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In vivo Study of the Appearance and Fluctuations of Insulin Binding Sites in Different Tissues During Rat Development

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The appearance and fluctuations of specific insulin binding sites in several tissues *in vivo* during rat development, have been determined. After intravenous administration of ^{125}I-insulin to fetal, suckling and adult rats, changes on specific hormone uptake were observed depending on the tissues tested and on the age of animals. Thus, in liver, specific insulin uptake was much greater in 19 day-old fetuses and 10 day-old suckling animals than in adult rats. By contrast, brown fat and spleen insulin uptake was undetected in fetal animals but present in suckling rats, while lung insulin uptake was absent in the adults but present in fetal and suckling animals. Of interest were the specific insulin uptakes by three different muscle tissues. In fact, heart insulin uptake was much higher in younger animals than in adult rats, while in the diaphragm it was significantly smaller in all groups and in skeletal muscles hormone uptake was much smaller than in the other two muscle tissues and was even absent in the fetuses. In those tissues that had previously been shown to exhibit a specific insulin uptake, the iodinated hormone uptake decreased proportionally with simultaneous injection of increasing amounts of unlabelled insulin. These results indicate that insulin binding sites appear at different times and fluctuate in a different manner according to the tissues tested during rat development; this might be important in the stimulation of the functional activities of those tissues during perinatal age.

Key words: Insulin binding sites, Appearance, Development, Rat ontogenia.

Insulin, a hormone with powerful anabolic effects on many mammal tissues, seems to be equally active during intrauterine life, according to the reports describing the physiological effects of this hormone on several fetal tissues (1, 5, 7, 14, 16, 19, 20). However, during rat development the time of appearance of these hormonal effects changes in relation with the tissues tested (6, 7, 9, 10), suggesting that these tissues have a different chronological pattern regarding the appearance of insulin receptors.

In an attempt to verify this possibility we studied simultaneously the insulin binding sites present in several tissues

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during rat ontogenic development. For this purpose an *in vivo* binding technique, applied in the past successfully to different proteins or hormones (4, 11, 15, 21), has been used.

Materials and Methods

Animals. — Wistar rats were housed under constant conditions of lighting and temperature and fed *ad libitum* a standard diet. The experimental groups of rats included: 19 and 21 day-old fetuses, 10 day-old suckling and adult rats. Female rats, weighing 200-250 g, were caged with males until mating had occured. Vaginal smears were examined for spermatozoa early each morning. Pregnancy was dated from the first day on which spermatozoa were indentified and the accuracy of this method, estimated to have 6-12 h error, was validated by the fact that all rats delivered 22 days after the spermatozoa finding.

Specific insulin uptake by different tissues. — Porcine insulin (biological potency: 26.8 I.U/mg; Novo Research Institute, Copenhagen, Denmark) was labelled with carrier-free Na^{125}I, according to the method of FREYCHET *et al.* (13), with specific activities of 280-350 μCi/μg. After iodination mono ^{125}I-insulin was purified by chromatography on a DEAE-cellulose (DE 52) column (0.9 × 30 cm) with 50 mM Tris-HCl buffer (pH 9.3) and a linear gradient of NaCl (0-0.1 M). Pure mono ^{125}I-insulin retains its ability to bind specifically to liver plasma membranes and isolated adipocytes.

Suckling and adult rats, lightly anaesthetized with ether, received an injection into the jugular vein of 20 ng, 6.3 μCi/100 g body weight of mono ^{125}I-insulin, with or without unlabelled insulin dissolved in sodium chloride (0.9%) with bovine serum albumin (0.1%). Hormone administration in experiments with fetuses was done with a Hamilton microsyringe through the umbilical vein. At the times indicated, blood samples were obtained from the jugular vein and pieces of tissues were removed. In the case of fetuses, blood samples were obtained by decapitation. The radioactivity present in blood plasma and pieces of tissues was measured with a Berthold gamma counter with 70% efficiency. Tissue uptake was expressed as the tissue plasma/ratio (counts per min/g tissue/cpm/g plasma) or as cpm/g dry tissue weight, calculated as described by RETEGUI-SARDOU *et al.* (17). Insulin degradation was studied in tissue homogenates and blood plasma by precipitation with 10% (w/v; final concentration) trichloroacetic acid (TCA). The percentage of hormone degraded was calculated by comparing the percentage of mono ^{125}I-insulin counts soluble in TCA before and after its intravenous administration. The percentage of TCA-precipitable non-injected mono ^{125}I-insulin was 95.

Results

Between 3-15 min after injection of mono ^{125}I-insulin alone, to 21 day-old fetuses, plasma radioactivity decreased significantly, while the concomitant injection of native insulin markedly retarded clearance of the tracer. However, the administration of radioactive insulin alone produces a gradual increase in the liver uptake of radioactivity, reaching a maximal value at 5 min and decreasing slowly thereafter, while the simultaneous administration of native insulin significantly reduced the uptake of radioactivity at any time tested. By contrast, in the kidney the injection of native insulin did not produce a displacement of radioactivity. Similar results were obtained in animals from the others experimental groups (fig. 1).

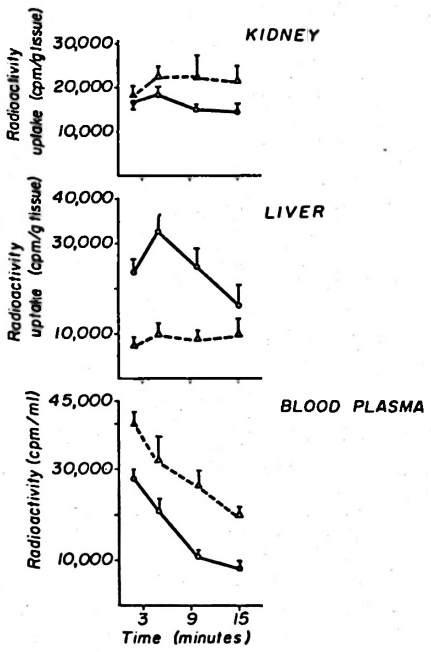

Fig. 1. *Tissue radioactivity uptake at different times after intravenous administration of mono ^{125}I-insulin alone (O—O) or mixed with an excess of unlabelled insulin (Δ- - -Δ) to 21 day-old fetuses. (Means ± SEM, n = 3).*

A single dose of 20 ng, 6.3 μCi/100 g b.w. of mono ^{125}-insulin was administered through the umbilical vein with or without unlabelled insulin (100 μg/100 g b.w.). Blood samples and pieces of tissues were taken at the indicated times. Radioactivity measurements were carried out with a Berthold gamma counter with 70% efficiency.

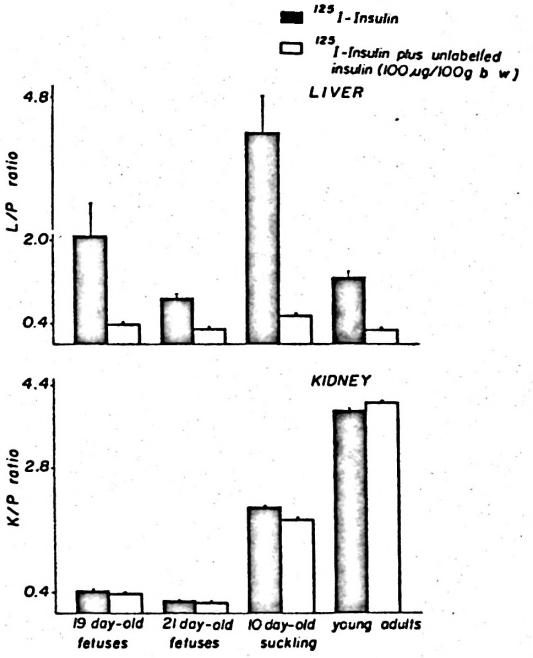

Fig. 2. *Uptake of mono ^{125}I-insulin by the liver and kidney of fetal, suckling and adult rats in the absence or presence of unlabelled insulin. (Means ± SEM, n = 6).*

Suckling and adult rats received an injection into the jugular vein of 20 ng, 6.3 μCi/100 g b.w. of mono ^{125}I-insulin, with or without unlabelled insulin (100 μg/100 g b.w.). Hormone administration in experiments with fetuses were performed through the umbilical vein. Blood samples and pieces of tissues were taken five minutes after hormone administration.

In the studies described below, tissue radioactivity was studied 5 minutes after intravenous injection of mono ^{125}I-insulin, since it was the time of maximal radioactivity. Similar findings were obtained when the results were expressed as cpm/ml plasma and cpm/g tissue, and as the tissues/plasma ratio (table I and figs. 1-4). In some tissues radioactivity uptake decreased significantly with simultaneous injection of unlabelled hormone (table I and figs. 2-6), while kidney radioactivity uptake was not reduced by the presence of the unlabelled hormone. Non-specific radioactivity uptake by the kidney was significantly smaller in fetal and suckling animals than in adult rats. However, in the liver, specific radioactivity was much greater in 19 day-old fetuses and 10 day-old suckling animals than in adult rats (fig. 2). Of interest was the specific radioactivity uptake by three different muscle tissues during rat ontogenic development (fig. 3). In fact, heart radioactivity uptake was much higher in fetal and suckling animals than in adult rats, while

Table I. *Organ distribution of radioactivity after the intravenous injection of mono ^{125}I-insulin alone or mixed with an excess of unlabelled insulin to fetuses, suckling and young adult rats. (Means ± SEM. n = 6).* RI: radioactivity insulin, UI: unlabelled insulin.

Biological specimens	Treatment	21 day-old fetuses	10 day-old suckling	Young adults
		Radioactivity uptake (cpm/g tissue or ml plasma)		
Liver	RI	34,349 ± 5,501	79,123 ± 8,690	27,136 ± 6,582
	RI plus UI	17,434 ± 2,566	16,744 ± 1,706	10,739 ± 1,607
Kidney	RI	10,167 ± 2,268	40,694 ± 5,638	78,716 ± 10,192
	RI plus UI	12,914 ± 2,691	57,347 ± 5,785	168,917 ± 17,710
Lung	RI	10,153 ± 1,816	12,413 ± 1,598	8,670 ± 1,131
	PI plus UI	8,967 ± 829	11,302 ± 796	16,698 ± 1,171
Blood plasma	RI	39,225 ± 4,857	19,831 ± 1,655	20,475 ± 3,139
	RI plus UI	63,393 ± 5,363	30,426 ± 1,722	40,741 ± 3,228

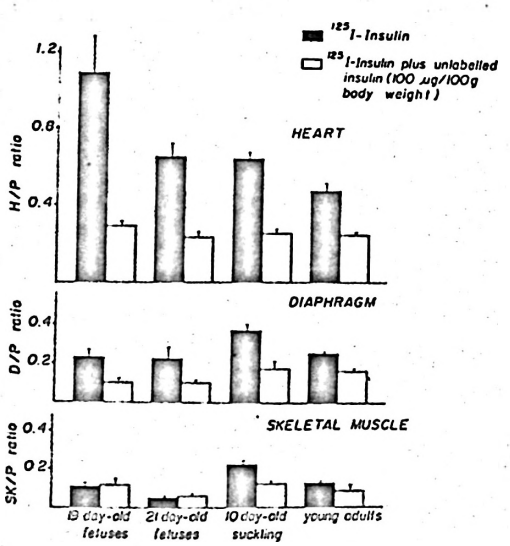

Fig. 3. *Uptake of mono ^{125}I-insulin by the heart, diaphragm and skeletal muscles of fetal, suckling and adult rats in the absence or presence of unlabelled insulin. (Means ± SEM, n = 6).*

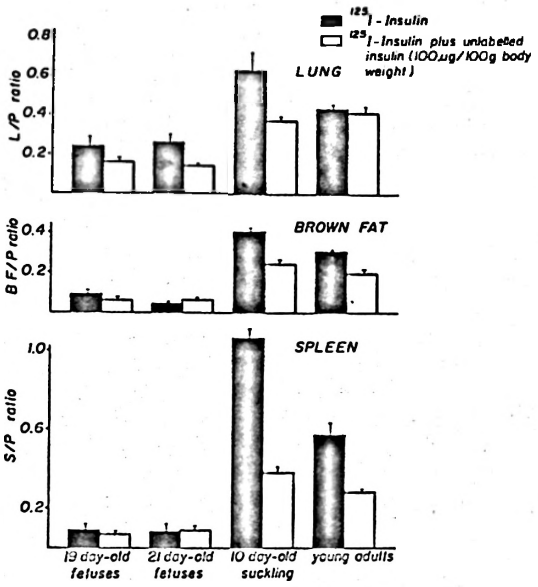

Fig. 4. *Uptake of mono ^{125}I-insulin by the lung, brown fat and spleen of fetal, suckling and adult rats in the absence or presence of unlabelled insulin. (Means ± SEM, n = 6).*

in the diaphragm it was significantly smaller in all the groups studied; in the skeletal muscles radioactivity uptake was much smaller than in the other two muscle tissues and was undetectable in the fetuses.

By contrast, lung radioactivity uptake was absent in adult animals but present in fetal and suckling rats (fig. 4), while brown fat and spleen specific radioactivity uptake was undetected in fetal rats

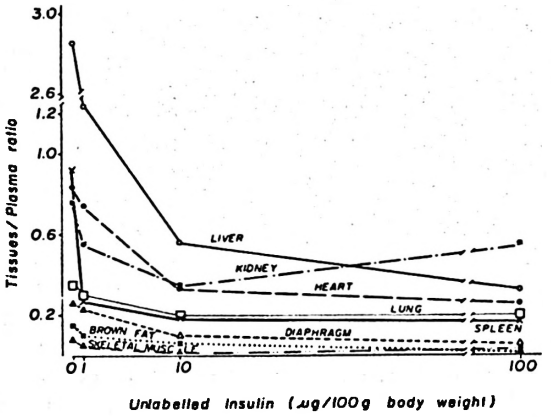

Fig. 5. *Inhibition by unlabelled insulin of mono ^{125}I-insulin uptake by several tissues of 21 day old fetuses (n = 3).*

A single dose of 20 ng, 6.3 µCi/100 g b.w. of mono ^{125}I-insulin was administered through the umbiblical vein, together with increasing doses of unlabelled insulin as indicated in the abscissa. Blood samples and pieces of tissues were taken five minutes after hormone administration.

but present in suckling and adults animals.

Insulin degradation as determined by its solubility in trichloroacetic acid, was smaller in the livers of 21 day-old fetuses (40%) as compared with the data obtained in adults rats (50%) 5 min after mono ^{125}I-insulin administration. Similar percentages of insulin inactivation by the tissues of both experimental groups were found.

Doses of 1 to 50 µg/100 g body weight of unlabelled insulin injected together with the corresponding mono ^{125}I-insulin produced a dose-response curve only in the tissues of 21 day-old fetuses and adult rats (figs. 5 and 6) that had previously been shown to exhibit a specific radioactivity uptake.

Since the water is greater in fetal than in adult tissues these results were also calculated to the tissue dry weight yielding radioactivity uptake values similar to those for fresh tissues.

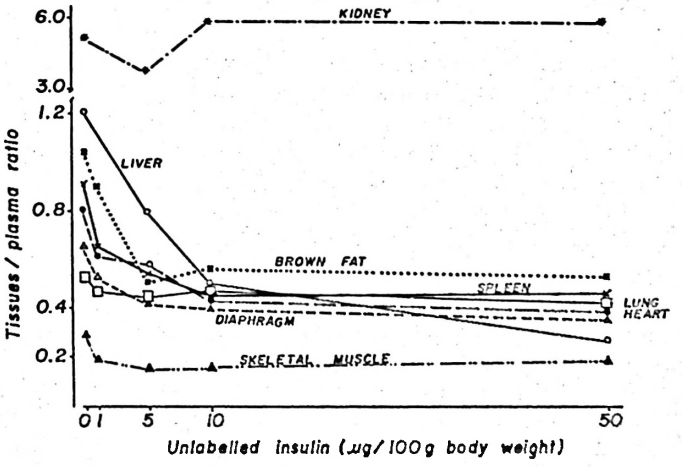

Fig. 6. *Inhibition by unlabelled insulin of ^{125}I-insulin uptake by several tissues of adult rats. (n = 3).*

A single dose of 20 ng, 6.3 µCi/100 g b.w. of mono ^{125}I-insulin was administered through the jugular vein, together with increasing doses of unlabelled insulin, as indicated in the abscissa. Blood samples and pieces of tissues were taken five minutes after hormone administration.

Discussion

Binding techniques for different proteins and hormones have succesfully been used *in vivo* (4, 15, 21), being very useful by making it possible to determine specific hormonal binding sites simultaneously in several tissues. Moreover an experimental design that detected *in vivo* the interactions of bGH with specific somatogenic binding sites in rat liver during ontogenic development had previously been used (11). In the present paper, in an attempt to simulate the physiological distribution of insulin during rat development, the radioactive hormone was administered intravenously to fetal, suckling and adult animals, the radioactivity distribution being studied over time in different tissues and blood plasma. The labelled hormone used was pure and fully active, since labelled impurities may interfere with the true profiles of distribution.

After injection of the tracer alone a considerable amount of radioactivity was trapped by several tissues, while after the administration of the tracer with the native hormone the radioactivity uptake by those tissues was dramatically reduces although the amount of radioactivity in circulating blood was significantly increased. These results suggest that the injected label was trapped in a saturable compartment, most likely in the receptor compartment, since the presence of native insulin is not expected to modify the distribution of radioactive insulin in the plasma and extracellular fluid compartments. The inhibition observed in the radioactivity uptake by the tissue was proportional to the dose of the unlabelled injected hormone, reinforcing thus the idea that radioactivity is bound to specific sites rather than simply absorbed by the tissues. It is noteworthy that the hormone doses that inhibit tissue radioactivity uptake are lower than those used in insulin bioassays.

Similar conclusions were obtained when the results were expressed as radioactivity per gram of tissue or as the ratios of the concentration of radioactivity in the tissues and in plasma, indicating that the differences observed between the experimental groups of animals were due to specific changes in the radioactivity uptake by the tissues rather than to a different speed in the disappearance of radioactivity from blood circulation.

In contrast to the maximal specific radioactivity uptake values showed by liver radioactivity uptake by the kidney was similar in the absence or presence of the unlabelled hormone. Nevertheless, nonspecific insulin uptake by the kidney was significantly smaller in fetal and suckling animals, pointing to the possible immaturity of this organ in younger animals, as demonstrated by a decreased glomerular filtration and by a proximal tubule immaturity (8, 18).

Like other polipeptide hormones, insulin is actively degraded both *in vivo* and *in vitro*. This hormone has been found to be inactivated to a lesser extent by the livers of fetuses than those of adult rats, confirming the data obtained *in vitro* by different authors (3, 22). This difference in insulin inactivation could affect the specific binding of this hormone to its receptors; however in purified liver membranes insulin receptors were found to be the same in fetal and greater in suckling rats as compared with adult animals (3, 22). Furthermore VINICOR and KIEDROWSKI (22) have shown that at an equivalent degree of hormone degradation, the liver membranes of newborn rats still bind significantly more insulin than those of adult animals. In addition, it is well known that degraded insulin is unable to bind to its receptors (12).

As expected, a specific insulin uptake in many tissues of adult rats was found, in agreement with the well-known existence of insulin receptors and the biological effects of this hormone in multiple

target organs. In fetal and suckling rats a different pattern in the appearance and development of specific insulin uptake was observed, which might be related to the physiological needs of different organs during intrauterine and perinatal ages. Accordingly, an insulin uptake by fetal livers and hearts greater than by those of adult animals could favour the anabolic processes and early mechanical activity during intrauterine life, respectively. Also, the appearance of insulin uptake in brown fat after birth could facilitate the adaptation of the newborn to a colder enviroment, while the existence of insulin uptake by the lungs of younger animals, but not of adult rats, could suggest that insulin may play a role in the development of these organs (19).

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Resumen

Se determina *in vivo* la aparición y fluctuaciones de los sitios de unión específicos para insulina en distintos tejidos durante el desarrollo de la rata. Después de la administración intravenosa de insulina-^{125}I a fetos, lactantes y ratas adultas se observan cambios en la captación tisular específica de la hormona, los cuales fueron dependientes de los tejidos estudiados y de la edad de los animales. La captación en hígado es mucho mayor en fetos de 19 días y ratas lactantes que en animales adultos en la grasa parda y el bazo no se detecta en los fetos pero sí en los lactantes y ratas adultas, mientras que en el pulmón sólo es patente en los fetos y lactantes. La captación por el corazón es mucho mayor en las ratas más jóvenes que en las adultas, por el diafragma es menor en todos los grupos y en el músculo esquelético aún es menor e incluso no se detectó en los fetos. En los tejidos en los que previamente se han encontrado sitios de unión para insulina, la captación específica de la hormona radiactiva disminuye proporcionalmente con la inyección simultánea de cantidades crecientes de insulina nativa. Estos resultados indican que los sitios de unión para insulina aparecen en distintos momentos y fluctúan de manera diferente según los tejidos, durante el desarrollo de la rata, lo cual puede ser importante para la estimación funcional de estos tejidos durante el período perinatal.

Palabras clave: Receptores insulina, Aparición, Desarrollo, Ontogenia, Rata.

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