EFFECT OF INSULIN AND ITS DERIVATIVES ON INCORPORATION OF [1-^{14}C]GLYCINE INTO TISSUE PROTEINS

S. A. Morenkova

UDC 615.357.379.015.42:612.015.348

The effect of parenteral injections of insulin and its polypeptide A and B chains on the rate of protein metabolism in various organs of rats was investigated. Insulin was shown to accelerate the incorporation of [1-^{14}C]glycine into proteins of the liver, kidneys, pancreas, spleen, skeletal muscle, and thyroid, thymus, and adrenal glands but to have no action on this process in heart muscle and the diaphragm. The A and B chains of insulin also activate protein synthesis in several organs. However, despite some specificity of their effect, the spectrum of their action is narrower than that of insulin.

KEY WORDS: insulin; A- and B-polypeptide chains of insulin; protein biosynthesis.

A stimulating action of insulin on protein biosynthesis has been demonstrated in investigations on isolated muscle tissues [10, 13]. The effect observed was independent of the action of the hormone on glucose transport and utilization [14]. Data on insulin-activated protein biosynthesis in the liver obtained from experiments in vitro are highly contradictory. There are some indications that exogenous insulin does not increase the incorporation of labeled amino acids into liver proteins of diabetic animals, synthesis of which is considerably inhibited compared with that in intact animals [2]. However, there is certain evidence that insulin stimulates the incorporation of labeled amino acids into proteins of subcellular fractions of the liver of diabetic animals [9]. An action of insulin has also been found in adipose tissue [11], kidneys [2], bone marrow [15], and pituitary [7], thymus [3], and thyroid glands [16].

However, no investigations of the effect of insulin on protein synthesis in the intact organism have yet been undertaken from the kinetic aspect. Yet the need for the study of such processes under dynamic conditions is obvious, for the permeability of cell membranes is affected by many substances, including insulin, and as a result the metabolic reserves of intracellular amino acids are altered.

In the investigation described below the rate of protein synthesis was studied in intact animals in vivo under the influence of exogenous insulin. Another essential part of the investigation was to study the role of polypeptide A and B chains of insulin in this process, for the free chains are found in several organs and tissues [1, 12], and changes in their concentration have also been observed in the blood stream in diabetes [14]. Hence it follows that the A and B chains are functionally active and, in particular, they may affect protein metabolism.

EXPERIMENTAL METHOD

Insulin (25 units/mg) from Eli Lilly, USA, was fractionated into A- and B- polypeptide chains by oxidative sulfitolysis [5] followed by gel filtration on a column (1×50) with Sephadex G-75 [17]. The purity of the chains was tested by Edman's method [6]. The first cycle of treatment led to the formation only of phenylthiohydantoin derivatives of glycine and phenylalanine, belonging to the A and B chains, respectively.

Experiments were carried out on male albino rats weighing 120-150 g. The animals of each of the three groups (12 rats in each case) received an intraperitoneal injection of insulin (1 mg/100 g body weight) or of its A or B chain (2 mg/100 g body weight). Animals of the control group received physiological saline. Immediately after injection of each substance, [1-^{14}C]glycine (specific activity 170 μCi/mg) also was injected intraperitoneally in a dose of 20 μCi/100 g body weight; the animals were decapitated 40, 80, and 120 min

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 M. I. Kuzin.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 85, No. 4, pp. 419-421, April, 1978. Original article submitted October 20, 1977.

0007-4888/78/8504-0454$07.50

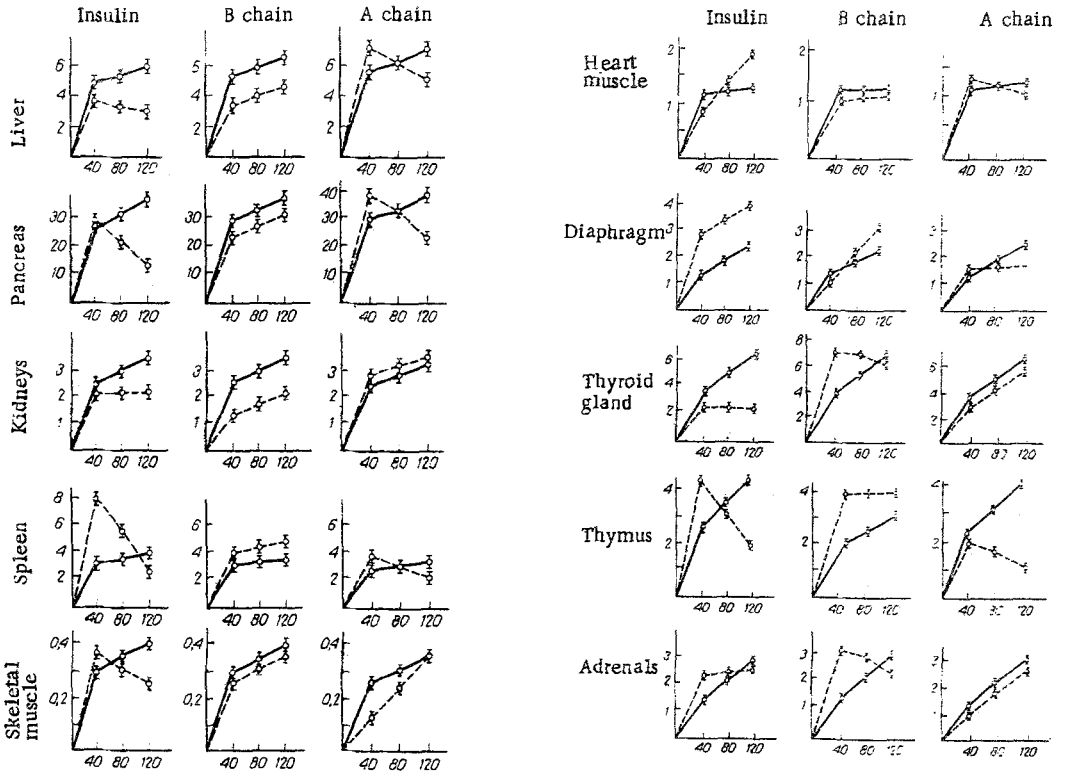

Fig. 1. Rate of incorporation of [1-^{14}C]glycine into proteins of organs after administration of insulin and of its A and B chain subunits. Abscissa, time (in min); ordinate, CPM/mg protein $\times 10^{-2}$. Continuous line, control; broken line, experiment.

later. Proteins were isolated from the organs as described in [1, 4] and their specific activity was determined.

EXPERIMENTAL RESULTS

As the results in Fig. 1 show, incorporation of [1-^{14}C]glycine into proteins of all the organs studied in the control animals increased progressively for 2 h. Under the influence of insulin the incorporation of labeled glycine into proteins of the various organs took place much faster. The maximum of incorporation of labeled glycine into proteins of the liver, kidneys, pancreas, spleen, skeletal muscle, and thyroid, thymus, and adrenal glands under the influence of insulin was observed 40 min after injection of the hormone. This means that parenteral injection of insulin leads to more rapid protein synthesis in the above-mentioned organs than in control animals. The radioactivities of proteins of heart muscle and diaphragm were still rising after 2 h, just as was observed in the control animals.

Under the influence of the B chain of insulin the rate of incorporation of the labeled amino acid into proteins of the thyroid, thymus, and adrenal glands was increased compared with its incorporation into proteins of the same organs of the control animals, for maximal incorporation of glycine under the influence of B chains was reached 40 min after the injection, whereas incorporation of [1-^{14}C]glycine into the proteins of these same glands in the control animals was still rising at that time. However, the rate of protein synthesis in other organs of animals receiving insulin B chain was indistinguishable from that in the controls.

Incorporation of [1-^{14}C]glycine into proteins of the liver, pancreas, and thymus also was accelerated by the A chain of insulin. Incorporation of the labeled amino acid into the proteins of these organs reached a maximum 40 min after injection, and a marked decrease in radioactivity was observed after 120 min. Meanwhile, radioactivity in the proteins of the same organs of the control animals continued to rise throughout the period of investigation. The rate of incorporation of labeled glycine into proteins of the kidneys, lungs, spleen, skeletal and heart muscles, thyroid gland, and adrenals after parenteral injection of the insulin A chain was unchanged compared with that in the control animals.

It can thus be concluded from these experimental results not only that the rate of protein synthesis in several organs in the intact animal is stimulated by parenteral injection of insulin, but also that the A and B chains of insulin have the ability to activate protein metabolism. Compared with insulin, however, the spectrum of their action is narrower. At the same time, the two polypeptide chains of insulin exhibit selectivity in their action on protein biosynthesis in individual organs, and only protein synthesis in the thymus is stimulated by insulin and by both of its chains.

LITERATURE CITED

1. A. S. Konikova, S. A. Morenkova, N. V. Petrova, et al., Metabolism, 17, 411 (1968).
2. M. G. Kritsman, A. S. Konikova, and D. G. Stepanyan, Biokhimiya, 16, 246 (1951).
3. V. G. Allfrey, R. Meudt, J. W. Hopkins, et al., Proc. Nat. Acad. Sci. USA, 47, 907 (1961).
4. K. Asplund, Hormone Res., 6, 12 (1975).
5. J. L. Baily and R. D. Cole, J. Biol. Chem., 234, 1733 (1959).
6. P. Edman, Acta Chem. Scand., 84, 283 (1964).
7. C. J. Goodner and J. T. Dowling, Diabetes, 12, 368 (1963).
8. P. Hohmann and R. D. Cole, Nature, 233, 1064 (1969).
9. A. Korner, J. Endocrinol., 20, 256 (1960).
10. M. E. Krahl, Science, 116, 524 (1952).
11. M. E. Krahl, Biochim. Biophys. Acta, 35, 556 (1959).
12. J. L. Kyner, J. Clin. Invest., 29, 659 (1969).
13. K. L. Manchester and F. G. Young, Biochem. J., 70, 353 (1958).
14. J. C. Meek, K. M. Doffing, and R. E. Bollinger, Diabetes, 17, 61 (1968).
15. T. F. Nechels, Am. J. Physiol., 203, 693 (1962).
16. V. N. Singh, P. M. Nataf, and I. L. Chaikoff, Life Sci., 4, 1603 (1965).
17. P. T. Varandani, Biochim. Biophys. Acta, 127, 246 (1966).