EFFECT OF NORADRENALIN, ACETYLCHOLINE, LIDASE, AND RUTAMINE ON INTRAVASCULAR LIFE SPAN OF GRANULOCYTES

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UDC 612.112.91:612.13.014.46

Intravital labeling of granulocytes with mepacrine has shown that intravenous injection of noradrenalin, acetylcholine, and rutamine (0.6 ml) shortens the intravascular life span of the granulocytes. Injection of rutamine in a dose of 2 ml was accompanied by lengthening of the intravascular life span of the granulocytes. Definite changes in this index were also observed after perivascular administration of these preparations and also of lidase.

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The intravascular life span of the granulocytes, the circulation time of mature granulocytes in the blood stream, has a normal value of 30-40 min (2, 8, 14, 15, 16), whereas their total life span, according to data given by various authors [13, 15, 17, 18], varies mainly between 7 and 13 days.

When the normal state of the organism is disturbed, a change in the rate in which granulocytes pass from the bloodstream into the organs and tissues is possible.

The object of the investigation was to study factors influencing these changes in the intravascular life span of granulocytes. The circulation of the granulocytes in the blood was investigated after administration of noradrenalin, acetylcholine, rutin, and lidase, substances formed locally in the tissues under physiological conditions [3, 4, 6, 12] and playing an important role in the circulation in individual organs.

EXPERIMENTAL METHOD AND RESULTS

Intravenous injection of mepacrine in a dose of 1.5 mg/kg into animals provides instantaneous labeling of granulocytes in the circulating blood [9, 14, 15]. By calculating the percentage of fluorescent granulocytes in blood films taken 2, 5, and every 10 min for 1 h after labeling, we determined the length of stay of the granulocytes in the blood stream. Altogether 78 experiments were performed on 35 rabbits.

In 8 experiments, 0.1 ml of solution of noradrenalin or acetylcholine (1:1 000 000), was injected by the perivascular route into the right and left ears of a rabbit, while in 5 analogous experiments 0.1 ml lidase (15% solution) or rutamine (a rutin–procaine mixture) was injected; the substances were given subcutaneously in the immediate neighborhood of the walls of blood vessels – the marginal veins of the right and left ear 2.5-3 min after injection of mepacrine into the vein at the base of the ear. The dynamics of the labeled granulocytes was studied in blood taken from the part of the vein near which the substances to be tested were injected.

The preparations were also injected intravenously 2.5-3 min after labeling: noradrenalin in a dose of 0.1 ml/kg (7 experiments), acetylcholine in doses of 0.1 and 0.3 mg (16 experiments), lidase in a dose of 30 mg (0.3 ml; 10 experiments), and rutamine in doses of 0.1, 0.3, 0.6, and 2 ml (21 experiments).

After subcutaneous injection of lidase in a dose of 5.5 mg/kg daily for 8 days, 6 experiments were performed.

Results. The experiments showed that noradrenalin, acetylcholine, rutamine, and lidase affect the intravascular life span of granulocytes (Table 1).

Faculty of Therapeutics, S. M. Kirov Gor'kii Medical Institute; Gor'kii Institute of Work Hygiene and Occupational Diseases (Presented by Active Member of the Academy of Medical Sciences of the USSR N. A. Fedorov). Translated from Byulleten' Eksperimental'noi Biologii i Meditsiny, Vol. 65, No. 6, pp. 23-28, June, 1968. Original article submitted November 17, 1966.

TABLE 1. Effect of Noradrenalin, Acetylcholine, Lidase, and Rutamine on Intravascular Life Span of Granulocytes

Series of experiments	No. of experiments	Percent of labeled granulocytes (M ± m) at following times after labeling with mepacrine						
		5 min	10 min	20 min	30 min	40 min	50 min	60 min
Control	40	80±1,2	53±2,3	17,5±1,3	6±0,7	2,05±0,5	0,7±0,21	0,6±0,15
Intravenous injection of								
noradrenalin, 0.1 ml/kg	7	52,5±5,6	19,3±4,4	4,8±1,3	1,8±1,5	1,8±0,8	0,5±0,6	0,6±0,7
acetylcholine, 0.1 mg	8	50,3±8,6	45,6±9,7	19,1±5,5	10,2±2,2	5,5±1,7	2,5±0,8	1,7±0,6
" , 0.3 mg	8	50,7±6,3	19,4±3,7	5,7±1,7	2,1±1,0	0,4±0,5	0	0
1% solution lidase, 30 mg	10	60,3±4,5	38,1±9,7	14,7±4,7	6,4±2,7	5,2±2,0	2,8±1,95	1,4±0,8
rutamine, 0.6 ml	6	51±9,5	15,2±6,5	2,8±1,2	9,4±1,0	0,4±0,4	0,6±0,4	0
" , 2 ml	5	92±2,46	66,4±5,40	34,4±6,8	19,0±5,7	11,0±3,7	10,0±3,3	11,0±5,8
Perivascular injection of noradrenalin, 0.1 ml of 1:10⁶ soln.	6	41,5±6,1	16,4±3,8	4,0±1,5	1,6±1,3	1,5±0,8	1,7±1,7	0,6±0,6
acetylcholine, 0.1 ml of 1:10⁶ soln.		81,2±4,7	48,7±7,7	22,5±6,2	4,5±2,04	1,8±0,8	1,4±0,6	1,4±0,8
lidase, 0.1 ml of 6% solution	5	89,4±5,0	53,2±10,7	55,6±12,8	18,0±4,35	8,4±4,4	11,8±5,7	15,2±11,2
rutamine, 0.1 ml		7,0±6,9	14,4±6,4	0,6±1,1	0	0	1,8±1,7	0
Subcutaneous injection of lidase, 3.5 mg/kg daily for 8 days	6	59,6±7,4	30,5±7,8	9,2±3,0	2,3±0,6	1,1±0,7	0	0,6±0,5

As Table 1 shows, intravenous injection of 0.1 ml/kg noradrenalin reduced this period to 20 min ($P < 0.001$). Injection of acetylcholine in a dose of 0.3 mg also led to more rapid removal of granulocytes from the blood stream ($P < 0.01$); however, after injection of acetylcholine in a dose of 0.1 mg, no consistent changes could be found ($P > 0.5$).

The speed with which the circulating time of granulocytes in the blood vessels was shortened (2.5-3 min after intravenous injection of noradrenalin or acetylcholine) demonstrated that this may be associated with a disturbance of capillary permeability, in which changes take place instantaneously [5, 7], whereas constriction or dilatation of the capillaries takes place very slowly [6]. Substances modifying permeability (lidase and rutamine) also affected the length of stay of the granulocytes in the circulating blood (Table 1).

The fact that labeled granulocytes in the experiments with perivascular injection moved toward the blood vessel near which lidase was injected ($P < 0.01$) but avoided the vessel near which rutamine ($P < 0.01$) or noradrenalin ($P < 0.001$) was injected, indicates that these substances may play a role in the regulation of granulocyte dynamics.

Intravenous injection of lidase within a few minutes caused spasm of the vessels, leukopenia, and a more rapid disappearance of fluorescent granulocytes from the blood stream ($P < 0.001$). However, after 10 min and later during the experiment, besides congestion of the blood vessels, in certain cases the number of labeled cells in the blood was actually increased as a result of the return of granulocytes rapidly removed from the blood stream and probably retained in the capillaries. After subcutaneous injections of lidase the intravascular life span of the granulocytes shortened to 20 min ($P < 0.02$).

After injection of rutamine into the rabbits in doses of 0.1 and 0.3 ml, the intravascular life span of the granulocytes was normal in most cases; an increase in this index was observed in two of five experiments when the dose given was 0.3 ml. Injection of 0.6 ml rutamine consistently increased the rate of removal of granulocytes from the blood stream ($P < 0.01$); with a dose of 2 ml an increase in the length of stay of the granulocytes in the blood stream as a whole to 50 min was observed ($P < 0.02$).

Hence, both mediators of the nervous system and the state of the vascular permeability influence the duration of stay of the granulocytes in the systemic blood stream.

LITERATURE CITED

1. S. I. Ashbel', S. A. Troitskii, V. C. Sokolova, et al., Klin. Med., No. 12, 62 (1964).
2. A. A. Bagdasarov, F. R. Vinograd-Finkel', and R. I. Rodina, Probl. Gematol., No. 4, 34 (1960).
3. V. G. Vogralik, Klin. Med., 23, No. 4-5, 36 (1945).
4. T. A. Grigor'eva, Innervations of the Blood Vessels [in Russian], Moscow (1954).
5. Kh. S. Koshtoyants, Proteins, Metabolism, and Nervous Regulation [in Russian], Moscow (1951).
6. G. I. Mchedlishvili, The Capillary Circulation [in Russian], Tbilisi (1958).
7. A. I. Smirnov-Zamkova, Klin. Med., No. 6, 11 (1957).
8. S. A. Troitskii and Z. G. Filyushina, Probl. Gematol., No. 7, 51 (1963).
9. Z. G. Filyushina, in: Methods of Physico-Chemical Analysis [in Russian], Rostov-on-Don (1965), p. 235.
10. Z. G. Filyushina, Gig. Truda, No. 3, 52 (1966).
11. Z. G. Filyushina, Gig. Truda, No. 9, 52 (1967).
12. B. V. Tsveifakh, Pat. Fiziol., No. 2, 6 (1964).
13. H. R. Bierman, K. H. Kelly, F. L. Cordes, et al., Blood, 7, 683 (1952).
14. P. Lawrence and M. D. White, Blood 9, 73 (1954).
15. J. Lissac, G. Mathe, and J. Bernard, Rev. Franc. Et. Clin. Biol., 1, 631 (1956).
16. J. Lissac, Rev. Franc. Et. Clin. Biol., 2, 176 (1957).
17. E. E. Osgood, Blood, 9, 1141 (1954).
18. J. Ottesen, Acta Physiol. Scand., 32, 75 (1954).
19. A. Vannoti, Helv. Med. Acta, 27, 469 (1960).