6. J. Mironneau, C. R. Acad. Sci. (Paris) D, 276, 1005 (1973).
7. J. Mironneau, J. Physiol. (London), 233, 127 (1973).
8. T. Osa, Jpn. J. Physiol., 23, 401 (1973).
9. T. Osa, Jpn. J. Physiol., 24, 101 (1974).

EFFECT OF DESYMPATHIZATION ON PLATELET AGGREGATION AND BLOOD COAGULATION POTENTIALS IN RATS

B. A. Kudryashov, A. Mukhammedov, A. V. Suvorov, L. V. Berdysheva, and G. D. Kurbanova

UDC 612.115-06:612.89

KEY WORDS: adhesion and aggregation of platelets; desympathization; hemostasis.

During formation of a blood clot, besides plasma components of blood coagulation cells also take part, principally the blood platelets. Adhesion and aggregation of platelets under the influence of collagen, thrombin, adrenalin, prostaglandins, ADP, and certain other agents can create a basis for the formation of an intravascular thrombus [9, 10]. There is no doubt that the nervous system also participates in the regulation of intravascular blood clotting. Dysfunction of the sympathetic or parasympathetic nervous system leads to changes in hemostasis [12, 13].

In previous investigations the writers showed that an experimental model of intracardiac thrombosis can be created [14]. This model was obtained in rats deprived of their sympathetic peripheral innervation since the time of birth. In stress situations the desympathized animals died, due to the formation of large thrombi in the chambers of the atria [6, 13, 14].

The object of the present investigation was to study the effect of chemical desympathization on platelet aggregation and the role of platelets in thrombus formation in desympathized rats.

EXPERIMENTAL METHOD

Noninbred rats were desympathized with the aid of guanethidine. If this substance is injected into newborn animals, they develop irreversible degeneration of sympathetic ganglion cell bodies [8, 14]. The following groups of animals were used: 1) partially desympathized rats receiving guanethidine for two weeks after birth. About 25% of uninjured neurons remained in the stellate ganglia of these animals; 2) rats with complete desympathization, receiving guanethidine for four weeks after birth. Only 0.5% of neurons were preserved in the stellate ganglia of these animals [3, 14]. ADP-induced aggregation was determined in both groups of rats on reaching the ages of 1.5, 2.5, and 4 months, and thrombin-induced aggregation was studied in the completely desympathized animals (CDS) only, at the ages of 1.5 and 2.5 months.

Blood for investigation was taken from the jugular vein and treated with 3.8% sodium citrate solution in the ratio of 9:1. The aggregating power of the platelets was determined by the method described in [7]. ADP (Reanal, Hungary) or thrombin (Sigma), in a final concentration of 10 μM and 0.1 unit, respectively, were used to induce aggregation. The recalcification time of the animals' blood was determined with the N333 coagulograph, and the thrombin time and adrenalin concentration were also measured [5]. The sensitivity of the vas deferens to adrenalin also was investigated by determining the apparent dissociation constant (K) of the adrenalin–adrenoreceptor complex, which is the reciprocal of sensitivity [1].

Department of Human and Animal Physiology, Biological Faculty, M. V. Lomonosov Moscow University. (Presented by Academician of the Academy of Medical Sciences of the USSR S. E. Severin.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 89, No. 4, pp. 395-397, April, 1980. Original article submitted April 20, 1979.

0007-4888/80/8904-0405$07.50

TABLE 1. Aggregating Power of Platelets, Recalcification Time, and Thrombin Time in Desympathized and Intact Rats

Group of animals	Age, months	Aggregating power, conventional units		Recalcification time, sec	Thrombin time, sec
		ADP-induced aggregation	thrombin-induced aggregation		
Control		20,5±6,3 (n=49)	22,5±4,8 (n=9)	153,6±18 (n=8)	31,2±3,1 (n=9)
PDA	1½	2,08±0,55 (n=11)	—	70±6,1 (n=10)	23,4±4,5 (n=9)
	2½	3,33±0,6 (n=12)	—	104±3,8 (n=9)	30,9±1,3 (n=13)
	4	13,3±0,75 (n=13)	—	150±11,3 (n=10)	—
CDA	1½	1,77±0,67 (n=9)	0 (n=15)	—	—
	2½	0 (n=14)	0 (n=9)	88,0±7,7 (n=12)	22,3±0,7 (n=10)
	4	1,58±0,32 (n=12)	—	88±4,1 (n=12)	23±3,1 (n=7)

Legend. Aggregating power of intact rats aged 1.5, 2.5, and 4 months was identical; the results are pooled.

EXPERIMENTAL RESULTS

The aggregating power of the platelets of the desympathized rats was significantly reduced (Table 1). Desympathization led to complete blocking of thrombin-induced and to sharp inhibition of ADP-induced aggregation.

The age of CDA had no effect on the degree of ADP- or thrombin-induced aggregation. With an increase in age of the partially desympathized animals (PDA), however, ADP-induced aggregation was increased: In the rats of this group aged 4 months the aggregating power was statistically significantly greater than in rats of other ages ($P < 0.001$).

The results thus indicate that desympathization of rats reduces the aggregating power of the platelets. During recovery of the density of the peripheral sympathetic innervation, which occurs in PDA [2], the degree of ADP-induced aggregation showed a tendency to return to normal.

A parallel study of the recalcification time and thrombin time showed that these parameters in CDA were significantly lower ($P < 0.01$) than in control animals. Partial desympathization led to a significant decrease in the values of these parameters in rats only at the age of 1.5 months; at the age of 2.5 months the recalcification time was higher than in similar rats aged 1.5 months (Table 1), i.e., with recovery of the innervation, taking place in PDA [2], the blood coagulation potential showed a tendency to return to normal.

The next step was to study the possible mechanisms whereby desympathization of rats inhibits platelet aggregation. Similar phenomena are known in intact animals, for example, after injection of large doses of adrenalin [4] or of certain other substances, including prostacyclin and aspirin [15], into the blood stream. We were interested in adrenalin in this instance, for its concentration in whole blood in CDA was found to be increased, to 5.836 ng/ml (n = 33) compared with 0.561 ng/ml in intact rats (n = 36). The sensitivity of the vas deferens to adrenalin in CDA also was shown to be increased by 11 times (the constant in the control group was $20.8 \pm 3.4 \times 10^{-7}$ mole, compared with $1.9 \pm 0.8 \times 10^{-7}$ mole in CDA. To test the hypothesis that the observed effects may perhaps be mediated by adrenalin, a situation was created experimentally in healthy rats in which the blood adrenalin concentration was raised, and the aggregating power of the platelets was determined under those conditions. The increase in the blood adrenalin concentration was produced by immobilizing the animals by securing them to a frame. As Fig. 1 shows, immobilization stress caused an increase in the blood adrenalin concentration which reached a maximum 10 min after the beginning of immobilization. The ADP-induced aggregation of platelets obtained from blood taken after 10 and 30 min was sharply reduced ($P < 0.001$). Inhibition of platelet aggregation by adrenalin has also been found in experiments by other workers [4, 11].

Considering the increase in the blood adrenalin concentration found in desympathized rats, the increase

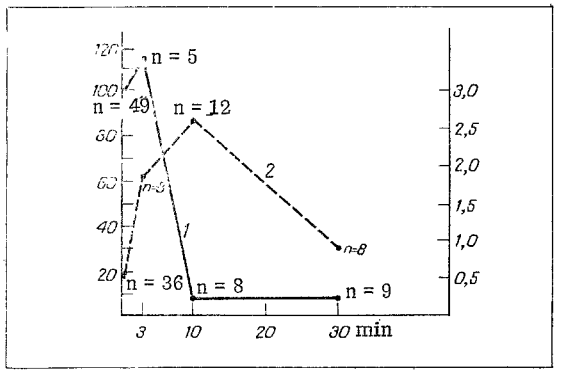

Fig. 1. Changes in aggregating power of platelets in intact animals with an increase in blood adrenalin concentration during immobilization stress. 1) change in aggregating power of platelets in animals at different times after beginning of immobilization stress; 2) changes in adrenalin concentration in animals at different times of immobilization stress. Abscissa, duration of immobilization of animals (in min); ordinate, on left – aggregating power of platelets (in percent). Aggregating power of platelets in animals taken before immobilization stress taken as 100%, on right – adrenalin concentration in blood (in ng/ml) taken from animals during immobilization stress.

in their sensitivity to adrenalin, and inhibition of platelet aggregation observed in the experiments with stress and probably due to elevation of the blood adrenalin level, it can be postulated that the reduction in the aggregating power of the platelets was caused by the action of adrenalin.

Further evidence of the participation of adrenalin in the changes in hemostasis induced by desympathization was given by the data showing the effect of the α-adrenoblocker phentolamine on the recalcification time of whole blood. Intact rats and CDA were given an intravenous injection of 0.5 ml of phentolamine solution (5 mg/100 g) or of 0.5 ml physiological saline 15 min before the blood sample was taken. Compared with rats receiving injections of physiological saline, in intact rats phentolamine shortened the recalcification time from 161 ± 6.8 to 138 ± 10 sec, whereas in the CDA, the α-adrenergic block caused a significant increase in the recalcification time from 88 ± 7.7 to 150 ± 7.6 sec.

It can thus be concluded that desympathization of rats causes sharp changes in hemostasis, increases the coagulation potential of the blood plasma, and inhibits platelet aggregation. These changes are probably due to an increase in the blood adrenalin concentration and to an increase in the sensitivity of the tissues to adrenalin.

LITERATURE CITED

1. L. V. Berdysheva, Fiziol. Zh. SSSR, No. 8, 1197 (1975).
2. M. M. Borisov, P. P. Doronin, L. V. Zueva, et al., Neirofiziologiya, No. 8, 84 (1976).
3. M. M. Borisov, A. Mukhammedov, I. M. Rodionov, et al., Ontogenez, No. 3, 311 (1977).
4. B. A. Kudryashov, V. E. Pastorova, and L. A. Lyapina, Probl. Gematol., No. 5, 28 (1973).
5. B. N. Manukhin, L. V. Berdysheva, and E. V. Volina, Vopr. Med. Khim., No. 3, 317 (1975).
6. A. Mukhammedov, Biol. Nauki, No. 12, 33 (1977).
7. G. V. Born, J. Physiol. (London), 161, 160 (1962).
8. O. Eranko and L. Eranko, Histochem. J., 3, 451 (1971).
9. S. J. Hafts, K. Gershengorn, and P. D. Kranz, Am. J. Cardiol., 30, 838 (1972).
10. H. Holmsen, H. Day, C. A. Setkowsky, Biochem. J., 129, 67 (1972).
11. S. K. Ju and J. G. Latour, Thrombos. Haemostas. (Stuttgart), 37, 413 (1977).
12. B. A. Kudryashov (B. A. Kudrjashov) and T. M. Kalishevskaya, Nature, 169, 647 (1962).
13. B. A. Kudryashov (B. A. Kudrjashov), I. M. Rodionov, L. A. Lyapina (L. A. Ljapina), et al., Folia Haemat. (Leipzig), 103, 252 (1976).

14. I. M. Rodionov, A. Mukhammedov, I. I. Poletaeva, et al., Experientia, 33, 39 (1977).
15. B. J. R. Whittle, S. Moncada, and J. R. Vane, Prostaglandins, 16, 373 (1978).

MECHANISMS OF REGULATION OF RESPIRATION UNDER A RESISTIVE LOAD

I. A. Breslav, N. Z. Klyueva, and Ê. A. Konza

UDC 612.2-063

KEY WORDS: respiration; resistive load; diaphragm; bilateral vagotomy.

An increase in the nonelastic resistance to respiration (resistive loading) is known to cause a greater or lesser increase in the activity of the inspiratory muscles in man and animals, which may partly or completely compensate the additional load on the ventilatory apparatus [1, 6-9]. However, the mechanism of this response has not been adequately studied, in particular, the role which the afferent system of the lungs plays in its formation.

In this investigation a physiological analysis was undertaken of responses of respiration of intact and vagotomized cats to resistive loading, acting against the background of quiet breathing or of hyperpnea caused by progressive hypercapnia.

EXPERIMENTAL METHOD

The following parameters were recorded in 33 tracheotomized cats under urethane-chloralose anesthesia, during quiet breathing and with an additional inspiratory resistance equal to 380 cm water/liter/sec: electrical activity of the phrenic nerve (APN), the peak inspiratory intrathoracic pressure (P_I), the respiratory volume (V_T), the duration of inspiration (T_I), the mean velocity of the inspiratory flow ($\dot{V}_I$), and the partial CO_2 pressure of the alveolar gas (P_{ACO_2}). The technique of recording these various parameters and the method of creating the resistive load were described previously [3-5]. In series I the animals breathed air and the additional resistance was introduced at the second minute. The responses to this load were recorded in the transitional (the first respiratory cycle after addition of the resistive load) and stable (end of the second minute of respiration against additional resistance) periods. In series II the animals breathed oxygen in a closed system, with a gradual increase in P_{ACO_2} from 35 to 57 mm Hg. Rebreathing lasted 3-4 min and took place under quiet breathing or resistive loading conditions. In both series each test was carried out before and after division of both vagus nerves in the neck. Statistically significant ($P \leq 0.05$) changes in the parameters tested due to the action of loading are given below.

EXPERIMENTAL RESULTS

In the experiments of series I (Fig. 1) introduction of the additional resistance caused a rapid rise in APN and P_I as well as a decrease in V_T and slowing of $\dot{V}_I$. Consequently, as early as during the first "loaded" inspiration, the mechanism increasing the inspiratory activity of the respiratory muscle had begun to act. The response observed was not due to intensification of chemoreceptor stimuli. Humoral changes caused by hypoventilation developed later: Toward the end of the second minute of respiration against the additional resistance P_{ACO_2} increased on average by 1 mm Hg. The further increase in APN and P_I, and also the restoration of V_T and $\dot{V}_I$ to their initial levels (during quiet breathing), observed by that time, must also be explained by intensification of chemoreceptor stimulation.

After vagotomy APN, P_I, V_T, and T_I were increased, but $\dot{V}_I$ showed no significant change under these conditions. Resistive loading no longer increased APN, whereas the increase in P_I was maintained. This sug-

Group for Physiology of Respiration, I. P. Pavlov Institute of Physiology, Academy of Sciences of the USSR, Leningrad. (Presented by Academician of the Academy of Medical Sciences of the USSR V. N. Chernigovskii.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 89, No. 4, pp. 397-400, April, 1980. Original article submitted June 26, 1979.

 0007-4888/80/8904-0408$07.50