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ORIGINAL ARTICLE

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Propranolol affects stress-induced leukocytosis and cellular adhesion molecule expression

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Abstract In this study, the impact of the β-adrenergic antagonist propranolol on resting and acute psychological- and physical-stress-induced circulating leukocyte numbers and the density of cellular adhesion molecules was investigated. In a randomized double-blind crossover design, 45 healthy volunteers performed a 15-min public speaking task and 21 subjects performed a 16-min bicycle exercise after 5 days of ingesting a placebo and after 5 days of ingesting 100 mg/day propranolol. One week of ingesting propranolol modestly elevated the numbers of $CD62L^+$ ($P < 0.019$) but not $CD62L^-$ T-lymphocytes. Moreover, propranolol preferentially blunted-psychological stress-induced increases in naïve T-helper ($CD4^+CD62L^+$; $P < 0.049$) and naïve T-cytotoxic lymphocytes ($CD8^+CD62L^+$; $P < 0.003$), as well as activated T-cytotoxic lymphocytes ($CD8^+CD29^+$; $P < 0.005$). However, exercise-induced increases in leukocyte numbers were enhanced following propranolol treatment ($P < 0.04$). In contrast to the effect on the numbers of adhesion-molecule-bearing cells, there was only a modest effect of propranolol on stress-induced alterations of the density of CD62L, CD54 and CD11a. In this study, propranolol treatment interfered with the adrenergic regulation of circulating leukocyte numbers by blunting psychological stress effects but enhancing exercise effects. Propranolol affected the cell activation status to a lesser extent, as reflected by the density of adhesion molecules.

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Introduction

About 30 years ago, β-blocking agents were introduced as a treatment for arrhythmia and angina pectoris. Since then, β-adrenergic antagonists have gained a significant role in the treatment of a wide range of cardiovascular disorders (Goldstein 1997). However, the reasons behind some of their beneficial therapeutic effects are not completely understood.

Sympathetic stimulation under stressful conditions or in some pathological conditions (e.g., congestive heart failure) leads to a transient leukocytosis. Upon adrenergic stimulation, predominantly natural killer (NK) cells and cytotoxic T-lymphocytes leave the marginal pools to increase cell numbers in the peripheral blood (for review, see Benschop et al. 1996a). Recent studies implicate changes in the expression of leukocyte adhesion molecules as a possible explanation for this cellular redistribution (Benschop et al. 1997; Carlson et al. 1996; Gabriel and Kindermann 1998; Kurokawa et al. 1995).

CD62L, a member of the selectin family, regulates the rolling of leukocytes along the endothelial wall. Following stress, its expression is reduced on circulating cytotoxic lymphocytes and NK cells following a preferential redistribution of $CD62L^-$ cells into the peripheral blood stream, as well as the possible shedding of CD62L from individual cells (Goebel and Mills 2000; Miles et al. 1998; Rehman et al. 1997). Moreover, the expression of integrin (CD11a, b and c/CD18), adhesion molecules that facilitate the aggregation and transmigration of leukocytes across the endothelium, increases on T-cytotoxic lymphocytes and neutrophils after physical exercise and infections (Gabriel and Kindermann 1998). Some adhesion molecules, such as the intercellular adhesion molecule (ICAM) CD54 (ICAM-1) are reportedly shed upon physical exercise cells (Rehman et al. 1997). These observations may help to

explain how lymphocytes demarginate into the peripheral blood stream and how more mature, activated lymphocytes are redistributed (Mills et al. 2000).

β-adrenergic antagonists interfere with stressor-induced leukocytosis. Ahlborg and Ahlborg (1970) found that propranolol could partially block the exercise-induced increase in leukocytes. Murray et al. (1992) refined these observations by showing that the β1-adrenergic antagonist metoprolol was not able to inhibit the lymphocyte release during exercise. Leukocyte changes induced by acute psychological stress are also responsive to β-adrenergic antagonists. Benschop at al. (1994, 1996b) showed that mental stress, as well as a first-time parachute jump, increased the number of circulating NK cells and cytotoxic T-lymphocytes; this phenomenon can be blocked by propranolol (Benschop et al. 1994, 1996b) and labelotol (Bachen et al. 1995). Isoproterenol, a β-adrenergic agonist, mimics the catecholamine-induced effects of acute stress on the immune system, an effect also mitigated by propranolol (Landmann et al. 1984; Mills et al. 1997, 2000).

Although the effects of β-blockade on the number of circulating cytotoxic lymphocytes and NK cells are well documented (Ahlborg and Ahlborg 1970; Benschop et al. 1996b; Murray et al. 1992), few studies have attempted to investigate the subsets of cytotoxic and helper T-lymphocytes with respect to adhesion molecules and cell activation status. The study presented here was designed to examine the impact of propranolol on lymphocyte subsets and the adhesion molecule expression induced by acute psychological stress and physical exercise in a group of healthy subjects. People routinely face acute psychological and physical stressors, however the two situations are characterized by different hormonal profiles, such as the marked norepinephrine surge that occurs during exercise but not during psychological stress (Kinderman et al. 1982). Based on the findings of recent studies on the differential effects of stressors on leukocyte adhesion molecule expression (Kurokawa et al. 1995; Miles et al. 1998), we hypothesized that β-blockade would preferentially blunt the stress-induced increase in $CD62L^-$ memory as compared to $CD62L^+$ naïve leukocytes. Thus, we looked at cell counts of various lymphocyte subsets bearing surface molecule combinations such as $CD62L^+CD45RA^+$ (naïve lymphocytes), $CD29^+CD45RO^+$ (activated memory cells) and CD62L and CD54. We also looked at the density of CD62L, CD54 and CD11a on mononuclear cells. In addition, in order to understand the possible mechanisms underlying the effects of the stressor and/or propranolol on immune cells, we also determined plasma norepinephrine and epinephrine levels.

Methods

Subjects

A group of 45 volunteers [mean (SD) age 36 (7) years] gave their written informed consent to participate in the study. All subjects were healthy, with normal electrocardiograms (ECGs). Volunteers were recruited from the local community and compensated financially for their participation. The protocol was approved by the Institutional Review Board of the University of California, San Diego.

Procedure

Subjects were studied in a randomized, double-blind, crossover design following 5 days each of placebo or 100 mg/day of the non-specific β-blocker propranolol (Inderal-LA). At least 2 weeks separated each testing condition. Subjects refrained from ingesting caffeinated beverages and smoking for 12 h prior to the study. Upon arrival at the laboratory, subjects were seated and a 19-gauge catheter was inserted into a forearm vein; they then rested for 30 min. Starting at 9:00 a.m., volunteers performed a standardized 15-min public speaking stressor (Mills et al. 1996). This consisted of two back-to-back speeches performed in a sitting position, whereby the subject prepared (3-min/speech) and then gave a speech (3-min/speech) on a hypothetical situation. Subjects were told that the speech would be recorded and evaluated by experts. The two topics were to defend oneself from being falsely accused of shoplifting, and a confrontation at an unscrupulous car dealership. If subjects stopped speaking before the time was up, they were reminded to continue to talk by reiterating and summarizing their points.

Following a 90-min rest period, a subset of subjects [$n = 21$; age 35 (8) years] was selected randomly to perform a 16-min bicycle ergometer exercise. Power analysis of our prior data (Goebel and Mills 2000) indicated that in response to exercise a sample size of $n = 19$ subjects would have 80% power to detect mean differences at a significance level of $p < 0.05$. The exercise task lasted approximately 16 min, comprising four 4-min stages marked by increasing resistance on a bicycle ergometer. Subjects were informed of the warning signs of excess exertion (e.g., faintness, shortness of breath, dizziness, muscle cramps). Subjects maintained a pedal rate of 70 rpm. Maximum oxygen uptake ($\dot{V}O_{2max}$) was estimated using heart rate, and the workload was adjusted so the exercise was completed at a level comparable to 75% of the estimated $\dot{V}O_{2max}$ for each subject. Following the test, the wheel resistance was removed and subjects continued to pedal freely against no resistance for 5 min as a cool-down period.

Leukocyte subsets and adhesion molecule expression

Whole blood was sampled prior to and immediately following each challenge. Blood was preserved with ethylenediaminetetraacetic acid and maintained at room temperature (23°C). Complete blood count (CBC) analysis was performed by using a Coulter STKS CBC Counter. Flow cytometry (FACSCalibur, Becton Dickinson, San Jose, Calif., USA) was carried out on blood processed within 3 h of collection using CellQuest software (Mills et al. 2000). After the lysing (FACS Brand lysing solution, Becton-Dickinson), positive four-color staining was used with monoclonal antibodies conjugated to either fluorescein isothiocyanate, phycoerythrin (PE), peridinin chlorophyll protein (PerCP), or allophycocyanin (APC) (Becton-Dickinson and PharMingen). The fluorescence compensation was performed using CaliBRITE beads (Becton-Dickinson) and FACSComp software. Isotypic controls were used for each assay to determine non-specific staining. Phenotypes were expressed as the percentages of total cells analyzed by flow cytometry. Gating strategies for multi-parameter analyses were performed using side scatter versus FL3- (PerCP), CD8-positive cells or side scatter versus FL4 (APC)-, CD4-positive cells using various combinations of monoclonal antibodies. For adhesion molecule density quantification, flow cytometric estimation of antibodies bound/cell (ABCs) was performed using Quantibrite PE beads (Becton-Dickinson). The Quantibrite PE beads were run at the same instrument settings as the assay. The number of PE molecules/bead was calibrated with the geometric means of the bead peaks in linear fluorescence, and the FL2 (PE) axis was converted into the number of PE molecules bound/cell. ABCs,

being the number of antibodies that bind to the specific cell, provide a good approximation of antigen density.

Catecholamines

Blood for catecholamine analyses was collected on ice before and immediately following the speech and exercise, separated in a refrigerated centrifuge (300 *g*) and the plasma stored at –80°C until assay. Epinephrine and norepinephrine were determined by radioenzymatic assay (Kennedy and Ziegler 1990). The intra- and inter-assay coefficients of variation were 6.5% and 11%, respectively.

Statistical analysis

In order to evaluate drug and task effects and their interactions, the data were analyzed using a 2×2 (placebo or propranolol × baseline or task) repeated-measure analysis of variance design (SPSS Statistical Software 9.0). Of the 45 subjects who completed the speech stressor and the 21 subjects who were assigned to the exercise challenge, cell adhesion density data were obtained from 40 and 17 subjects, respectively. Data are presented as the mean (SD).

Results

Psychological stress

Heart rate

Propranolol led to a significant decrease in the pre-speech values of resting heart rate [placebo 73.61 (9) beats/min versus propranolol 63.74 (9)beats/min; $F=103$, $P<0.001$]. Propranolol also significantly attenuated the speech-induced increase in heart rate [+4.51 beats/min placebo versus +1.16 beats/min propranolol: placebo 78.12 (9) beats/min versus propranolol 64.9 (8) beats/min; drug × speech interaction, $F=12.6$, $P<0.001$].

Immune cell numbers

Consistent with prior reports, psychological and physical challenge resulted in a marked elevation of all leukocyte subsets and affected the density of adhesion molecules on leukocytes (Tables 1, 2, 3). Also consistent with prior reports, β-blockade tended to blunt the psychological stress-induced increase in all examined cell populations (see Tables 1, 2). The effect was statistically significant for mixed lymphocytes ($F=6.39$, $P<0.015$), NK lymphocytes ($F=31.17$, $P<0.001$), $CD62L^-$ NK lymphocytes ($F=26.47$, $P<0.001$), $CD54^+$ lymphocytes ($F=7.72$, $P<0.008$), $CD8^+$ lymphocytes ($F=9.19$, $P<0.004$), $CD8^+CD62L^+CD45RA^+$ lymphocytes ($F=5.19$, $P<0.028$), $CD8^+CD29^+CD45RO^+$ lymphocytes ($F=4.44$, $P<0.041$), and $CD4^+CD62L^-$ ($F=4.09$, $P<0.049$) and $CD8^+CD62L^-$ ($F=9.84$, $P<0.003$) lymphocytes.

Propranolol also affected baseline white blood cell numbers ($F=7.43$, $P<0.009$) by significantly increasing mixed lymphocytes ($F=7.15$, $P<0.010$), predominantly $CD4^+$ lymphocytes ($F=4.83$, $P<0.033$), naïve T-helper lymphocytes ($CD4^+CD62L^+$; $F=5.97$, $P<0.019$) and naïve T-cytotoxic lymphocytes ($CD8^+CD62L^+$; $F=4.09$, $P<0.049$).

Density of adhesion molecules on mononuclear cells

Propranolol significantly diminished the stress-induced increase of CD11a density on lymphocytes ($F=10.07$,

Table 1 The effect of propranolol on the alteration in circulating leukocyte numbers in response to acute psychological and physical stress. Data are presented as the mean (SD) number of cells/μl. (*WBC* White blood cells, *NK* natural killer)

Cell type	Condition	Psychological stress		Physical stress	
		Baseline	Public speaking	Baseline	Exercise
WBC	Placebo	6074 (2100)	6380 (2198)$^{\#\#}$	6590 (2138)	8502 (2718)$^{\#\#}$
	Propranolol	6472 (1896)*	6682 (2010)	6916 (2067)**	9871 (2758)^{II}
Monocytes	Placebo	437 (124)	475 (169)$^{\#}$	488 (138)	662 (190)$^{\#\#}$
	Propranolol	479 (145)	516 (160)	547 (159)**	849 (241)^{II}
Mixed lymphocytes	Placebo	1845 (692)	2044 (717)$^{\#}$	2141 (730)	3024 (950)$^{\#\#}$
	Propranolol	2081 (655)*	2142 (562)^{I}	2333 (664)**	3635 (1102)^{II}
B-lymphocytes ($CD19^+$)	Placebo	249 (201)	317 (350)	301 (232)	356 (219)$^{\#\#}$
	Propranolol	271 (152)	274 (145)	305 (177)*	449 (226)^{II}
T-helper lymphocytes ($CD4^+$)	Placebo	871 (355)	979 (440)$^{\#}$	974 (386)	1220 (476)$^{\#\#}$
	Propranolol	1006 (355)*	1033 (314)	1102 (373)**	1520 (525)^{II}
Cytotoxic T-lymphocytes ($CD8^+$)	Placebo	462 (224)	543 (276)$^{\#\#}$	543 (262)	809 (443)$^{\#\#}$
	Propranolol	538 (233)	556 (215)^{I}	606 (253)**	974 (536)^{II}
NK cells ($CD16^+CD56^+$)	Placebo	217 (267)	280 (290)$^{\#\#}$	294 (322)	606 (459)$^{\#\#}$
	Propranolol	227 (199)	235 (212)^{II}	290 (260)	721 (492)^{II}
L-selectin-negative NK cells ($CD16^+CD56^+CD62L–$)	Placebo	60 (156)	83 (158)$^{\#\#}$	83 (185)	94 (202)$^{\#\#}$
	Propranolol	42 (35)	42 (35)^{II}	224 (253)	251 (280)
$CD54^+$ lymphocytes	Placebo	910 (355)	1020 (393)$^{\#\#}$	1061 (446)	1620 (682)$^{\#\#}$
	Propranolol	1012 (378)	1048 (364)^{I}	1159 (399)**	1898 (654)^{II}
$CD54^+$ monocytes	Placebo	427 (123)	466 (167)	466 (155)	636 (211)$^{\#\#}$
	Propranolol	553 (636)	505 (156)	534 (155)**	829 (239)^{II}

Drug effect: $^{*}P<0.05$, $^{**}P<0.001$;
task effect: $^{\#}P<0.05$, $^{\#\#}P<0.001$;
drug × task interaction: $^{I}P<0.05$, $^{II}P<0.001$

Table 2 The effect of propranolol on naïve and memory T-lymphocyte subsets in response to acute psychological and physical stress. $CD4^+CD62L^+CD45RA^+$ and $CD8^+CD62L^+CD45RA^+$ are naïve lymphocytes, and $CD4^+CD29^+CD45RO^+$ and $CD8^+CD29^+CD45RO^+$ are activated memory cells

Cell type	Condition	Psychological stress		Physical stress	
		Baseline	Public speaking	Baseline	Exercise
$CD4^+CD62L^+$	Placebo	722 (321)	786 (352)$^{\#}$	800 (337)	983 (396)$^{\#\#}$
	Propranolol	815 (303)*	839 (270)	883 (313)**	1206 (422)^{II}
$CD4^+CD62L^-$	Placebo	150 (95)	198 (169)	178 (116)	246 (183)$^{\#\#}$
	Propranolol	173 (128)	176 (107)^{I}	193 (138)	277 (213)
$CD4^+CD62L^+CD45RA^+$	Placebo	367 (217)	427 (295)	390 (231)	493 (282)$^{\#\#}$
	Propranolol	423 (227)	421 (210)	435 (219)**	600 (311)^{II}
$CD4^+CD29^+CD45RO^+$	Placebo	421 (192)	460 (200)$^{\#}$	464 (233)	615 (309)$^{\#\#}$
	Propranolol	487 (226)	506 (233)	534 (268)**	782 (421)^{II}
$CD8^+CD62L^+$	Placebo	287 (139)	331 (176)$^{\#}$	327 (148)	436 (222)$^{\#\#}$
	Propranolol	334 (150)*	346 (144)	357 (142)*	536 (316)^{I}
$CD8^+CD62L^-$	Placebo	177 (133)	215 (133)$^{\#}$	221 (164)	377 (296)$^{\#\#}$
	Propranolol	195 (129)	201 (118)^{I}	225 (149)	425 (302)^{I}
$CD8^+CD62L^+CD45RA^+$	Placebo	231 (123)	270 (155)$^{\#}$	263 (117)	360 (179)$^{\#\#}$
	Propranolol	265 (116)	269 (108)^{I}	288 (115)*	428 (268)^{I}
$CD8^+CD29^+CD45RO^+$	Placebo	113 (60)	128 (71)	122 (70)	181 (123)$^{\#\#}$
	Propranolol	129 (89)	128 (85)^{I}	135 (93)*	230 (188)^{I}

Drug effect: $*P<0.05$, $**P<0.001$;
task effect: $^{\#}P<0.05$, $^{\#\#}P<0.001$;
drug × task interaction: $^{I}P<0.05$, $^{II}P<0.001$

Table 3 The effect of propranolol on the density of adhesion molecules on mononuclear cells in response to acute psychological and physical stress. Data are presented as the mean (SD) of the number of receptors per cell. (*CAM* Cell adhesion molecule)

Type of CAM	Condition	Psychological stress		Physical stress	
		Baseline	Public speaking	Baseline	Physical exercise
CD62L on lymphocytes	Placebo	12936 (3879)	12247 (3651)$^{\#}$	11979 (3647)	10570 (3544)$^{\#}$
	Propranolol	12651 (3851)	12136 (3458)	11779 (3554)	10239 (3276)
CD54 on lymphocytes	Placebo	1091 (310)	1064 (281)$^{\#}$	1045 (282)	1056 (270)$^{\#}$
	Propranolol	1100 (254)	1070 (238)	1070 (272)	1090 (264)
CD54 on monocytes	Placebo	4633 (1001)	4561 (995)$^{\#}$	4662 (1042)	4720 (1049)$^{\#}$
	Propranolol	5083 (1248)*	4932 (1308)	4998 (1550)	5127 (1546)
CD11a on lymphocytes	Placebo	22781 (6463)	23838 (6763)$^{\#}$	23758 (7052)	27499 (8102)$^{\#}$
	Propranolol	23573 (6298)	23439 (5989)I	24062 (6693)	27872 (8031)
CD11a on monocytes	Placebo	37705 (6821)	37335 (7220)$^{\#}$	36196 (6712)	35907 (6409)
	Propranolol	40327 (7796)**	39090 (7262)I	39298 (8493)**	39349 (8075)

Drug effect: $*P<0.05$, $**P<0.001$;
task effect: $^{\#}P<0.05$, $^{\#\#}P<0.001$;
drug × task interaction: $^{I}P<0.05$, $^{II}P<0.001$

$P<0.003$). However, it led to a decreased density of CD11a on monocytes ($F=4.59$, $P<0.038$). Propranolol did not significantly interfere with stress-induced changes in the density of CD62L or CD54 on mononuclear cells (Table 3). At rest, propranolol increased the density of CD54 ($F=5.63$, $P<0.024$) and CD11a ($F=13.55$, $P<0.001$) on monocytes. However, there was no significant effect of propranolol at rest on the density of CD62L, CD54 and CD11a on lymphocytes.

Hormone levels

The results from the analysis of the hormone data in response to psychological stress are presented in Table 4. Propranolol did not significantly affect the increase in either norepinephrine or epinephrine in response to the speech stressor.

Physical exercise

Heart rate

Propranolol led to a significant decrease in the pre-exercise values of resting heart rate [placebo 75.88 (10) beats/min versus propranolol 65.06 (9.9) beats/min; $F=143$, $P<0.001$]. Propranolol also significantly attenuated the exercise-induced increase in heart rate [+49.32 beats/min placebo versus +36.25 beats/min propranolol: placebo 125.20 (15) beats/min versus

Table 4 Changes in levels of catecholamines in response to acute psychological and physical stress following the administration of a placebo or propranolol

Catecholamine	Condition	Psychological stress		Physical stress	
		Baseline	Public speaking	Baseline	Physical exercise
Norepinephrine (pg/ml)	Placebo	294 (117)	333 (135)$^{\#\#}$	436 (175)	727 (351)$^{\#\#}$
	Propranolol	318 (114)	332 (91)	432 (139)**	1131 (546)^{II}
Epinephrine (pg/ml)	Placebo	41 (42)	73 (150)$^{\#}$	53 (33)	99 (97)$^{\#\#}$
	Propranolol	45 (76)	62 (54)	79 (90)**	215 (191)^{II}

Drug effect: $*P<0.05$, $**P<0.001$;
task effect: $^{\#}P<0.05$, $^{\#\#}P<0.001$;
drug × task interaction: $^{I}P<0.05$, $^{II}P<0.001$

propranolol 101.31 (13) beats/min; drug × exercise interaction, $F=143$, $P<0.001$].

Immune cell numbers

In contrast to the case for psychological stress, propranolol treatment significantly enhanced the effect of the bicycle exercise on leukocytes, including T-helper and T-cytotoxic subset responses ($F>4.9$, $P<0.05$ in all cases; Table 1). In addition, naïve and activated memory T-helper cells ($F>7.3$, $P<0.01$ in all cases) and naïve activated memory T-cytotoxic cells ($F>5.4$, $P<0.05$ in all cases) were increased disproportionately following exercise under the propranolol condition (Table 2). The effects of exercise on $CD16^{+}CD56^{+}CD62L^{-}$ cells were not different under the propranolol condition (Table 1).

β-blockade also caused circulating baseline cell numbers to increase. This effect was modest and comparable to the corresponding data of the speech task. It was significant in all examined populations ($F<6.06$, $P<0.02$ in all cases) except for NK cells and naïve T lymphocytes.

Density of adhesion molecules on mononuclear cells

Propranolol treatment did not significantly alter the effect of exercise on the density of CD11a, CD54 or CD62L on mononuclear cells beyond the exercise effect (Table 3).

Hormone levels

Propranolol led to an increase in norepinephrine levels ($F=15.36$, $P<0.001$) and epinephrine levels ($F=5.00$, $P<0.038$) in response to exercise (Table 4). Resting epinephrine levels ($F=6.14$, $P<0.023$) were increased following propranolol as compared to placebo treatment. Under both propranolol and placebo treatment, exercise baseline catecholamine levels were non-significantly elevated as compared to speech baseline catecholamine levels, an effect attributable to the change in posture with exercise (Ziegler 1980).

Discussion

In this study we examined the impact of relatively short-term β-blockade on psychological and physical stressor-induced alterations in circulating immune cell numbers and adhesion molecule expression. Consistent with prior studies (Bachen et al. 1995; Benschop et al. 1994), propranolol significantly blunted the psychological-stress-induced increases in the numbers of mixed lymphocytes, particularly NK cells and memory T cells. However, with physical exercise, β-blockade tended to enhance the increase in circulating leukocytes. Interestingly, in contrast to the effects on the numbers of circulating cells, there was only a modest drug–stress interaction on the density of adhesion molecules on mononuclear cells. Five days of propranolol ingestion also affected modestly the number of circulating leukocytes at rest, primarily elevating the number of naïve, non-activated lymphocytes and monocytes.

The results of our study confirm the effects of β-blocking agents in terms of psychological stressors, however, under propranolol treatment the number of circulating cells tended to increase in response to exercise. The 100-mg dose of propranolol used in this study is not significantly different from what has been used in other studies. Following 1 week of propranolol treatment, subjects expended more effort on the bicycle for the given time than without β-blockade. The hormone data lends support to this observation, as under propranolol treatment catecholamine levels rose to levels twice as high as under placebo treatment during exercise. In prior studies, the duration of the exercise was typically shorter under the propranolol condition because the subjects fatigued more readily (Mills et al. 1999; Murray et al. 1992). In our study, despite the fatigue, we motivated subjects to continue on the bicycle until the prescribed 16 min were completed. It was extremely difficult for our subjects to complete the entire allotted exercise period while on propranolol. We speculate that this much greater effort (and the significantly greater catecholamine levels associated with that effort) led to the greater leukocytosis that occurred under the propranolol condition.

Numbers of circulating $CD62L^-$ lymphocytes and CD54+ mononuclear cells were increased upon stress. Both effects were blunted by propranolol during the public speaking task but were amplified in the physical exercise condition. However, these effects were not as evident when looking at the density of CD62L, CD54 and CD11a. Thus, it seems that β-blockade may interfere with the regulation of circulating leukocyte numbers, but might not affect to the same extent the density of adhesion molecules on individual cells. The CD62L-regulated rolling along the endothelium is a prerequisite to the extravasation of immune cells to sites of inflammation and immune activation. The shedding of CD62L from lymphocytes has been associated with activation of the cells. At the same time, CD11a and CD54 are upregulated to enhance the interaction between leukocytes and with the endothelium. In our study, increasingly activated lymphocytes were released into the peripheral blood pool. Propranolol treatment reduced the redistribution of the cells to the peripheral blood pool, but it did not significantly modify their activation status.

Certain limitations of this study need to be addressed. Due to the difficulty of obtaining enough antibody, we were not able to get complete adhesion molecule density data on all of the subjects tested, missing 4–5 subjects in each testing group. In addition, this study was designed to examine the effects of β-blockade in a relatively young and healthy group of subjects, with the intention to expand subsequent studies to examine clinical populations. Thus, while the subjects were a random selection from the general population, the group was not necessarily representative of patients receiving β-antagonist treatment who, for example, may be older than our subjects. Finally, given that we did not assess extended post-stressor recovery measures, we cannot make a determination of the potential longer-term physiological implications of the findings.

Despite the potential limitations of our subjects in terms of being able to make generalizations, it may be worth commenting on the potential clinical implications of these data. Cardiologists treat hypertension, coronary heart disease and chronic heart failure (CHF) with β-blocking agents (Gottlieb et al. 1998; Lechat et al. 1998). CHF patients have fewer circulating lymphocytes due to chronic adrenergic stimulation (Maisel et al. 1990). β-adrenergic antagonists reduce mortality among high-risk and low-risk patients after myocardial infarction (Gottlieb et al. 1998) and are beneficial in the treatment of CHF (Lechat et al. 1998). Furthermore, non-selective β-blocking agents, as used in this study, are associated with a larger survival benefit than are $\beta 1$-selective antagonists (Lechat et al. 1998).

Animal studies have shown that propranolol treatment delays the onset of coronary atherosclerosis in dominant macaque monkeys housed under stressful conditions (Kaplan et al. 1987). In our study we found that β-blockade diminished the stress-induced changes in activated cytotoxic T-lymphocytes, which are among the first cells in atherosclerotic plaques (Watanabe and Fan 1998). Adhesion molecules also play a crucial role in the trafficking of lymphocytes to inflammatory sites. Antibodies against CD11b and CD54 reduce intimal thickening after injury to rat carotid arteries (Rogers et al. 1998). Antibodies against CD11a/CD18 and CD54, injected into hypercholesterolemic rats, reduce the number of mononuclear cells adhering to the aortic intima (Nie et al. 1997). In this study, propranolol reduced the density of CD11a on mononuclear cells following psychological stress. These findings suggest that some of the benefits of β-adrenergic antagonists are related to their non-cardiovascular effects, such as on immune cells.

In conclusion, we have shown that treatment with the β-adrenergic antagonist propranolol modifies stress-induced leukocytosis. Whereas psychological stress effects were blunted, exercise effects may be enhanced under propranolol due to greater exertion. To a lesser extent, propranolol affected the density of adhesion molecules on mononuclear cells. Considering the wide usage of β-adrenergic antagonists in the treatment of cardiovascular disorders, the results of this study may help us to appreciate further the mechanisms that may contribute to the benefits of treatment with β-blocking agents.

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