12	♂	30	−9·6	14·5
7	♀	30	−14·4	21·2
9	♂	45	−15·8	5·6
5	♀	45	−10·0	16·8

The animals were subjected to work (using a treadmill) for various periods of time. Blood samples were collected 6 days before by heart puncture or immediately before start of work (from the tail) (control) and immediately after stop of work.

Effect of adrenalin and ACTH

Because adrenalin is known to be released during conditions involving stress,[7, 8, 20] the effect of this hormone on the arylesterase activity was tested both *in vitro* and *in vivo*. Adrenalin (0·1 mM) had no effect on rat plasma arylesterase *in vitro*. Fig. 2 shows the effect of adrenalin on the arylesterase level of rat plasma *in vivo*. In both sexes this hormone resulted in a statistically significant increase in the activity of about 20 per cent within 6 hr after the treatment with 0·01 mg adrenalin per animal. Then the activity decreased in the females and was unaltered or increased slightly more in the male rats within 12–16 hr. Six days after the treatment the activities had returned to original levels in both sexes.

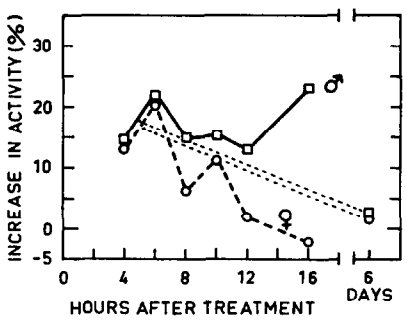

FIG. 2. Effect of adrenalin (0·01 mg adrenalin bitartrate per animal i.m.) on the arylesterase activity of rat plasma *in vivo*. The values indicate the means, based on 4–6 animals at each point, of per cent alteration in arylesterase activity compared with control values taken 8–20 days before the animals were treated with adrenalin. Each animal was treated only once. Each point represents one separate group of animals, except the "6 days" values which were obtained from the same animals (altogether 11 males and 10 females) as those used in the 4 and 6 hr experiments. Control animals belonging to the same litters showed constant values during the same period of time.

The effect of adrenalin in releasing adrenocortical hormones, which has been shown to cause an increase in the arylesterase activity of castrated male pigs,[12] is mediated by ACTH from the anterior pituitary.[7, 8, 20] ACTH has now been shown to produce no effect on this enzyme in rats. When given together with adrenalin, ACTH neutralized the effect of increasing the esterase activity of the former hormone. Cortisone and hydrocortisone (5 mg per animal) had no effect on rat plasma arylesterase.

Effect of cyclic 3′,5′-AMP

In preliminary experiments with this nucleotide it was shown that 1 mM of cyclic 3′,5′-AMP had no effect on the arylesterase activity of rat plasma *in vitro*. *In vivo* also this nucleotide (5 mg per animal) seemed to be without effect on this enzyme. These experiments, however, were carried out with exogenous cyclic 3′,5′-AMP, which is known to enter cells poorly (see further below).

DISCUSSION

The great variation of the activity level of plasma arylesterase among individuals can be explained, at least in certain animals (e.g. the rat), by poorly standardized handling of the animals. Particularly noticeable is the relationship to consumption of

food. There is, for instance, a marked increase in activity following the transition from the fed to the fasting condition.[1]

Factors contributing to individual variation were examined further in the present investigation. The results presented provide evidence that emotional stress produces an increase in the plasma arylesterase activity in rats, more pronounced in males than in females. Muscle work had the opposite effect. The mechanism of the stress-induced elevation in esterase activity might be of hormonal origin. Arylesterase has been shown previously to be under the control of testosterone and cortisone, the former producing a decrease, the latter an increase in activity.[12, 13] The additional observation described above that adrenalin also produced an increase in the arylesterase activity level supports the view of hormonal control of this activity. The effect of adrenalin could be neutralized by ACTH probably as a result of feed-back inhibition. Moreover, evidence has been presented[10] that the effects of adrenalin and a number of other hormones are mediated by cyclic 3′,5′-AMP which is released from the effector cells by the stimulation of the activity of adenyl cyclase. The preliminary experiments reported above show that this nucleotide was without effect on arylesterase both *in vivo* and *in vitro*. This does not exclude, however, a possible indirect effect of cyclic 3′,5′-AMP because the experiments were carried out with a derivative which enters the cells poorly and is highly susceptible to inactivation by a phosphodiesterase. The better suitable N^6-2′-*O*-dibutyryl derivative was not available.[11, 21] A direct effect of cyclic 3′,5′-AMP on arylesterase is unlikely.

A number of previous observations indicate that arylesterase may be involved in the metabolism of FFA, primarily in transesterification reactions between acids of long carbon chains and acetic acid. The phenolic moiety involved is considered to be tyrosine, the *O*-acetate of which appears to be a natural substrate for arylesterases.[16–18] No direct proof has yet been presented for this attractive hypothesis. Preliminary experiment in our laboratory with the rats subjected to emotional stress have recently demonstrated that the composition of FFA in the plasma is changed in such a way that the content of oleic acid (but not palmitic acid) is significantly decreased as a result of stress.[22] This stress-induced effect was neutralized when the animals were treated with ACTH before being subjected to emotional stress. It was also shown that a decrease in arylesterase activity, observed when the animals were subjected to work, was followed in most cases by a decrease in FFA content. Whether arylesterase is related to these alterations in the composition of FFA of plasma and involved in their binding to plasma albumin[14, 15] is still an open question. In this connection it is pertinent to refer to a hypothesis, recently discussed by Pilz *et al.* implying that arylesterase is necessary for hydrolysing lipoproteins by means of a lipase, i.e. arylesterase is functioning as a co-factor for this enzyme.[23]

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