Modification of Ethylmethane Sulfonate-Induced Mytagenesis with Adrenaline

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Experiments with *Crepis capillaris* dry seeds show that pretreatment with adrenaline hydrochloride and adrenaline hydrotartrate significantly reduces the number of aberrations induced by the supermutagen ethylmethane sulfonate. The effective concentration ranges for adrenaline adrenaline hydrotartrate and hydrochloride are 10^{-1}-10^{-7} M and 10^{-3}-10^{-7} M, respectively. Adrenaline hydrochloride is more effective than adrenaline hydrotartrate (79.1 *vs.* 65%, respectively).

Key Words: *antimutagens, mutagenesis; receptor; adrenaline*

Endogenous cAMP modulates various metabolic processes in cells including the development of cell resistance to mutagens [3]. Intracellular cAMP concentration depends on the intensity of membrane processes, in particular, activity of the adenylate cyclase complex coupled to receptors. Hence, bioactive substances (for example adrenaline) interacting with adenylate cyclase-coupled receptors can regulate the content of endogenous cAMP, thus modulating gene-protecting systems and acting as antimutagens.

Adrenaline induces metabolic changes in animal cells via interaction with membrane α_1-, α_2-, β_1-, and β_2-adrenoreceptros [6]. β-Adrenoreceptros are coupled to adenylate cyclase that regulates the concentration of intracellular cAMP.

The aim of the present study was to assess the effect of various pharmaceutical forms of adrenaline on mutagenic effect of the alkylating agent ethylmethane sulfonate (EMS).

MATERIALS AND METHODS

Experiments were carried out with *Crepis capillaris* seeds. Catecholamine receptors in plant cells are poorly studied. However, there is data suggesting that the effect of physiologically active substances is principally the same in animal and plant cells [2].

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Two pharmaceutical forms of adrenaline: adrenaline hydrotartrate (AHT) and adrenaline hydrochloride (AHC) were tested in a wide concentration range [1]. Mutations were induced by the supermutagen EMS (1.6×10^{-5} M, Sigma). *Crepis capillaris* dry seeds were wetted in distilled water for 2 h (control) or treated with different concentrations of adrenaline for 2 h. The seeds were then washed for 20 min with flow water, treated with EMS for 2 h, and after 20-min washout placed in 0.01% colchicine (Sigma) for germination (26°C). Twenty-four-36-h germs with 1.0-1.5-mm roots were taken, root apexes were cut off, fixed in Carnoy's fixative (absolute ethanol:glacial acetic acid, 3:1), stained with acetocarmine in a water bath for 10 min, and after a 30-min incubation with chloralhydrate squash preparations were prepared. All types of chromosomal and chromatid aberrations were counted. The data were processed statistically using the Student *t* test. The antimutagenic effect was calculated from the following formula [5]: $(M_1-M_2)/M_1\times100\%$, where M_1 and M_2 are the percentage of aberrant metaphases in the presence of mutagen alone and mutage+protecter, respectively.

RESULTS

Both adrenaline forms markedly reduced the number of EMS-induced aberrations (Table 1), AHC being

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TABLE 1. EMS-Induced Mutagenesis in *Crepis Capillaris* Cells Pretreated with AHC and AHT (*M±m*)

Concentration, M		Total number of		Aberration rate, %	Effect, %
		metaphases	aberrations		
AHC					
Control		1400	13	0.93±0.26	—
ENS		1378	64	4.64±0.57*	—
AHT	4.6×10^{-3}	1360	22	1.62±0.34+	65.1
	4.6×10^{-4}	1340	13	0.97±0.27+	79.1
	4.6×10^{-5}	1346	15	1.11±0.28+	76.1
	4.6×10^{-6}	1650	23	1.39±0.29+	70.0
	4.6×10^{-7}	1610	45	2.80±0.41++	39.6
	4.6×10^{-8}	1542	73	4.73±0.54+	—
AHT					
Control		561	4	0.71±0.35	—
ENS		900	36	4.00±0.65*	—
AHT	5.4×10^{-1}	1002	14	1.40±0.37+	65.0
	3.0×10^{-2}	838	12	1.43±0.41+	64.3
	3.0×10^{-3}	1044	18	1.72±0.40++	57.0
	3.0×10^{-4}	688	12	1.74±0.50++	56.5
	3.0×10^{-5}	1007	22	2.18±0.46+++	45.5
	3.0×10^{-6}	759	16	2.11±0.52+++	47.5
	3.0×10^{-7}	1203	29	2.41±0.44+++	39.8
	3.0×10^{-8}	985	31	3.15±0.56	21.3

Note. *$p<0.001$ compared with the control, +$p<0.001$, ++$p<0.01$, +++$p<0.05$ compared with EMS.

a more potent antimutagen that AHT (79.1 vs. 65%, respectively). On the other hand, AHT was active in a broader concentration range (5.4×10^{-1}-3.0×10^{-7} M) than AHC (4.6×10^{-3}-4.6×10^{-7} M). The maximum antimutagen activity of AHT and AHC was observed at concentrations of 5.4×10^{-1} and 4.6×10^{-4} M, respectively.

Taking into account the fact that adrenaline reduces the level of induced mutagenesis, this substance can be recognized as an antimutagen.

Interestingly, adrenaline exhibits its antimutagenic effect at low (10^{-7} M) concentrations characteristic of ligand-receptor interactions [2]. Moreover, adrenaline acts through a receptor-coupled adenylate cyclase which is responsible for the rise of endogenous cAMP. It can be hypothesized that the antimutagenic effect of adrenaline is realized though specific receptors and is mediated by the second messenger cAMP.

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