Effect of Interleukin-1β on Platelet Aggregation in August, Wistar, and WAG Rats in Acute Emotional Stress

S. S. Pertsov, A. S. Sosnovskii, A. A. Kubatiev, and G. V. Pirogova

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 124, No. 8, pp. 144-147, August, 1997
Original article submitted March 2, 1996

Acute emotional stress inhibits platelet aggregation in August and WAG rats and reduces it in Wistar rats. Functional parameters of platelets are altered predominantly in passive rats. Interleukin-1β reduces the rate of platelet aggregation in nonstressed August and WAG rats and elevates it in Wistar rats. Preliminary injection of interleukin-1β reduces the stress-induced changes in platelet aggregation in August and WAG rats; while in Wistar rats this effect is not observed.

Key Words: *interleukin-1β; emotional stress; platelet aggregation; August, Wistar, and WAG rats*

Clinical and experimental studies show that circulatory disturbances associated with changes in platelets play an important role in the genesis of numerous diseases caused by emotional stress (ES). Platelets modify hemostasis both locally [5] and through secretion of vasoactive substances [11]. Different models of ES are accompanied by increased [9] or decreased [7] rate of platelet aggregation.

It was established that different rat strains are characterized by different resistance to ES [4]; individual resistance to ES greatly varies between individual animals of the same species [3].

Interleukin-1β (IL-1β) is a mediator of the acute phase of stress reaction [6]. Injection of IL-1β induces activation of the hypothalamus-pituitary-adrenal axis [10] accompanied by production of the endogenous platelet activation factor in response to cytokine injection [14]. On the other hand, ES modulates production and secretion of cytokines by different cells [17] and stimulates expression of IL-1β mRNA in the brain [12]. The effect of IL-1β on the blood and its participation in the regulation of cell-mediated [15] and humoral [8] immunity was previously demonstrated. However, the effect of IL-1β on functional activity of platelets in ES is been poorly understood.

The aim of the present study was to investigate the effect of IL-1β on platelet aggregation in August, Wistar, and WAG rats in acute ES.

MATERIALS AND METHODS

Experiments were carried out on male August (196.1± 4.1 g, n=17), Wistar (180.4±11.2 g, n=15), and WAG (268.5±13.9 g, n=13) rats. The rats were kept in cages, 4 animals per each, at 20-22°C and natural illumination with free access to food and water.

Five days before the tests, a hollow steel guide 3 mm long and 0.8 mm in diameter was implanted into the skull bone (A-P=-1 mm, L=1 mm) under Nembutal anesthesia (40 mg/kg intraperitoneally). The guide did not penetrate the brain tissue or the lateral ventricle cavity.

Rat behavior was tested in an open field 4 days postoperation; rearing, ambulation, and the number of fecal boluses and urinations for 15 min were evaluated for each animal.

On day 5 after implantation immediately before the experiment, IL-1β or physiological saline was

P. K. Anokhin Institute of Normal Physiology, Russian Academy of Medical Sciences, Moscow

0007-4888/97/0008-0747$18.00

TABLE 1. Amplitude of Platelet Aggregation in August, Wistar, and WAG Rats Injected with IL-1β or Physiological Saline (%)

Rat strain**	Control		Stress*	
	IL-1β (group 2)	Physiological saline (group 4)	IL-1β (group 1)	Physiological saline (group 3)
ADP, 5.0 μM				
August	31.38±2.13	50.33±11.78	25.13±9.23	26.33±9.33
Wistar	60.50±20.78	44.00±1.00	46.17±10.80	50.25±2.73
WAG	45.75±5.75	74.00±1.00	40.63±7.03	49.25±19.25
ADP, 2.5 μM				
Wistar	60.25±5.75	37.00±0.00	58.50±0.00	50.17±5.09
WAG	32.50±7.50	64.50±8.50	23.00±9.57	33.75±25.25
Collagen, 9 μg/ml				
Wistar	48.17±18.88	22.75±22.75	41.50±21.01	35.88±12.17
WAG	30.00±30.00	75.50±6.50	28.13±16.28	31.00±31.00

Note. ANOVA: *$p<0.05$: differences between strains; **$p<0.01$: differences between stressed and control animals.

injected in the lateral ventricle by means of a microsyringe inserted via the guide 3 mm deep into the brain.

The animals were divided into 4 groups. Group 1 rats were injected with IL-1β in 10 μl physiological saline (10 μg/ml) and subjected to stress; group 2 rats were injected with IL-1β and returned to their cages; groups 3 and 4 were treated like groups 1 and 2, respectively, except that they received only the vehicle (10 μl physiological saline). Human recombinant IL-1β (activity 3×10^7 U/μl) was obtained from the Institute of Immunology.

The rats were deprived of food for 24 h prior to the tests but had free access to water. After injection of IL-1β or saline, group 1 and 3 rats were subjected to acute ES as described elsewhere [13]. The animals were immobilized in plastic tubes (16.5 cm long with an internal diameter of 5.5 cm) and immersed in water (23°C) up to the metasternum for 2 h and then placed in their cages for another 2 h.

Blood was taken from the abdominal aorta under light ether anesthesia. Platelets were isolated by differential centrifugation (190*g*, 10 min at 22°C), washed with buffered saline and resuspended in a buffer.

Platelet aggregation was recorded in a dual-channel PICA Lumi-Aggregometer (Chrono-Log), in 250-μl thermocontrolled cells (37±0.2°C). Aggregation was induced by adenosine diphosphate (ADP, Sigma) in concentrations of 2.5 and 5.0 μM or collagen (Chrono-Log) in a concentration of 9 μg/ml. The amplitude and rate of platelet aggregation were recorded into computer via an Aggro-Link interface (Chrono-Log).

The data were processed by multifactor dispersion analysis (stress/control×IL-1β/physiological saline×rat strain) with subsequent multiple comparison of the experimental groups. Correlation matrices were composed using the Kendall correlation test. The data are presented as means and their standard deviations.

RESULTS

Effect of IL-1β on platelet aggregation. In the control (unstressed) August and WAG rats intraventricular injection of IL-1β reduced the amplitude of platelet aggregation in comparison with group 3 rats injected with physiological saline. The amplitude of platelet aggregation (Table 1) decreased by 37.65% in August rats (5.0 μM ADP), and by 38.18, 49.61, and 60.26% in WAG rats (5.0 and 2.5 μM ADP and 9 μg/ml collagen, respectively). The rate of aggregation (Table 2) decreased by 26.62% in August rats (5.0 μM ADP), and by 28.86, 42.83, and 68.88% in WAG rats (5.0 and 2.5 μM ADP and 9 μg/ml collagen, respectively).

In unstressed Wistar rats IL-1β increased the amplitude of platelet aggregation induced by ADP (5.0 and 2.5 μM) and collagen (9 μg/ml) by 37.5, 62.84, and 111.74%, respectively, in comparison with controls receiving physiological saline. The amplitude of platelet aggregation induced by 5.0 μM ADP was maximal in Wistar rats in comparison with that in WAG and August rats ($F=5.71$ (1), $p<0.01$). IL-1β increased the rate of platelet aggregation induced by 5.0 and 2.5 μM ADP and 9 μg/ml collagen by 46.87, 53, and 73.82%, respectively, in comparison with control animals injected with physiological saline.

Thus, intraventricular injection of IL-1β reduced the rate and amplitude of platelet aggregation in

TABLE 2. Rate of Platelet Aggregation in August, Wistar, and WAG Rats Injected with IL-1β or Physiological Saline (%/min)

Rat strain	Control		Stress*	
	IL-1β (group 2)	Physiological saline (group 4)	IL-1β (group 1)	Physiological saline (group 3)
ADP, 5.0 μM				
August	72.65±0.13	99.00±15.67	57.13±18.27	58.50±10.58
Wistar	97.67±4.49	66.50±0.50	85.17±17.59	99.00±5.07
WAG	88.75±3.25	124.75±4.25	70.00±13.36	91.25±40.25
ADP, 2.5 μM				
Wistar	101.75±9.75	66.50±0.00	100.50±0.00	84.67±8.14
WAG	69.75±16.75	122.00±4.00	61.00±23.28	84.00±36.50
Collagen, 9 μg/ml				
Wistar	48.67±15.73	28.00±28.00	41.17±21.67	31.00±11.02
WAG	27.00±27.00	86.75±0.75	24.63±14.28	27.50±27.50

Note. ANOVA: *$p<0.09$: differences between stressed and control animals.

August and WAG rats and increased these parameters in Wistar rats.

Effect of ES on platelet aggregation. Acute ES reduced the amplitude ($F=5.70$ (1), $p<0.03$) and the rate of platelet aggregation in August and WAG rats of group 3 (saline injection) in comparison with unstressed animals of group 4. The amplitude of platelet aggregation (Table 1) decreased by 47.69% in August rats (5.0 μM ADP) and by 33.45, 47.67, and 58.94% in WAG rats (5.0 and 2.5 μM ADP and 9 μg/ml collagen, respectively). The decrease in platelet aggregation (Table 2) in ES constituted 40.91% in August rats (5.0 μM ADP) and 26.85, 31.15, and 68.30% in WAG rats (5.0 and 2.5 μM ADP and 9 μg/ml collagen, respectively).

In contrast, in Wistar rats injected with physiological saline (group 3), acute ES increased the amplitude of platelet aggregation induced by ADP (5.0 and 2.5 μM) and collagen (9 μg/ml) by 14.20, 35.59, and 57.71%, respectively, in comparison with the unstressed rats of group 4. However, these changes were statistically insignificant. In Wistar rats injected with physiological saline, acute stress also increased the rate of platelet aggregation induced by ADP (5.0 and 2.5 μM) and collagen (9 μg/ml) by 48.87, 27.32, and 10.71%, respectively, in comparison with unstressed rats of group 4.

Thus, in August and WAG rats, acute ES reduced the amplitude and rate of aggregation of isolated platelets in response to various activators. This effect was most pronounced in August rats characterized by low resistance to ES. No decrease in the rate and amplitude of platelet aggregation was noted in Wistar rats characterized by high resistance to ES. These data agree with our previous results [16] on platelet aggregation in platelet-rich plasma after ES induced by long-term (24 h) immobilization.

The ES-induced decrease in the amplitude and rate of platelet aggregation in August and WAG rats of group 1 (injection of IL-1β) was less pronounced in comparison with the animals of group 3 (injection of physiological saline). In group 1, ES reduced the amplitude of platelet aggregation by 19.92% in August rats (5.0 μM ADP) and by 11.91, 29.23, and 6.23% in WAG rats (5.0 and 2.5 μM ADP and 9 μg/ml collagen, respectively). In August and WAG rats injected with IL-1β, ES also produced a less pronounced decrease in the rate of platelet aggregation in comparison with animals given physiological saline. This parameter decreased in ES by 21.36% in August rats (5.0 μM ADP) and by 21.13, 12.54, and 8.78% in WAG rats (5.0 and 2.5 μM ADP and 9 μg/ml collagen, respectively).

In Wistar rats injected with IL-1β (group 1), ES lowered the amplitude of platelet aggregation induced by ADP (5.0 and 2.5 μM) and collagen (9 μg/ml) by 23.69, 2.90, and 13.85%, respectively. The rate of platelet aggregation induced by ADP (5.0 and 2.5 μM) and collagen (9 μg/ml) also decreased in ES by 12.80, 1.23, and 15.41%, respectively.

Thus, injection of IL-1β to August and WAG rats reduces the ES-induced decrease in the amplitude and rate of platelet aggregation, while in Wistar rats these relationships were not observed. Our previous experiments [2] showed that acute ES induces accumulation of 2-thiobarbituric acid-reactive substances in rat hypothalamus. However, no ES-induced activation of lipid peroxidation was noted in the brain against the background of IL-1β pretreatment. Moreover, IL-1β decreased the number of ES-

induced gastric lesions in August, Wistar, and WAG rats [1]. Thus, IL-1β has a protective effect on the organism in acute ES.

In WAG rats injected with IL-1β, a positive correlation was noted between peripheral rearing in the open field test and the amplitude ($p<0.01$) and rate ($p<0.04$) of platelet aggregation induced by ADP (5.0 μM) and collagen (9 μg/ml). In unstressed August rats pretreated with IL-1β, the rate and amplitude of platelet aggregation induced by ADP (5.0 μM) directly correlated with ambulation ($p<0.05$) and the number of fecal boluses ($p<0.01$), and inversely correlated with the number of urinations ($p<0.01$) in the open field test.

The significant correlation of platelet aggregation with initial motor activity in an open field suggests that ES primarily modulates functional parameters of platelets in passive rats. This is consistent with our previous studies demonstrating that thymus involution and inhibition of platelet aggregation in stress are more pronounced in passive animals [16]. These experiments also revealed some differences in platelet aggregation in ES between rats of various strains injected with IL-1β or physiological saline.

The study was supported by International Scientific Foundation (grant MNY000).

REFERENCES

1. S. S. Pertsov, V. M. Abramov, A. S. Sosnovskii, *et al.*, *Byull. Eksp. Biol. Med.*, **117**, No. 3, 238-239 (1994).
2. S. S. Pertsov, T. S. Balashova, A. A. Kubatiev, *et al.*, *Ibid.*, **120**, No. 9, 244-247 (1995).
3. K. V. Sudakov, *Pat. Fiziol.*, No. 4, 86-93 (1992).
4. K. V. Sudakov, *Byull. Eksp. Biol. Med.*, **123**, No. 2, 124-130 (1997).
5. M. Capelli-Bigazzi, K. G. Lamping, D. W. Nuno, and D. G. Harrison, *Am. J. Physiol.*, **259**, H1161-H1170 (1990).
6. A. J. Chovergonzalez, M. S. Harbuz, and S. L. Lightman, *J. Neuroimmunol.*, **42**, 155-160 (1993).
7. J. I. Haft and Y. S. Arkel, *Chest*, **70**, 501-505 (1976).
8. M. Irwin, *Endocrinology*, **133**, 1352-1360 (1993).
9. P. T. Larson, P. Hjemdahl, G. Olsson, *et al.*, *Clin. Sci.*, **76**, 369-376 (1989).
10. G. Mastoracos, G. P. Chrousos, and J. S. Weber, *J. Clin. Endocrinol. Metab.*, **77**, 1690-1694 (1993).
11. W. G. Mayhan, *Brain Res.*, **545**, 97-102 (1991).
12. M. Minami, Y. Kuraishi, T. Yamaguchi, *et al.*, *Neurosci. Lett.*, **123**, 254-256 (1991).
13. J. Overmier, R. Murison, *et al.*, *Behav. Neural Biol.*, **46**, 372-386 (1986).
14. C. Rougeot, C. Tiberghein, P. Minary, and F. Dray, *J. Lipid Mediator*, **4**, 45-60 (1991).
15. A. Saperstein, H. Brand, T. Audhya, *et al.*, *Endocrinology*, **130**, 152-158 (1992).
16. A. S. Sosnovsky and A. A. Kubatiev, *Stress Medicine*, **9**, 207-214 (1993).
17. D. H. Zhou, A. W. Kusnecov, M. R. Shurin, *et al.*, *Endocrinology*, **133**, 2523-2530 (1993).