INFLUENCE OF NON-SELECTIVE BETA-ADRENOSTIMULATION AND BETA-ADRENOBLOCKADE ON ^{75}SELENOMETHIONINE INCORPORATION IN RAT THROMBOCYTES

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The problem of neuro-humoral thrombocytopoiesis regulation remains still unsatisfactorily clarified despite its theoretical and practical interest. Data about the participation of adrenoceptors in this process are incomplete and contradictory (15).

The interest in β-adrenoceptors rises also because of the circumstance that they are proved on the membrane of pluripotent stem cells (8) and of thrombocytes themselves (7, 18).

Proceeding from the fact that ^{75}selenomethionine (^{75}Se-M) incorporation into newly formed thrombocytes (6, 16) presents a main quantitative index for thrombocytopoiesis evaluation we decided to study this process in relation to stimulating and blocking β-adrenergic effects. This will enable us to clarify more precisely some aspects of the control function of the β-adrenergic system (and β-adrenoceptors, respectively) on thrombocyte formation.

Material and methods

Our observation covered 84 white male rats of Wistar breed with 160—180 g b. w. divided into 4 equal groups. Animals were injected as folowed: 1st group — with Isoprenaline hydrochloride (IP) (a non-selective β-adrenostimulator) at dosis 3 mg/kg b. w.; IInd — with Propranolol hydrochloride (PR) (a non-selective β-adrenoblocker) at dosis 5 mg/kg b. w. followed by an IP injection at dosis 3 mg/kg b. w. after 1 hour; IIIrd — with PR at dosis 5 mg/kg b. w. alone, and IVth — with saline in the same volume. Animals were sterilly injected twice daily in 12 hour intervals during a 3 dayperiod. One hour after the first injection all the animals were injected intraperitoneally 15 μCi ^{75}Se-M each in a volume of 0.5 ml. On the 72th hour after this injection we aspirated by means of puncture of the abdominal aorta under ether narcosis 1.00 ml blood from all the animals each. Further processing for thrombocyte separation and calculation was carried out according to the method of Penington (16) whereas radiometrics was done on the apparatus N/K 350 (Hungary). Data obtained were processed by using of the variation analysis.

Results and discussion

One can see on fig. 1 that β-adrenostimulation with IP enhances isotope incorporation by 58.87 per cent ($p < 0.001$) whereas PR β-adrenoblockade reduces significantly the incorporation in newly formed thrombocytes by 55.70 per cent

($p<0.001$). Preliminary β-adrenoblocker treatment reduces completely IP stimulating effect. It is stressing that both kinds of adrenergic influences act statistically considerably on ^{75}Se-M incorporation in newly formed thrombocytes.

The increased ^{75}Se-M incorporation in thrombocytes under IP influence is a sure indicator for its stimulating effect on thrombocytopoiesis. In our opinion, these changes can be mainly due to the direct stimulating IP effect on β-adrenoceptors of stem cells and of megakaryocyte line in so far as such effect of IP on other blood-cell system is already documented (2).

It is proved that IP stimulates β-adrenoceptors of early precursors of committed stem erythroid cells and of more mature erythroid ones (8). As consequence of that a comprising of cells into cell cycle, an increased DNA synthesis and cell cycle shortening can be observed. In seems rather probable that IP exerts a similar effect on megakaryocyte precursors and on megakaryocytes themselves as well. It can result in an increase of the percentage of ^{75}Se-M incorporation and of thrombocyte count.

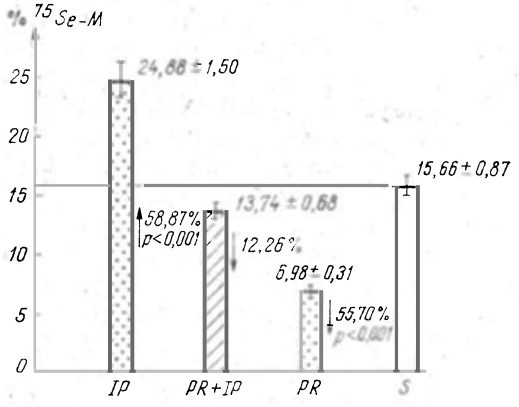

Fig. 1. Influence of IP and PR on ^{75}Se-M incorporation in newlyformed rat thrombocytes
IP — group treated with IP; PR — group treated with PR; PR+IP — group of consecutive treatment with both adrenergic agents in one hour interval. Data are presented as $\bar{x} \pm S\bar{x}$. Percentage difference is calculated towards control values

We suggest also that it is possible that IP effect stimulating the total metabolism and known concerning other processes and cellular systems (5, 11, 12, 17) is favourable when thrombocyte precursor metabolism is concerned, too. It can lead to an increased amino acid ^{75}Se-M incorporation and thrombocyte formation, respectively. When explaining these changes one should have in mind that IP stimulates presynaptic β-adrenoceptors which enhances noradrenalin liberation. That can induce an adrenergic activity increase and it accelerates coagulation and extends thrombocyte number (13). Our data concerning an increased ^{75}Se-M incorporation confirm the latter results and specify them to be a result of β-adrenostimulation.

Concerning the intimate mechanism of influence we suppose that the system adenylate cyclase — cAMP is stimulated similarly to that event established for erythroid stem cells and for bone marrow cells (9). The suppression of IP stimulating effect on ^{75}Se-M incorporation after preliminary PR treatment supports definitely our statement about a direct positive relation between β-adrenoceptor stimulation and increased ^{75}Se-M incorporation in newly formed thrombocytes. The last circumstance as already outlined is a basic criterion for stimulated thrombocytopoiesis. A similar dependence (suppression of IP effect) is established in other processes and cellular systems (1—3).

In our oipnion the considerable reduction of ^{75}Se-M incorporation in newly formed thrombocytes can be due to the following reasons when PR influence is concerned:

First of all, proceeding from the PR suppressive effect on a series of proces ses and enzymes (4, 10, 13) we accept that it is possible that it inhibits the cellu lar metabolism of stem and megakaryocyte cells and suppresses their prolifera tion and differentiation in the concrete example. Undoubtedly, it will reduc ^{75}Se-M incorporation in thrombocytes which argues for thrombocytopoiesis in hibition. These data confirm in fact our previous investigation (3) that there is thrombocyte count decrease after one-day and three-day β-adrenoblockade.

Having in mind PR stabilizing effect on the cell membrane (19) we assum that on this basis a difficult assimilation of the labelled amino acid by precur sors and megakaryocytes seems rather possible. We can take into consideratio other data, too (14) according to which PR changes thrombocyte membran expressed with integrity loss and vacuolar system rupture. Together with thes two considerations mentioned it is also possible that thrombocyte lysis contri butes additionally to thrombocyte count reduction.

Summarizing the results obtained we would like to emphasize that IP non selective β-adrenostimulation increases considerably ^{75}Se-M incorporation i thrombocytes which is an indicator for thrombocytopoiesis stimulation. The iso lated application of PR non-selective β-adrenoblockade inhibits strongly isotop incorporation, i. e. thrombocytopoiesis itself. Simultaneously, PR pretreatment reduces completely IP stimulating effect on ^{75}Se-M incorporation in thrombo cytes. These findings allow us to accept that ^{75}Se-M incorporation in newly for med thrombocytes is most probably a β-adrenodependent process. It can be con cluded that β-adrenoceptors participate in thrombocytopoiesis regulation.

REFERENCES

1. Ганчев, Т. С. Канд. дис. Варна, ВМИ, 1975. — 2. Ганчев, Т. С., Н. М Новиков. В: Кислородный режим организма и механизмы его обеспечения. Тезисы докладов Всесоюзн. конф. Барнаул. 1978, 86—87. — 3. Ганчев, Т. С., Е. Дянков, Н. Негрев, Р. Станева. В: Обед. науч. сес. Окр. болница — Варна, 1979 г. *Научни трудове, Варна*, 1979, 3, 33—34. — 4. Глезер, Г. А., Н. П. Москаленко, Р. И. Мегралишвили, Н. Л. Зильберг. *Тер. арх.*, 1974, № 9, 48—57. — 5. Неусыпина, Е. Е., В. А. Смирнов. *Тр. Ленингр. санит. гиг. мед. инст.*, 145, 1982, 60—62. — 6. Путинцева, Е. Г. *Патол. физиол. эксперим. тер.*, 1979, № 6, 66—68. — 7. Bradshaw, D., P. H. Franz, L. A. Sugden. *Brit. J. Pharmacol.*, 78, 1983, Suppl. 183. — 8. Byron, J. W. *Experim. Haematol.*, 3, 1975, No 1, 44—53. — 9. Guthberg, A. W., P. Pic. *Brit. J. Pharmacol.*, 49, 1973, 134—137. — 10. Harrison, M. H. *J. Appl. Physiol.*, 35, 1973, 794—797. — 11. Hims-Hagen, J. In: Catecholamines. Berlin, etc., 1972, 363. — 12. Johns, E. J., H. K. Richards, B. Singer. *Brit. J. Pharmacol.*, 53, 1975, 67—73. — 13. Langer, S. Z. In: The Release of Catecholamines from Adrenergic Neuron. Oxford, etc., Pergamon Press, 1979, 59—82. — 14. Lemmer, B. G., D. Wiethold, J. Hellenbrecht, I. Bak, H. Grobecker. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, 275, 1972, 299—313. — 15. Lipski, S. *Internat. J. Radiat. Biol.*, 29, 1976, 359—363. — 16. Penington, D. S. *Brit. Med. J.*, 4, 1969, 782—784. — 17. Rothwel, N. J., M. J. Stocu, P. D. Winter. *J. Physiol.*, 340, 1983, 62 P. — 18. Tawara, K., E. Steiner, G. Bahr. *Brit. J. Clin. Pharmacol.*, 14, 1982, 589—592. — 19. Weksler, B., M. Gillick, J. Pink. *Blood*, 49, 1977, 185—195.

ВЛИЯНИЕ НЕСЕЛЕКТИВНОЙ БЕТА-АДРЕНОСТИМУЛЯЦИИ И АДРЕНОБЛОКАДЫ НА ИНКОРПОРАЦИЮ ^{75}СЕЛЕНОМЕТИОНИНА В ТРОМБОЦИТЫ

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РЕЗЮМЕ

Исследовано влияние неселективной бета-адреностимуляции изопреналином (2×3 мг/кг в день) и бета-адреноблокады пропранололом (2х5 мг/кг в день) на включение ^{75}селенометионин (^{75}Se-M) в новообразовавшиеся тромбоциты в течение трех дней. Устанавливается, что инкорпорация у животных, которым вводился пропранолол, понижается значимо на 55,70 % ($p<0,001$), а изопреналин приводит к увеличению включения на 58,87 % ($p<0,001$). Предварительное введение пропранолола полностью снимает эффект изопреналина. Полученные результаты дают основание считать, что включение ^{75}Se-M в новообразовавшиеся тромбоциты является по всей вероятности бета-адренозависимым процессом. Делается заключение, что бета-адренорецепторы участвуют в регуляции тромбоцитопоэза.