The Content of Serotonin in Human Platelets is Unstable: Facts and Possible Mechanisms

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Human blood platelets are used with justification as an extracerebral model of the serotoninergic neuron [2,6]. In numerous reports the content of serotonin in human platelets under normal conditions and in psychopathological states has been determined [5,9]. It was assumed in these experiments that the quantity of serotonin remains unchanged at least during the time of the experiment. The aim of this investigation was to study the dynamics of serotonin content in human platelets and factors affecting this dynamics.

MATERIALS AND METHODS

Blood samples were taken from 66 healthy donors. The content of serotonin in the platelets (Spl) and plasma (Sp) and the total serotonin (St) level were assayed by the spectrofluorimetric method [3]. The intensity of fluorescence was recorded on Elyumin 2M spectrofluorimeter using the 365 nm wavelength for activation and the 480 nm wavelength for emission. In order to study the dynamics of the serotonin content in the platelets, tubes with platelet-enriched plasma were placed one after the other at 20-sec intervals into ice-cold water for 5 min, after which the tubes were processed simultaneously. Such a cooling procedure blocks the processes of serotonin release and uptake by platelets, and thus the Spl and Sp indexes can be fixed at a certain time point. The serotonin content was evaluated in ng/ml when comparing Spl and Sp, and in ng/10^9 platelets when estimating platelet serotonin.

In corresponding experiments the plasma proteins were removed by two methods: by resuspending the platelet sediment in buffered (protein-free) solution or by filtration of platelet-enriched plasma through sepharose in a vertical chromatographic column. For reconstitution of the initial conditions, 0.5 ml of platelet-free native plasma was added. Calcium was removed by addition of 16 mM EDTA to the platelet-enriched plasma with further incubation for 10 min at 37°C. The calcium content was restored by incubation with 0.1 M $CaCl_2$ solution.

Statistical analysis of the results was performed using the Statgraphics software package.

RESULTS

The total serotonin content in the blood of 6 healthy donors is on average 329±54 ng/ml, varying from 287 to 419 ng/ml. St variability in each individual is not large; the coefficient of variation (CV) does not exceed 7.4%. However, unlike St, Spl is an unstable value.

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TABLE 1. Serotonin Content (ng/ml) in Platelets and Blood Plasma

Platelets					Plasma				
time points			mean	CV	time points			mean	CV
1	2	3			1	2	3		
339	390	454	394	14.7	140	116	72	109	31.2
396	399	253	349	23.8	81	93	205	126	54.0
122	166	273	187	41.7	144	77	27	83	71.1
232	112	242	195	36.9	53	89	52	65	32.3
140	267	121	176	44.9	68	0	56	41	87.8
311	229	180	240	27.5	10	42	42	31	58.1

The serotonin content was recorded in parallel in platelets and plasma three times at 20-sec intervals. During this period the Spl of each donor changed 1.4-2.2 times (Table 1). The CV of Spl ranged within 14.7-44.9% and was 32% on average. The CV of Sp is even higher (evidently due to the much lower average content of serotonin) and is 56% for the donor group studied. The coefficient of correlation between standardized values of Spl and Sp is -0.98 ($p<0.001$). The stable level of St and strongly correlated changes in Spl and Sp content make it possible to state that the fluctuations of these indexes are related to the transport of serotonin from platelets to plasma and vice versa.

Spl level instability is further proved in an assay of blood of 25 donors 6-8 times consecutively at 20-sec intervals. The mean Spl value varies from 383 to 1516 ng/10^9 platelets, and CV from 16% to 89%, on average 45%. The rise of CV in comparison to the first experimental series (32%) may be related to the increase in the number of Spl determinations and presents additional indirect evidence of the existence of Spl fluctuations.

If the serotonin transport in each platelet were an independent process, then the registered Spl value should correspond to a certain mean level without marked fluctuations. The visible Spl changes imply the synchronous change of this value in a significant number of platelets.

The presumed factors able to mediate the functional synchronization of platelets *in vitro* were studied using blood specimens from 18 donors. For this purpose plasma was first depleted of and then reconstituted with the factors in question (Table 2).

Removal of plasma proteins, as well as Ca^{2+} results in a significant decrease of the Spl CV, despite the unchanged mean level. Restoration of the protein and Ca^{2+} content leads to a rise of the Spl fluctuations up to a level statistically indistinguishable from the control level. Thus, plasma proteins and Ca^{2+} are necessary for the appearance of the registered Spl fluctuations, i.e., for the synchronization of Spl transport.

The mean content of serotonin in the platelets and blood determined in this study is fully consistent with the results of other workers [3,4]; however, significant fluctuations of the Spl value within short periods of time are described for the first time. A hypothesis about the alternation of serotonin release and uptake by platelets was advanced by Pletscher [7] as early as in 1967. We have disclosed a stable level of St and a reciprocal relationship between Sp and Spl, which proves this hypothesis. Spontaneous serotonin secretion and uptake should be in equilibrium. Therefore, *in vitro* a permanent serotonin exchange between platelets and plasma in the submembrane zone is possible.

Visible Spl fluctuations require synchronous transport of serotonin in a significant number of

TABLE 2. Relationship Between Plasma Proteins and Ca^{2+} Presence and Dynamics of Serotonin Content in Platelets ($M \pm m$)

Factor	Experimental conditions	Spl, ng/10^9 platelets	CV, %	Significance of CV variations, p
Proteins	Control	692±313	45.3	<0.01
	Removal	549±82	15.0	>0.05
	Addition	653±345	52.8	<0.01
Ca^{2+}	Control	687±192	27.9	<0.01
	Removal	697±77	11.1	>0.05
	Addition	655±310	47.4	<0.01

platelets. This synchronization may be mediated by fibrinogen molecules, which bind to the specific receptors (GPIIb/GPIIIa), forming a kind of loose network. Here the second type of secretion and irreversible platelet aggregation do not take place [1]. Ca^{2+} ions are required for the formation of receptor heterodimer and its binding to fibrinogen [8]. Thus, the requirement, shown in this study, of plasma proteins and Ca^{2+} for recording fluctuations in Spl confirms the hypothesis mentioned above.

This investigation sheds new light on earlier results on the Spl level under normal and pathological conditions. The St of the blood may be considered to be comparatively constant. However, the Spl variability in short periods of time reflects the large measure of chance in the estimation of this index. A study of Spl instability and the factors influencing it is necessary for the investigation of peculiarities of the serotoninergic system under normal and pathological conditions.

REFERENCES

1. N. V. Belitser, M. G. Anishchuk, T. M. Pozdnyakova, *et al., Vestn. Akad. Med. Nauk SSSR,* № 10, 45-56 (1991).
2. A. A. Zozulya, *Zh. Nevropatol. Psikhiatr.*, № 4, 599-603 (1986).
3. N. L. Rabinovich, V. M. Makhotchenko, I. M. Kozinets, *et al., Lab. Delo,* № 12, 729 (1985).
4. A. P. Solomonov and Z. G. Larskii, *Ibid.*, № 2, 80-83 (1973).
5. A. Coppen, C. Swade, and K. Wood, *Brit. J. Psychiat.*, **136**, 235-238 (1980).
6. J. R. Ieni and L. P. Meyerson, *Life Sci.*, **42**, № 3, 311-320 (1988).
7. A. Pletscher, *Experientia*, **23**, 289-291 (1967).
8. E. W. Salzman and J. A. Ware, *Methods for Studying Platelets and Megakaryocytes*, New York (1987), pp. 419-430.
9. S. Stahl, D. Woo, I. Mefford, *et al, Amer. J. Psychiat.*, **140**, 26-30 (1983).

Bioenergetic Parameters of the Brain in Rats with Different Resistance to Hypoxia

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Today we have a body of evidence supporting the notion that the different brain resistance to hypoxia in highly resistant and susceptible animals correlates with peculiarities of the energy-synthesizing function of the brain, which is connected with the oxidation of different substrates [4,7,8,10,11]. In the brain energetic substrates undergo oxidation mostly via the NAD-dependent pathway, which is a limiting link of the respiratory chain during hypoxia [2,5-8,17]. Differences in the degree of its inhibition during oxygen deprivation in resistant and susceptible animals may be a decisive factor in the formation of the initial resistance of the brain to hypoxia.

The goal of our study was to obtain direct confirmation of this assumption by studying the enzyme composition of the respiratory chain in brain mitochondria, as well as some other biochemical param-

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