It must also be pointed out that oxidation of adrenalin into adrenochrome in membranes containing LPP may be the source of errors in the analytical procedure of determination of SOD. The velocity of the adrenalin–adrenochrome reaction in the presence of membrane fragments will in fact be determined not only by the ability of SOD to catalyze the reaction of dismutation of superoxide anion-radicals:

$$O_2^{\cdot} + O_2^{\cdot} \xrightarrow[SOD]{2H^+} H_2O_2 + {}^3O_2,$$

but also by conversion of adrenalin into adrenochrome on account of LPP contained in the membrane, independent of the superoxide anion-radical.

LITERATURE CITED

1. A. I. Archakov, V. M. Devichenskii, I. I. Karuzina, et al., Biokhimiya, 33, 497 (1968).
2. G. E. Adams and B. D. Michael, "Radicals," Trans. Faraday Soc., 63, 1171 (1967).
3. W. Bors, M. Saran, C. Michel, et al., Int. Radiat. Biol., 2B, 353 (1975).
4. J. Folch, M. Lee, and G. H. S. Stanley, J. Biol. Chem., 226, 497 (1957).
5. S. Green, A. Mazur, and E. Shorr, J. Biol. Chem., 220, 237 (1956).
6. F. J. Hawko, C. R. O'Brien, and P. J. O'Brien, Biochem. Biophys. Res. Commun., 76, 354 (1977).
7. H. J. Kohn and M. Liversedge, J. Parmacol. Exp. Ther., 82, 292 (1944).
8. A. Hoffer, H. Osmond, and J. Smythies, J. Ment. Sci., 100, 29 (1954).
9. N. I. Krinsky, Trends Biochem. Sci., 4, 35 (1977).
10. H. P. Misra and I. Fridovich, J. Biol. Chem., 247, 3170 (1972).
11. P. Neta and R. W. Fessenden, J. Phys. Chem., 78, 523 (1974).
12. C. Richter, A. Assi, V. Wesser, et al., J. Biol. Chem., 252, 5061 (1974).
13. J. Strosznaider and Z. Dobowiecki, Bull. Acad. Pol. Sci., 32, 647 (1975).
14. E. Walaas, O. Walaas, and S. Haavaldsen, Arch. Biochem., 100, 97 (1963).

ROLE OF ATP IN SPECIFIC BINDING OF ^{125}I-INSULIN WITH CYTOPLASMIC RECEPTORS OF LIVER AND MUSCLE MEMBRANES OF CONTROL ANIMALS AND OF RATS WITH DIABETES

S. A. Morenkova and A. A. Karelin

UDC 616.379-008.61-092.9-07:616-008.943.79-073.916

KEY WORDS: diabetes; insulin and its receptors; liver; muscles.

The mechanism of the decrease in sensitivity of the cells of various organs and tissues to insulin in diabetes is not yet clear. There is ample evidence to indicate a reduction in the binding of insulin with its receptors in some tissues in states characterized by resistance to endogenous or exogenous insulin [2, 5, 7, 8, 10]. However, insular insufficiency may be manifested in the presence of normal or even increased binding of the hormone with its specific receptors, and it is probably caused by disturbances of glucose metabolism at stages following activation of insulin receptors. The possibility cannot be ruled out that the processes responsible for tissue sensitivity to insulin are regulated by ATP, the effector component of the plasma membrane, in both normal and resistant states or against the background of deficient production of the hormone. It has been suggested that the ability of receptors to bind insulin is regulated by phosphorylation of membrane proteins, possibly even of the receptors themselves, and that ATP has a modulating influence on the binding of insulin, and in that way it affects its biological action [3, 13].

Laboratory of Biochemistry, A. V. Vishnevskii Institute of Surgery, Academy of Medical Sciences of the USSR, Moscow.

(Presented by Academician of the Academy of Medical Sciences of the USSR S. S. Debov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 90, No. 11, pp. 557-559, November, 1980. Original article submitted December 29, 1979.

0007-4888/80/9011-1528$07.50

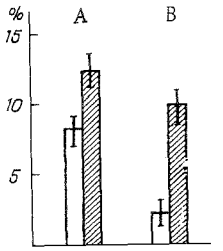

Fig. 1. Specific binding of ^{125}I-insulin with cytoplasmic membranes of liver (A) and muscles (B) of control animals (unshaded columns) and rats with diabetes (shaded columns). Ordinate, % of specific binding of ^{125}I-insulin.

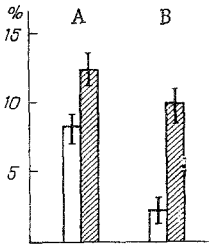

Fig. 2. Effect of ATP on specific binding of ^{125}I-insulin with cytoplasmic membranes of liver (A) and muscles (B) of control animals (1) and of rats with diabetes (2). Abscissa, ATP concentrations (in moles); ordinate, % of specific binding of ^{125}I-insulin. Unlabeled insulin — 500 ng/ml, labeled insulin — 100 pg/ml, membrane protein concentration 400 µg/ml.

In accordance with the facts described above it was decided to study the effect of ATP on the ability of insulin to bind with cytoplasmic membranes of liver and skeletal muscle cells of control animals and of rats with streptozotocin diabetes.

EXPERIMENTAL METHOD

Experiments were carried out on male rats weighing 140-160 g. Streptozotocin was injected intraperitoneally into the animals in a dose of 65 mg/kg body weight. Immediately before injection, the preparation was dissolved in citrate buffer, pH 4.6. The blood sugar of these animals reached 340 ± 60 mg% after 2 days.

Cytoplasmic membranes of control rats and of animals with diabetes were isolated from the liver and muscles by the method in [11] and kept at −40°C. Some of the membranes were preincubated with ATP for 10 min at 20°C. Solutions of ATP were added to the membranes in a volume of 50 µl in a final concentration of 10^{-3}, 10^{-6}, or 10^{-12} M. Control samples were treated with 50 µl of buffer and were kept under identical conditions.

Binding of ^{125}I-insulin with its receptors in the tissues was studied by the method in [12]. The reaction mixture contained labeled insulin in a dose of 100-500 pg/ml (specific activity 180 µCi/µg, about 7000 cpm), a suspension of plasma membranes — 400 µg protein/ml, and nonradioactive insulin — 500 ng/ml. Each reagent was added in a volume of 50 µl in Krebs–Ringer buffer, pH 7.4. The labeled insulin also contained 3% serum albumin. The samples were incubated for 60 min at 30°C. At the end of incubation the samples were centrifuged for 15 min at 1500g and radioactivity was determined in the residue. The re-

sults were expressed as percentages of specific binding of labeled insulin, the value of which was calculated as the difference between the radioactivity of samples not containing insulin and samples containing insulin, relative to total radioactivity of the incubation medium.

EXPERIMENTAL RESULTS

As Fig. 1 shows, specific binding of labeled insulin with receptors on the cytoplasmic membranes of the liver and muscles was increased in animals with streptozotocin diabetes compared with the control. This effect was most marked in muscle membranes. Preincubation of membranes with ATP did not affect binding of insulin with the liver and muscle receptors of control animals (Fig. 2). However, binding of the hormone with the liver receptors of rats with diabetes was sharply inhibited by ATP in a concentration of 10^{-3} M and was maintained at this level by ATP in a concentration of 10^{-6} M. Lower concentrations of ATP (10^{-12} M) had an additional, although weaker, inhibitory action.

ATP likewise did not affect binding of insulin with muscle receptors of control animals, just as was observed in the liver. Preincubation of muscle membranes of rats with diabetes with ATP led to a reduction in the specific binding of insulin with receptors, only in the presence of low ATP concentrations (10^{-12} M).

Increased binding of insulin with plasma membranes from the liver of rats with streptozotocin diabetes also was observed by Davidson and Kaplan [4], who showed that in diabetes the receptor binding capacity is considerably increased, although the affinity of the receptors for insulin is not increased. An increase in the number of insulin receptors in adipocytes, but no increase in their affinity for the hormone, also was observed in rats with streptozotocin diabetes by Masato et al. [9]. They observed no change in the degree of association between receptor binding and the glucose transport system, on account of which the sensitivity of the adipocytes to insulin was reduced.

In rats with alloxan diabetes we also observed a twofold increase in insulin binding with receptors of hepatic cytoplasmic membranes [1]. Streptozotocin diabetes led to an increase in binding of insulin in membranes of the liver and, in particular, of the muscles. Preincubation of liver and muscle membranes from control animals with ATP for 10 min did not disturb receptor binding with the hormone subsequently. However, interaction between receptors and insulin was sharply inhibited in both tissues in the membranes of animals with diabetes that bound insulin more intensively after preincubation with ATP. Chang and Cuatrecasas [3] found no effect of ATP on specific binding of insulin with fat cells of intact animals, although under these circumstances ATP inhibited insulin-stimulated glucose transport. Inhibition of insulin-stimulated transport of sugars in muscle tissue after preincubation under anaerobic conditions was observed by Yu and Gould [13] and Gould [6]. Loss of sensitivity to insulin was associated with a decrease in binding of ^{125}I-insulin. In this case the ATP reserves in the muscle were exhausted.

These results may be evidence in support of the view that ATP has a modulating influence on the biological action of insulin. The fact that in our observations ATP did not affect the binding of insulin with membranes of healthy animals, but inhibited this process in membranes of liver and muscle tissue of animals with diabetes, may probably be attributable to the presence of heterogeneous receptors in these tissues, and also to the fact that more intensive phosphorylation of some membrane proteins or of the group of receptors responsible for inhibiting binding of the hormone with some of those receptors, takes place in diabetes.

Another interesting fact is that inhibition of binding of insulin in muscle tissue took place in the presence of ATP concentrations significantly below physiological (10^{-12} M). In the liver, these same ATP concentrations gave an additional inhibitory effect, although maximal inhibition of insulin binding was observed with physiological ATP concentrations (10^{-3} M). This difference in ATP concentrations inhibiting binding of insulin in liver and muscle tissues may also be evidence that binding in these tissues takes place in several ATP-dependent stages.

LITERATURE CITED

1. S. A. Morenkova and A. A. Karelin, Vopr. Med. Khim., No. 5, 604 (1979).
2. J. A. Archer, P. Gorden, J. R. Gavin, et al., J. Clin. Endocrinol., 36, 627 (1973).
3. K. Chang and P. Cuatrecasas, J. Biol. Chem., 249, 3170 (1974).

4. M. B. Davidson and S. A. Kaplan, J. Clin. Invest., 59, 22 (1977).
5. J. D. Goldfine, C. R. Kahn, and D. M. Neville, Biochem. Biophys. Res. Commun., 53, 582 (1973).
6. M. K. Gould, Trends Biochem. Sci., 4, 10 (1979).
7. C. R. Kahn, D. M. Neville, S. P. Gorden, et al., Biochem. Biophys. Res. Commun., 77, 203 (1977).
8. C. R. Kahn, D. M. Neville, and J. Roth, J. Biol. Chem., 248, 244 (1973).
9. K. Masato, A. Yasuo, N. Yasuhiko, et al., Am. J. Physiol., 233, 175 (1978).
10. T. H. Maugh, Science, 193, 220 (1976).
11. D. M. Neville, Biochim. Biophys. Acta, 154, 540 (1968).
12. D. M. Neville, In Vitro, 9, 445 (1974).
13. K. T. Yu and M. K. Gould, Biochem. Biophys. Res. Commun., 77, 203 (1977).

HYBRIDIZATION PROPERTIES OF 4S RNA OF INFLUENZA VIRIONS

A. Z. Plyusnin and É. A. Ratovitskii

UDC 576.858.75.095.58

KEY WORDS: influenza virus; low-molecular-weight RNA; RNA—DNA and RNA—RNA hybridization.

Much experimental evidence has now been obtained to show that virions of a number of influenza viruses contain fractions of low-molecular-weight RNA (lmwRNA) which play an important role in virus reproduction processes [3]. In virions of influenza serotype A the writers discovered an lmwRNA with sedimentation constant of 4S and studied its priming properties in a cell-free reverse transcription system [2].

In the investigation described below, a method of molecular hybridization was used to study the nature of the lmwRNA fraction and the possibility of its interaction with the high-molecular-weight RNA (hmwRNA) of the virions.

EXPERIMENTAL METHOD

Accumulation and purification of influenza virus (strain A/Texas/1/77a), extraction of RNA from the virions, its electrophoresis in 8% polyacrylamide gel (PAG), and isolation of the 4S lmwRNA and hmwRNA fractions were carried out as described previously [2]. By electrophoresis of RNA in 8% PAG followed by extraction of individual fractions, 4S RNA and other forms of lmwRNA, including 4.5S, 5S, and so on, can be clearly separated [1]. The 4S RNA of the virions which was investigated was thus an electrophoretically pure fraction. DNA from chick embryonic skin and muscle tissue cells was isolated by the phenol-detergent method, using RNase A (Sigma, USA) and pronase (Ferak, Berlin). RNA was labeled with ^{125}I with the aid of chloramine T [4]. The specific activity of the preparations reached $2 \cdot 10^5$ cpm/μg RNA. Reassociation of DNA and hybridization of ^{125}I-4S RNA with a large excess of cellular DNA and hmwRNA of the virions was carried out as described in [10]. Competitive hybridization of ^{125}I-4S RNA with a large excess of cellular DNA in the presence of unlabeled 4S RNA from chick embryos was carried out in a solution of 0.4 M Na-phosphate buffer, pH 6.8, containing 40% formamide and 0.2% sodium dodecylsulfate [8] at C_0t = 8-10 moles·sec/liter.

EXPERIMENTAL RESULTS

It will be clear from Fig. 1 that virion 4S lmwRNA formed duplexes with DNA from chick embryonic cells within the range of concentrations of the latter corresponding to $C_0t_{1/2}$ = 8 moles·sec/liter (curve 1). Comparison of curves 1 and 3 shows that DNA sequences hy-

Leningrad Research Institute of Vaccines and Sera, Ministry of Health of the USSR.
(Presented by Academician of the Academy of Medical Sciences of the USSR A. A. Smorodintsev.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 90, No. 11, pp. 559-561, November, 1980. Original article submitted October 16, 1979.

