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EFFECT OF PLANT GROWTH REGULATORS — HYDRAZINE DERIVATIVES — ON MICROSOMAL SYSTEMS OF THE LIVER

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Among the plant growth regulators used in agriculture, the hydrazine derivatives are attracting increasing interest. However, the mechanism of their toxic action on the body has not been adequately studied. In particular, there are no data on their effect on microsomal mono-oxygenase systems of the liver. However, these systems are known to perform the function of chemical protection of the organism.

Hydrazines belong to the class of inhibitors of microsomal cytochrome P-450, and they act indirectly through their metabolites [11]. It has been shown that the toxic effect of hydrazine is realized through the formation of intermediate compounds of free-radical nature in the course of oxidative metabolism [9]. During microsomal oxidation of hydrazine derivatives, oxygen radicals are formed [4]. The effect of several plant growth regulators which are hydrazine derivatives was studied on hydroxylase reactions of microsomes, on their superoxide dismutase (SOD) activity, and on lipid peroxidation (LPO).

EXPERIMENTAL METHOD

Experiments were carried out on noninbred male albino rats weighing 200 ± 20 g. Animals of the experimental groups were given Gidrel (hydrazinium 2-chloroethyl-bis-phosphonic acid), LD_{50} 2200 mg/kg body weight, Digidrel (dimethylhydrazinium 2-chloroethyl-bis-phosphonic acid), LD_{50} 3350 mg/kg, DSA (Daminozide; N,N'-dimethylhydrazide of succinic acid), LD_{50} 10,000 mg/kg, and MH (maleic hydrazide sodium salt), LD_{50} 15,000 mg/kg, in doses corresponding to 0.1 and 0.05 LD_{50}, perorally, once only.

The animals were decapitated 24 h after administration of the compounds. The microsomal fraction was obtained from liver homogenate by differential centrifugation [2]. The velocity of p-hydroxylation of aniline in the microsomes was determined from the quantity of p-aminophenol formed [10]. Cumyl hydroperoxide (CHP) was used as the cosubstrate [2]. The velocity of N-demethylation of aminopyrine in a CHP-dependent system was estimated from the accumulation of formaldehyde in the incubation medium, using the color reaction in [6]. SOD activity was determined by the method in [9], based on the ability of microsomes to inhibit the color reaction of reduction of nitroblue tetrazolium with the formation of formazan. The state of LPO was determined by the level of malonic dialdehyde (MDA) [1]. The protein concentration in the microsomal suspension was determined by Lowry's method [5], using bovine serum albumin as the standard. Binding of substrates with the oxidized form of cytochrome P-450 was determined by measuring the differential absorption spectra of the microsomes.

EXPERIMENTAL RESULTS

The hydrazine derivatives of phosphonic acid, Gidrel and Digidrel, in a dose of 0.1 LD_{50} slowed the velocity of aniline hydroxylation by more than half and demethylation of aminopyrine by 1.5-2 times. At the same time, SOD activity of the microsomes was inhibited and LPO decreased.

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TABLE 1. Effect of Single Action of Hydrazine Derivatives on Microsomal Systems of the Liver (M ± m, n = 6)

Preparation	Dose, mg/kg (fraction of (LD_{50})	p-hydroxylation of aniline, a	N-demethylation of aminopyrine, a	LPO b	SOD activity, c
Control	—	0,172±0,017	2,67±0,27	8,10±0,95	0,30±0,07
Gidrel	220 (1/10)	0,080±0,010*	1,65±0,15*	13,52±1,8*	0,74±0,03*
	110 (1/20)	0,180±0,02	3,11±0,15	10,45±1,9	0,66±0,06*
Digidrel	335 (1/10)	0,072±0,009*	1,35±0,09*	14,60±1,64*	0,74±0,03*
	167 (1/20)	0,142±0,023	3,30±0,30	17,07±3,77*	0,51±0,05*
DSA	1000 (1/10)	0,101±0,016*	2,09±0,14*	13,87±0,64*	0,72±0,06*
	500 (1/20)	0,190±0,011	3,11±0,18	11,74±1,33	0,60±0,06*
MH-Na	1500 (1/10)	0,181±0,018	2,67±0,14	6,79±0,94	0,27±0,05

Legend. *p<0.05 compared with control. a) nmoles metabolizing substrate/mg protein/min, b) nmoles MDA/mg protein/min, c) quantity of enzyme required to inhibit reaction of formazan formation by 50% (in conventional units).

DSA had a similar weaker action (Table 1). Spectral investigations of binding of these substrates with cytochrome P-450 in experiments in vitro showed that Gidrel, Digidrel, and DSA formed enzyme–substrate complexes with cytochrome P-450, by inducing spectral changes of type II. On the basis of these data it is easy to understand the strong inhibition of the hydroxylation reaction of the type II substrate aniline under the influence of hydrazine derivatives as a result of competition for the common binding site on cytochrome. Under these circumstances, the rate of demethylation of aminopyrine, a type I substrate, also was reduced.

These substrates, in a dose of 1/20 LD_{50} had only an inhibitory effect on microsomal SOD activity, but Digidrel also led to an increase in LPO of the microsomes, whereas the velocity of hydroxylase reactions was unchanged. It will be clear from Table 1 that inhibition of microsomal SOD activity by Digidrel, Gidrel, and DSA is dose-dependent in character.

It can be postulated on the basis of these results that under the influence of these compounds one of the first stages to be affected is the microsomal superoxide dismutase system.

MH-Na salt, in a dose of 1500 mg/kg (1/10 LD_{50}) had no effect on the hydroxylase systems of the liver. It likewise did not form an enzyme–substrate complex with cytochrome P-450 in experiments in vitro. These results are in agreement on the whole with data in the literature showing that MH-Na salt in a dose of 2000 mg/kg or less, has no damaging effect on microsomal enzymes of the rat liver. It is concluded that maleic hydrazide itself is evidently not metabolized by microsomal enzymes and does not modify the metabolism of other chemical substances which may be present in that system [7].

The results of the present investigations showed that if larger doses of MH-Na salt are administered and, in particular, 7500 mg/kg, processes of microsomal oxidation are nevertheless inhibited, SOD activity is reduced, and microsomal LPO intensified.

Thus besides direct interaction with cytochrome P-450 with the formation of type II enzyme–substrate complexes, a factor of no less importance in the mechanism of inhibition of microsomal oxidation by hydrazine derivatives is their effect on microsomal SOD activity and LPO. Inhibition of superoxide dismutase in the microsomes may be considered to be one of the primary reactions taking place under the influence of hydrazine derivatives.

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CHANGES IN POTASSIUM ION HOMEOSTASIS IN THE LENS OF FRASER MICE WITH HEREDITARY CATARACT (LINE Cat^{Fr})

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Among human diseases that most often lead to blindness, the first place is occupied by cataract, or opacity of the lens of the eye [3]. Although there may be different causes of different types of cataract, the mechanisms of development both of the commonest type of cataract, namely senile, and the less common hereditary type of cataract, are most probably the same. We know that genetically determined cataract is characteristic not only of man, but also of other mammals: mice [12], guinea pigs [11], rats [14], and monkeys [8]. One line of mice in which opacity of the lens develops in the 2nd week after birth is the Cat^{Fr} line [12]. The morphology of the lens in these animals has been well studied [4, 7, 10], and work has recently been published on changes in crystalline structure [5, 6]. However, K^+ homeostasis in the lens of Cat^{Fr} mice has not been investigated.

The development of cataract in mice of another line (Nakano) is linked with a disturbance of K^+, Na^+-ATPase function in the epitheliocytes of the lens [12]. It has been shown [13] that that preservation of transparency of the lens in mammals is due to a high K^+ level in the cytoplasm of the fibers. The aim of the present investigation was accordingly to study the distribution of K^+ in the lens and aqueous humor of Cat^{Fr} mice.

EXPERIMENTAL METHOD

A strain of mice homozygous for the autosomally dominant Cat^{Fr} gene, generously provided by the staff of the Institute of General Genetics, Academy of Sciences of the USSR, was used. Noninbred albino mice of the same age served as the control. The animals were given a normal diet. The lenses were removed after the mice were killed by cervical dislocation.

To obtain histological specimens the lenses were fixed in 4% formalin (pH 7.4) and sections were stained with hematoxylin and eosin.

The OP-K-07118 valinomycin electrode and OP-267 ionograph (Radelkis, Hungary) were used to measure K^+ activity. An OP-0830P Ag-AgCl electrode from the same firm was used as comparison electrode. K^+ activity was estimated from the change in electrode potentials, and the steepness of the electrode function was taken into account each time. All measurements were made in a solution containing 0.15 M NaCl and 0.2 mM KCl [1].

To assess K^+ activity in the aqueous humor, it was sampled by means of a Hamilton microsyringe (USA) in a volume of 3-5 µl; the K^+ level in the lens was determined after preparation of homogenates.

EXPERIMENTAL RESULTS

A morphological manifestation of senile cataract is the appearance of so-called bullous cells, degeneration of the epithelium, and edema of the lens fibers [9]. In month-old Cat^{Fr}

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