similar time course of threshold voltage was encountered in the E unit. It is quite likely that with increasing stimulus strength, recruitment of additional descending or ascending fibres occurred, excitation of which facilitated antidromic spike invasion in RMN. If facilitation is synaptic in nature and 0.5 msec are allowed for synaptic transmission, these facilitatory fibres must conduct faster than the axons emerging from the facilitated RMN. If the facilitatory action potentials are supposed to travel antidromically, they may travel along axons originating from faster conducting RMN which send an axon collateral to the facilitated unit. The mean latency of antidromic spike invasion of RMN was found to be 1.27 msec and the range ($\bar{X} \pm SD$) was 0.65–1.89 msec[7]. On the other hand, they may travel along axons originating from other types of bulbar neurons, the discharge of which is not modulated with respiration. Mean latency of antidromic spike invasion in this type of cell was found to be 0.47 msec and the range was 0.22–0.72 msec[7] which leaves enough time for facilitation of all RMN. The facilitating spikes, however, may well travel orthodromically along spinal ascending fibres. The time course of excitability determined from threshold voltages was somewhat different from the time course of latencies of antidromic spike invasion; threshold voltage was lower during the later part of the silent period.

In addition to the above mentioned mutual inhibitory mechanism, inspiratory activity is inhibited by excitation of pulmonary stretch afferents (Hering-Breuer inflation reflex). Single electrical pulses delivered to the vagal nerves immediately entail lengthening of one interspike interval in more than $^1/_3$ of all RMN tested[8]. Close examination then revealed that lengthening was more pronounced in I than in E neurons, and in the former cell type mainly in the later part of burst discharge; lengthening was modest in the middle part of bursting activity of E units (figure). Variability in the lengthening was high at respiratory phase transitions. Lengthenings of interspike intervals, and thus the inhibitory state of RMN, apparently do not reflect the time course of the resting membrane potential of I and E neurons, but rather parallel that of the central inspiratory 'off-switch' mechanism[9,10].


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## Effect of sympathectomy on platelet aggregation and blood coagulation in rats


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*Summary.* It is shown that the ability of blood platelets to aggregate in partially and completely sympathectomized rats is significantly lower than in intact animals. The blood clotting system of sympathectomized rats is hyperactive. The sympathectomy-provoked changes may be due to the increased content of adrenaline in the blood.


It is known that in addition to involving plasma components, blood clot formation also involves blood cells, predominantly platelets. The adhesiveness platelets and their ability to aggregate, stimulated by collagen, thrombin, adrenaline, ADP and some other substances, can favour thrombosis in blood vessels[1,2]. An indispensable regulator of intravascular blood coagulation is the nervous system; the dysfunctions of the sympathetic or parasympathetic nervous systems cause changes in hemostasis[3].

In previous experiments we demonstrated an experimental model of heart cavity thrombosis, developed in rats with a congenital lack of sympathetic peripheral innervation[4–6]. Under stress conditions the sympathectomized animals perished due to extensive thrombosis in the auricular cavity.

The aim of the present work is to investigate the aggregation of blood platelets in sympathectomized rats.

*Materials and methods.* Sympathectomy was performed on mongrel rats by injecting newborn animals with guanethidine, which causes irreversible degeneration of sympathetic ganglia cell organelles[7,8]. The experimental animals were divided into 2 groups. The 1st group included rats with partial sympathectomy (PSR), caused by injecting newborn rats with guanethidine daily for 2 weeks. The stellate ganglia of these animals contained only 25% of the normal amount of neurons. The 2nd group included animals with total sympathectomy (TSR) caused by a 4-week administration of guanethidine; in this case the content of neurons in the stellate ganglia was only 0.5%. In both experimental groups the ADP-induced aggregation was determined in 1.5, 2.5 and 4-month-old animals; the thrombin-induced aggregation was measured in 1.5 and 2.5-month-old animals of group 2 alone.

Blood samples were collected from the jugular vein, using 3.8% sodium citrate solution (9:1). Platelet aggregation was assayed according to Born[10]. The aggregation was induced by ADP ("Reanal") or thrombin ("Sigma") at final concentrations of 10 mkM and 0.1$\mu$, respectively. The recalcification time, thrombin time and the blood adrenaline content of experimental animals were determined.

*Results and discussion.* As can be seen from the table, the platelet aggregation in sympathectomized animals is significantly decreased. Sympathectomy results in a total blocking of the thrombin-provoked aggregation and a marked inhibition of the ATP-induced aggregation. The number of platelets in whole blood samples of intact and sympathectomized animals in practically the same.

The degree of aggregation induced by ADP or thrombin in TSR does not depend on age. However, in the case of PSR the ADP-provoked platelet aggregation is age-dependent, e.g. in 4-month-old rats the aggregation is statistically significantly higher than in animals from other age groups ($p < 0.001$).

Platelet aggregation, recalcification time and thrombin time in intact and sympathectomized rats

| | Age (months) | Platelet aggregation (mV) ADP-induced | Thrombin-induced | Recalcification time (sec) | Thrombin time (sec) |
|---|---|---|---|---|---|
| Intact rats | * | 20.5 ±6.3 n=49 | 22.5±4.8 n=9 | 153.6±18 n=8 | 31.2±3.1 n=9 |
| Partially sympathectomized rats | 1.5 | 2.08±0.55 n=11 | - | 70±6.1 n=10 | 23.4±4.5 n=9 |
| | 2.5 | 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 | - |
| Totally sympathectomized rats | 1.5 | 1.77±0.67 n=9 | 0 n=15 | 88±7.7 n=12 | 22.3±0.7 n=10 |
| | 2.5 | 0 n=14 | 0 n=9 | - | - |
| | 4 | 1.58±0.32 n=12 | - | 88±4.1 n=12 | 23.0±3.1 n=7 |

* Platelet aggregation in intact rats at the ages of 1.5, 2.5 and 4 months are the same and results are combined.

Thus, the results obtained suggest that sympathectomy decreases the ability of blood platelets to aggregat. As the density of peripheral sympathetic innervation is restored, which takes place only in PSR[9], the level of ADP-induced aggregation tends to return to normal.

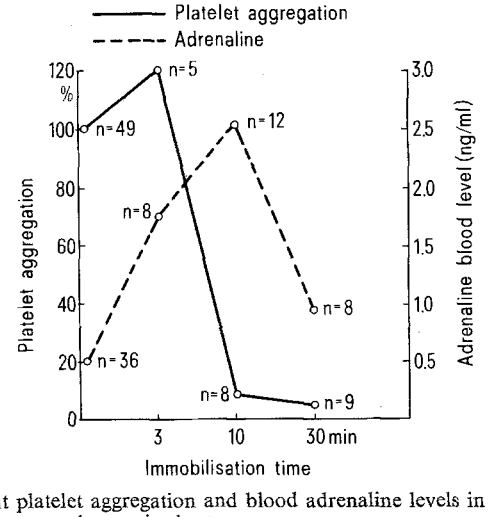
Parallel analysis of the recalcification and thrombin times shows that in TSR these parameters are significantly lower ($p < 0.001$) than in the controls. Partial sympathectomy considerably decreases the values of the given parameters only in 1.5-month-old animals; in 2.5-month-old animals the recalcification time is higher than that in 1.5-month-old animals (table).

Further experiments were carried out to determine how sympathectomy causes changes in the coagulating potential of the blood and inhibition of platelet aggregation. According the literature, platelet aggregation in healthy animals can be inhibited by aspirin, catecholamines and other substances[11,12]. Our special interests were focused on the effects of adrenaline, since we discovered that the adrenaline content in the blood of TSR is increased (5.836 ng/ml of blood, n=33), whereas in the control animals its content is 0.561 ng/ml, n=32. Besides, we showed that the sensitivity of TSR to adrenaline (in the case of the deferent duct) increases 11-fold, the control constant being $20.8 \pm 3.4 \cdot 10^{-7}$ M, the TSR value $1.9 \pm 0.8 \cdot 10^{-7}$ M.

When studying the effect of the $\alpha$-adrenergic phentolamine blocker on recalcification time we obtained some evidence for participation of adrenaline in the sympathectomy-provoked changes in the coagulating system. Phentolamine (5 mg/100 g b.wt) was administered i.v. to intact animals and TSR 15 min prior to the collection of blood samples. In intact animals phentolamine had no effect on the recalcification time, whereas in TSR adrenergic blocking increased the recalcification time from 88±7.7 sec to 150±7.6 sec. Thus, $\alpha$-adrenoreceptor blocking completely stabilizes the coagulating activity of the blood in TSR.

In order to test the possible effect of adrenaline on platelet aggregation we caused an increase of adrenaline in the blood by immobilizing the animals, and then measured the amount of platelet aggregation. As can be seen from the figure, the adrenaline content in the blood reaches its maximum 10 min after the onset of immobilization, and the ADP-induced aggregation of blood platelets is sharply decreased ($p < 0.001$).

The increase in the adrenaline content of the blood of sympathectomized rats, the enhanced sensitivity of tissues to adrenaline and the inhibition of platelet aggregation under restraint stress suggest that the decrease in platelet aggregation and the increase of the coagulating potential of the blood of sympathectomized animals are due to the effect of excess adrenaline. Thus, sympathectomy produces drastic changes in hemostasis, i.e. increase in the coagulating potential of the blood and inhibition of platelet aggregation. The latter process does not affect thrombus formation in sympathectomized animals under stress conditions.




Percent platelet aggregation and blood adrenaline levels in immobilized, sympathectomized rats.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