Accelerated Publications

^{13}C NMR for the Assessment of Human Brain Glucose Metabolism in Vivo†

Nicolau Beckmann,‡ Igor Turkalj,§ Joachim Seelig,*,‡ and Ulrich Keller§

MR Center and Biocenter of the University of Basel, Klingelbergstrasse 70, CH-4056 Basel, Switzerland, and Division of Endocrinology and Metabolism, University Hospital, Petersgraben 2, CH-4031 Basel, Switzerland

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ABSTRACT: Proton-decoupled ^{13}C NMR spectra of the human head were obtained during hyperglycemic glucose clamping using intravenous infusions of [1-^{13}C]glucose in normal volunteers. In addition to ^{13}C signals of mobile lipids, a variety of new metabolite resonances could be resolved for the first time in the human brain. At an enrichment level of 20% [1-^{13}C]glucose, the signals of α- and β-glucose at 92.7 and 96.6 ppm, respectively, could be detected in the human brain after only an infusion period of 15 min. The spatial localization of the different regions of interest was confirmed by ^{13}C NMR spectroscopic imaging with a time resolution of 9 min. Increasing the enrichment level to 99% [1-^{13}C]glucose not only improved the time resolution but allowed the detection of metabolic breakdown products of [1-^{13}C]glucose. The time course of ^{13}C label incorporation into the C_2, C_3, and C_4 resonances of glutamate/glutamine and into lactate could be recorded in the human brain. These results suggest the possibility of obtaining time-resolved, spatially selective, and chemically specific information on the human body.

In the past few years the interest in applying ^{13}C magnetic resonance spectroscopy to analyze metabolic processes in humans in vivo has grown considerably. Despite the inherent difficulties presented by this nucleus (natural abundance of only 1.1%, low magnetogyric ratio), several metabolites can be detected by ^{13}C NMR in reasonable measurement times of about 20–30 min (Avison et al., 1988; Beckmann et al., 1990; Heerschap et al., 1989; Jue et al., 1989). Indeed, broad-band decoupled natural abundance ^{13}C spectra of the human calf, the abdominal region, and the head have been reported recently (Beckmann et al., 1990; Heerschap et al., 1989) and now are obtained routinely in our laboratory.

The information content of a ^{13}C NMR spectrum can be substantially increased if substrates enriched with ^{13}C are administered via the oral or intravenous route. During the metabolism of the externally supplied ^{13}C-labeled compounds (typically 90% enriched in ^{13}C), the ^{13}C label is transferred to various intermediates that, in part, may become detectable with ^{13}C NMR. Hence metabolic pathways and fluxes may become amenable to an in vivo analysis. Until now, most in vivo studies with ^{13}C-enriched substrates and ^{13}C NMR were restricted to animals, with particular emphasis to the head and the liver [for reviews, see Cerdan and Seelig (1990) and London (1988)].

Applications to humans have also been reported. Shulman et al. assessed the rate of incorporation of intravenously infused [1-^{13}C]glucose into the gastrocnemius muscle of normal subjects and of patients with non-insulin-dependent diabetes (Shulman et al., 1990). Brainard et al. measured glucose production rates in humans by continuously infusing [U-^{13}C]glucose over a 4-h period, followed by ^{13}C glucose analysis of blood plasma with in vitro NMR (Brainard et al., 1989). Kalderon et al. (1989) and Gopher et al. (1990) obtained ^{13}C spectra from human blood plasma after injection of [U-^{13}C]glucose and [U-^{13}C]fructose into patients with type I and type III glycogen storage disease and into fructose-intolerant children, respectively. Rothman et al. (1990) observed lactate production in infarcted human brain by applying ^{1}H spectroscopy after the infusion of [1-^{13}C]glucose, their results being consistent with the hypothesis that persistently elevated lactate is present in actively metabolizing cells of infarcted brain.

Here we present broad-band proton-decoupled ^{13}C spectra of the human brain obtained during the intravenous infusion of [1-^{13}C]glucose in the arm of normal volunteers. Furthermore, the potential of ^{13}C spectroscopic imaging (SI) (Beckmann & Müller, 1991; Müller & Beckmann, 1989) in following the incorporation of ^{13}C-labeled substances in the human body is demonstrated with a temporal resolution of only 9.0 min for the human head.

Materials and Methods

Studies were performed on three healthy male overnight-fasted volunteers (mean age, 24.7 ± 0.3 years; mean body weight, 65.7 ± 2.8 kg; mean fasting plasma glucose, 82.0 ± 3.0 mg/dL). None of the subjects was taking any medication. Before inclusion in the study, the volunteers were screened to be healthy, and they gave their written consent after being informed about the procedure and the potential risks of the study. The study protocol was reviewed and approved by the Ethical Committee of the Basel University Hospital.

^{13}C measurements were performed with a Siemens Helicon whole body scanner operating at 1.5 T, equipped with ^{1}H and ^{13}C radio-frequency (rf) channels. Two coplanar and concentric ^{1}H and ^{13}C surface coils of diameters 13.0 and 8.0 cm, respectively, tuned to 63.7 and 16.0 MHz, were used for transmission and receiving. The surface coils were positioned parallel to the periphery of the body. The excitation of the ^{13}C nuclei was accomplished by a rectangular pulse of 200-μs duration. During signal collection broad-band decoupling with a Waltz-8 sequence (Levitt et al., 1983) was applied during 44 ms. Decoupling was performed discontinuously; i.e., intervals of 500 μs were intercalated between the rectangular ^{1}H decoupling pulses. The ^{13}C pulse power was adjusted to

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‡ MR Center and Biocenter of the University of Basel.

§ Division of Endocrinology and Metabolism, University Hospital.

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give a 200° flip angle at the center of the surface coil. All spectra shown below were line-broadened by a Gaussian filter of 15 Hz applied to the time domain signal before Fourier transformation. Baseline correction was performed on all the spectra.

The spectroscopic imaging experiment (Brown et al., 1982; Maudsley & Hilal, 1985) was restricted to one spatial and one spectroscopic axis, with the phase-encoding gradient perpendicular to the plane of the surface coil. The slices closest to the coil reflect mainly subcutaneous adipose tissue. Signals from deeper lying organs can be found in the remaining sections of the spectroscopic image (Beckmann & Müller, 1991). The map presented in this paper is the result of real part calculations and has a dimension of 256 × 1024 points. The raw data were acquired in a 64 × 256 complex matrix, zero-filled along both the spatial and spectroscopic axes and further processed with the FTNMR software by Hare (1988). A 10-Hz exponential line broadening was applied prior to Fourier transformation along the spectroscopic dimension. The spatially resolved spectra were baseline-corrected with a cubic spline function.

The phase-encoding gradient followed a trapezoidal wave form with 500-μs rise time and 200-μs decay. It was switched on already during the application of the 100-μs-long ^{13}C radio-frequency pulse in order to generate efficient phase encoding following the nonselective rf pulse. The strength varied between −5.0 and 5.0 mT/m in 64 phase-encoding steps.

The volunteers were admitted to the Magnetic Resonance Center at 9:00 a.m. after a 14-h overnight fast. An initial spectrum was acquired prior to the infusion of glucose. Next, a butterfly needle (G21) was then inserted into a dorsal foot vein in retrograde fashion for drawing arterialized-venous blood samples throughout the study. A warming cushion was wrapped around the foot to keep its temperature at approximately 40 °C, allowing arterialization of venous blood. Cannulating a foot vein made it possible to sample blood without the need to remove the volunteers from the spectrometer. ^{13}C NMR spectra of blood samples could thus be obtained simultaneously during the whole 120-min infusion procedure. Blood samples were drawn in 10-min intervals for measurement of plasma glucose and every 30 min for determination of plasma concentrations of insulin and C-peptide.

An anticubital vein was also cannulated (Venflon catheter) for the administration of infusions (somatostatin, insulin, and glucose). The subjects remained in a supine position throughout the infusion procedure.

The hyperglycemic glucose clamp was performed with a modification of the procedure described by DeFronzo et al. (1979). Ten minutes before starting the glucose infusion, endogenous insulin secretion was suppressed by administration of somatostatin (Stilamin Serono) at a rate of 0.1 μg kg^{-1} min^{-1}. Thereby, hyperglycemia-induced insulin secretion was avoided, and less intravenous glucose was required to maintain hyperglycemia. Five minutes later insulin (Actrapid HM Novo) was infused at a priming rate of 80 milliunits m^{-2} min^{-1} during 4 min and afterward at 20 milliunits m^{-2} min^{-1} to induce acute physiological (fed state) hyperinsulinemia, thereby suppressing endogenous glucose production. Five minutes later an infusion of glucose containing [1-^{13}C]glucose was started. In the first experiments 20%-enriched [1-^{13}C]-glucose was administered to the three volunteers. The same clamp procedure was then repeated once with 99% $^{13}C_1$-enriched glucose.

Plasma glucose concentration was raised within 30 min to the desired value of 10–11 mmol/L; it was maintained at this level until the end of the study (120 min) by varying the glucose infusion rate in 10-min intervals according to the instantaneously measured plasma glucose level (Yellow Springs glucose analyzer). Plasma insulin and C-peptide concentrations were determined by radioimmunoassays.

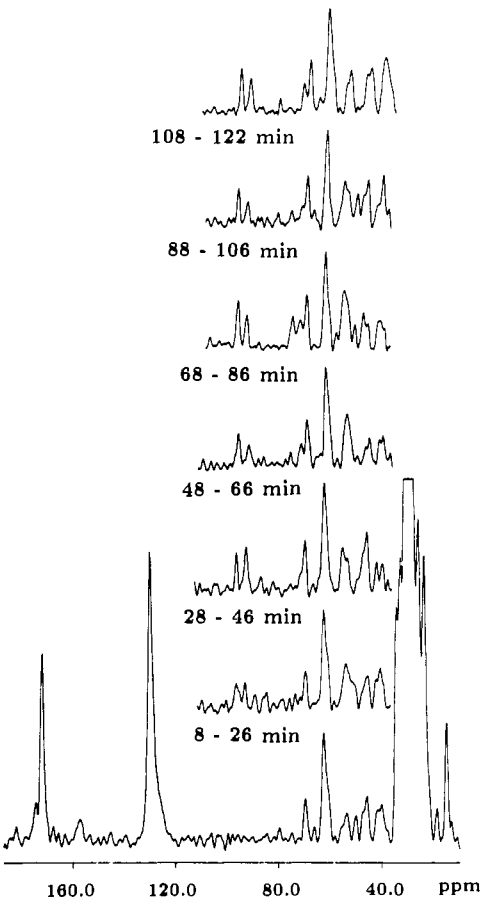

FIGURE 1: Broad-band decoupled ^{13}C spectra of the head of a normal volunteer during intravenous infusion of [1-^{13}C]glucose. Each spectrum represents the average of 640 acquisitions. The time scale is relative to the beginning of the glucose infusion. The resonances at 92.7 ppm correspond to C_1 of α-glucose, while the resonances at 96.6 ppm arise from C_1 of β-glucose.

Results

In the present studies the mean initial glucose concentration was normal in all volunteers (4.8 ± 0.2 mmol/L). After the start of the clamp, the plasma glucose concentration was raised within 30 min to reach a plateau value of 10.7 ± 0.3 mmol/L. The mean glucose concentrations recorded in 30-min intervals from 30 to 120 min after the beginning of the glucose infusion were 10.4 ± 0.2, 10.9 ± 0.1, 10.8 ± 0.2, and 10.5 ± 0.2 mmol/L. Plasma C-peptide concentrations decreased from 381 ± 34 (0 min) to 165 ± 28 pmol/L (120 min). Plasma insulin concentrations increased from 8 ± 2 (0 min) to 16 ± 4 microunits/mL (120 min).

Figure 1 contains $^{13}C\{^1H\}$ spectra of the human head, obtained before and during the infusion of [1-^{13}C]glucose. Each spectrum represents the average of 640 acquisitions, distributed in five blocks of 128 acquisitions (TR = 0.94 s, 2-min measurement time), with a 2-min interval between each block; i.e., the time resolution of the spectra is 18 min. With the present concentration of enriched glucose (20%) the signals of C_1 of α- and β-glucose at 92.7 and 96.6 ppm, respectively, can be clearly resolved.

The spatial localization of the above spectra was confirmed with ^{13}C spectroscopic imaging (Müller & Beckmann, 1989).

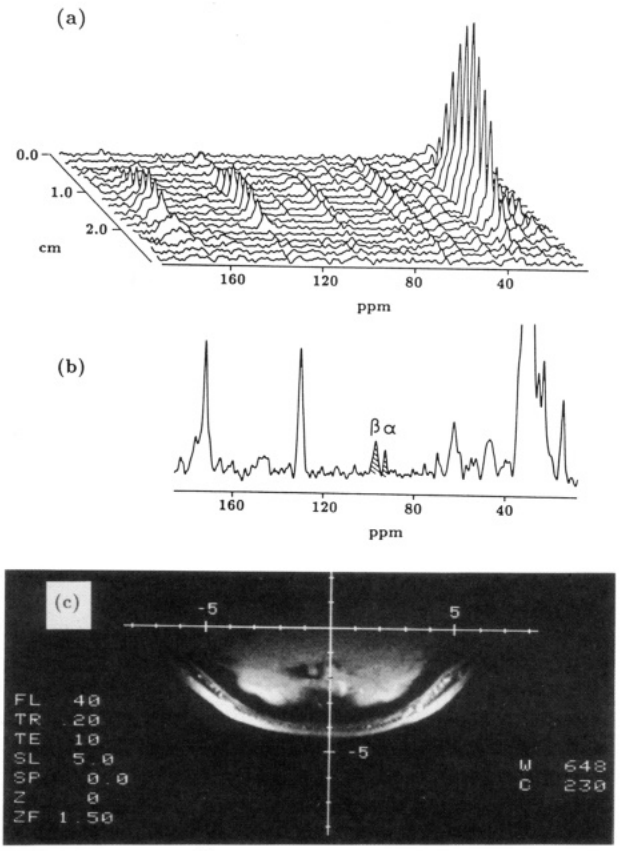

FIGURE 2: Surface coil ^{13}C SI applied to the head of a normal volunteer after injection of glucose labeled at the C_1 position. (a) Real part map. Conditions: acquisition time, 9.0 min; TR = 1.05 s, eight averages; 64 phase-encoding steps (−5.0 to 5.0 mT/m); decoupling with Waltz 8. (b) Spectrum extracted from the ^{13}C SI map (a), showing the good signal-to-noise ratio of the resonances corresponding to the enriched substance, despite the low measurement time. (c) FLASH image of the same region obtained with the ^1H surface coil.

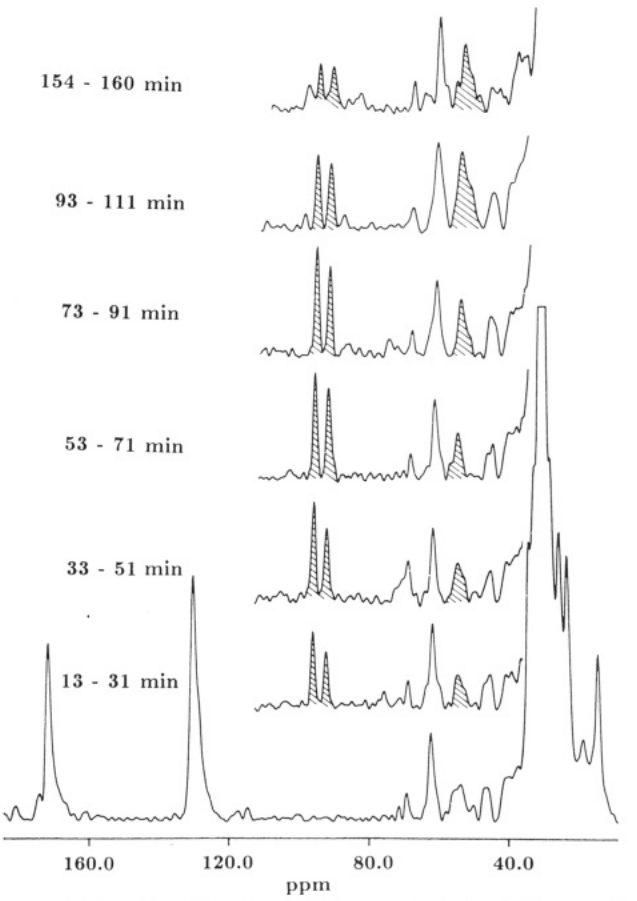

FIGURE 3: Broad-band decoupled ^{13}C spectra of the head of a normal volunteer during intravenous infusion of [1-^{13}C]glucose. Each spectrum represents the average of 640 acquisitions. The time scale is relative to the beginning of the glucose infusion. The resonances at 92.7 ppm correspond to C_1 of α-glucose, while the resonances at 96.6 ppm arise from C_1 of β-glucose.

The ^{13}C SI sequence was applied at the end of the infusion experiment, and Figure 2a displays the ^{13}C SI spectra as a function of the slice position. The C_1 resonances of α- (92.7 ppm) and β-glucose (96.6 ppm) are not observed in slices immediately adjacent to the surface coil. However, for slices deeper inside the brain (0.8–2.0-cm distance) the ^{13}C SI spectra are characterized by significant contributions of [1-^{13}C]glucose (Figure 2b). This demonstrates that the simultaneous ^{13}C NMR examination of serial adjacent brain regions is possible within less than 10 min. The direct correlation of the ^{13}C SI map to anatomical information is furnished by a ^1H image taken with the ^1H surface coil (Figure 2c). The results shown in Figure 2 were confirmed in two further experiments. Detection of [1-^{13}C]glucose in the brain was always easily possible but no other glucose metabolites were clearly observable at this enrichment (20%).

In order to increase the sensitivity of the measurements, the 20%-enriched glucose was replaced by 99%-enriched [1-^{13}C]glucose. The spectra shown in Figure 3 were obtained with this higher enrichment level, keeping all other experimental conditions identical with those in the previous experiments. As expected, the C_1 resonances of α- and β-glucose are much increased in intensity compared to the experiments with 20% enrichment. These resonances remained detectable for at least 30 min after the end of the infusion. Figure 3 further demonstrates a distinct increase of the 55.0 ppm resonance after about 60-min infusion time. This resonance is attributed to C_2 of glutamate/glutamine (Bárány et al., 1985). Figure 4 shows the same data, this time presented as difference spectra (spectra at time of infusion minus starting spectrum). This comparison clearly demonstrates the appearance of resonances at 55.0 and 34.4 ppm, attributed to C_2 and C_4 of glutamine/glutamate, respectively (Bárány et al., 1985), at about 60 min after the start of the infusion. There are also indications of additional resonances at 27.8 ppm (C_3 of glutamate/glutamine) and 21.0 ppm (C_3 of lactate). These results are comparable to analogue measurements performed by Behar et al. on the rabbit brain after the scalp and the temporalis muscles had been removed (Behar et al., 1986).

Figure 5 shows the time course of the glutamate/glutamine production, based on the ^{13}C spectra. The data express the signal intensity in percentage of the maximum intensity reached by each of the metabolite resonances, i.e., C_2, C_3, and C_4 of glutamate/glutamine. Figure 5 demonstrates that the ^{13}C label reaches the C_4 position earlier (~30–40 min) than the C_2 and C_3 positions of glutamate/glutamine (~50–70 min).

Discussion

The present work describes the application of ^{13}C NMR spectroscopy to study the dynamics of [1-^{13}C]glucose incorporation and its metabolism in human brain. In the absence of ^{13}C label the ^{13}C NMR brain spectra were dominated by intense signals at 30, 130, and 180 ppm, which can be assigned to the natural abundance methylene, olefinic, and carbonyl carbons, respectively, of mobile triglyerides. When 20%-enriched [1-^{13}C]glucose was infused intravenously under hyperglycemic clamp conditions in the arm of a normal volunteer,

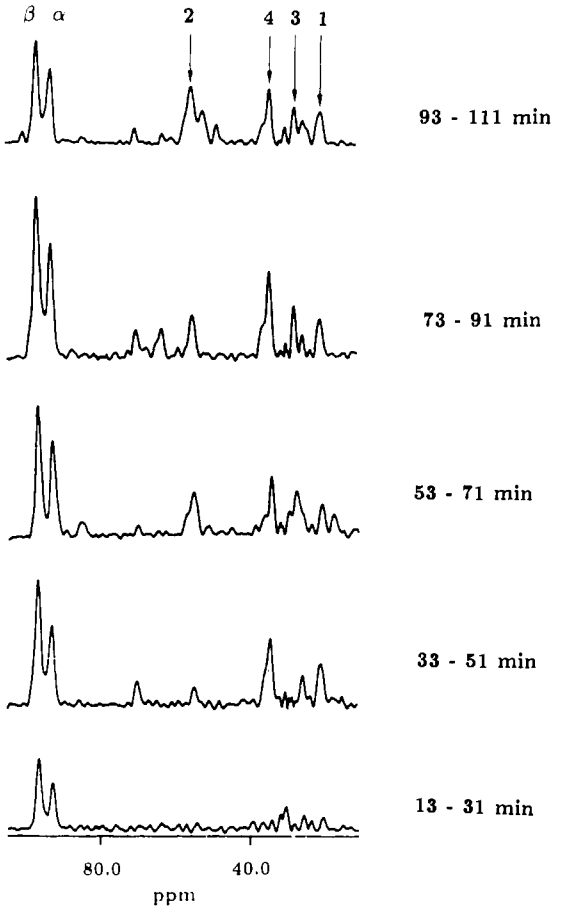

FIGURE 4: Spectra obtained from the difference of the signals during and before the infusion for the experiment with the 99%-enriched glucose. The lettering indicated in the spectra correspond to the following: β, C_1 of β-glucose (96.6 ppm); α, C_1 of α-glucose (92.7 ppm); 2, C_2 of glutamate/glutamine (55.0 ppm); 4, C_4 of glutamine/glutamate (34.4 ppm); 3, C_3 of glutamine/glutamate (27.8 ppm); 1, C_3 of lactate (21.0 ppm).

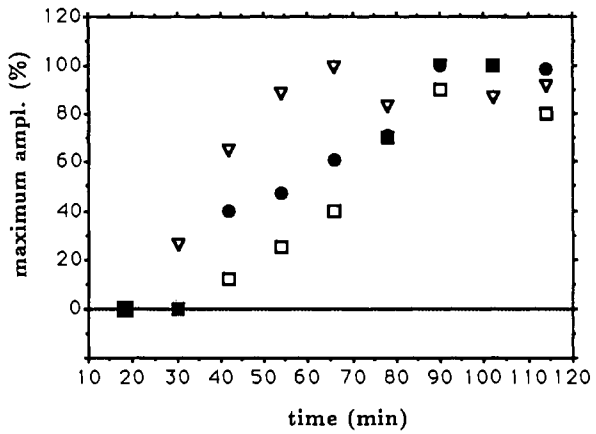

FIGURE 5: Time course of the glutamate/glutamine production in the human brain, derived from the ^{13}C spectra. The intensities are expressed in percentage of the maximum intensity reached for each resonance [C_2 (●), C_3 (□), and C_4 (▽)] of glutamate/glutamine.

the resonances corresponding to C_1 of α- and β-glucose could be differentiated 15–20 min after the beginning of the infusion. This time interval was reduced to about 5 min by using the 99%-enriched glucose. It is interesting to note that the glucose signals persisted even 30 min after the end of the infusion. This observation, together with the glutamate/glutamine and lactate signals detected after the injection of 99%-enriched glucose, indicates that the measured signals were indeed arising from glucose and glucose metabolites in the brain. Closer inspection of the difference spectra of Figure 4 demonstrates that signals of glutamate/glutamine are first observed at C_4 (34.4 ppm) and later at C_3 (27.8 ppm) and C_2 (55.0 ppm). The C_3 signal of lactate was detectable after only about 30 min. The time course for the appearance of the glutamate and glutamine resonances (around 30–40 min for the C_4 and 50–60 min for C_2 resonance) is comparable to the results presented by Behar et al. (1986) for the rabbit brain.

The hyperglycemic glucose clamp was chosen in the present studies to increase glucose uptake by the brain due to mass action (Baron et al., 1988). Modest hyperinsulinemia was produced to suppress hepatic glucose output, thereby minimizing dilution of infused [^{13}C]glucose with endogenous [^{12}C]glucose (Gottesman et al., 1982). Without simultaneous somatostatin administration, hyperglycemia would have resulted in increased plasma insulin levels, producing [^{13}C]-glucose uptake in insulin-sensitive tissues such as skeletal muscle (DeFronzo et al., 1981).

A limiting factor to the clinical application of ^{13}C-enriched substrates is certainly their high costs. The price of 1 g of 99%-enriched [1-^{13}C]glucose is at present around $100.00. A 2-h clamp as described herein necessitates between 20 and 50 g of tracer, so that the costs range from $2000.00 to $5000.00 per experiment. This is about 10–30 times the cost of contrast media for NMR imaging.

In conclusion, ^{13}C NMR spectroscopy is well suited to monitor the incorporation of intravenously infused [1-^{13}C]-glucose into the human brain. The broad-band decoupled ^{13}C spectra show clearly the C_1 resonances of α- and β-glucose at 92.7 and 96.6 ppm, respectively. In addition, the formation of C_2, C_3, and C_4 of glutamate/glutamine, as well as of C_3 of lactate, could be followed as a function of time. ^{13}C spectroscopic imaging in combination with [1-^{13}C]glucose suggests the possibility for obtaining time-resolved, spatially selective, and chemically specific information on the human body.

References

Avison, M. J., Rothman, D. L., Nadel, E., & Shulman, R. G. (1988) *Proc. Natl. Acad. Sci. U.S.A. 85*, 1634–1636.

Bárány, M., Arûs, C., & Chang, Y.-C. (1985) *Magn. Reson. Med. 2*, 289–295.

Baron, A. D., Brechtel, G., Wallace, P., et al. (1988) *Am. J. Physiol. 255*, E769–E774.

Beckmann, N., & Müller, S. (1991) *J. Magn. Reson.* (in press).

Beckmann, N., Seelig, J., & Wick, H. (1990) *Magn. Reson. Med. 16*, 150–160.

Behar, K. L., Petroff, O. A. C., Prichard, J. W., Alger, J. R., & Shulman, R. G. (1986) *Magn. Reson. Med. 3*, 911–920.

Brainard, J. R., Downey, R. S., Bier, D. M., & London, R. E. (1989) *Anal. Biochem. 176*, 307–312.

Brown, T. R., Kincaid, B. M., & Uğurbil, K. (1982) *Proc. Natl. Acad. Sci. U.S.A. 79*, 3523–3526.

Cerdan, S., & Seelig, J. (1990) *Annu. Rev. Biophys. Chem. 19*, 43–67.

DeFronzo, R. A., Tobins, J. E., & Andres, R. (1979) *Am. J. Physiol. 237*, E214–E223.

DeFronzo, R. A., Jacot, E., Jequier, E., et al. (1981) *Diabetes 30*, 1000–1007.

Gopher, A., Vaisman, N., Mandel, H., & Lapidot, A. (1990) *Proc. Natl. Acad. Sci. U.S.A. 87*, 5449–5453.

Gottesman, I., Mandarino, L., Verdonk, C., Rizza, R. A., & Gerich, J. E. (1982) *J. Clin. Invest. 70*, 1310–1314.

Hare, D. (1988) *FTNMR, User Documentation*, Version 5.2,

Hare Research, Inc., Philadelphia, PA.
Heerschap, A., Luyten, P. R., van der Heyden, J. I., Oosterwaal, L. J. M. P., & den Hollander, J. A. (1989) *NMR Biomed. 2*, 124-132.
Jue, T., Rothman, D. L., Tavitian, B. A., & Shulman, R. G. (1989) *Proc. Natl. Acad. Sci. U.S.A. 86*, 1439-1442.
Kalderon, B., Korman, S. H., Gutman, A., & Lapidot, A. (1989) *Proc. Natl. Acad. Sci. U.S.A. 86*, 4690-4694.
Levitt, M. H., Freeman, R., & Frenkiel, T. (1983) in *Advances in Magnetic Resonance* (Waugh, J. S., Ed.) Vol. 11, pp 47-110, Academic Press, New York.
London, R. E. (1988) *Prog. NMR Spectrosc. 20*, 337-383.
Maudsley, A. A., & Hilal, S. K. (1985) *Magn. Reson. Med. 2*, 218-233.
Müller, S., & Beckmann, N. (1989) *Magn. Reson. Med. 12*, 400-406.
Rothman, D. L., Howseman, A. M., Graham, G. D., Lantos, G., Petroff, O. A. C., Brass, L. M., Fayad, P. B., Shulman, G. I., Shulman, R. G., & Prichard, J. W. (1990) in Proceedings of the Society on Magnetic Resonance in Medicine, 9th Annual Meeting, New York, p 107.
Shulman, G. I., Rothman, D. L., Jue, T., Stein, P., DeFronzo, R. A., & Shulman, R. G. (1990) *N. Engl. J. Med. 322* (4), 223-228.

Articles

Cytoplasmic Juxtamembrane Region of the Insulin Receptor: A Critical Role in ATP Binding, Endogenous Substrate Phosphorylation, and Insulin-Stimulated Bioeffects in CHO Cells†

Jonathan M. Backer,*,‡ Gregory G. Schroeder,‡ Deborah A. Cahill,‡ Axel Ullrich,§ Kenneth Siddle,‡ and Morris F. White‡

Research Division, Joslin Diabetes Center, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, Massachusetts 02215, and Max Planck Institut fur Biochemie, 8033 Martinsried bei Munchen, West Germany

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ABSTRACT: We have expressed in CHO cells a mutant receptor ($IR_{\Delta 960}$) from which 12 amino acids in the juxtamembrane region (A954-D965), including Tyr_{960}, have been deleted. The mutant receptor bound insulin normally but exhibited an increased K_m for ATP during autophosphorylation. Upon prolonged incubation in vitro, or at high ATP concentrations such as those observed in vivo, autophosphorylation of $IR_{\Delta 960}$ was similar to wild type, and the in vitro phosphotransferase activity of the autophosphorylated $IR_{\Delta 960}$ was normal. These results suggest that the deletion did not cause a nonspecific structural disruption of the catalytic domain of $IR_{\Delta 960}$. In vivo autophosphorylation of the $IR_{\Delta 960}$ receptor was reduced by 30% after 2 min of insulin stimulation and was similar to the wild-type receptor after 30 min of insulin stimulation. However, the mutant receptor was defective in insulin-stimulated tyrosyl phosphorylation of the endogenous substrate pp185. In additon, $IR_{\Delta 960}$ was deficient in mediating insulin stimulation of glycogen and DNA synthesis. Thus, autophosphorylation of the insulin receptor is necessary but not sufficient for signal transmission. These data extend the hypothesis that the cytoplasmic juxtamembrane region of the insulin receptor is important for its interactions with ATP, intracellular substrates, and other proteins and is broadly necessary for biological signal transmission.

Insulin binding to the insulin receptor causes the autophosphorylation and activation of its β-subunit tyrosyl kinase (Kahn & White, 1988). In intact cells, the tyrosine kinase is responsible for the phosphorylation of endogenous substrates such as pp185 (White et al., 1985). Inactivation of the receptor by mutagenesis of its ATP binding site abolishes both receptor autophosphorylation and biological activity, suggesting that the kinase is essential for signal transmission (Chou et al., McClain et al., 1987). However, in mutant receptors from which tyrosyl phosphorylation sites required for full activation have been removed, some biological responses are retained despite reduced kinase activity (Ellis et al., 1986; Wilden et al., 1990). In light of these studies, as well as the insulin-mimetic properties of anti-receptor antibodies which weakly stimulate receptor autophosphorylation, the relation between kinase activity and biological activity of the insulin receptor remains unclear (Sung et al., 1989).

Previous studies in our laboratory suggest that the juxtamembrane region of the insulin receptor β-subunit, located just beyond the membrane-spanning region, is important for biological signaling. Conservative point mutations in this region had no effect on receptor autophosphorylation yet blocked endogenous substrate phosphorylation and biological activity (White et al., 1988). In addition, we have shown that the deletion of 12 amino acids (A954-D965) from this region results in a mutant receptor ($IR_{\Delta 960}$) which is markedly de-

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* Address correspondence to this author at the Research Division, Joslin Diabetes Center, One Joslin Place, Boston, MA 02215.
‡ Brigham and Women's Hospital and Harvard Medical School.
§ Max Planck Institut fur Biochemie.

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