EXPERIMENTAL BIOLOGY

Interactions Between Erythrocyte and Tissue Insulin Receptors

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The formation of membranous insulin receptor complexes in hepatic, cardiac, and pancreatic tissue of frogs was studied after complete replacement of the blood with blood substitute containing insulin-free or insulin-containing solution with or without washed autogenous erythrocytes. The effect of the erythrocyte insulin receptors on the dissociation and formation of the complexes in the pancreas and other tissues is demonstrated.

Key Words: *insulin; insulin receptors; insulin circulation*

Insulin transport is believed to be mediated by tissue receptors [1,6]; however, the role of erythrocyte reception of the hormone is unclear. Its high activity, a potent insulin-degrading mechanism, and a low level of externalization of the inactivated hormone products [4,5] cannot be explained in terms of the modern concept of insulin transport, at least in the energy aspect. These facts are sufficient to regard the insulin receptors of erythrocytes as a specific type. Since free metabolic cycles are blocked in principle, the discovery of interactions between erythrocyte and tissue receptors becomes an urgent problem.

MATERIALS AND METHODS

Insulin receptors (IR) were detected on cell membrane by their ligand complexes in specially fixed tissue sections and blood smears [2]. The fixation was based on the effect of selective binding of heavy metal-containing anions with surviving cell complexes. Subsequent treatment of tissue samples in hypotonic solutions and alcohols did not lead to any appreciable desorption of ions fixed in IR, and they could be detected by any ion-sensitive stain before the hardening of the block and its sectioning [3]. The detected IR may be assayed by the dark-field reflector microspectrophotometry of preparations. Two rays spectrally corresponding to the appropriate absorption zones and to the transparency zones of the stain used have been employed. The percent ratio of the coefficients of reflection of the reference rays illuminating one and the same photometry field in turn is the metrological factor of analysis (*D*).

In order to provide specific measuring of the insulin complexes, the experiment was carried out under conditions of complete replacement of the blood of an experimental animal with a blood substitute containing one or no ligands. Experiments were performed on frogs (*Rana ridibunda*) surviving total insulin-free perfusion. Perfusion solutions were as follows: 1) Ringer solution — R; 2) Ringer solution with physiological excess of insulin — RI (bovine crystalline, 0.1 ng/ml); 3) insulin-free and insulin-containing solutions with washed autogenous erythrocytes (REc and RIEc), the erythrocyte titer being 10% of the whole blood titer.

The number of experimental animals and perfusion procedure were as follows: 1) 5 animals per composition $(R)_v$, $(RI)_v$, and $(RIEc)_v$ — perfusion through the portal vein; 2) 5 and 7 animals, re-

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TABLE 1. Formation of Insulin Ligand-Receptor Complexes in Tissues ($D\pm\Delta D$)

Composition of perfusates and method of perfusion	Liver	Heart	Pancreas
$(P)_v$	11.35±0.20	12.26±0.23	13.50±0.20
$(PI)_v$	9.00±0.30	9.47±0.23	13.92±0.22
$(PEc)_a$	10.60±0.32	—	—
$(PEc)_v$	9.58±0.28	10.39±0.17	15.53±0.26
$(PIEc)_a$	9.87±0.33	—	—
$(PIEc)_{a+v}$	8.55±0.27	9.65±0.16	14.12±0.25

spectively, with compositions $(REc)_a$ and $(RIEc)_a$ — perfusion through the aortic arch; and 3) 4 animals with composition $(RIEc)_{a+v}$ — simultaneous perfusion in the aortic arch and portal vein.

Specimens of the pancreas, liver, and heart for analysis of IR were collected 90 min after elimination of the circulation system with $(R)_v$ or elimination followed by 20-min perfusion with another composition. Heart work was monitored by ECG throughout the experiment. Sections 600 nm thick were prepared and examined under photometer at ×200.

RESULTS

The D values in the examined tissues are presented in Table 1. Each value is the mean of photometry of 5 sections in 5 fields of view. As Table 1 shows, after perfusion with (R)v the D values in the cells of all tissues were significantly different. After $(RI)_v$ perfusion, the content of dissociated receptors in the liver and heart decreases at the expense of insulin, because this perfusate contains no other ligand except the hormone. The following equation is within the experiment error:

$$L(RI)_v - L(R)_v = H(RI)_v - H(R)_v = -2.56\pm0.23 \quad (1),$$

where L and H are the group indices for the liver and heart. Here and further on the result (1) was calculated by the similarly ranged data mode.

In the pancreas, $(RI)_v$ perfusion led to degradation of IR, because the corresponding difference had a sign other than in (1).

After perfusion with insulin-free solution $(REc)_v$, IR were formed in tissues. Their content in the liver was as high as after $(RI)_v$ perfusion:

$$L(REc)_v + L(REc)_a - 2L(R)_v = -2.52\pm0.30 \quad (2).$$

The result (2) is statistically justified. The correlation coefficient between the experimental blocks (1) and (2) is equal to 0.99. The appearance of insulin in insulin-free perfusate REc is due to the effect of erythrocytes on the pancreatic gland (RG) IR:

$$RG(REc)_v - RG(R)_v = 2.03\pm0.25;$$

$$L(REc)_v - L(R)_v = H(REc)_v - H(R)_v = -1.82\pm0.25 \quad (3).$$

An amendment is necessary for system (3). It is obvious that perfusion $(..)_{a+v}$ is identical to perfusion $(..)_v$ for the heart and to perfusion $(..)_a$ for the pancreas gland. If RI is physiologically excessive for insulin, then $(RI)_v = (RI)_{a+v}$. Thus:

$$RG(RIEc)_{a+v} - RG(RI)_v = 0.20;$$
$$H(RIEc)_{a+v} - H(RI)_v = 0.18 \quad (4).$$

The differences (4) are amendments for the presence of erythrocyte in erythrocyte perfusates. Neglecting the amendments, we equalize the IR dissociation in the pancreas and the formation of IR in other tissues. Their balance indicates saturation of erythrocytes with the ligand. As for the threshold saturation with insulin, for example, of liver tissue, it is much higher. The mean D values evidence it:

$$L(RIEc)_{a+v} - L(RI)_v.$$

The conclusion that erythrocytes carry the insulin ligand from tissues to the pancreas has been validated by a special study of IR on erythrocytes (Table 2).

Despite the fact that erythrocyte and tissue IR are forming similarly, the receptors on erythrocytes are not identical to the tissue ones, because submerging of erythrocytes in insulin solutions virtually did not change the content of IR on them, in contrast to other tissues.

Hence, experimental findings permit the following conclusions:

1. The pancreas releases (with the help of circulating erythrocytes) active I^+ insulin in the blood

TABLE 2. Ligand-Receptor Complexes in Perfusion Solutions

Value	(PEc)	(PIEc)
Initial	4.21±0.12	4.65±0.16
In pancreatic veins	7.72±0.35	7.32±0.26
In hepatic vein	6.12±0.30	5.97±0.17
In the aorta	5.35±0.16	5.14±0.25

plasma, which forms IR^+ complexes on dissociated R receptors in the tissues utilizing the hormone.

2. Insulin initiates intracellular metabolism and degrades in IR^+ complexes, the I^- products of its degradation forming transport complexes I^-R° with dissociated R° erythrocyte receptors.

3. Erythrocytic insulin-receptor complexes are dissociated into R° and I^- in the pancreas; the degraded hormone is repaired with the formation of active I^+ insulin in addition to the produced hormone.

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Effect of Tetrapeptide A10, an Agonist of μ-Opioid Receptors, on DNA Synthesis in the Myocardium and Liver of Albino Rats in Early Postnatal Ontogeny

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The DNA synthesis in the myocardium and liver of 5-7-day-old albino rats after intraperitoneal injection of selective antagonist of μ-opioid receptors A10 (H-Tyr-D-Orn-Phe-Gly-OH) are studied using ^{3}H-thymidine autography. In the myocardium, both single (70 μg/kg) and 5-fold (100 μg/kg) administration of A10 significantly increases the intensity of labeling. The 5-fold administration of A10 induces an increase in absolute and relative mass of the heart. In the liver, the number of DNA-synthesizing hepatocytes rises in all experimental series. These data attest to a stimulating effect of μ-agonist on physiological regeneration of the myocardium and liver of albino rats in early postnatal ontogeny.

Key Words: *opioid peptides; DNA synthesis; myocardium; liver; ontogeny*

Opioid peptides contribute to the maintenance of structural homeostasis in the organism [14]. The effect of opioids on cell proliferation in the nervous tissue [13], surface epithelium [6-8], and lymphoid [12] and hemopoietic [1] tissues is well established. However, little is known about the effect of opioid peptides on cell reproduction in such vital organs as the heart and liver. Since cardiomyocytes and hepatocytes of mature mammals exhibit no proliferative activity under physiological conditions [5], the processes of cell proliferation in these populations may be evaluated *in vivo* only during early ontogeny.

The aim of the present study was to analyze the effect of A10, an agonist of opioid receptors (OR), on DNA synthesis in the myocardium and liver of albino rats during early ontogeny.

MATERIALS AND METHODS

Structural analog of dermorphin tetrapeptide A10 (H-Tyr-D-Orn-Phe-Gly-OH) was used. The affinity of A10 for μ-OR is higher than that of DAGO, a usual μ-OR agonist [9]. A10 synthesized at the La-

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