EFFECT OF INSULIN ON Na,K-ATP-ASE ACTIVITY IN RAT BRAIN MICROSONES

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KEY WORDS: Na,K-ATPase; brain; insulin; microsomal fraction.

Hormones activating the genetic apparatus of the cell affect excitability, permeability, and energy-yielding processes [5].

The role of Na,K-ATPase in cell metabolism and excitability is responsible for interest in the study of the action of hormones on this enzyme. However, the results of a study of this action are few in number and contradictory in nature. Some workers state that for insulin to exert its influence on Na,K-ATPase activity, integrity of the cell is an essential factor [13, 14]. Other workers have observed changes in activity of the Na-pump in plasma membrane in the presence of insulin [7, 15].

The small number of investigations so far undertaken on brain plasma membranes justified a study of the effect of different doses and durations of incubation of insulin on Na,K-ATPase activity in vitro, and the investigation described below was undertaken for this purpose.

EXPERIMENTAL METHOD

Experiments were carried out on the brain microsomal fraction obtained from Wistar rats by differential centrifugation. Na,K-ATPase activity, inhibited by strophanthin K ($10^{-3}$), was determined by the method in [10], and protein was estimated by Lowry's method [6]. Bovine insulin (Darnitsa Pharmaceutical Chemical Combine) was added to the incubation medium in doses of 0.18 and 0.36 U/ml. Simultaneous incubation with the hormone and strophanthin lasted 10 and 30 min, preincubation of the enzyme with insulin 5 min, and combined incubation with the hormone and strophanthin another 5 min.

The experimental results were subjected to statistical analysis by the Wilcoxon-Mann-Whitney method [3].

EXPERIMENTAL RESULTS

The experiments of series I showed (Table 1) that addition of insulin to the incubation medium in a dose of 0.18 U/ml did not lead to any significant change in Na,K-ATPase activity, whereas in a dose of 0.36 U/ml insulin reduced it by 59%. Prolonging incubation to 30 min had no significant effect on activity the Na-pump compared with that after incubation for 10 min.

Preincubation of the rat brain microsomal fraction for 5 min with insulin (0.36 U/ml) significantly increased ($P < 0.01$) Na,K-ATPase activity from 21.81 µg P_i/mg protein/h ($n = 13$) to 29.75 µg P_i/mg protein/h ($n = 10$).

The experimental results thus showed that Na,K-ATPase activity depends both on the experimental conditions and on the dose of insulin added to the incubation medium. Most investigations into the effect of insulin on the Na-pump have been conducted on muscle fibers, liver cells, kidneys, and isolated dog's heart [8, 11, 13]. They have shown an increase in ouabain binding by 41-113% [8]. It has been suggested that this effect is the result of the direct action of the hormones on the Na-pump [9].

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Table 1. Na,K-ATPase Activity (µg P1/mg protein/h) of Rat Brain Microsomal Fraction depending on Dose of Insulin and Incubation Time

| Statistical Index | Incubation for 10 min | Incubation for 30 min |
|-------------------|-----------------------|-----------------------|
|                   | dose of insulin, U/ml |                       |
|                   | 0.18                  | 0.36                  | 0.36                  |
| M                 | 23.66                 | 26.16                 | 14.15                 |
| n                 | 14                    | 8                     | 6                     |
| P                 | >0.05                 | <0.005                | 0.005                 |

The response to addition of the hormone was consistently activating regardless of dose, which varied in different experiments from 0.01 to 100 U/mol [9, 13]. The duration of incubation (2 min to 4 h) likewise had no significant influence on the trend of the observed effect [8, 13], for binding of the hormone with the cells is considered to take place after 2 min, and this level of binding thereafter persists for 90 min [12]. Only in one or two publications was the effect observed to depend on dose and on duration of incubation of the hormone [11].

In some of the investigations mentioned above it is suggested that for the effect of insulin on Na,K-ATPase activity to be revealed, integrity of the cell is essential, for it is impossible to obtain any effect in homogenized tissue or isolated preparations of Na,K-ATPase [13, 14]. Some workers, however, observed changes in activity of the Na-pump in the presence of insulin in plasma membranes from bovine, pig, and rat liver [11, 15].

Our own results also showed that insulin can affect enzyme activity in plasma membrane of rat brain in vitro.

When undertaking experiments of this kind we set out from the following propositions. First, Na,K-ATPase is distributed exclusively in plasma membranes and is one of a group of membrane-bound enzymes [2, 4]. Glycoside acceptors and K⁺-binding groups of the enzyme lie on the outer surface of the plasma membrane, the substrate site on its inner surface. Second, the existence of "reserves" of enzyme molecules in the membrane has been demonstrated [1]. Third, there is evidence that insulin activates pre-existing enzymes and does not include Na,K-ATPase synthesis de novo [13]. The presence of galactose, an essential component of the insulin binding site [2, 16], in the β-subunit of the enzyme also provides a basis for interaction of the hormone with it.

Our experiments showed that the effect depends on dose of the hormone and sequence of incubation. Small doses (0.18 U/ml) were insufficient to reveal significant changes in Na,K-ATPase activity. Doubling the dose of the hormone led to a decrease in enzyme activity in the case of simultaneous incubation with strophanthin K and insulin, and to an increase activity in the case of preincubation of the enzyme with insulin.

Dependence of the results on the conditions of incubation have been demonstrated elsewhere [9, 14]. Oubain (strophanthin), for instance, can prevent the insulin effect or, conversely, insulin inhibits the effect of ouabain.

Strophanthin K is known to inhibit the ATPase reaction at the dephosphorylation stage, preventing removal of phosphate from the enzyme protein and thereby leading to accumulation of an inactive form of the enzyme [2, 4]. Inhibition of enzyme activity by strophanthin is accompanied by changes in the conformational properties of this enzyme, with the result that its affinity for insulin is reduced [16]. This may probably explain the decrease in Na,K-ATPase activity in the case of simultaneous incubation of the enzyme with strophanthin K and insulin.

Preincubation of the enzyme with insulin stimulates phosphorylation [17]. Activation of the phosphokinase stage of the reaction promotes the formation of a strophanthin-sensitive phospho-form [4] and an increase in Na,K-ATPase activity under the influence of the hormone. The change in enzyme activity probably takes place through conversion of existing molecules into the activated state, for in experiments on microsomes, synthesis can be virtually disregarded.
The investigations thus showed that insulin can influence Na,K-ATPase activity of the microsomal fraction of rat brain in vitro. The results depend both on the dose of hormone added to the incubation medium and on the experimental conditions.

**LITERATURE CITED**

1. S. N. Airapetyan, S. S. Dadalyan, et al., Dokl. Akad. Nauk SSSR, 258, No. 4, 1003 (1981).
2. A. A. Boldyrev, Usp. Fiziol. Nauk, 12, 91 (1981).
3. E. B. Gubler and A. A. Genkin, The Use of Nonparametric Statistical Criteria in Medical and Biological Research [in Russian], Leningrad (1973).
4. V. K. Lishko, The Sodium Pump of Biological Membranes [in Russian], Kiev (1977).
5. V. V. Frolikis, O. A. Martynenko, et al., Dokl. Akad. Nauk SSSR, 232, No. 5, 1201 (1977).
6. A. A. Shatkin, in: Methods in Virology and Molecular Biology [in Russian], Moscow (1972), pp. 187-189.
7. P. Baldini and S. Inserpi, Boll. Soc. ital. Biol. Sper., 55, 1060 (1979).
8. T. Clausen and O. Hansen, J. Physiol. (London), 270, 415 (1977).
9. M. Gracium and S. T. Agrigercael, Rev. Roum. Biol. Anim., 23, 143 (1978).
10. C. H. Fiske and J. Subbarov, J. Biol. Chem., 66, 375 (1926).
11. J. R. Flatman and T. Clausen, Nature, 281, 580 (1979).
12. J. D. Goldfine, G. J. Smith, et al., Proc. Natl. Acad. Sci. USA, 74, 1368 (1977).
13. T. J. Hougen, B. E. Hopkins, and T. W. Smith, Am. J. Physiol., 234, 59 (1978).
14. I. F. Manery, E. E. Dryden, J. S. Still, et al., Can. J. Physiol., 55, 21 (1977).
15. P. Rosen, M. Simon, H. Reinauer, et al., Biochem. J., 186, 945 (1980).
16. P. Zengbush, Molecular and Cellular Biology [Russian translation], Moscow (1982).
17. O. Walaas, E. Zystad, and R. S. Horn, Acta Endocrinol. (Copenhagen), 94, 87 (1980).

PHOSPHOLIPIDS, CEREBROSIDE, AND CEREBROSIDE SULFATE LEVELS IN THE CNS OF MICE WITH ACUTE EXPERIMENTAL VIRAL DEMYELINATION

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side sulfates.

One of the most urgent problems in modern neurology is that of the demyelinating diseases. The etiology and pathogenesis of this group of diseases, which includes multiple sclerosis, multifocal leukoencephalopathy, etc., have not yet been completely explained.

However, opportunities for the intravital study of pathological changes in the nerve tissue of human patients are limited. Because of this, it is particularly valuable to study experimental models of the demyelinating process in animals. Accumulation of data on the possible role of viral infection in the genesis of demyelination [1, 2, 10] had led to the development of adequate experimental viral models.

One such model is encephalomyelitis caused by the neurotropic ghm strain of murine hepatitis coronaviruses (EMH). These viruses, it has been suggested, cause destruction of oligodendrocytes and the appearance of demyelinated regions in the white matter of the brain and spinal cord [2, 10]. Considering that lipids are the main structural component of myelin sheaths, which are the main structure destroyed during demyelination, their study in EMH would seem to be particularly important. There are no data in the literature on biochemical changes taking place in nerve tissue in this disease.

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