IMMUNOHISTOCHEMICAL STUDY OF INSULIN RECEPTORS OF THE LIVER

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Insulin is the principal regulator of insulin receptors (IR) in insulin-sensitive tissues [7]. It has been shown in experiments both in vivo and in vitro that when surrounding insulin concentrations are high the number of IR on membranes is reduced, but when the insulin concentration falls, their number conversely rises [8]. The leading peripheral mechanism of these changes is internalization of the receptors as a result of conjugation of the ligand with the α-subunit of the receptor molecule [6]. Meanwhile there is evidence in the literature of a central regulating action of insulin. This hormone can directly influence specific receptor structures in the brain, causing changes in the internal medium of the body [3]. Thus insulin not only plays the role of peripheral regulator of tissue metabolism in the CNS, but it may also have an effect similar to that of the regulatory peptides of the brain. The present immunohistochemical investigation was devoted to the study of the mechanisms of these effects on peripheral IR in the liver.

EXPERIMENTAL METHOD

The histochemical localization of hepatic IR was determined at the light- and electron-microscopic levels with the aid of an indirect immunoperoxidase method. Antisera (AS) of rabbits immunized with synthetic peptides, with an amino acid sequence corresponding to the 9-25 (AS-1) and 48-77 (AS-3) regions of the α-subunit of the insulin receptor of the human placenta, and to the 736-760 region (AS-31) of the β-subunit of the placental IR, were used in a dilution of 1:400 as the primary antibodies. The sera were obtained by N. Yanaihara and co-workers [15]. Nonimmune rabbit serum was used as the negative control. The secondary antibodies were Fab-fragments of donkey antirabbit immunoglobulins in a dilution of 1:200, conjugated with horseradish peroxidase (HRP, "Sigma VI," USA) by the periodate method [14]. Experiments were carried out on 24 Wistar rats aged 21 days, in 12 of which the central insulin-sensitive neurons of the arcuate nucleus of the hypothalamus had been destroyed by injections of sodium glutamate in the early postnatal period [4]. In the experiments of series I, insulin was injected intraperitoneally into 12 rats in a dose of 1 U in 200 μl (Humalin RU-100, "Sinogi Pharm. Co.," Japan) or they were given the same value of physiological saline. In series II, insulin in a dose of 1 U in a volume of 10 μl or 10 μl of physiological saline was injected stereotaxically into the right lateral ventricles of 12 rats.

In each series of experiments there were three control animals, receiving injections of insulin and physiological saline, and three experimental rats with destruction of the hypothalamus, receiving insulin or physiological saline, respectively. The tissues were fixed in 4% paraformaldehyde in 0.1 M phosphate buffer. Immunohistochemical investigations for light and electron microscopy were carried out on 6-μ frozen sections by the method in [9].

EXPERIMENTAL RESULTS

The light-optical immunohistochemical investigation showed that after treatment of the sections with all the test antisera from rabbits immunized with synthetic peptides with an amino acid sequence corresponding to that of placental IR, unlike those immunized with nonimmune sera, the distribution of insoluble products of the diaminobenzidine (DAB) reaction could be detected in the liver of all groups of experimental animals. The distribution

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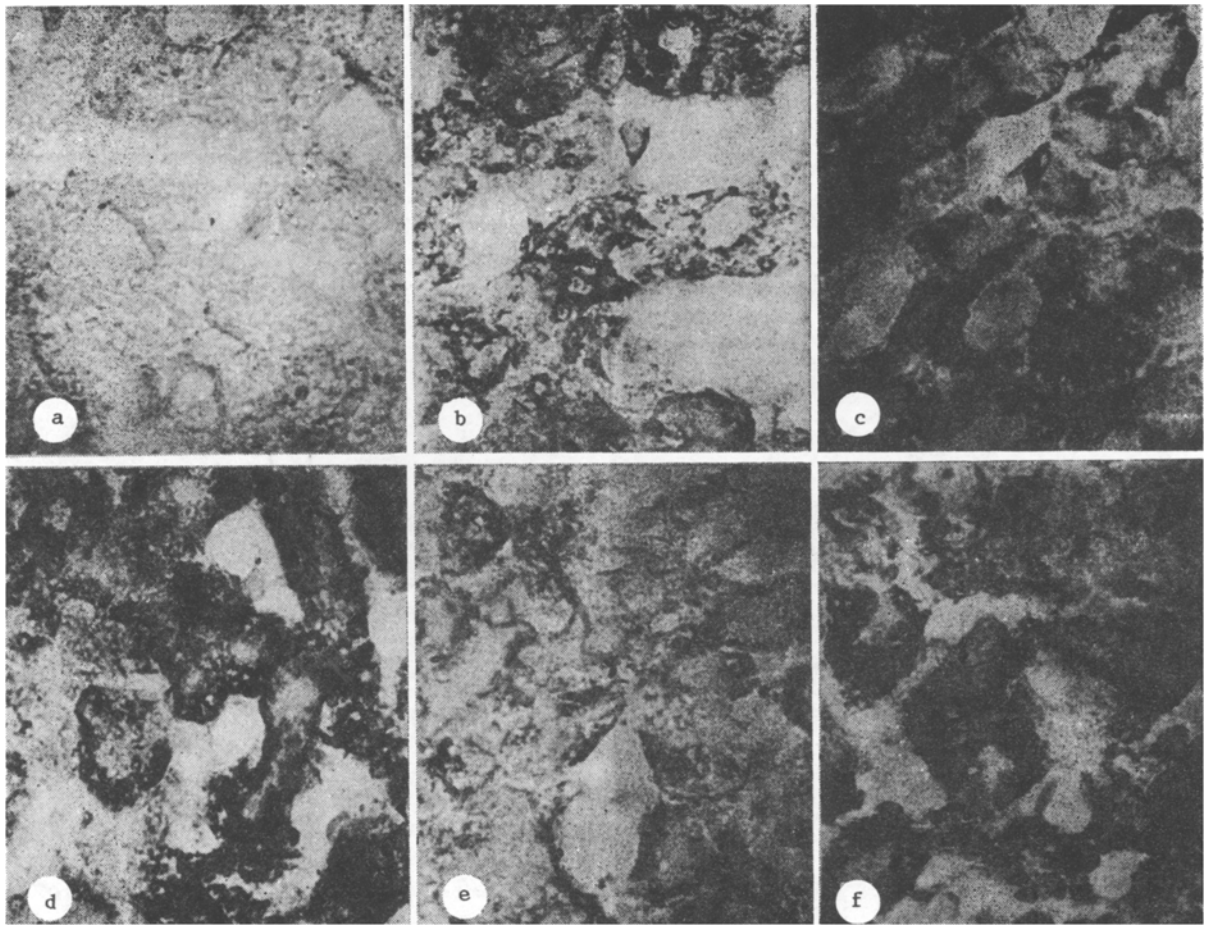

Fig. 1. Particular features of immunohistochemical reaction with nonimmune serum (a) and with antiserum AS-31 (b-f) in liver of control (a-d) and experimental animals (e, f). Magnification 1300; b) 30 min after intraperitoneal injection of 1 U insulin; d, e) 30 min after intraventricular injection of 1 U insulin.

of the immunoperoxidase reaction products were similar for antisera AS-1, AS-3, and AS-31, and it was found in hepatocytes and endothelial cells of small vessels and sinusoidal capillaries. However, definite differences in the distribution of the DAB reaction products were found in the hepatocytes of the experimental animals. For instance, in control and experimental rats receiving injections of sodium glutamate, after intraperitoneal injection of physiological saline a low density of distribution of DAB reaction products was found mainly along the cytoplasmic membrane, adjacent to the sinusoidal capillaries (Fig. 1c). In the control rats and in experimental animals with destruction of the hypothalamic centers, an increase in the intensity of the immunoperoxidase reaction was found 30 min after intraperitoneal injection of 1 U insulin, and at the light-optical level this increase was characterized by the appearance of large concentrations of granules in the cytoplasm of the hepatocytes (Fig. 1b).

On electron-microscopic study of the liver from animals of both experimental groups, after intraperitoneal injection of insulin, concentrations of electron-dense immunoperoxidase reaction products were seen in ultrathin unstained sections in the region of invaginations of the cytoplasmic membrane of the hepatocytes (Fig. 2a), representing the initial stage of a process of endocytosis, and on large secondary lysosomes in the cytoplasm of the hepatocytes (Fig. 2b). Considering the fact that a similar ultrastructural localization of IR was found by other workers [2] who used autoradiography with ^{125}I-labeled insulin and who studied internalization of IR in adipose tissue with the aid of ferritin-labeled antibodies [11], the increase in the intensity of the immunoperoxidase reaction which we described in liver sections and the particular features of the ultrastructural distribution of the DAB-reaction products in the hepatocytes most probably reflect a process of peripheral internalization of IR.

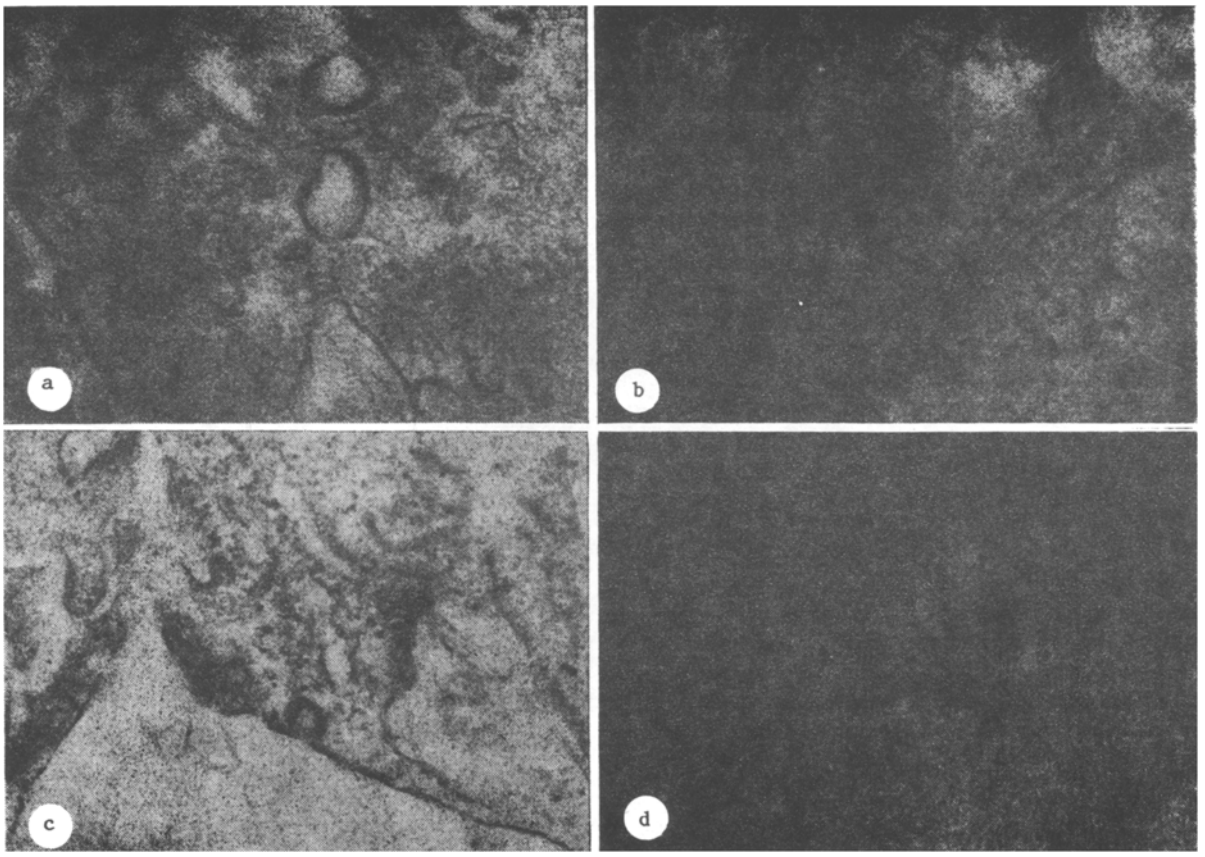

Fig. 2. Distribution of electron-dense products of immunohistochemical reaction in liver of animals after intraperitoneal (a, b) and intraventricular (c, d) injection of 1 U insulin into control (c) and experimental (a, b, d) animals. a, b, d) with antiserum AS-31 [magnification: a) 72,000, b, d) 57,000]; c) with antiserum AS-3 (magnification: 54,000).

Immunohistochemical investigations with antisera to different regions of the human placental IR molecule in the experiments of series II, with intraventricular injection of 1 U insulin, revealed similar patterns of distribution of the DAB-reaction products (Fig. 1d). At the light-optical level, injection of insulin into the cerebral ventricles of the control animals and experimental animals after destruction of their hypothalamic structures, caused an increase in the intensity of the immunoperoxidase reaction on the membranes and in the cytoplasm of the hepatocytes (Fig. 1f).

Electron-microscopic investigation of the liver of animals receiving an intraventricular injection of insulin revealed electron-dense DAB-reaction products in ultrathin sections on membranes of compound vesicles (Fig. 2c), in lysosomes, and in small secretory granules with a dense core. The latter as a rule were distributed around elements of the lamellar complex of the hepatocytes (Fig. 2d).

Thus in the two experimental series, after application of antisera to placental IR to rat liver sections after intraperitoneal and intraventricular injection of 1 U insulin, an increase in the intensity of the DAB reaction was observed at the light-optical level compared with control animals which received an injection of physiological saline under similar conditions instead of insulin. At the submicroscopic level, signs of internalization of IR were found in the liver cells from animals of both experimental series. The subcellular distribution of immunohistochemical reaction products in vesicles surrounding elements of the lamellar complex of hepatocytes was described in the present study for the first time. Since we know that the definitive formation of the IR molecules takes place in elements of the lamellar complex [2], this kind of ultrastructural localization of IR evidently reflects activation of intracellular synthetic mechanisms. The increase in the intensity of the DAB reaction after intraventricular injection of insulin, and also the presence of ultrastructural signs of internalization of the receptors, are most probably

linked with the central regulating action of insulin, causing an increase in the secretion of endogenous insulin by the pancreas [3].

The question of the localization of insulin-sensitive centers in the brain is more complex. In autoradiographic studies, after injection of labeled insulin into the CSF two regions of uptake were discovered: the first in the arcuate nucleus of the hypothalamus, the second in the area postrema of the brain stem [1]. In adult rats, neonatally receiving injections of sodium glutamate, insulin-specific binding in the arcuate nucleus of the hypothalamus was significantly reduced [13]. Considering that we could find no differences in the intensity of the immunohistochemical reactions in intact animals and in rats after destruction of the arcuate nucleus of the hypothalamus, it can be tentatively suggested that the principal receptor structures are distributed in the area postrema, which, because of reciprocal connections with vagal centers [10], is a component of the single parasympathetic center of the brain stem, involved in the regulation of glucose metabolism in the liver [12]. Quantitative autoradiographic analysis of the brain showed that the region of binding of ^{125}I-insulin in the hypothalamus is more widely represented [5], and the possibility accordingly cannot be ruled out that other structures may also be involved in central mechanisms of regulation of hepatic IR.

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