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Insulin-stimulated glucose uptake in rat diaphragm during postnatal development: Lack of correlation with the number of insulin receptors and of intracellular glucose transporters

(skeletal muscle/glucose transport/regulation/hormone)

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ABSTRACT **The insulin responsiveness of the membrane transport system for glucose (2-deoxy-D-glucose) in diaphragm was measured during postnatal development of the rat. At birth, the basal rate of 2-deoxy-D-glucose transport is 3 nmol/min·g and it gradually decreases to 1 nmol/min·g over a period of 40 days. On the other hand, the insulin-stimulated rate of transport is 6 nmol/min·g at birth, it increases to 9 nmol/min·g in 16- to 20-day-old rats, and it decreases again to ≈4 nmol/min·g in the 40-day-old rats. The stimulation of 2-deoxy-D-glucose transport by insulin is 2-fold at birth and increases to 4- to 5-fold 20 days after birth. The number of insulin receptors in the plasma membrane and the number of intracellular glucose transporters was also measured as a function of age to determine if there might be a correlation between these components of the insulin responsive system and the development of the increased insulin stimulation of 2-deoxy-D-glucose transport. The number of insulin receptors per g of wet weight decreased continuously with increasing age; the diaphragm of 40-day-old rats had about 50% of the receptors present in the diaphragm of the newborn rat. Similarly, the number of intracellular D-glucose transporters per g of wet weight decreased with increasing age; for adult rats, the number of transporters per g of diaphragm was 60% of that of newborn rats. The results indicate that the extent of insulin stimulation of glucose (2-deoxy-D-glucose) transport in the diaphragm during the first 20 days of life is not directly or simply related to the number of insulin receptors or the number of intracellular glucose transporters. The extent of the insulin response depends on some other factor that activates or is part of the machinery for translocation of the transporter.**

One of the primary effects of insulin on muscle and adipose tissue is to stimulate glucose uptake. Recent experimental evidence indicates that in rat adipocytes insulin causes an increase in the number of glucose transporters in the plasma membrane fraction with a concomitant decrease of transporters in Golgi-enriched fractions (1–5). A similar result was obtained with rat diaphragm (6). These results suggested that insulin stimulates glucose uptake by causing translocation of the transporters from an intracellular site to the plasma membrane. Thus, there are at least four components required for the stimulation of glucose transport by insulin: the insulin receptor, the pool of glucose transporters in intracellular membranes, the machinery that brings about translocation of the intracellular pool to the plasma membrane, and the signal transducer from receptor to translocase.

The question of interest here is which of these components is or are different in tissues that are insulin sensitive as compared to those in which insulin does not stimulate glucose transport.

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Results in the literature do not provide a clear answer to this question. On the one hand, the inability of insulin to stimulate glucose transport in guinea pig adipocytes has been related to a small intracellular pool of glucose transporters (7). On the other hand, changes in insulin responsiveness of glucose uptake during the induced differentiation of 3T3-L1 fibroblasts into adipocytes (8) and in the nonfusing muscle cell line BC3H-1 (9) are accompanied by an increase in the number of insulin receptors, although in the former case increased glucose uptake precedes the change in insulin receptors. Furthermore, insulin stimulates glucose uptake by 50% in chicken and rat embryonic heart at about the time that insulin can be detected in fetal tissue (10–12).

In an effort to obtain information on this question, I have studied the change in insulin responsiveness of rat diaphragm during the postnatal development of the animal. This tissue was chosen because muscle is known to have insulin receptors (13–15) and because the parameters of glucose transport have been well described (16–18). In this report, I show that while the basal rate of glucose uptake decreases, the insulin-stimulated rate of glucose uptake in diaphragm follows a complex pattern during postnatal development. To determine whether this growth-dependent effect correlates with insulin-binding capacity, a method was established to recover the insulin receptors from muscle tissue. No correlation was found between the pattern of insulin binding and insulin stimulation of glucose uptake at various ages. Furthermore, no appreciable changes were observed in the intracellular pool size of glucose transporters in diaphragm during development. These results suggest the insulin stimulation of glucose transport in diaphragm is not simply a function of the concentrations of insulin receptors and/or of the intracellular glucose transporters but is dependent on some other factor that activates or is part of the machinery for translocation.

EXPERIMENTAL PROCEDURES

Assay of 2-Deoxy-D-Glucose Uptake. Intact rat diaphragms were isolated according to the method of Kipnis and Cori (16) and incubated in Hepes-buffered saline (25 mM Hepes/120 mM NaCl/5 mM KCl/1.5 mM $CaCl_2$/1.0 mM $MgCl_2$/1.2 mM KH_2PO_4/10 mM D-glucose/1 mM sodium pyruvate/0.002% phenol red, pH 7.4) bubbled with O_2 (19) for 45–60 min at 37°C. The diaphragms were then transferred to glucose-free buffered saline with or without 330 nM (45 milliunits/ml) insulin for 10 min. At this time, 2-deoxy-D-[^{3}H]glucose (New England Nuclear; specific activity, 5 Ci/mmol; 1 Ci = 37 GBq) and 2-deoxy-D-glucose (Sigma) were added to final concentrations of 0.25 μCi/ml and 100 μM, respectively (17). After 20 min, the diaphragms were rinsed three times with ice-cold buffer over a period of 1–2 min, blotted with filter paper, weighted, and homogenized in 5% trichloroacetic acid. An aliquot of the supernatant was mixed with 10 vol of Aquasol (New England Nuclear) for liquid scintillation spec-

trophotometry. Under these experimental conditions, the uptake of 2-deoxy-D-glucose in diaphragm was linear for at least an hour (data not shown, see also ref. 17).

Isolation and Measurement of Insulin-Binding Capacity in Diaphragm. The diaphragms isolated from 6–24 rats were pooled, washed once with ice-cold homogenization buffer (0.25 M sucrose/2 mM EGTA/2 mM EDTA/1 mM sodium tetrathionate/0.1 M sodium phosphate buffer, pH 7.7), minced with scissors, and homogenized on ice in 5 vol of the same buffer with a Polytron (PTA 10S) (Brinkmann) at maximal speed for 2–3 sec. The homogenate was centrifuged at 5000 × *g* for 15 min. The pellet was extracted with 1% Triton X-100/0.1 M phosphate buffer, pH 7.7, containing 2 mM EGTA, 2 mM EDTA, and 1 mM sodium tetrathionate at room temperature for 45 min (5–10 ml of buffer per g of tissue). The suspension was centrifuged at 20,000 × *g* for 15 min, and the pellet was reextracted with the Triton X-100 buffer. The supernatants were pooled and centrifuged at 100,000 × *g* for 30 min. The clear supernatant was divided into aliquots and immediately frozen and stored at −70°C. The insulin receptors are stable for at least 3 months.

Insulin-binding capacity was measured by incubating Triton X-100-soluble material with ^{125}I-labeled insulin (^{125}I-insulin) (New England Nuclear) at room temperature for 2 hr in 120 mM phosphate buffer (pH 7.7) containing 0.15% Triton X-100, 0.1% bovine serum albumin, and 1 mM EGTA. Then the insulin and insulin–receptor complex were separated from insulin by polyethylene glycol precipitation with bovine gamma globulin as added carrier as described by Cuatrecasas (20). The ^{125}I-insulin bound in the presence of 3 μM nonradioactive insulin was regarded as nonspecific binding.

Assay of D-Glucose-Displaceable Cytochalasin B Binding. The supernatant solution of the 5000 × *g* centrifugation was centrifuged at 100,000 × *g* for 30 min. The pellet of the 100,000 × *g* centrifugation, presumably composed of microsomes, broken mitochondria, and some plasma membrane, was washed once and resuspended in homogenization buffer. The suspension was mixed with an equal volume of 100 mM NaH_2PO_4/100 mM NaCl/5 mM dithiothreitol/2.5 mM $MgCl_2$. [^{3}H]Cytochalasin B (New England Nuclear; specific activity, 10–15 Ci/mmol), cytochalasin E, and D-glucose or L-glucose were added to final concentrations of 5–50 nM, 10 μM, and 400 mM, respectively, in a total volume of 250 μl. After 20 min at room temperature, the suspension was centrifuged at 25,000 × *g* for 45 min. The clear supernatant was withdrawn and the pellet was solubilized with 150 μl of 1% $NaDodSO_4$ in 150 mM NH_4HCO_3 before subjecting to liquid scintillation spectrophotometry. The difference in ^{3}H radioactivity between L-glucose- and D-glucose-containing samples was taken as D-glucose-inhibitable cytochalasin B binding. Usually, it accounted for 10–20% of the total cytochalasin B bound.

Enzymatic Assays. The plasma membrane enzyme marker 5′ nucleotidase was assayed by the method of Avruch and Wallach (21).

The enzyme marker for the Golgi apparatus UDPgalactose:*N*-acetylglucosamine galactosyltransferase was assayed by the method of Kono *et al.* (4) with slight modifications. The homogenates (300–600 μg of protein) or microsomes (200–400 μg of protein) were incubated with 1 mM UDP[^{3}H]galactose (3 μCi/ml), 20 mM dithiothreitol/20 mM $MnSO_4$/1% Triton X-100/0.2% bovine serum albumin/50 mM 2-(*N*-morpholino)ethanesulfonic acid, pH 6.5, without or with 50 mM *N*-acetylglucosamine in a total volume of 100 μl at 37°C. After 3 hr, 40 μl of 0.2 M EDTA (pH 7.8) was added, and an aliquot (100 μl) was applied to a Dowex AG1-X8 column (0.8 ml of resin in a Pasteur pipet) and washed twice with 0.6 ml of 1 mM EDTA (pH 7.8). The eluate was collected for liquid scintillation spectrophotometry. The difference between the amount of radioactivity in the presence and in the absence of *N*-acetylglucosamine was taken as transferase activity.

RESULTS

2-Deoxy-D-Glucose Uptake in Diaphragm. Fig. 1*A* shows the average rate of basal and insulin-stimulated 2-deoxy-D-glucose uptake in intact diaphragm at different stages of postnatal development (from birth to 45 days). The uptake measured here represents actual entry of 2-deoxy-D-glucose into the intracellular space, with minimal contributions from trapping of the solute in the extracellular space. This conclusion is supported by the following observations. The maximal amount of 2-deoxy-D-glucose that can equilibrate with the extracellular space would make a contribution of 0.7 nmol/min·g to the measured rate of uptake; this is a small fraction of the insulin-stimulated rate. In the second place, the washout time for 2-deoxy-D-glucose is between 1 and 2 min, and most of the trapped 2-deoxy-D-glucose is removed by the washing procedure.

The important feature of the data shown in Fig. 1 is that the extent of stimulation of 2-deoxy-D-glucose uptake by insulin is low in the newborn rat (from 3 to 6 nmol/min·g of tissue) and reaches a maximum in rats 15–20 days of age (from 2 to 9 nmol/min·g of tissue). This result cannot be explained by the hypothesis that uptake of the sugar is controlled by diffusion into the extracellular space. If this were the situation, the larger diaphragms would be more greatly affected than the smaller ones and the true rate of 2-deoxy-D-glucose uptake in the larger muscles would be underestimated. This

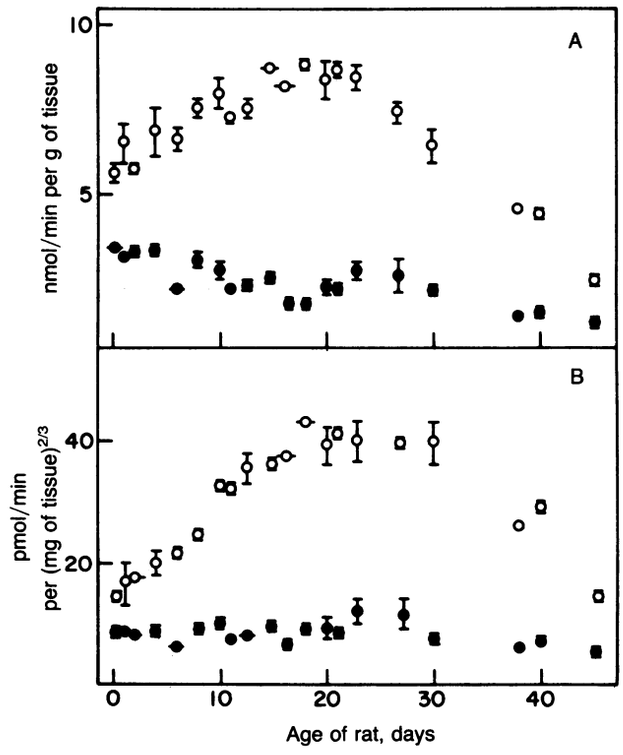

FIG. 1. 2-Deoxy-D-glucose uptake of intact rat diaphragm during postnatal development. The average rate of transport was measured with or without insulin pretreatment. The bar gives the range of duplicate determinations. Open circles and closed circles represent the results obtained in the presence and absence of insulin, respectively. (*A*) Uptake is shown as a function of the wet weight (*M*) of the tissue. (*B*) Uptake is related to $(M)^{2/3}$. The latter curve presumably shows the relative density of transporters (number per unit surface area; in arbitrary units).

would not alter the conclusion that there would be substantially more stimulation of 2-deoxy-D-glucose uptake in the 15- to 20-day-old rats than in the newborn ones.

Another feature of the data is that the basal level of glucose uptake decreases with increasing age, so that in 45-day-old rats the rate is lower by a factor of 3–4 than in the newborn rat.

Assuming that the rate of glucose uptake is proportional to the number of active transporters in the plasma membrane and that the surface area (S) is proportional to the two-thirds power of wet weight (M) of the tissue—i.e., $S = k(M)^{2/3}$—the data obtained (Fig. 1*A*) can be recast to show the number of transporters per surface area before and after insulin treatment. The results of such a calculation are given in Fig. 1*B*. The data indicate that the number of transporters per surface area remains more or less the same in the basal state as a function of age. However, in the presence of insulin, the number of transporters per surface area shows a profound variation as a function of age. In particular, it reaches a maximal level for 15- to 30-day-old rats and is lower for younger and older rats.

To determine whether or not the insulin-stimulated glucose uptake can be correlated with a variation in the numbers of insulin receptors and of intracellular glucose transporters at different stages of postnatal development, the insulin-binding capacity and the size of the latent pool of glucose transporters of diaphragm were measured.

Insulin-Binding Capacity of Diaphragm. Intact diaphragm was used first to quantitate the total number of insulin receptors, but the large amount of nonspecific binding ruled out this approach. The results of cell fractionation shown in Table 1 indicate that the pellet of a low-speed centrifugation contains 90% or more of the total 5′ nucleotidase activity. Since the insulin receptors are stable in Triton X-100, the pellet was extracted with the detergent to solubilize the receptors. After high-speed centrifugation of the Triton X-100 extract, the clear receptor-containing supernatant was utilized for the insulin-binding assay. The results of such experiments done on diaphragms from rats over the age period of 2–45 days are shown in Fig. 2. Here, the concentration of insulin is 800 pM. A similar pattern was observed when 100 pM insulin was used for the binding assay, except that the insulin bound was only 10–20% of that obtained with 800 pM insulin (data not shown).

From Fig. 2*A*, it appears that the insulin-binding capacity, normalized to the wet weight of muscle, gradually decreases with increasing age. At the age of 45 days, the insulin-binding capacity is about 50% of that at the neonatal state. However, the reduction in insulin receptors per unit surface area is not as dramatic, if present at all (Fig. 2*B*). In any event, it is clear

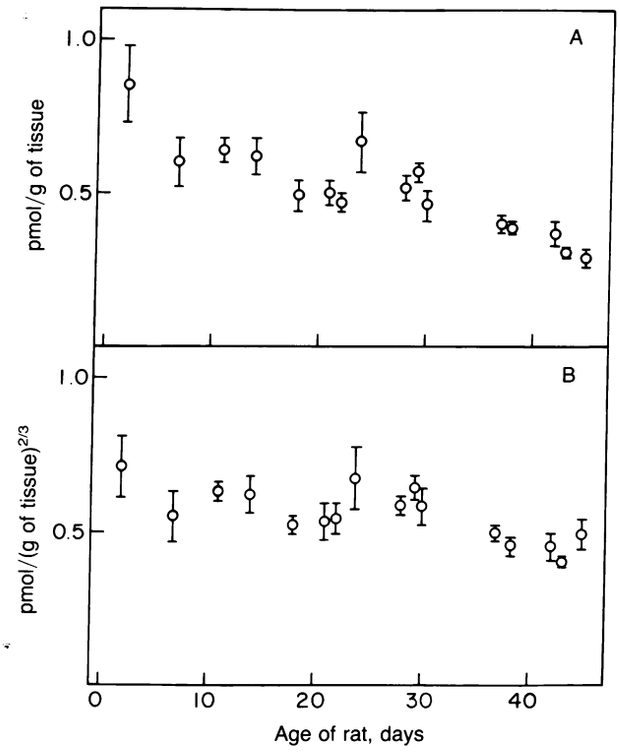

FIG. 2. Variation of insulin-binding capacity of rat diaphragm during development. Aliquots of Triton X-100-solubilized diaphragm were utilized for insulin-binding assays by the polyethylene glycol precipitation method. The total concentration of insulin was 800 pM and the total binding was <3%, judging by ^{125}I radioactivity in the precipitate. Usually, the nonspecific binding was 30–40% of the total. Triplicate determinations of the specific binding are shown here.

that no correlation exists between insulin-binding capacity and stimulation of glucose uptake (compare Figs. 1 and 2).

Assuming that the affinity of insulin for insulin receptors of diaphragm is identical to that for receptors of human placenta (22)—i.e., $K_a = 1.4 \times 10^9\ M^{-1}$—one can estimate that there is 0.5–1 pmol of insulin receptors per g of tissue, which is comparable to the amount in human placenta (23). Since it was not possible to measure the number of insulin receptors in the intact diaphragm, the question remains as to the yield of insulin receptors extracted from the membrane by the method described here. This number is not known exactly but is likely to be comparable to the yield of 5′ nucleotidase activity extracted by Triton X-100 from the low-speed pellet: this yield is 70%. In any event, it is not likely that the yield

Table 1. Recovery of enzymatic activity during cell fractionation

Enzyme	Age, days	Enzyme activity in fraction		
		Homogenate	Low-speed pellet	Microsome
Experiment 1*				
5′ Nucleotidase, nmol/hr per g	15	236 ± 7	254 ± 11	16 ± 1
	40	410 ± 13	362 ± 13	9.8 ± 0.4
Galactosyltransferase, μmol/3 hr per g	15	0.87 ± 0.20	ND	0.33 ± 0.01
	40	0.56 ± 0.01	ND	0.16 ± 0.02
Experiment 2†				
5′ Nucleotidase, nmol/hr per g	15	262 ± 9	257 ± 13	14 ± 0.5
	40	522 ± 10	449 ± 9	14.3 ± 0.3
Galactosyltransferase, μmol/3 hr per g	15	0.87 ± 0.06	ND	0.37 ± 0.01
	40	0.50 ± 0.21	ND	0.24 ± 0.02

Diaphragms were isolated and fractionated, and the enzymatic activities were assayed. Values are given as mean ± SD. ND, not determined.

*Ten diaphragms of 15-day-old rats and six diaphragms of 40-day-old rats were pooled.

†Eleven and five diaphragms from 15- and 40-day-old rats were used, respectively.

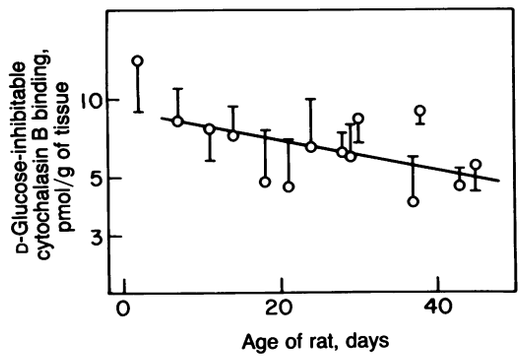

FIG. 3. Intracellular pool size of D-glucose transporters. The intracellular pool of transporters was measured by D-glucose-inhibitable cytochlasin B binding in the presence of an equal amount of L-glucose. The initial concentration of cytochalasin B was 50 nM. The total binding varied from 10% to 30% of the originally added cytochlasin B; D-glucose-displaceable binding comprised 10–25% of the total binding. The average of the triplicate determinations is given here.

of receptors will differ depending on the age of the animal or the size of the diaphragm.

D-Glucose-Displaceable Cytochalasin B Binding in Microsomes. In order to measure the intracellular pool of glucose transporters in the diaphragms of rats at different times after birth, crude microsomes were prepared and used to assay D-glucose-displaceable cytochalasin B binding. This microsomal preparation contained 30% ± 10% of the total galactosyltransferase activity present in the muscle homogenate. The results, shown in Fig. 3, indicate that there is a slight decrease in the number of intracellular D-glucose transporters with increasing age of the rat. It is clear, however, that the variation in the stimulation of glucose transport by insulin during development is not directly related to the size of the intracellular pool of transporters.

It has been shown that insulin causes a translocation of D-glucose transporters from an intracellular site to the plasma membrane in rat diaphragm (6). From the data shown in Table 2, it appears that insulin treatment indeed reduced the amount of D-glucose transporters in these microsomal fractions. These results are consistent with the assumption that the D-glucose transporters in these crude preparations are not derived from contaminating plasma membrane. If they were, one should expect to observe an increase in D-glucose-inhibitable cytochalasin B binding instead of a decrease.

DISCUSSION

Although it has been known for decades that skeletal muscle is one of the target tissues for insulin, it was not known previously whether or not insulin responsiveness of glucose transport was dependent on development in muscle cells. Here, I demonstrate that insulin-stimulated glucose transport follows a complex pattern in diaphragm during postnatal development, while the rate of glucose uptake per unit wet weight in the absence of insulin decreases continuously as growth proceeds (Fig. 1).

Table 2. Reduction of D-glucose transporters in microsomes after insulin treatment

	D-Glucose-inhibitable cytochalasin B binding, pmol/g of tissue	
Age, days	Insulin-treated	Untreated
12	5.0 ± 0.3	6.4 ± 0.4
36	1.8 ± 0.6	3.0 ± 1.0

The intact diaphragms were dissected and treated with 330 nM insulin for 30 min; then the microsomes were isolated and assayed for D-glucose-inhibitable cytochalasin B binding. Values represent the mean and the range of three determinations. The initial concentrations of [^{3}H]cytochalasin B used were 50 nM and 30 nM for the diaphragms from 12-day-old and 36-day-old rats, respectively.

The results shown in Fig. 1*A* can be analyzed and interpreted in several ways. In the first place, it is likely that the rate of transport measured in the basal state is proportional to the number of transporters per unit area of plasma membrane. Since the diaphragms of rats at different ages have the same number of muscle fibers, one can obtain an estimate of the surface area of the muscle cells by assuming that the cylindrical fibers grow by a proportional increase in length and radius. Under the conditions that the ratio of length to radius of the fibers is a constant, the surface area (S) is related to the volume (mass, M) of the muscle cells by the relationship: $S = k(M)^{2/3}$. Using this value for the surface area and recasting the data in terms of rate of transport per surface area, it is apparent that the basal rate of transport in the diaphragm of the rat is constant from birth to 40 days of age (Fig. 1*B*). The simplest explanation of these data is that the surface density of transporters in the basal state is constant with age. Expressing the insulin-dependent rate of transport per unit surface area, as shown in Fig. 1*B*, reveals that the stimulation of the glucose transport rate by insulin increases from birth to 20 days of age, is constant between 20 and 30 days, and then decreases between 30 and 40 days of age. The central question here is whether the insulin-dependent increase in the rate of transport is caused by an increase in the surface density of transporters, by an increase in the rate of transport of the transporters already in the membrane, or by a combination of these events. Although the evidence presented in Table 2 suggests that insulin does cause translocation of intracellular transporters, a conclusion supported by the experiments of Wardzala and Jeanrenaud (6), one cannot exclude the possibility that an increased activity of the transporters accounts for most of the increase in rate seen in Fig. 1*B*. If one assumes, however, that the mechanism of action of insulin in diaphragm is similar to that in the adipocyte, the conclusion is that the insulin-dependent translocation of glucose transporters from the intracellular pool to the plasma membrane is small at birth and increases to the maximal value at 15–20 days of age.

There are several possible explanations for the growth-dependent increase in stimulation of glucose transport by insulin. Two simple possibilities are that the effect depends directly on the number of insulin receptors in the plasma membrane or that it is directly related to the ability of the insulin receptor to generate an intracellular signal. These explanations are valid whether transporter translocation is involved or transporter activation is dominant. Two other possible reasons for the developmental effect are that the pool of intracellular transporters increases or the machinery for translocation develops with age. These explanations are valid only if transporter translocation has the major role in the phenomenon.

An attempt was made to measure the number of insulin receptors in the plasma membrane and the size of the intracellular pool of glucose transporters as a function of age in order to support or exclude two of the explanations suggested above.

The simplest explanation of the results of the insulin-binding experiments, shown in Fig. 2, is that the surface density of insulin receptors changes by <50% from birth to 45 days of age. This conclusion is based on the assumption that the affinity of the receptors for insulin is constant with age and that the fraction of receptors extracted from the membrane by Triton X-100 is also constant with age. Since these

assumptions are likely to be correct, the results indicate that the level of insulin-stimulated glucose uptake is not correlated with the insulin-binding capacity. Furthermore, the insulin receptors solubilized from the diaphragms of rats at all of these stages of growth all have insulin-dependent protein kinase activity that is proportional to the amount of insulin bound by the receptor (unpublished observations). This result suggests that the protein kinase activity of the insulin receptor is also not correlated with the level of stimulation of glucose uptake by insulin.

The evidence that in the guinea pig adipocyte the minimal stimulation of glucose transport by insulin is directly correlated and apparently causally related to the small pool of intracellular glucose transporters (7) was the basis for the measurements of the intracellular pool of transporters in the diaphragm as a function of the age of the rat. The results of these experiments show, however, that there is no obvious increase or change in the number of glucose transporters in the diaphragm as a function of age. Thus, the situation in the rat diaphragm is different from that in the guinea pig adipocyte. In the diaphragm the stimulation of glucose transport by insulin is blunted in the newborn rat and reaches a maximum in the 15- to 20-day-old rat, even though the pool of intracellular transporters is as large or larger in the newborn as compared to the 15- to 20-day-old rat. Hence, the lower level of insulin stimulation of glucose uptake in the newborn rat cannot be explained by a smaller intracellular pool of glucose transporters. Taken together, the results presented here indicate that the stimulation of glucose transport in the diaphragm of the newborn rat is minimal compared to that in the diaphragm of the 15- to 20-day-old rat in spite of the fact that the number of insulin receptors with protein kinase activity and of intracellular glucose transporters is the same in the diaphragms of newborn and older rats.

The most likely explanation of this observation is that the translocation of the intracellular pool of transporters to the plasma membrane in the diaphragm is defective at birth and becomes progressively more active during the first 15 days of life. This explanation is not unequivocal because it was not possible to demonstrate that translocation actually does take place in the diaphragm of 15- to 20-day-old rats. The difficulty of isolating sufficient pure plasma membrane in the absence of Triton X-100 made it impossible to measure the number of glucose transporters in the plasma membrane. Thus, it is possible, although unlikely, that the insulin-dependent increase in glucose transport in the diaphragm is brought about by increased activity of the transporters in the membrane in the absence of translocation.

In any event, two of the four essential components for the stimulation of glucose transport by insulin are present in equal amounts in newborn and older rat diaphragms: intracellular glucose transporters and insulin receptors. It is likely that either the signal transducer from the receptor to the membrane and to the translocation machinery or the translocation machinery itself is defective or not completely developed in the newborn rat. It remains to be seen if small messenger molecules, protein kinases or phosphatases, GTP-binding protein, or other components still to be imagined are found to be part of the signaling device or the translocation machinery.

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1. Suzuki, K. & Kono, T. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 2542–2545.
2. Cushman, S. W. & Wardzala, L. J. (1980) *J. Biol. Chem.* **255**, 4758–4762.
3. Karnieli, E., Zarnowski, M. J., Hissin, P., Simpson, I. A., Salans, L. B. & Cushman, S. W. (1981) *J. Biol. Chem.* **256**, 4772–4777.
4. Kono, T., Suzuki, K., Dansey, L. E., Robinson, D. W. & Blevins, T. L. (1981) *J. Biol. Chem.* **256**, 6400–6407.
5. Kono, T., Robinson, F. W., Blevins, T. L. & Ezaki, O. (1982) *J. Biol. Chem.* **257**, 10942–10947.
6. Wardzala, L. J. & Jeanrenaud, B. (1981) *J. Biol. Chem.* **256**, 7090–7093.
7. Horuk, R., Rodbell, M., Cushman, S. W. & Wardzala, L. J. (1983) *J. Biol. Chem.* **258**, 7425–7429.
8. Resh, M. D. (1982) *J. Biol. Chem.* **257**, 6978–6986.
9. Standaert, M. L., Schimmel, S. D. & Pollet, R. J. (1984) *J. Biol. Chem.* **259**, 2337–2349.
10. Guidotti, G. & Foa, P. P. (1961) *Am. J. Physiol.* **201**, 869–872.
11. Clark, C. M., Jr. (1971) *Am. J. Physiol.* **220**, 583–588.
12. Kutchai, H., King, S. L., Martin, K. & Daves, E. D. (1977) *Dev. Biol.* **55**, 92–102.
13. Newerly, K. & Berson, S. A. (1957) *Proc. Soc. Exp. Biol. Med.* **94**, 751–755.
14. Olefsky, J., Bacon, V. C. & Baur, S. (1976) *Metabolism* **25**, 179–191.
15. Sandra, A. & Przybylski, R. J. (1979) *Dev. Biol.* **68**, 546–556.
16. Kipnis, D. M. & Cori, C. F. (1957) *J. Biol. Chem.* **224**, 681–693.
17. Kipnis, D. M. & Cori, C. F. (1959) *J. Biol. Chem.* **234**, 171–177.
18. Kipnis, D. M. & Parrish, J. E. (1965) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **24**, 1051–1059.
19. Morgan, H. E. & Whitfield, C. F. (1973) *Curr. Top. Membr. Transp.* **4**, 255–303.
20. Cuatrecasas, P. (1972) *Proc. Natl. Acad. Sci. USA* **69**, 318–322.
21. Avruch, J. & Wallach, D. F. H. (1971) *Biochim. Biophys. Acta* **233**, 334–347.
22. Kohanski, R. A. & Lane, M. D. (1983) *J. Biol. Chem.* **258**, 7460–7468.
23. Fujita-Yamaguchi, Y., Choi, S., Sakamoto, Y. & Itakura, K. (1983) *J. Biol. Chem.* **258**, 5045–5049.