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Study of Some Biological Indices of the State of the Sympathoadrenaline System under the Effect of Polychlorocamphene

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The effect of exposure to different amounts of polychlorocamphene (toxaphene) on the level of catecholamines (noradrenalin and adrenalin), their precursors (DOPA and dophamine), and a metabolite (vanillylmandelic acid) in tissues (adrenals, brain, heart) and daily urine in white male rats has been studied.

It was established that the single administration of 120 mg/kg toxaphene (half the LD_{50}) as well as 2.4 mg/kg daily (1/100 of LD_{50}) for 1 and 3 months produced a disturbance of catecholamine metabolism.

The absolute level of ratio of separate components of the sympathicoadrenalic system is unequally changed in tissues, the breakdown of catecholamines is increased, and the specificity of their excretion is destroyed.

The widespread use of organochlorine pesticides in agriculture in the USSR makes it necessary to determine the nature of the response reactions of an organism to these substances.

The sympatho-adrenaline system, one of the most important of the regulating systems of an organism, plays an important role in the response reaction of an organism to the effect of harmful factors.

The basic components of the system are pyrocatecholamines or catecholamines, compounds incorporating in their structure the nature of the primary stimulants of protoplasm, and amines and oxidation-reduction systems, quinonepyrocatechols (*1*).

Hormones produced in the chromaffinic cells of the suprarenal glands, the paraganglia of the sympathetic nerve nodes, sympathetic nerve endings and other accumulations of chromaffinic tissues are referred to as catecholamines. The biosynthesis and decomposition of catecholamines is quite well known (*1–6*).

The initial product for the biosynthesis of catecholamines is the amino acid tyrosine, which, under the effect of the highly specific enzyme tyrosinehydroxylase, is changed into dihydroxyphenylalanine (DOPA). During the participation of dopadecarboxylase, DOPA decarboxylates, forming dopamine. The latter hydroxylyzes and forms noradrenaline, which is transferred to adrenaline as the result of methylation.

The decomposition of catecholamines basically occurs by means of *O*-methylation under the effect of catechol-*O*-methyltransferase and subsequent deamination in the presence of monoaminoxydase. Vanillylmandelic acid is formed under these conditions which is common both for adrenaline and noradrenaline as the terminal product of metabolism. In addition to this, adrenaline may be subjected to oxidation with the subsequent formation of quinoid and indole type structures: adrenochrome, adrenolutin, and 5,6-dioxy-*N*-methylindole.

Catecholamines, their precursors and metabolites, being biologically active substances, take part in regulating physiological processes and adaptive reactions. Because of this, changes in the catecholamine content in tissue can be one cause of the disturbance of these processes.

The aim of the present work is the study of the functional state of the sympatho-adrenaline system under conditions of administering polychlorocamphene (toxaphene) to white rats. Polychlorocamphene is an organochlorine pesticide which is widely used both in the United States and the Soviet Union.

The following serve as the indices of the state of the sympatho-adrenaline system: the content in the adrenal gland, cerebrum and the myocardium of

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catecholamines (adrenaline and noradrenaline) and their precursors (DOPA and dopamine) and also the amount of catecholamines excreted with urine, precursors of the catecholamines and the metabolite (vanillylmandelic acid).

The polychlorocamphene was administered orally to the experimental animals either in single doses of 120 mg/kg (half the LD_{50}) or as 2.4 mg/kg (0.01 LD_{50}) daily for 1 and 3 months. In the single dose, the studies were conducted 1, 5, and 15 days after the introduction of the substance.

Amounts of adrenaline, noradrenaline, DOPA, and dopamine were determined fluorometrically in tissues by the method of Matlina and Rakhamanova (*7*). The method of Matlina et al. (*8*) was used for the analysis of urine. The vanillylmandelic acid content was determined by the paper chromatography method (*9*). Data on the catechol metabolite content in the tissues and urine of the control animals are given in Table 1.

Our studies show that a single large dose of polychlorocamphene (120 mg/kg) results in decreasing the amount of adrenaline in the adrenal glands to 955 ± 100 μg/g, i.e., by 24% with respect to the control group and an increase in the adrenaline (by 122%) and noradrenaline (by 94%) in the daily urine during the first 24 hr. No changes were observed in the cerebrum. In the myocardium a verifiable reduction was observed in the DOPA level (down to 0.362 ± 0.082 μg/g, i.e., a reduction of 40%) (Table 2).

After 5 days, the level of adrenaline increases in the adrenal glands up to 1879 ± 186 μg/g (by 50%) and the amount of noradrenaline falls in all of the studied tissues: adrenal glands, cerebrum, and the myocardium (by 50% on the average). Under these conditions, the amount of dopamine in the myocardium increases by 97% and constitutes 6.848 ± 1.163 μg/g.

After 15 days, the adrenaline in the adrenal glands increases to 2111 ± 109 μg/g (by 68%), in the cerebrum the adrenaline increases to 0.094 ± 0.024 μg/g (by 235%), the noradrenaline increases to 0.391 ± 0.017 μg/g (by 66%) and dopamine increases to 4.200 ± 0.593 μg/g (by 56%). In the myocardium the dopamine increases to 7.458 ± 1.377 μg/g (by 115%)

Table 1. Catecholamines, their precursors and metabolites in the tissues and urines of control animals.

	Catecholamines			
Index studied	Adrenal gland, μg/g	Brain, μg/g	Heart, μg/g	Urine, μg/day
DOPA	109 ± 7	0.183 ± 0.035	0.608 ± 0.064	0.556 ± 0.079
Dopamine	126 ± 27	2.692 ± 0.307	3.462 ± 0.563	3.597 ± 0.641
Noradrenaline	558 ± 62	0.236 ± 0.028	0.968 ± 0.174	0.627 ± 0.107
Adrenaline	1250 ± 72	0.028 ± 0.004	0.138 ± 0.018	0.225 ± 0.033
Vanillylmandelic acid	—	—	—	22.4 ± 1.5

Tabel 2. Changes in the level of catecholamines, their precursors and metabolites, in the tissues and urine after a single dose of polychlorocamphene.

Index	Time after dose of polychlorocamphene, days	Change in catecholamine level, %[a]			
		Adrenals	Brain	Heart	Urine
DOPA	1	—	—	−40	—
Dopamine	1	—	—	—	—
Noradrenaline	1	—	—	—	94
Adrenaline	1	−24	—	—	122
Vanillylmandelic acid	1	—	—	—	—
Dopa	5	—		—	—
Dopamine	5	—		97	—
Noradrenaline	5	−48	−50	−58	—
Adrenaline	5	50	—	—	—
Vanillylmandelic acid	—	—	—	—	
DOPA	15	—	—	−46	—
Dopamine	15	—	56	115	—
Noradrenaline	15	—	66	—	—
Adrenaline	15	68	235	—	—
Vanillylmandelic acid	15	—	—	—	—

[a] Values given are percent with respect to control group. A positive value indicates an increase with respect to controls; a negative value indicates a decrease. Only significant ($p < 0.05$) changes are given.

against a background of a decreased DOPA level of 0.334 ± 0.092 μg/g (a decrease of 46%). The excretion with urine, the same as after 5 days, remains within normal limits.

As a result, a large single dose of a large polychlorocamphene stresses the sympatho-adrenaline system which during the first 24 hr results in an increased release of catecholamines with urine and in a reduction of the adrenaline level in the adrenal glands. The manifested changes can be considered either as the result of an intensified discharge of catecholamines or as the result of the disturbance of the synthesis of catecholamines in the adrenal glands and of the catabolism in the tissues. The increase in the amount of adrenaline noted after 5 days in the adrenal glands is obviously compensatory in nature and is conditioned by the activation of noradrenaline methylation which results in the observed reduction in its level in all tissues.

The data obtained on day 15 after the single dose administration of polychlorocamphene are of interest. These data attest to the accumulation of dopamine in the myocardium and the intensification of the biosynthesis of catecholamines in the cerebrum. This results in a sharp increase of the catecholamine level, particularly of adrenaline. Because of this, the ratio of adrenaline to noradrenaline is more than doubled. Such a late reaction of cerebral tissue can obviously be related to particulars associated with the permeation of polychlorocamphene into the central nervous system. In our earlier experiments (*10*), it was shown that the single-dose administration of polychlorocamphene to rats in a dose of 120 mg/kg (0.5 LD_{50}) results in observing the pesticide in the lipids of the brain 15 days after introduction.

Consequently, the organism reacts with a stress reaction to a single large dose of toxin. This type of stress reaction is characterized by an intensified release of adrenaline and the reaction is directed towards maintaining homeostasis. Nevertheless, the process does not terminate with this. Later on disturbances arise in catecholamine metabolism in such vital organs as the brain and heart. These disturbances serve as the reason for the clinically observed damage to the nervous system and the myocardium (*11–14*) for polychlorocamphene exposure. Long-term introduction of 2.4 mg/kg (0.1 LD_{50}) polychlorocamphene evokes significant changes in the functional activity of the sympatho-adrenaline system (Table 3). After 1 and 3 months of the daily exposure to the substance, the adrenaline level in the urine rises to 0.321 ± 0.030 μg/day (by 42%) after a month and twice the initial level after 3 months, constituting 0.450 ± 0.050 μg/day. An average increase of 44% was observed in the metabolite of catecholamines-vanillylmandelic acid (32.6 ± 5.5 μg/day after 1 month and 31.9 ± 6.9 μg/day after 3 months). After 1 month, the amount of DOPA released falls to 0.317 ± 0.036 μg/day (a reduction of 43%) and after 3 months the amount of dopamine increases to 5.668 ± 0.641 μg/day (an increase of 57%). The persistent increase in the amount of vanillylmandelic acid in the urine provides a basis for considering that the long-term administration of polychlorocamphene results in the intensification of catecholamine break-down.

The amount of adrenaline doubles in the brain (0.056 ± 0.007 μg/g after 1 month and 0.051 ± 0.004 μg/g after 3 months). As a result, the ratio between the catecholamines (adrenalin/noradrenaline) increases by a factor of three after 1 month and by a factor of two after 3 months.

Significant changes are detected in the myocardium. Long-term administration of polychlorocam-

Table 3. Changes in the level of catecholamines, their precursors and metabolites in tissues and urine during long-term administration of 0.01 LD_{50} (2.4 mg/kg) polychlorocamphene.

Index	Time after initial dose polychlorocamphene, months	Change in catecholamine level, %[a]			
		Adrenals	Brain	Heart	Urine
DOPA	1	—	—	−32	−43
Dopamine	1	—	—	78	—
Noradrenaline	1	—	—	−41	—
Adrenaline	1	—	100	−38	42
Vanillylmandelic acid	1	—	—	—	45
DOPA	3	—	—	−45	—
Dopamine	3	73	—	56	57
Noradrenaline	3	—	—	−46	—
Adrenaline	3	51	82	—	100
Vanillylmandelic acid	3	—	—	—	42

[a] Values given are percent with respect to control group. A positive value indicates an increase with respect to controls; a negative value indicates a decrease. Only significant ($p < 0.050$) changes are given.

phene most likely results in the activation of DOPA-decarboxylase which results in reducing DOPA to 0.418 ± 0.057 μg/g (by 32%) after 1 month and to 0.338 ± 0.074 μg/g (by 45%) after 3 months, and in increasing dopamine to 6.083 ± 1.074 μg/g (by 78%) after 1 month and 5.304 ± 0.740 μg/g (by 56%) after 3 months, i.e., the same as was observed 15 days after the single-dose administration of the substance. Nevertheless, in the case of multiple introductions of the substance, an augmenting factor appears to be the observed reduction in the amount of noradrenaline (0.571 ± 0.067 μg/g after 1 month and 0.529 ± 0.096 μg/g after 3 months, a reduction of 45% on the average) and a reduction of adrenaline of 38% (0.086 ± 0.11 and 0.098 ± 0.015 μg/g). The latter may be considered from one point of view as the result of the inhibition of catecholamine biosynthesis at the dopamine-β-hydroxylase level, from another point of view, it may be considered the result of the reduced capacity of the myocardium for absorbing the catecholamines from blood. As the results of the shifts which took place in the myocardium, the ratio of the amount of catecholamines and their precursors was reduced three times: $(A + NA)/(D + D_N)$ is 0.27 for the control group; after 1 and 3 months of polychlorocamphene introduction, this ratio was −0.10.

The obtained data attest to the high sensitivity of myocardium to the entrance of polychlorocamphene. This is in agreement with the clinically observed results and with studies of the isoenzyme spectra of lactate dehydrogenase (LHD) of blood serum, where the most characteristic changes were changes in the LHD-LDH_1 and LDH_2 heart fractions (*15*). It can be assumed that tachycardia, angina pains, and changes in the EKG which take place during exposure to polychlorocamphene and are characteristics for diffusion hypoxy of the cardium (*16*) are conditioned by the significant accumulation of polychlorocamphene in the myocardium (*17*) and are related to the development of an imbalance of catecholamines. On the one hand, an increase of the dopamine level in the myocardium increases the heart rate and changes the rhythm (*18*); on the other hand, a reduction in the amount of catecholamines (adrenaline and noradrenaline) reduces the activity of the metabolic processes, ensuring the necessary energy potential (*19*).

Damage to the nervous system which is characteristic for exposure to polychlorocamphene can be related to the build-up of catecholamines in brain tissue, since it is known that an excess of adrenaline reduces the excitability of the hypothalamic region and the sympatheric adjoining trunk (*19*). According to previous data (*12–14*), the following were observed in the case of polychlorocamphene exposure: headaches, paresthesia, insomnia, sleep disorders of the narcolepsy type, anisocoria, nyosis, intermittent disturbances in the blood circulation of the brain, and other sign of vegetative-vascular disturbances, indicating a functional inadequacy of the hypothalamus trunk of the brain.

We can conclude on the basis of the obtained data that the ingestion of polychlorocamphene distorts catecholamine metabolism. Atypical changes take place in tissue of the absolute amount and ratio between the individual components of the sympatho-adrenaline system, the nature of their release is disturbed, and catecholamine breakdown is intensified in the case of the long-term introduction of the substance. All of the above may be the reason for the manifestation of inadequate reactions, disturbed metabolism, and inadequate function of vital organs and systems.

It should be noted that the nature of the excretion of the components of the sympatho-adrenaline system with urine is not always correlated with the state of catecholamine metabolism in tissue, particularly in the case of the single large dose of polychlorocamphene. We must study not only the nature of the excretion of catecholamines with urine, but also their metabolism in tissues in order to uncover the pathogenesis of the untoward effects of chemical factors.

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