Pergamon

Pharmacology Biochemistry and Behavior, Vol. 51, Nos. 2/3, pp. 217–221, 1995

0091-3057/95 $9.50 + .00

0091-3057(94)00432-4

Lowering of Body Temperature Affects Human Platelet Functions and Norepinephrine Release

CLAUS OPPER,* JÜRGEN HENNIG,† CHRISTINA CLEMENT,* ULRICH LASCHEFSKI,† DANIELA DEY,† JUTTA DIECKWISCH,† PETRA NETTER† AND WOLFGANG WESEMANN*[1]

*Institute of Physiological Chemistry, University of Marburg, Hans-Meerwein-Str., D-35033 Marburg, Germany
†Department of Psychology, University of Giessen, Otto-Behaghel-Str. 10, D-35385 Giessen, Germany

Received 1 September 1994; Revised 14 November 1994; Accepted 14 November 1994

OPPER, C., J. HENNIG, C. CLEMENT, U. LASCHEFSKI, D. DEY, J. DIECKWISCH, P. NETTER AND W. WESEMANN. *Lowering of body temperature affects human platelet functions and norepinephrine release.* PHARMACOL BIOCHEM BEHAV 51(2/3) 217–221, 1995.—The effect of lowering body temperature on plasma epinephrine, norepinephrine, and platelet density distribution and volume was studied in a placebo-controlled double-blind study. Lowering of body core temperature was induced by either exposure to a cold environment at a temperature of 5°C (CT) or by a single dose of the 5-HT_{1A} agonist ipsapirone (IPS). A third group exposed to an ambient temperature of 28°C was given placebo (PLAC). All of the three groups were investigated in a climate chamber. In the CT group the density distribution of blood platelet subpopulations was shifted to an increase in less dense platelets that were more sensitive towards aggregation-inducing agents. The mean platelet volume in this subpopulation was decreased. Epinephrine was not affected, whereas the increase of norepinephrine was correlated with an increase of platelets that were more sensitive to aggregation-inducing agents in the CT group but not in the PLAC and IPS groups.

Platelet function Mean platelet volume Epinephrine Norepinephrine Cold exposure
Body core temperature Platelet density distribution Ipsapirone

DURING the last decade an increasing interest in the properties of platelets was observed within different disciplines. Some results from these studies reveal that platelets respond to emotional stress (2,14,15,17). A possible link between platelet activation and coronary heart disease was mentioned frequently and it was hypothetized that stress-induced changes in platelet behaviour may be a major mechanism in coronary events (17). An increase in mean platelet volume (MPV) in patients suffering from myocardial infarction (5,6,18,19) and a correlation of MPV to psychological parameters was reported (2). On the other hand, fitness positively influences platelet epinephrine accumulation in subjects with low trait anxiety (25). Platelet activation during moderate noise stress is accompanied by secretion of epinephrine (1), and platelet number is slightly increased during exposure to cold stress in healthy subject (26). In recent papers we reported a different sensitivity of heterogeneous human blood platelets towards aggregation-inducing substances (20,21). Less dense platelets (subpopulation I, SP I), obtained by density gradient centrifugation, showed a higher sensitivity when stimulated with low concentrations of aggregation-inducing agonists. Furthermore, this subpopulation was increased in patients suffering from myocardial infarction or angina pectoris (27).

Taken together, this suggests that there may be a relationship between platelet response to stress and to epinephrine, and that the change in percentages of SP I seems to be relevant.

However, so far it is unknown a) how physiological stress can change number and activation of platelets, b) how the different subpopulations are involved, and c) if release of catecholamines is an essential mechanism for the platelet-related changes. To investigate the role of physical load with a concomitant norepinephrine release, we used a model of thermoregulatory responses. Exposure to a cold environment leads to discomfort and norepinephrine release due to an increase in muscle activity for heat production (22). To control for the possible influence of a decrease in body core temperature, we examined another group receiving the 5-HT_{1A} receptor agonist ipsapirone (IPS), which also causes a decrease in body core temperature without shivering for heat production (10,13). Thermoregulatory mechanisms influence the catecholamine response (11), and these mechanisms may play an important

[1] To whom requests for reprints should be addressed.

role in platelet activation. In this study we investigated platelet parameters and catecholamines during and after reduction of the body temperature.

METHOD

Experimental Design and Subjects

The conditions in the climatic chamber during the experiment and the times of blood sampling are described in detail elsewhere (10,22) and are shown in Fig. 1. Briefly, all experiments were carried out in the climatic chamber between 1500 and 1800 h. During the experiment, subjects, just wearing bathing trunks, rested in a plastic mesh chair in a supine position. Sixty healthy male volunteers were randomly assigned to one of the following three treatment groups: 1) exposure to an ambient temperature of 5°C (N = 20; CT), 2) intake of 10 mg IPS and exposure to a temperature of 28°C (N = 20; IPS), and 3) a placebo group exposed to a temperature of 28°C (N = 20; PLAC). Blood was drawn four times from the vena basilica with an indwelling catheter (Fig. 1). Citrate was used as an anticoagulant. A baseline sample was drawn immediately after drug intake (time 1). Further samples were drawn after 55 min (end of cold exposure, time 2), 90 min (temperature restored to 28°C, time 3), and after 110 min (end of the session, time 4). The study was approved by the ethical committee of the German Psychological Association.

Platelet-Rich Plasma (PRP)

PRP was obtained by centrifugation of the blood at 350 × g_{max}, at 15°C, for 15 min with a Beckman centrifuge and immediately used for the analysis described below.

Distribution Pattern of Platelet Subpopulations and Determination of Mean Platelet Volume (MPV)

The density distribution pattern of platelet subpopulations was obtained from 2.5 ml PRP after centrifugation (3500 × g_{max} for 15 min, +4°C) on a Percoll density gradient (2.0 ml of each density 1.08 g/cm^3, 1.07 g/cm^3, 1.065 g/cm^3, 1.04 g/cm^3). Three platelet subpopulations were collected from the gradient: subpopulation I (SP I, ρ = 1.04–1.065 g/cm^3), subpopulation II (SP II, ρ = 1.065–1.07 g/cm^3), and subpopulation III (SP III, ρ = 1.07–1.08 g/cm^3), and washed twice with modified Gaintners solution, pH 6.4 (8). Cell counts and mean

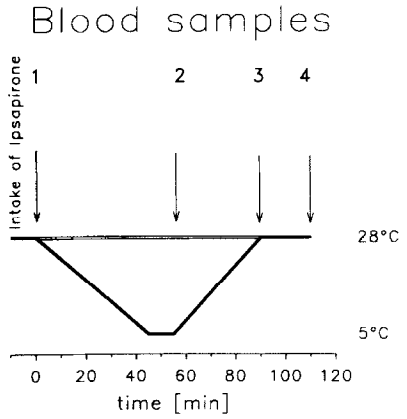

FIG. 1. Experimental design. Intake of placebo or ipsapirone was immediately prior to the first time of blood sampling.

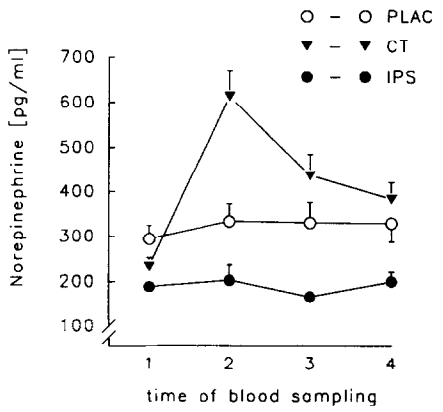

FIG. 2. Time course of norepinephrine concentration. Data expressed as means ± SEM in the three experimental (CT, PLAC, IPS) groups. Covariance analyses with repeated measurements indicated a significant main effect for treatment group ($p < 0.01$) and a time effect ($p < 0.01$).

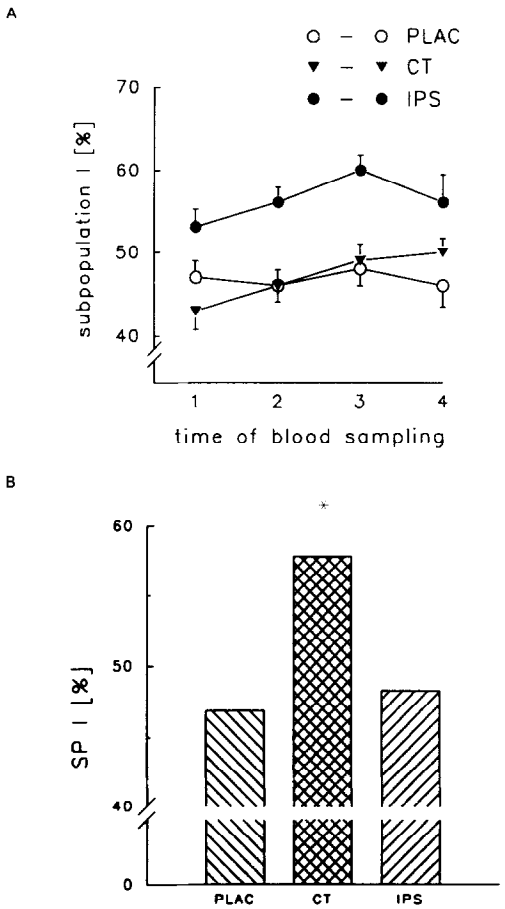

FIG. 3. (a) Time course of SP I in the three treatment groups. Data expressed as means ± SEM in the three experimental groups. Analyses of covariance with repeated measurements indicated a significant main effect for treatment group ($p < 0.01$). (b) Main treatment effect on SP I. Baseline adjusted means across time points 2–4 of the three experimental groups are shown. *$p < 0.01$, post hoc Scheffè test, CT group as compared to IPS group and PLAC group.

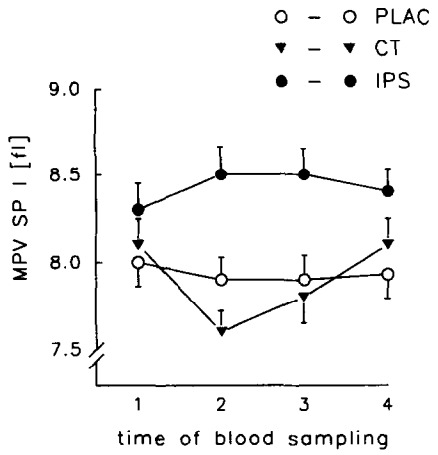

FIG. 4. Time course of mean platelet volume (MPV) in SP I. Data expressed as means ± SEM in the three experimental groups. In tendency analyses of covariance with repeated measurements a significant main treatment effect is indicated ($p = 0.053$).

platelet volume (MPV) were determined in a Sysmex F 800 cell counter. The distribution was expressed as the percentage of SP I, SP II, and SP III (sum = 100%).

Catecholamine Determination

Epinephrine (E) and norepinephrine (NE) concentrations were determined using the method described by Fruhstorfer et al. (7) with modifications. E and NE were extracted together with 1 ng 3,4-dihydroxybenzylamine (Sigma, Germany, Taufkirchen) as an internal standard from 1 ml plasma with 1.5 ml extraction buffer (1.4 M ethylenedinitrilo tetraacetic acid, 50 mM trishydroxymethyl aminomethane, pH 8.6) and 10 mg AL_2O_3. The catecholamines were eluted with 100 μM 0.1 M perchloric acid. HPLC with electrochemical detection was used for analysis (column: Superspher 100 RP 18c, Merck, Germany; mobile phase: 34.9 mM citric acid, 90.2 mM sodium acetate, 0.67 mM trishydroxymethyl aminomethane, 0.23 mM octanesulfonic acid, 11.6 mM diethylamine, and 2.6 v/v methanol, pH 4.7; detectors: ESA Coulochem, model 5100, USA, guard cell +0.35 V, detector 1 +0.35 V, detector 2 −0,36 V, gain 500).

Statistical Analysis

For treatment effects analyses of covariance (ANCOVA) for repeated measurements were computed with baseline values as covariates. Baseline adjusted means were calculated for main treatment effects and significant effects were located by post hoc Scheffé test. Furthermore, mean changes (mean of +55 min, +90 min, +110 min corrected for baseline values) in all of the described parameters were computed and analyzed by a multiple stepwise regression model.

RESULTS

NE concentration showed a significant main treatment effect (Fig. 2) and a time effect in the CT group. Cold exposure resulted in a marked increase of NE at the end of the exposure to 5°C (time 2). No time effects on NE were observed in the IPS group and in the PLAC group. No main treatment or time effect was observed on E concentrations (data not shown).

The density distribution of heterogeneous human blood platelets was only slightly influenced by IPS but was markedly influenced by cold exposure. Physically lowering of body core temperature (CT) leads to an increase in the relative number of SP I [Fig. 3(a)] whereas SP II and SP III were decreased (data not shown). For detailed demonstration of the main

TABLE 1

RESULT OF A STEPWISE MULTIPLE REGRESSION ANALYSIS

	PLAC	CT			IPS
	r	r	R	R^2	r
Step 1					
Skin temperature	0.34	0.07			−0.106
Body core temperature	0.58	−0.371			0.297
Metabolic rate	0.206	0.178			−0.038
Heart rate	−0.061	0.086			0.127
Muscle activity	−0.046	0.307			0.071
Norepinephrine	0.187	0.509*			−0.186
			0.509	0.26	
Step 2					
Skin temperature	0.34	0.063			−0.106
Body core temperature	0.58	−0.409*			0.297
Metabolic rate	0.206	0.006			−0.038
Heart rate	−0.061	0.075			0.127
Muscle activity	−0.046	0.048			0.071
			0.653	0.42	

Step 1 and step 2 for each treatment group, respectively, with changes in subpopulation I as dependent and all of the other changes described above as independent variables (r = correlation, R = multiple correlation coefficient, R^2 = amount of explained variance).

*Indicates significant correlation, $p < 0.05$. ANOVA data for skin temperature, body core temperature, metabolic rate, heart rate, and muscle activity are less than $p < 0.01$ (treatment and time effect) and described in detail elsewhere (22).

effect in CT, baseline adjusted means are shown in Fig. 3(b). Mean platelet volume was not influenced, neither in PRP nor when analyzed in whole blood (data not shown). However, a decrease was observed in MPV in platelets of SP I in the CT group (Fig. 4) at time 2.

Because of the high intercorrelations between the changes mentioned in Table 1, a multiple stepwise regression analysis with changes in SP I as dependent, and all of the other changes as independent, variables was computed for each group, respectively. Table 1 indicates that only in the CT group a significant multiple correlation based on the influence of NE and in part of changes on body core temperature can be demonstrated.

DISCUSSION

The present study shows an influence of decreased body core temperature on platelet parameters. The physically induced decrease of body core temperature in contrast to the pharmacologically induced one was accompanied by a decrease of skin temperature and a significant increase of muscle activity. A decreased body core temperature after a single oral dose of IPS was described by other authors (13), but without changes in parameters observed for treatment with cold environment (13). Furthermore, in our study NE was increased only in the CT group. This may be the result of a higher muscle activity in this group. E was not influenced either in the PLAC group or in the experimental groups. The neuroendocrine response after physical exercise is known to result in an increase of NE and only in a small increase in E, whereas a more pronounced increase in E as compared to NE is known after psychological stress conditions (3,4). In addition to an increase in NE plasma levels, we also found an increase in SP I, the platelet subpopulation with the highest sensitivity to aggregation-inducing agents. A regression analysis demonstrates a significant correlation between changes in NE and increase of SP I in the CT group (Table 1). Therefore, there is evidence that changes in body core temperature without changes in skin temperature and muscle activity are of less importance to the parameters analyzed because in the IPS group only minor changes in SP I can be observed. Mean platelet volume in SP I was reduced in the CT group, which may reflect a change in the status of platelet activation in this subpopulation.

The results obtained suggest that in thermoregulation platelets are affected and this effect is dependent on decreased body core temperature and decreased skin temperature. Platelets are known to play a role in the development of atherosclerosis (23,24), and psychological stress has been assumed to be a potential risk factor in both the development and clinical sequelae of coronary artery diseases (16,17). A change to a higher responsiveness of platelets after mental, psychological, or emotional stress is well documented (2,9,14,15,17), whereas the influence of aerobic fitness may prevent platelet activation (12,25). Activation of platelets seems to be parallel with increase in muscle activity and NE release. Decrease of body core temperature alone did not affect platelet function.

ACKNOWLEDGEMENTS

The expert technical assistance of Ute Beck and Kirsten Fischer is gratefully acknowledged. This project was supported by the Hessian Ministry of Science and Arts (LFSP).

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