SEX DIFFERENCES IN THE CHOLINERGIC STATUS OF ALBINO RATS

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UDC 616.453-008.6-055.2-092.9]-02:613.863]-07

KEY WORDS: sex differences; cholinergic status; cholinesterase; stress; corticosterone.

Adrenocortical sensitivity to stress, which is higher in women than in men [1], is combined with a higher resistance of the cardiovascular system of women to excessive emotional strain [10, 16]. Sex differences in stress-reactivity and stress-resistance may be based on sex differences in cholinergic status. This suggestion is based on the fact that cholinergic mechanisms not only induce stress activation of the hypothalamo–hypophysen–adrenal system (HHAS) [9, 14], but they also increase the resistance of the cardiovascular system to prolonged and intensive stresses [3, 4, 7, 12]. The role of the cholinergic system in a living organism may be characterized by its functional reserves and the degree of its involvement in the regulation of the HHAS, a vital component of stress-induced and adaptive processes.

The aim of the investigation was to study sex differences in the cholinergic status of albino rats. To do this, the sensitivity of females and males to the organophosphorus anticholinesterase agent chlorophos (Dipterex) and also the effect of atropinization on basal and stress-related corticosterone levels were studied.

EXPERIMENTAL METHOD

To assess the functional reserves of the cholinergic system changes in the blood cholinesterase level were determined by the method in [15] in animals of both sexes 5 and 20 min after intramuscular injection of chlorophos in doses of 10 and 360 mg/kg. Indicators of the animals' resistance to chlorophos were clinical manifestations of the degree of poisoning and the value of LD_{50}, determined as in [2]. The sensitivity of HHAS to atropine was estimated (fluorometrically) by measuring changes in basal corticosterone levels in the adrenals and plasma 30 min after intraperitoneal injection of atropine in doses of 0.1, 0.3, and 0.6 mg/100 g body weight. Control animals were given an injection of physiological saline. To assess stress reactivity under atropinization, 30 min after injection of atropine some of the animals were subjected for 10 min to a combined form of stress, namely strict immobilization accompanied by an interrupted noise. Blood for biochemical tests was collected at decapitation of the animals. The results were subjected to statistical analysis by Student's test.

EXPERIMENTAL RESULTS

The basal blood level of cholinesterase activity was significantly higher in females than in males (0.23 ± 0.005 compared with 0.20 ± 0.003 mg/ml·min). Resistance of the enzyme to the action of chlorophos also was higher in females than in males (Fig. 1). Cholinesterase activity 5 min after injection of a small dose of chlorophos, insufficient to cause clinical symptoms of poisoning, fell in the females by 19% and in the males by 49%. After 20 min the

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(Presented by Academician of the Russian Academy of Medical Sciences K. V. Sudakov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 114, No. 10, pp. 351-353, October, 1992. Original article submitted January 27, 1992.

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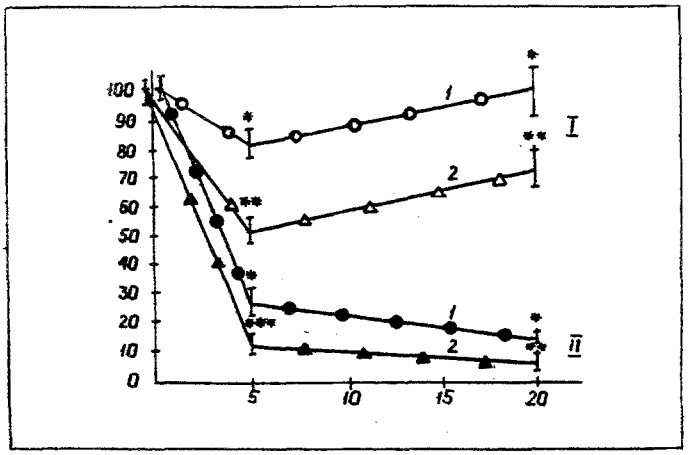

Fig. 1. Changes in blood cholinesterase activity of rats receiving chlorophos in doses of 10 mg/kg (I) and 360 mg/kg (II). Abscissa, time (in min); ordinate, cholinesterase activity (in % of control). Change is significant ($0.001 < p < 0.05$): *) compared with control, **) between females (1) and males (2). Data for eight animals are given.

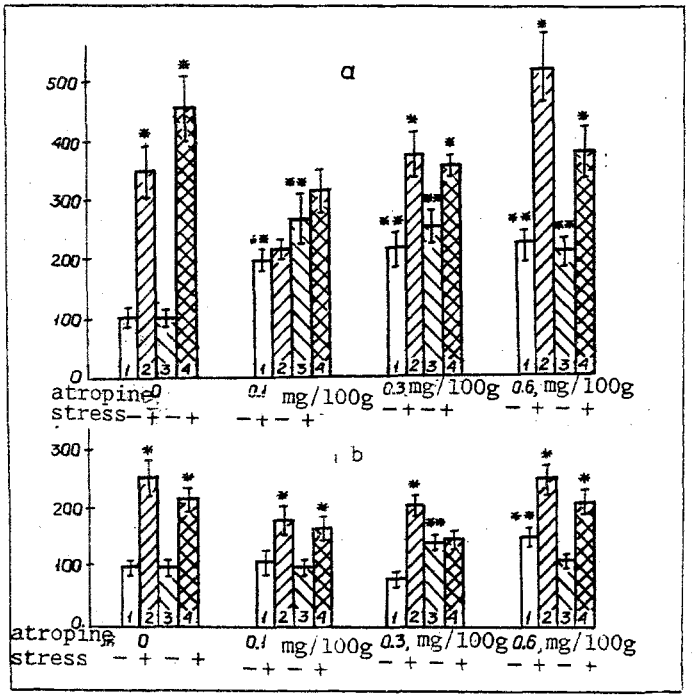

Fig. 2. Effect of atropine on basal and stress-related corticosterone levels in adrenals (1, 2) and plasma (3, 4) of females (a) and males (b). Ordinate, corticosterone concentration in % of control. Change is significant ($0.001 < p < 0.05$): *) compared with corresponding control; **) compared with intact animals. Each point represents M ± m for 8-16 animals.

enzyme level was back to normal in the females, but remained low in the males. Cholinesterase activity 5 min after injection of a large dose of chlorophos was 27% of the initial level in females but only 13% in males, and after 20 min it was 14% in females but only 5% in males. Clinical signs of poisoning after a large dose of chlorophos were limited in females to some degree of restraint in behavior, whereas in 38% of males general convulsions were noted.

Salivation was observed in only 12% of females but in 100% of males. LD_{50} for females was 860 mg/kg but for males 700 mg/kg. Thus the biochemical and physiological parameters of resistance to chlorophos correlate with each other and reflect increased functional reserves of the cholinergic system in females compared with males.

The results of the atropinization experiments revealed sex differences also in the degree of involvement of cholinergic mechanisms in the regulation of basal and stress-related levels of steroid production. Under the influence of increasing doses of atropine, an approximately equal increase was observed in females in the basal corticosterone levels in the adrenals (by 1.9-2.2-2.3 times) and plasma (by 2.7-2.3-2.0 times). Activation of steroid production under the influence of atropine is a known fact [8] and may be due to an increase in activity of the sympathoadrenal system when the cholinergic system is blocked by atropine [13]. Meanwhile atropine reduced adrenocortical sensitivity of the females to stress, and a small dose produced the greatest decrease. Whereas in intact females the corticosterone level in the adrenals and plasma increased during stress by 3.6 and 4.6 times respectively, atropine in a dose of 0.1 mg/100 g completely suppressed the stress reaction. After injection of 0.3 mg/100 g of atropine, the corticosterone level in the adrenals and plasma increased during stress by only 1.7 and 1.4 times, but in a dose of 0.6 mg/100 g by 2.3 and 1.9 times. Suppression of stress reactivity in atropinized females has been described in the literature [14]. The mechanism of suppression may be partly connected with blockade of the emotional component of the stress reaction in response to injection of atropine, for we know that fear reactions are cholinergic in nature [5]. Thus both basal and stress-related levels of activity of steroid production in females are sensitive to suppression of cholinergic influences, evidence of the important role of cholinergic mechanisms in the regulation of adrenocortical activity at rest and during stress.

A different picture was observed in males (Fig. 2b). The stimulating effect of atropine on basal levels of steroid production was negligible and was manifested only if the drug was given in average and high doses. In the first case, the plasma hormone level rose by 1.4 times, in the second case its level in the adrenals rose by 1.5 times. The suppressive effect of atropine on stress reactivity in males was significantly lower than in females. The hormone concentration in the adrenals and plasma of intact males rose during stress by 2.6 and 2.2 times, which, as in our previous experiments [1], is significantly lower than in females. With atropine in a dose of 0.1 mg/100 g the corticosterone level in stress rose in the adrenals and plasma by 1.8 and 1.7 times, with a dose of 0.3 mg/100 g by 2.5 and 1.1 times, and with a dose of 0.6 mg/100 g, by 1.9 and 2.0 times respectively. Thus only with an average dose of the preparation was the stress-induced increase in the plasma corticosterone concentration blocked in males. With small and large doses of atropine, stress-reactivity of the males did not change significantly. Consequently, inhibition of cholinergic influences in males, unlike in females, is not accompanied by any constant and substantial effects in relation to basal and stress-related corticosterone levels. These results are evidence that cholinergic mechanisms in females play a significantly greater role in females than in males, and ensure a higher level of adrenocortical sensitivity to stress in females than in males.

The higher functional reserves of the cholinergic system in females than in males, together with increased sensitivity of HHAS to its influences, lead to the conclusion that the cholinergic status is higher in females. These differences may play a decisive role in the maintenance of greater stability of the cardiovascular system to prolonged and intensive forms of stress in women compared with men, due to predominant activation of cholinergic mechanisms in women and adrenergic in men [6]. The important role of glucocorticoids in systemic regulation of cardio-hemodynamic shifts suggests that the positive effect of cholinergic mechanisms on the stress-resistance of the cardiovascular system in women is largely mediated through their higher adrenocortical sensitivity to stress. This hypothesis is confirmed by the fact that within the same (male) sex predominance of the parasympathetic type of regulation also correlates with increased amplitude of the glucocorticoid response and higher sensitivity of the cardiovascular system in stress situations [11].

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