SHORT COMMUNICATION

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IN VITRO ANALYSIS OF CLASTOGENIC EFFECTS OF ADRENALINE ON HUMAN LYMPHOCYTES.

N. Djelić[1], Biljana Spremo-Potparević[2], Lada Živković[2], Biljana Marković[1], and S. Dačić[3]. *[1]Department of Biology, Faculty of Veterinary Medicine, University of Belgrade,* 11000 Belgrade, Serbia; *[2]Department of Biology and Human Genetics, Institute of Physiology, Faculty of Pharmacy, University of Belgrade*, 11000 Belgrade, Serbia; *[3]Department of Stomatology, School of Medicine, University of Niš*, 18000 Niš, Serbia.

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Despite abundant published data concerning the biochemical and physiological effects of adrenaline and other catecholamines, little is known about possible genotoxic and mutagenic effects. There is an interesting experimental finding that dopamine induces DNA strand breaks in human skin fibroblasts and gene mutations in mouse lymphoma cells (Moldeus et al., 1983). However, dopamine did not exhibit genotoxic effects in the *Salmonella*/mammalian-microsome mutagenicity test, the sex-linked recessive lethal test in *Drosophila melanogaster*, the sister chromatid exchange (SCE) test in human lymphocytes , or the *in vivo* micronucleus assay in mouse and rat (Moldeus et al., 1983). Likewise, in cytogenetic analysis, adrenaline did not exhibit genotoxic effects on cultured human peripheral blood lymphocytes (Djelić, 1997). On the other hand, mutagenic effects of various catecholamines on mouse lymphoma cells resulted from the creation of superoxide anion (McGregor et al., 1988). In addition, catechol derivates, including adrenaline, induce DNA strand breakage by ferryl species, whereas the induction of 8-hydroxyguanine (8OHG) is due to a hydroxyl radical (˙OH) (Miura et al., 2000).

More recent studies revealed that noradrenaline induces primary DNA damage in the Comet assay on purified human lymphocytes (Djelić and Anderson, 2003) and sperm (Dobrzynska et al., 2004), most likely due to formation of reactive oxygen species (ROS). This result is in agreement with findings that adrenaline and other catecholamines can be involved in redox cycling under the influence of superoxide anion (Genova et al., 2006). Interestingly, superoxide anion may induce chromosome breakage and sister chromatid exchange (SCE) (M'bemba-Meka et al., 2007).

The cytogenetic effects of adrenaline were evaluated on cultured human peripheral blood lymphocytes. Lymphocyte cultures were set up according to the protocol of Rooney and Czepulkowski (1986), as described previously (Djelić et al., 2007). Two adrenaline exposure times were used (24 and 48 h), appropriate concentrations (0.01 to 150 μM, Table 1) of adrenaline hydrochloride (Jugoremedija, Zrenjanin, Serbia) being added to the culture vials at 24 h of incubation for the experiment with 48-h exposure and at 48 h for the one with 24-h exposure. A negative control was prepared by Jugoremedija

Table 1. Analysis of chromosome gaps and break frequencies and the mitotic index in whole-blood cultures of human lymphocytes treated with adrenaline for 24 or 48 h: results from three donors. G – gaps (not included in statistical data); CB - chromatid breaks; IB - isochromatid breaks; CE - chromatid exchanges; IE - isochromatid exchanges; MI – mitotic index; A total of 300 well-spread metaphases per each concentration were determined. *P< 0.05, **P< 0.01, and ***P< 0.001 ($\chi 2$ test).

Adrenaline concentrations (μM) plus negative and positive controls	24 h exposure							48 h exposure						
	Types of chromosomal changes					Breaks per metaphase		Types of chromosomal changes					Breaks per metaphase	
	G	CB	IB	CE	IE	(%) ± SD	MI (%)	*G*	CB	IB	CE	IE	(%) ± SD	MI (%)
Untreated	8	4	1			1.67 ± 0.13	4.70	6	2	2	1		2.00 ± 0.16	5.11
Negative control	10	4				1.33 ± 0.11	5.08	7	5				1.67 ± 0.13	6.22
Adrenaline 0.01 μM	7	5				1.67 ± 0.13	4.65	6	3	1			1.33 ± 0.11	5.17
Adrenaline 0.2 μM	5	2	1			1.00 ± 0.10	4.12	12	3		1		1.33 ± 0.15	5.94
Adrenaline 1 μM	6	4			1	2.00 ± 0.16	4.78	7	2				0.67 ± 0.08	4.43
Adrenaline 5 μM	11	3				1.00 ± 0.10	3.36*	10	4	1			1.67 ± 0.13	3.70**
Adrenaline 50 μM	8	6	1			2.33 ± 0.15	2.25***	9	5	1			2.00 ± 0.14	2.42***
Adrenaline 150 μM	9	5				1.67 ± 0.13	2.09***	10	6	1	1		2.67 ± 0.19	1.98***
Positive control (MNNG)	26	17	9	4	2	12.67 ± 0.39***	1.89***	38	31	11	2	3	17.33 ± 0.42***	1.46***

as a solution which contains all compounds except the active ingredient – adrenaline. A well-known mutagen, N-methyl-N'-nitro-N-nitrosoguanidine (MNNG), was used as a positive control, at a final concentration of 1 μM. Two hours before harvesting, colcemid (0.5 μg/mL) (Ciba, Basel, Switzerland) was added to the cultures. Hypotonic treatment, fixation, and staining were described elsewhere (Djelić et al., 2007). We scored 100 mitotic plates per donor for each experimental concentration and controls. Scoring of gaps and breaks was performed according to Brogger (1982), and the mitotic index (MI) was calculated as the percentage of cells in mitosis based on at least 2000 cells per donor. Statistical analysis was performed with the χ^2 test.

Experimental values of chromosome breakage and mitotic indices are presented in Table 1. It is evident that under the experimental conditions of this investigation, adrenaline did not cause significant changes in breaks per cell in comparison with either the negative control or untreated cultures at either exposure time (24 and 48 h). As expected, the positive control (MNNG) caused significant clastogenic effects in the guise of an increase in breaks and chromatid and isochromatid exchanges ($P < 0.001$) at both exposure times (24 and 48 h), thereby indicating that the experimental conditions were suitable for detection of clastogenic effects. On the other hand, there is a decrease in the mitotic index at the three highest concentrations of adrenaline (5, 50, and 150 μM).

The lack of clastogenic effects of adrenaline observed in this investigation is in accordance with results of genotoxicity evaluation of chemically similar dopamine, which did not induce sister chromatid exchanges in cultured human lymphocytes (Moldeus et al., 1983). On the other hand, noradrenaline had profound DNA damaging effects in the Comet assay on purified human lymphocytes (Djelić and Anderson, 2003). However, it should be pointed out that effects of noradrenaline in the Comet assay were monitored on purified lymphocytes, whereas in the present investigation we used whole-blood lymphocytes. This is important to mention because purified lymphocytes are more susceptible to DNA damage by ROS than lymphocytes in whole-blood cultures (Andreoli et al., 1999). Therefore, had we used purified lymphocytes, the effects of adrenaline might have been significant. This is conceivable because catechol groups can be involved in redox cycling accompanied by ROS generation, which favors oxidative stress (Dobrzynska et al., 2004). Indeed, Mc Gregor et al. (1988) investigated catecholamines and related substances and showed that although the phenolic group was not mutagenic, the addition of a catehol-forming second hydrohyl group was sufficient to create a mutagenic agent.

Finally, decrease in the mitotic index is in accordance with findings that teophylline inhibits proliferation of cultured human lymphocytes (Kotecki et al., 1989). To be specific, the molecular mechanism of signal transduction of both adrenaline and teophylline implies increase in the concentration of intracellular cAMP. Increased cAMP may have contributed to the mitotic index decrease observed in this investigation. Moreover, it has been shown that stimulation of β-adrenoceptors with isoproterenol decreases the proliferative response of lymphocytes to mitogens. Also, there is a marked increase of intracellular cAMP in lymphocytes exposed to isproterenol and mitogens (Carlson et al., 1994). Since naturally occuring catecholamines inhibit the proliferation of T-lymphocytes (Bartik et al., 1994), we assume that stimulation of adrenergic receptors by adrenaline may have been responsible, at least in part, for MI decrease. In addition, arrest of mitosis due to repair of genetic damage may have contributed to decreased values of MI.

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