Weight loss in rats following intraventricular transplants of pancreatic islets

Richardson, Ralph D.; Ramsay, Douglas S.; Lernmark, Åke; Scheurink, Anton J.W.; Baskin, Denis G.; Woods, Stephen C.

Published in:
The American Journal of Physiology - Regulatory, Integrative and Comparative Physiology

Weight loss in rats following intraventricular transplants of pancreatic islets

RALPH D. RICHARDSON, DOUGLAS S. RAMSAY, ÅKE LERNMARK,
ANTON J. W. SCHEURINK, DENIS G. BASKIN, AND STEPHEN C. WOODS
Departments of Psychology, Orthodontics, Pediatric Dentistry, Medicine, and Biological Structure at the University of Washington, Seattle 98195; and the Seattle Veterans Affairs Medical Research Center, Seattle, Washington 98108

Richardson, Ralph D., Douglas S. Ramsay, Åke Lernmark, Anton J. W. Scheurink, Denis G. Baskin, and Stephen C. Woods. Weight loss in rats following intraventricular transplants of pancreatic islets. *Am. J. Physiol.* 266 (*Regulatory Integrative Comp. Physiol.* 35): R59–R64, 1994.— Because of the body's resistance to permanent weight change, obesity remains a major health problem in modern society. It is hypothesized that the regulatory system defending body weight utilizes pancreatic insulin as an indicator of adiposity to the brain. To take advantage of this negative feedback system, we transplanted neonatal (*experiment 1*) or adult (*experiment 2*) pancreatic islets containing insulin-secreting cells into the 3rd ventricle of syngeneic Lewis rats. This resulted in an elevation of the insulin signal within the brain and a significant long-term reduction of body weight. Changes in food intake were consistent with the changes of body weight. The implantation of more islets resulted in a greater reduction of body weight, and changes in weight were inversely correlated with the level of insulin achieved in the cerebrospinal fluid. After the two studies were completed, histological examination revealed the presence of insulin-containing cells within the 3rd ventricle and adjacent hypothalamus. These studies suggest that transplanted insulin-secreting cells may provide a potential therapeutic strategy for maintenance of weight loss.

brain insulin; insulin; food intake; transplantation

OBESITY AND ITS ASSOCIATED medical and social consequences present a major health problem to modern society (6, 26, 29). Despite numerous experimental and therapeutic approaches that have been applied to reduce the incidence and severity of obesity over the past 50 yr, the fact remains that most overweight individuals are likely to remain so throughout their adult lives (6, 11, 26, 29). One popular explanation is that adiposity appears to be tightly regulated; i.e., behavioral and physiological controls exist that maintain body fat at some predetermined level or "set point." Hence, attempts to lower weight and maintain weight loss through such diverse means as dieting, behavioral modification, use of anorexigenic drugs, lipectomy, or surgery designed to reduce the absorptive area of the gut all work against this robust innate control system. One consequence is that over time, individuals who lose weight by any of these means tend to regain it. The converse is also true because the regulatory system controlling body weight is symmetrical. When excess fat is experimentally induced, and subjects are subsequently allowed to feed freely, food intake is reduced until adiposity returns to normal levels (5, 25).

The presumption underlying all of these findings is that a signal proportional to body fat interacts with fat-controlling systems in the brain to contribute to the homeostatic maintenance of weight. When an individual is below his or her normal or defended level of adiposity, the adiposity-regulating areas of the brain presumably receive a reduced or altered adiposity feedback signal that serves to increase appetite and adjust metabolism until fat is restored (21, 30). An obvious strategy to lower body weight would therefore be to elevate the adiposity signal experimentally (or therapeutically) without changing the actual fat content of the body and thereby activate intrinsic adiposity-reducing mechanisms.

Although the nature of the adiposity signal is poorly understood (26), there is compelling evidence from studies on parabiotic animals to suggest that an adiposity feedback signal circulates in the blood and that its administration to animals results in reduced food intake and weight loss (9, 13). The pancreatic hormone insulin is a strong candidate for the elusive adiposity-related signal (21, 29, 30). Insulin is secreted into the blood in direct proportion to the degree of adiposity (25), and a specific transport system exists that passes insulin from the blood into the brain (20, 23). Within the brain, specific insulin receptors are found on neurons in areas known to be important in the control of food intake and body weight (3, 10), and the synthesis of at least one brain neuropeptide important in feeding, neuropeptide Y, appears to be under the direct control of brain insulin levels (22, 24).

When exogenous insulin is administered directly into the central nervous system, it elicits decreased feeding and body weight. This phenomenon has been demonstrated in numerous species, including rats (1, 7, 14, 17), baboons (31), and marmots (12). The reductions of food intake and body weight are dose dependent (7, 31) and reliant on insulin administration into appropriate central locations because some sites appear nonresponsive (17). The most robust demonstrations of the phenomenon have occurred when insulin is administered into the 3rd ventricle (1, 7, 14) or directly into the ventral hypothalamus of rats (17).

In the past, central administration of insulin has been accomplished via cannulation of the central nervous system coupled with continuous or intermittent administration of insulin from an exogenous source. Although the suppression of food intake and/or body weight elicited by the infusion of exogenous insulin does not reverse immediately when the infusion is stopped (7, 31), these parameters do return to control levels within

a few days. In an attempt to establish a long-term and potentially therapeutically useful method for introducing insulin into the brain and hence causing a chronic reduction in body adiposity, we have developed a method for transplanting viable insulin-secreting pancreatic cells into the ventricular system of rats. The results suggest that such cells thrive and secrete insulin locally within the brain and that this may provide a strategy to achieve permanent weight loss.

METHODS

General Procedures

Surgical procedures. For third ventricular cannulation, rats were anesthetized with Equithesin (3.0 ml/kg ip) and placed in a stereotaxic apparatus (Kopf). The animal's head was adjusted in the horizontal plane so that bregma and lambda had the same dorsal-ventral coordinate. A 22-gauge guide cannula with a 28-gauge internal obturator (Plastics One, Roanoke, VA) was lowered ventrally at coordinates 7.2 mm anterior to the interaural line and on the midline. The tip was lowered 7.0 mm below the cortical surface directly ventral to the midsagittal sinus. To accomplish this, the midsagittal sinus was retracted laterally and then allowed to settle against the guide cannula. The cannula was anchored to the skull with dental acrylic and stainless steel anchor screws.

Animals received a prophylactic injection of gentamicin (300,000 U/ml im; 0.2 ml/rat) immediately after surgery. All subjects were allowed to recover to presurgery body weight before receiving experimental or control treatments. To confirm placement of the cannulas, rats were injected with 4 μl of 10 μg/ml angiotensin II (ANG II). To be eligible for inclusion in the transplantation studies, a rat had to consume at least 5 ml of water within 30 min after the injection intracranially of the ANG II.

Islet preparation. Pancreatic islets were isolated from neonatal (*experiment 1*) or adult (*experiment 2*) Lewis rats after collagenase digestion of the pancreas and separation of the islets from the exocrine cells by Percoll gradient centrifugation as previously described (8). The islets were suspended in RPMI-1640 culture medium (GIBCO, Grand Island, NY) supplemented with 10% (wt/vol) heat-inactivated fetal calf serum (GIBCO), 20 mM *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid, 2 mM/L-glutamine, 24 mM Na-HCO_3, 100 U/ml penicillin, and 100 μg/ml streptomycin (RPMI medium) and individually selected under a stereomicroscope. The yield was ~100 islets/pancreas. After culture overnight in RPMI medium at 37°C in a humidified atmosphere of 5% CO_2-95% air, healthy-appearing islets were carefully reselected for implantation under the stereomicroscope before being implanted.

Islet implantation. Individual pancreatic islets were counted through the tip of an injection cannula into polyethylene tubing (PE-20, Clay Adams) filled with synthetic cerebrospinal fluid (CSF) and attached to a microsyringe (Hamilton). Rats were anesthetized with halothane and received islets or control solution into the 3rd ventricle. The total volume necessary to collect and infuse the islets never exceeded 12 μl in any given condition and was administered over an interval of ~6 min. Penicillin G (Bicillin, 300,000 U/ml) was administered (0:2 ml/rat im) immediately after each procedure.

Dependent measures. Body weight was measured each day for the duration of the experiment. Because food intake was depressed for several days after the combined surgical and implantation procedures, we do not report values until they had stabilized (3–4 days). At the end of the experiment, continuing animals were anesthetized with ketamine (100 mg/kg ip) and cisternal CSF samples were taken as previously described (27). Blood samples were taken by cardiac puncture. The rat was then perfused intracardially with saline, and the brain was removed and fixed in Bouin-Hollande solution for subsequent immunocytochemical analysis. Blood was centrifuged and plasma was frozen at −20°C until assayed for immunoreactive insulin (IRI) by a modification of the double-antibody method (18) and for glucose by the glucose oxidase method. Cell-free CSF samples were frozen, and IRI was assayed by the highly sensitive method of Baskin et al. (4).

Histology. Brains were embedded in paraffin, and serial 5 μm-thick coronal sections were mounted on slides. After removal of paraffin and hydration, slides were placed in distilled water for 30 min at room temperature (RT). Sections were covered with 0.5-ml drops of immunostaining reagents, starting with 1% normal sheep serum in tris(hydroxymethyl)-aminomethane (Tris) buffer (0.5 m) containing 0.9% NaCl for 30 min at RT. After a rinse in Tris buffer saline (TBS), they were covered with undiluted mouse monoclonal antibody to insulin (AM029-5M, Biogenex Laboratories, San Ramon, CA) for 48 h at 6°C in a covered humid chamber. They were then rinsed in TBS and covered with biotinylated anti-mouse immunoglobulin (Ig)G (Biogenex) for 20 min (RT), followed by a TBS rinse and alkaline phosphatase-streptavidin conjugate (20 min; RT) from a Biogenex StrAviGen detection kit. Alkaline phosphatase was detected with a fast red chromogen system (Biogenex) for 5 min at RT, and the sections were cover slipped with glycerol. Control sections were treated with nonimmune mouse IgG. As a positive control, we demonstrated that this procedure intensely stained β-cells in the islets of rat pancreas sections that were measured in parallel with the brain sections.

Statistics. All data were analyzed with *t* tests or repeated measures analysis of variance.

Experiment 1

Adult male syngeneic Lewis rats (Simonsen) weighing ~300 g served as subjects. Animals were individually housed in standard stainless steel hanging cages in a light- and temperature-controlled room. Food (Wayne pellets) and water were available ad lib.

Islets were provided by neonatal Lewis rats. There were two implantations of islets (or control solution), the first (50 islets) occurring 1 wk after cannula implantation and the second (100 additional neonatal islets) occurring 27 days later. The animals were monitored for an additional 30 days after the second implantation.

Control animals received one of three treatments: an equivolumetric third ventricular injection of the islet culture medium diluted with synthetic CSF (the vehicle); bilateral ventromedial hypothalamic injections (27-gauge guide cannula with a 30-gauge internal cannula at coordinates: anteroposterior (AP) 5.8, dorsoventral (DV) −3.5, and 1.0 mm lateral from midline) of the vehicle mixture. This site is reported to be particularly sensitive to local insulin administration (17). The final site received a bilateral injection of 50 pancreatic islets into the lateral hypothalamic area at coordinates AP 5.0, DV −3.0, and 2.0 mm lateral from midline. This area is reported not to be responsive to local application of insulin (17). Only the first of these three control groups was continued to the end of the experiment. The others were terminated at the time of the second implantation.

Experiment 2

Male Lewis rats (Simonsen) weighing 250–300 g served as subjects. Animals were maintained on ad lib water and pelleted

diet before islet or control implantation. After implantation they were given ad lib water plus the liquid diet, Ensure (Ross Laboratories, Columbus, OH) to determine if the phenomenon might be enhanced with a more palatable diet.

For this experiment, the identical procedure used in *experiment 1* was followed except that the islets were obtained from adult rather than neonatal donors. Experimental animals received one implantation of 150 islets into the 3rd ventricle. Control animals received an isovolumetric injection of the incubation medium for the islets into the 3rd ventricle (as in the previous study) or an isovolumetric injection of a suspension of small pieces of exocrine tissue into the 3rd ventricle. The pieces of exocrine tissue were harvested from the same pancreas as the endocrine islets. Their size was comparable with that of average islets, and they were treated identically as the endocrine islets.

RESULTS

Experiment 1

Animals in the three control conditions (n = 11) did not differ significantly on any dependent measure and were combined for subsequent analyses. Rats receiving islets into the 3rd ventricle (n = 5) had a significant and persistent reduction of body weight relative to controls that became apparent within 2 days of receiving islets [F (1, 14) 12.70, $P < 0.01$, Fig. 1]. Food intake was also reduced but was more variable and did not attain statistical significance.

The effect of receiving the additional 100 islets is depicted in Fig. 2. It is important to note that only one control condition (n = 4) is included in this part of the experiment and that the absolute weights of the two groups differed on the day of the second transplantation: *day 0* (controls 432.4 ± 20.2 g vs. islet group 407.5 ± 4.8 g; NS). Experimental animals receiving islets (n = 5) experienced an additional reduction of weight relative to controls, and the controls continued to weigh more than the transplanted animals for the duration of the study (F (1, 9) 5.11, $P < 0.05$, one tailed).

Glucose and IRI. The number of rats with sufficient CSF and plasma samples for assay at the end of 57 days was low (exptl n, 5; control n, 3). Hence, comparisons between two groups were difficult. Median plasma glucose was comparable for the experimental and control animals when killed (control rats median, 123 mg/dl; experimental rats median, 121 mg/dl). Median plasma IRI levels were also not different (control rats median, 10 μU/ml; experimental rats median, 10 μU/ml). Despite the few number, experimental rats had elevated CSF IRI (0.73 ± 0.09 vs. 0.47 ± 0.03 (SE) μU/ml; t = 2.16, $P < 0.05$, by one-tailed t test) relative to controls. Importantly, the change of weight over the course of the experiment was inversely correlated with CSF IRI for these rats when killed ($r = -0.75$, df = 6, $P < 0.05$).

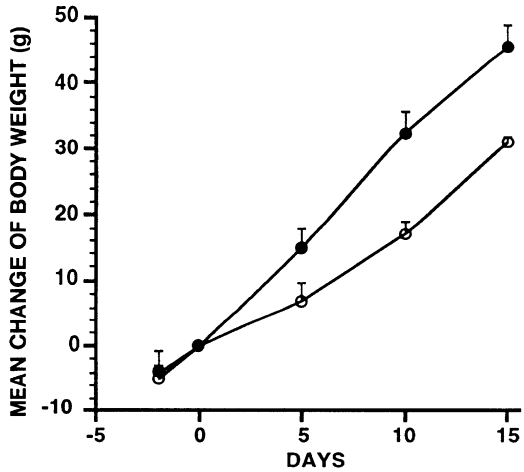

Fig. 1. Mean + SE change of body weight for control rats (●, n = 11) and rats receiving 3rd ventricular islets (○, n = 5) over first 15 days of *experiment 1*.

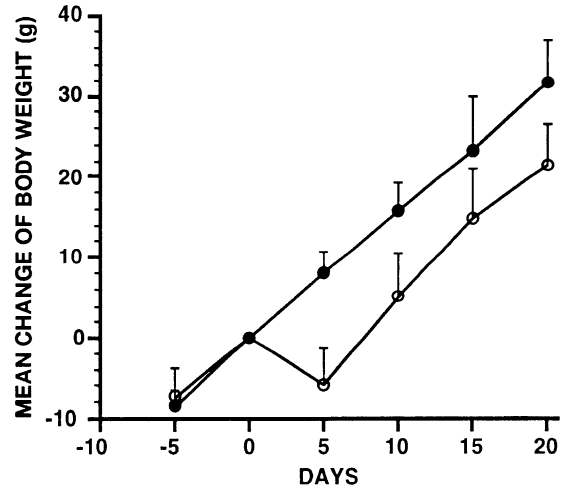

Fig. 2. Mean + SE change of body weight for control rats (●) and rats receiving additional 100 transplanted islets 37 days after initial transplant (○). Absolute weights of 2 groups differed on day of transplantation (*day 0*). Only 1 subset of control rats depicted in Fig. 1 was continued for this part of experiment.

Histology. Histological examination of the brains by light microscopy revealed viable transplanted cells within close proximity to the 3rd ventricle of experimental animals. Immunocytochemical analysis indicated that these cells contained IRI-like substance. The goal in this analysis was to identify the presence of insulin-containing cells within the the hypothalamus. Although a detailed mapping of the location was not made, some generalizations can be made. Insulin-positive cells were attached to the ependymal lining of the floor and walls of the 3rd ventricle of experimental rats. In some experimental rats, other insulin-positive cells appeared to have invaginated into the neuropil in the arcuate-median eminence area (Fig. 3). No insulin-positive cells were observed in vehicle-injected control animals.

Experiment 2

There was no reliable difference between the two control groups (n = 7) on any measure. Therefore the two groups were combined for the analyses. As seen in Fig. 4, the effect of receiving 150 adult islets into the 3rd ventricle on body weight (n = 8) was comparable with what was observed in *experiment 1*. Animals receiving 150 adult islets of Langerhans significantly reduced their body weights relative to controls [F (1, 13) 6.95, $P < 0.05$]. The effect on body weight was persistent

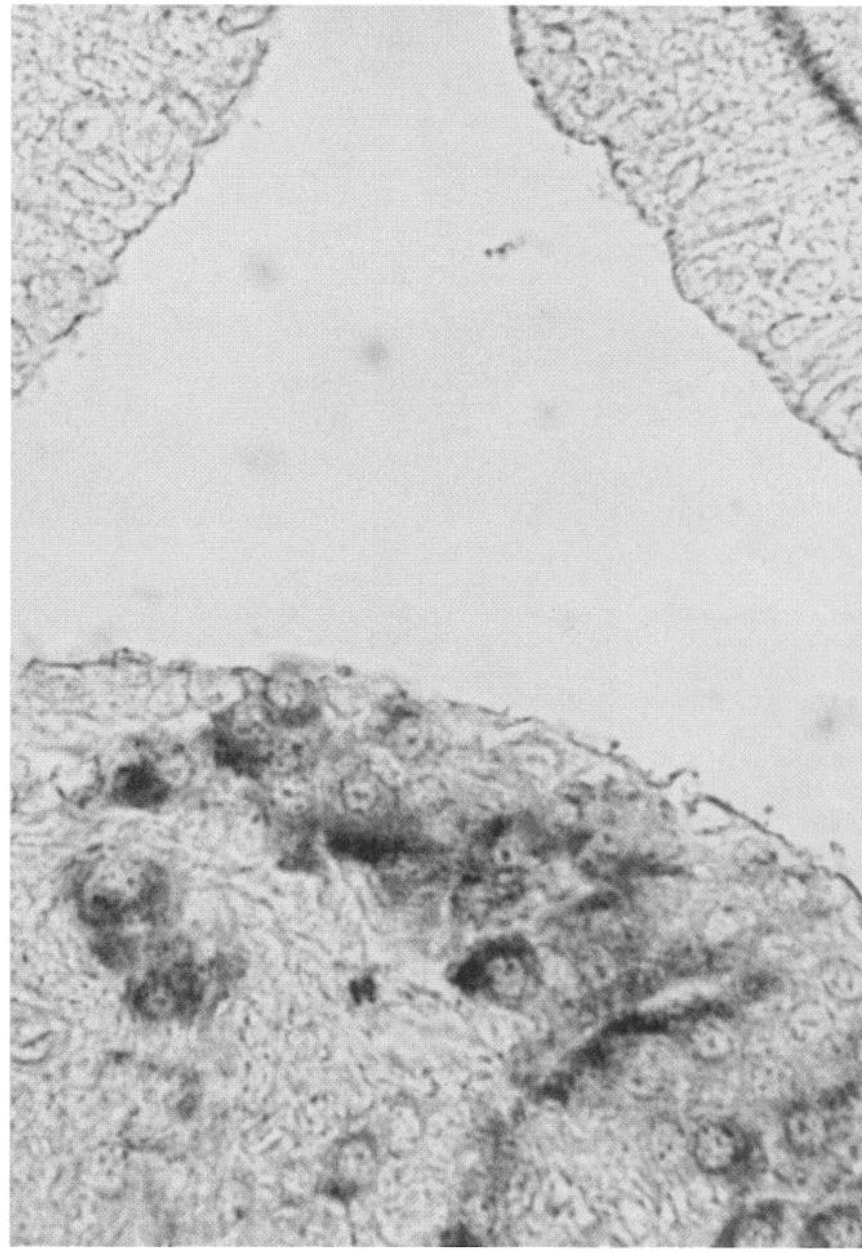

Fig. 3. Section through floor of 3rd ventricle of rat that received a total of 150 transplanted islets (magnification ×400). Section was immunostained to reveal insulin-containing cells (darker appearing). Insulin-containing cells are located within arcuate nucleus.

such that the values for experimental animals paralleled those for the controls for the duration of the experiment.

As in *experiment 1,* food intake was quite variable. The experimental and control rats were not different before the transplantation procedure (islet group, 30.9 ± 5.5 g diet; controls, 26.9 ± 2.1 g diet, NS). On the 5th day post-transplantation, which was the 1st day that food intake was recorded, animals receiving islets consumed 69.7 ± 6.5 ml Ensure and controls consumed 84.4 ± 3.1 ml [t (11) 2.04, $P < 0.05$). Thereafter, the differences lessened and did not attain significance on any other individual day.

At the termination of the experiment rats with transplanted islets had slightly higher CSF IRI than controls (2.12 ± 0.22 and 1.67 ± 0.33 μU/ml, respectively, NS). All brains appeared healthy at autopsy, and as was the case in *experiment 1,* light microscopy revealed the presence of transplanted cells within the 3rd ventricle of experimental but not control animals. Because of the accidental thawing of a freezer, subsequent immunohistochemical analysis could not be performed on these samples.

DISCUSSION

Although the total number of subjects in each experiment is small, the results of the two experiments are consistent. Animals that received transplanted islets into the 3rd ventricle had a persistent and significant reduction of body weight. They maintained a weight that was reduced yet parallel to that of controls over time. Food intake data were consistent but far more variable. In both experiments animals receiving islets

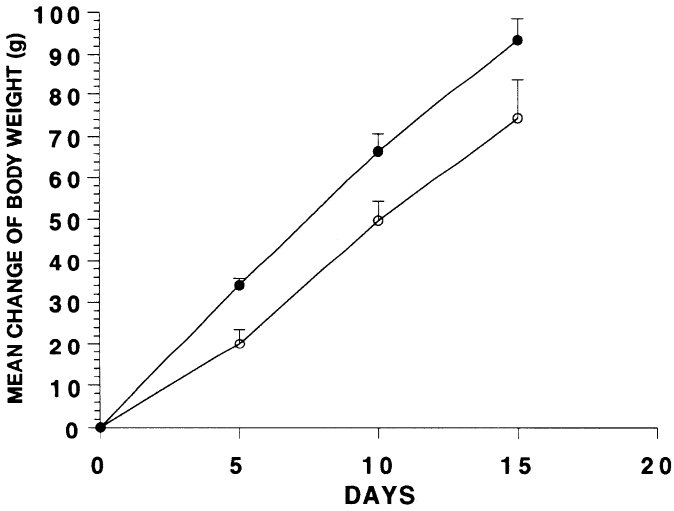

Fig. 4. Mean + SE change of body weight for control rats (●, $n = 7$) and rats receiving 150 3rd ventricular adult islets (○, $n = 8$) over first 15 days of *experiment 2*.

had reduced intake relative to controls, but the differences reached significance only in *experiment 2* and for only a brief interval after the transplantation procedure.

In preliminary studies not reported here but addressing the same question (19), Long-Evans rats served as subjects. In those studies experimental animals received third ventricular injections of up to 150 islets isolated from neonatal Wistar rats. Although the food intake and body weight of animals receiving islets became significantly reduced relative to controls, the effect peaked within the 1st wk and began to diminish by the 2nd wk of the study. Histological examination after 2 wk failed to reveal viable insulin-containing granules in the brains of experimental animals, and the presumption was that the transplanted cells had been rejected by the host. We therefore utilized syngeneic Lewis rats for the present studies.

Successful central nervous system (CNS) transplantation of pancreatic islets has been previously reported (16, 28). In those studies, large numbers of islets ($\sim$2,500) were transplanted into the lateral ventricles of streptozotocin-diabetic rats in an attempt to reduce the degree of glucose intolerance. The transplanted cells were functional and viable for $>$175 days. Similar to what we observed in *experiment 1,* histological examination of the transplant site revealed cells that were well vascularized, that had invaginated into the neuropil, and that stained positive for insulin. It is noteworthy that reductions of body weight were not observed in those experiments. However, in those experiments the islets had been implanted into the lateral ventricles, and it has been reported that in rats, third (1, 7, 14) but not lateral (15) ventricular infusions of insulin reduce food intake and body weight. Furthermore, all treated rats had severe diabetes mellitus and were underweight relative to controls before the transplantations. The present results are therefore consistent with the earlier findings that islets transplanted into the CNS survive for long intervals and that third ventricular administration of insulin into otherwise normal animals is effective at reducing body weight. Considering the stable and long duration of weight loss obtained with the syngeneic rats, there is every indication that the transplanted islets might survive indefinitely and that the weight loss might be maintained.

It is noteworthy that rats receiving islets had elevated CSF IRI, although with the few number of subjects the difference was only significant in *experiment 1.* Although the absolute magnitude of the difference was small, it was apparently effective because CSF insulin levels when the rats were killed were inversely correlated with weight gain in *experiment 1.* Furthermore, there is now compelling evidence that CSF insulin values are lower than what is actually seen by neurons in the CNS (20, 21, 23). This is important because many insulin-secreting cells in the present experiment were actually found to reside within the neuropil of the ventral hypothalamus. Interstitial fluid insulin levels might have been quite high in some regions of the ventral hypothalamus, and this area is known to be responsive to the suppressive effects of insulin on food intake (17). The absolute level of CSF IRI in all rats was higher in *experiment 2* than in *experiment 1.* This is presumably because of the diet (Ensure) of the rats in the second experiment relative to the first.

Although the decrease of body weight observed in the present experiment was modest, it was highly reliable and sensitive to critical manipulations. In *experiment 1,* the decrease in weight was enhanced when additional islets were implanted. In *experiment 2,* comparable results were obtained with a different diet and with use of adult instead of neonatal islets. Every indication is that the islets were viable and releasing insulin and that this is what caused the observed results.

It is not clear what constitutes an appropriate control condition for studies of this type. In the present experiments, we administered the incubation medium as well as islet-sized pieces of exocrine pancreas into the 3rd ventricle. We also administered islets into an area of the brain demonstrated to be insensitive to exogenous insulin. None of these conditions altered the feeding or growth curves of the rats, and implantation of viable islets into the 3rd ventricle resulted in reduced body weight relative to all of these.

These studies confirm and are in agreement with past experiments that have demonstrated the ability of exogenous insulin to lower an animal's body weight (1, 7, 12, 14, 17, 31). In past studies, intraventricular insulin has been administered via infusion pumps (31), osmotic pumps (1, 7, 14), or by repeated acute injections (22, 24). Because all of the above-mentioned systems of elevating CNS insulin levels are temporally limited, the termination of each administration regimen typically returns animals to preinfusion body weight within a short period of time. In summary, these studies provide evidence that central transplants of pancreatic islets might provide a means of chronically exposing the brain to elevated insulin levels and thus cause a decrease in body weight that is maintained.

We thank William Hogopian, Collin Park, Cindy Wilson, Tim Boswell, Daniel Porte, Jr., and Jon Kott for assistance. The immuno-

chemistry was done with the technical assistance of Martha DuRuz and Chore Vathanaprida. The Diabetes/Endocrinology Research Center Cell Sort Satellite provided the islets of Langerhans.

This work was supported by National Institute of Diabetes and Digestive and Kidney Diseases Grants DK-17844, DK-12829, and DK-17047, and National Institute of Dental Research Grant DE-00161.

Address for reprint requests: R. D. Richardson, Dept. of Psychology NI-25, Univ. of Washington, Seattle, WA 98195.

Received 5 March 1993; accepted in final form 18 June 1993.

REFERENCES

1. **Arase, K., J. S. Fisler, N. S. Shargill, D. A. York, and G. A. Bray.** Intracerebroventricular infusions of 3-OHB and insulin in a rat model of dietary obesity. *Am. J. Physiol.* 255 (*Regulatory Integrative Comp. Physiol.* 24): R974–R981, 1988.
2. **Bagdade, J. D., E. L. Bierman, and D. Porte, Jr.** The significance of basal insulin in the evaluation of the insulin response to glucose in diabetic and non-diabetic subjects. *J. Clin. Invest.* 46: 1549–1557, 1967.
3. **Baskin, D. G., D. P. Figlewicz, S. C. Woods, D. Porte, Jr., and D. M. Dorsa.** Insulin in the brain. *Annu. Rev. Physiol.* 49: 335–347, 1987.
4. **Baskin, D. G., D. Porte, Jr., K. Guest, and D. Dorsa.** Regional concentrations of insulin in the rat brain. *Endocrinology* 112: 898–903, 1983.
5. **Bernstein, I. L., E. C. Lotter, P. J. Kulkosky, D. Porte, Jr., and S. C. Woods.** Effect of force-feeding upon basal insulin levels of rats. *Proc. Soc. Exp. Biol. Med.* 150: 546–548, 1975.
6. **Bray, G. A.** *The Obese Patient.* Philadelphia: Saunders, 1976.
7. **Brief, D. J., and J. D. Davis.** Reduction of food intake and body weight by chronic intraventricular insulin infusion. *Brain Res. Bull.* 12: 571–575, 1984.
8. **Brunstedt, J., J. H. Nielsen, Å. Lernmark, and the Hagedorn Study Group.** Isolation of islets from mice and rats. In: *Methods in Diabetes Research,* edited by J. Larner and S. Pohl. New York: Wiley, 1984, vol. 1, pt. C, 245–258.
9. **Coleman, D. L., and K. P. Hummel.** Effects of parabiosis of normal with genetically diabetic mice. *Am. J. Physiol.* 217: 1298–1304, 1969.
10. **Corp, E. S., S. C. Woods, D. Porte, Jr., D. M. Dorsa, D. P. Figlewicz, and D. G. Baskin.** Localization of 125-I-insulin binding sites in the rat hypothalamus by quantitative autoradiography. *Neurosci Lett.* 70: 17–22, 1986.
11. **Drenick, E. J., and D. Johnson.** Weight reduction by fasting and semistarvation in morbid obesity: long-term follow-up. In: *Obesity: Comparative Methods of Weight Control,* edited by G. A. Bray. Westport, CT: Technonomic, 1980, p. 25–44.
12. **Florant, G. L., L. Singer, A. J. W. Scheurink, C. R. Park, R. D. Richardson, and S. C. Woods.** Intraventricular insulin reduces food intake and body weight of marmots during the summer feeding period. *Physiol. Behav.* 49: 335–338, 1991.
13. **Hervey, G. R.** The effects of lesions in the hypothalamus in parabiotic rats. *J. Physiol. Lond.* 145: 336–352, 1952.
14. **Ikeda, H., D. B. West, J. J. Pustek, D. P. Figlewicz, M. R. C. Greenwood, D. Porte, Jr., and S. C. Woods.** Intraventricular insulin reduces food intake and body weight of lean but not obese zucker rats. *Appetite* 7: 381–386, 1986.
15. **Manin, M., M. Balage, C. Larue-Achagiotis, and J. Grizard.** Chronic intracerebroventricular infusions of insulin failed to alter brain insulin-binding sites, food intake, and body weight. *J. Neurochem.* 51: 1689–1695, 1988.
16. **McEvoy, R. C., and P. E. Leung.** Transplantation of fetal islets into the cerebral ventricles of alloxan-diabetic rats. *Diabetes* 32: 852–857, 1983.
17. **McGowan, M. K., K. M. Andrews, J. Kelly, and S. P. Grossman.** Effects of chronic intrahypothalamic infusion of insulin on food intake and diurnal meal patterning in the rat. *Behav. Neurosci.* 104: 373–385, 1990.
18. **Porte, D., Jr., A. L. Graber, T. Kuzuya, and R. H. Williams.** The effect of epinephrine on immunoreactive insulin levels in man. *J. Clin. Invest.* 45: 228–236, 1966.
19. **Ramsay, D. S., R. D. Richardson, J. Kott, Å. Lernmark, and S. C. Woods.** Intraventricularly transplanted pancreatic islets reduce body weight of rats (Abstract). *Appetite* 12: 233, 1989.
20. **Schwartz, M. W., R. N. Bergman, S. E. Kahn, G. J. Taborsky, Jr., L. D. Fisher, A. J. Sipols, S. C. Woods, G. M. Stein, and D. Porte, Jr.** Evidence for entry of plasma insulin into cerebrospinal fluid through an intermediate compartment in dogs. *J. Clin. Invest.* 88: 1271–1281, 1991.
21. **Schwartz, M. W., D. P. Figlewicz, D. G. Baskin, S. C. Woods, and D. Porte, Jr.** Insulin in the brain: a hormonal regulator of energy balance. *Endocr. Rev.* 13: 1–27, 1992.
22. **Schwartz, M. W., J. L. Marks, A. J. Sipols, D. G. Baskin, S. C. Woods, S. E. Kahn, and D. Porte, Jr.** Central insulin administration reduces neuropeptide Y mRNA expression in the arcuate nucleus of food-deprived lean (*Fa/Fa*) but not obese (*fa/fa*) Zucker rats. *Endocrinology* 128: 1081–1083, 1991.
23. **Schwartz, M. W., A. J. Sipols, S. E. Kahn, D. F. Lattemann, G. J. Taborsky, Jr., R. N. Bergman, S. C. Woods, and D. Porte, Jr.** Kinetics and specificity of insulin uptake from plasma into cerebrospinal fluid. *Am. J. Physiol.* 259 (*Endocrinol. Metab.* 22): E378–E383, 1990.
24. **Schwartz, M. W., A. J. Sipols, J. L. Marks, G. Sanacora, J. D. White, A. J. W. Scheurink, S. E. Kahn, D. G. Baskin, S. C. Woods, D. P. Figlewicz, and D. Porte, Jr.** Inhibition of hypothalamic neuropeptide Y gene expression by insulin. *Endocrinology* 130: 3608–3616, 1992.
25. **Sims, E. A. H.** Experimental obesity, dietary-induced thermogenesis, and their clinical implications. *Clin. Endocrinol. Metab.* 5: 377–395, 1976.
26. **Stallone, D. D., and A. J. Stunkard.** The regulation of body weight: evidence and clinical implications. *Ann. Behav. Med.* 13: 220–230, 1991.
27. **Stein, L. J., D. M. Dorsa, D. G. Baskin, D. P. Figlewicz, D. Porte, Jr., and S. C. Woods.** Reduced effect of experimental peripheral hyperinsulinemia to elevate CSF insulin concentrations of obese Zucker rats. *Endocrinology* 121: 1611–1615, 1987.
28. **Tze, W. J., and J. Tai.** Successful intracerebral allotransplantation of purified pancreatic endocrine cells in diabetic rats. *Diabetes* 32: 1185–1187, 1983.
29. **Woods, S. C., E. Decke, and J. R. Vasselli.** Metabolic hormones and regulation of body weight. *Psychol. Rev.* 81: 26–43, 1974.
30. **Woods, S. C., D. P. Figlewicz-Lattemann, M. W. Schwartz, and D. Porte, Jr.** A reassessment of the regulation of adiposity and appetite by the brain insulin system. *Int. J. Obesity* 14, *Suppl.* 3: 69–76, 1990.
31. **Woods, S. C., E. C. Lotter, L. D. Mckay, and D. Porte, Jr.** Chronic intracerebroventricular infusion of insulin reduces food intake and body weight of baboons. *Nature Lond.* 282: 503–505, 1979.