Adrenergic Regulation of Lymphocyte Proliferative Response in Cultures with T-Cell Mitogens

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The effects of epinephrine, β-adrenergic agonist terbutaline sulfate, and cAMP phosphodiesterase inhibitor theophylline on proliferative response of peripheral blood lymphocytes from healthy subjects were studied in cultures with phytohemagglutinin and concanavalin A. Both adrenergic agonists inhibited lymphocyte blastogenesis, but the effect of epinephrine was more pronounced.

Key Words: *epinephrine; β-adrenergic compounds; lymphocyte blastogenesis*

Immune cells possess β_1, β_2, α_1, and α_2 adrenoceptors [4-7]. Stimulation of these receptors can produce opposite effects [4,9], and therefore the total effect of natural adrenergic compounds, *e. g.* epinephrine acting through all types of adrenoceptors, is not clear.

We examined *in vitro* effects of epinephrine, β-adrenomimetic terbutaline sulfate, and cAMP phosphodiesterase inhibitor theophylline on proliferative response of lymphocytes induced by T-cell mitogens in different concentrations.

MATERIALS AND METHODS

Peripheral blood leukocytes of healthy men aged 18-43 years were cultured with phytohemagglutinin (PHA, Olaina Chemical Plant) in different concentrations (12.5, 25, 50, 100, and 200 μg/ml) and concanavalin A (ConA, Calbiochem, 0.5, 5, and 50 μg/ml) in 96-well round-bottom plates. Each culture contained 2×10^5 cells in 0.2 ml complete nutrient medium prepared on medium 199 with 10 mM HEPES (Serva), 2 mM L-glutamine (Reanal), 100 μg/ml gentamicin, and 10% autoplasma. Cultures were incubated in a humid atmosphere with 5% CO_2 at 37°C for 72 h. Eighteen hours before the end of culturing 2 μCi ^{3}H-methylthymidine (10 μl) was added to each well. Radioactivity was measured on a Beta-2 scintillation counter (Medapparatura, Kiev). Epinephrine hydrochloride (final concentrations 0.0001-1.0 μg/ml), terbutaline sulfate (final concentrations 10^{-4}-10^{-9} M), and theophylline (final concentration 3 mM) were added to the cultures at the beginning.

The results were statistically processed using Student's *t* test for paired data, Wilcoxon paired *T* test, and Wilcoxon rank *W* test [1].

RESULTS

Epinephrine inhibited proliferative response of cultured lymphocytes to T-cell mitogens (Table 1). The effect depended on the type of mitogen and its concentration. In all tested cultures the mitogen significantly suppressed the proliferative response of lymphocytes in cultures with optimal (25 and 50 μg/ml) and superoptimal concentrations of PHA (200 and 100 μg/ml). In cultures with suboptimal concentration of PHA (12.5 μg/ml) epinephrine had a pronounced effect only in a high concentration (1 μg/ml). In cultures with ConA the hormone suppressed the proliferative response to both optimal (50 μg/ml) and suboptimal (0.5 and 5 μg/ml) concentrations of the mitogen. The studied concentrations of epinephrine are close to its normal level in the organism. The concentration of 0.0001-0.01 μg/ml is needed for smooth muscle con-

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TABLE 1. Effects of Epinephrine and Theophylline on Proliferative Response of Normal Human Peripheral Blood Lymphocytes (lg cpm, *M±m*)

Agent and its concentration	Without mitogen	Mitogen and its concentration, μg/ml							
		PHA					ConA		
		12.5	25	50	100	200	0.5	5	50
Control	3.5715±	4.2297±	4.5346±	4.5311±	4.3508±	3.9954±	3.5420±	3.7982±	4.1520±
	0.0499	0.1063	0.1122	0.0851	0.0906	0.1120	0.0552	0.0809	0.0672
	3729)	(16972)	(34244)	(33974)	(22430)	(9894)	(3484)	(6284)	(14189.5)
Epinephrine, μg/ml									
1	3.4732±	4.1090±	4.3006±	4.2281±	4.0608±	3.6992±	3.4378±	3.7281±	3.9037±
	0.0764	0.1255*°	0.1185**°	0.1430**°+	0.1364**°+	0.1210**°+	0.0792	0.0967	0.1163*°+
	(2973)	(12853)	(19981)	(16908)	(11502)	(5003)	(2741)	(5347)	(8011.0)
0.1	3.5063±	4.1553±	4.3187±	4.2389±	4.0488±	3.7254±	3.4799±	3.6967±	3.9444±
	0.0649	0.1302	0.1225***°	0.1499**°	0.1281**°+	0.1244*°+	0.0609	0.0602	0.1197°
	(3208)	(14298)	(20830)	(17334)	(11188)	(5314)	(3019)	(4974)	(8799.1)
0.01	3.4556±	4.2506±	4.3786±	4.2449±	4.0178±	3.6115±	3.4231±	3.6253±	3.9279±
	0.0695°	0.1245	0.1115***°	0.1452**°+	0.1347**°+	0.1270***°+	0.0613°	0.0731*°	0.1221°+
	(2855)	(17806)	(23909)	(17574)	(10418)	(4088)	(2649)	(4220)	(8470.8)
0.001	3.3665±	4.1924±	4.3090±	4.1946±	3.9649±	3.5911±	3.4278±	3.7059±	3.8422±
	0.0508*°+	0.1257	0.1210**°	0.1301***°+	0.1433***°+	0.1337***°+	0.0543°	0.0730	0.1378*°+
	(2326)	(15575)	(20369)	(15652)	(9223)	(3900)	(2678)	(5081)	(6953.5)
0.0001	3.4640±	4.1850±	4.4125±	4.2961±	4.0170±	3.6428±	3.4129±	3.6818±	3.9296±
	0.0842°	0.1261	0.0918*°	0.1121**°	0.1386***°+	0.0977***°+	0.0428*°+	0.0641	0.0428°+
	(2911)	(15312)	(25855)	(19773)	(10399)	(4394)	(2588)	(4806)	(2587.6)
Theophylline, 3 mM	3.4572±	3.2637±	3.4532±	3.3581±	3.2692±	3.4112±	3.2788±	3.4206±	3.3829±
	0.07530+	0.0891***°+	0.0926***°+	0.0699***°+	0.0591***°+	0.0538***°+	0.0825**°+	0.1057*°+	0.0907***°+
	(2865)	(1835)	(2839)	(2281)	(1859)	(2578)	(1900)	(2634)	(2415.2)

Note. Here and in Table 2: geometric mean of dpm (*M* antilogarithm) is in parentheses; the number of observations in the control and experimental samples is 10. *$p<0.05$; **$p<0.01$; ***$p<0.01$ *vs.* control (Student *t* test); +$p<0.05$ *vs.* control (Wilcoxon rank W test); °$p<0.05$ *vs.* control (Wilcoxon paired sign *T* test).

TABLE 2. Effect of Terbutaline Sulfate on Proliferative Response of Normal Human Peripheral Blood Lymphocytes (lg cpm, $M \pm m$)

Terbutaline, M	Without mitogen	Mitogen and its concentration, μg/ml							
		PHA					ConA		
		12.5	25	50	100	200	0.5	5	50
Control	3.4925± 0.0584 (3108)	4.1393± 0.1306 (13783)	4.4049± 0.1581 (25406)	4.4487± 0.1389 (28102)	4.3248± 0.1235 (21124)	3.9967± 0.0919 (9924)	3.4716± 0.0607 (2962)	3.8378± 0.0828 (6884)	4.1966± 0.0978 (15725)
10^{-4}	3.4418± 0.0562 (2766)	4.0785± 0.1418 (11980)	4.3090± 0.1378*° (20371)	4.3751± 0.1274*° (23722)	4.2491± 0.1338 (17745)	3.8213± 0.1128*° (6626)	3.3870± 0.0621 (2438)	3.7788± 0.1158 (6009)	4.1528± 0.1066 (14216)
10^{-5}	3.3701± 0.0639+ (2345)	4.1460± 0.1577 (13996)	4.2614± 0.1634*° (18254)	4.3855± 0.1542 (24295)	4.2941± 0.1149 (19685)	3.8846± 0.0898° (7667)	3.4454± 0.0694 (2789) (n=9)	3.7748± 0.0704 (5953)	4.2245± 0.1002 (16770)
10^{-6}	3.3766± 0.0710 (2380)	4.0766± 0.1538 (11930)	4.2826± 0.1424° (19170)	4.3453± 0.1374*° (22146)	4.1912± 0.1483 (15532)	3.7929± 0.0884*° (6209)	3.4150± 0.0749 (2600)	3.6959± 0.0801*° (4964)	4.1515± 0.1198 (14175)
10^{-7}	3.4281± 0.0702 (2680)	4.0690± 0.1667 (11723)	4.2848± 0.1583*° (19265)	4.3574± 0.1218*° (22772)	4.2007± 0.1171*° (15875)	3.7437± 0.0803**°+ (5543)	3.4158± 0.0771° (2605)	3.6798± 0.0816 (4784) (n=9)	4.1115± 0.1289 (12926)
10^{-8}	3.4052± 0.0903 (2542)	4.0918± 0.1433 (12353)	4.3176± 0.1339 (20776)	4.3489± 0.1337*° (22332)	4.1538± 0.1101° (14249)	3.7963± 0.0876*° (6256)	3.3788± 0.0450° (2392)	3.7311± 0.1025 (5384) (n=9)	4.2303± 0.1341 (16993)
10^{-9}	3.3293± 0.1236 (2135)	4.0515± 0.1664 (11259)	4.2990± 0.1369*° (19905)	4.3620± 0.1385 (23013)	04.1993± 0.0952*° (15825)	3.8420± 0.0702*° (6951)	3.4031± 0.0703 (2530)	3.7576± 0.0801 (5723)	4.1346± 0.1175 (13633)

Note. *n*: number of cases.

traction; the concentration of norepinephrine (whose effect is similar to that of epinephrine) in mouse spleen is 1.219-0.608 μg/ml [3]. Theophylline sharply suppressed lymphocyte blastogenesis induced by PHA or ConA irrespective of the mitogen concentration (Table 1). This effect seems to be realized through accumulation of intracellular cAMP, because the mechanism of theophylline effect is associated with inhibition of phosphodiesterase which catalyzes its transformation into inactive 5'-AMP [2,6].

Theophylline concentration of 3 mM increased the level of intracellular cAMP and suppressed CD2 expression on some T lymphocytes [2]. Epinephrine also increased the level of cAMP through β_1- and β_2-adrenoceptors [5,10]. A β-adrenergic agonist terbutaline sulfate acting mainly on β_2-adrenoceptors significantly suppressed lymphocyte blastogenesis mainly in the cultures with PHA in superoptimal (200 and 100 μg/ml) and optimal (50 and 25 μg/ml) concentrations (Table 2). No significant suppression was observed in cultures with suboptimal concentrations of PHA (12.5 μg/ml) and in the majority of cultures with ConA. In general, the effect of this agonist in concentrations of 10^{-6}-10^{-9} M was less expressed than that of epinephrine in similar concentrations (0.0001-1.0 μg/ml or 4.55×10^{-6}-4.55×10^{-10} M, respectively, Table 1). Taking into account the higher affinity of terbutaline for β-adrenoceptors and published data [6,8] that the immunosuppressive effect of epinephrine mediated mainly through these receptors one can expect a more pronounced effect. It is generally believed that the immunomodulating effects of adrenergic compounds are realized predominantly through β-adrenoceptors and increased level of cAMP [6,8]; however, our findings indicate that they can act through a greater variety of adrenoceptors. A less pronounced inhibitory effect in cultures with ConA in comparison with PHA can be due to a lower level of proliferative response of lymphocytes to ConA and to the fact that these mitogens activate cells expressing different adrenoceptors.

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