Role of the Oropharynx in Regulation of Glycemia

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SUMMARY

Previous studies have demonstrated that reflexes originating from the oral cavity at the start of food intake are necessary to ensure a normal glucose tolerance. In our experiment, the underlying mechanisms of these reflexes were studied in conscious, freely moving rats bearing chronic catheters. A double-isotope technique was used to measure, under non-steady-state conditions, rates of total glucose appearance (total R_a), total glucose disappearance (R_d), gut glucose absorption (gut R_a), hepatic glucose production (HGP), and the metabolic clearance rate of glucose (MCR_g). In random order, 1 wk apart, rats either spontaneously drank 1 ml of a 60% glucose solution or were given the same dose into the stomach via a chronic gastric catheter. Glycemia and insulinemia were lower when glucose was taken orally than when the same amount of the substrate was administered intragastrically. Total R_a after glucose administration was the same in both groups throughout the experiment. Despite lower insulin and glucose values, the increase in R_d was initially higher in the oral group than in the intragastric group. This was accompanied by initial higher MCR_g values in the oral group than in animals that received the glucose load directly into their stomachs. We conclude that a series of reflexes elicited by oral glucose ingestion improve glucose tolerance by increasing the efficiency of glucose disposal in the early stages after a glucose load, with a smaller amount of insulin released. *Diabetes* 36:791–95, 1987

Previous studies have demonstrated that when a meal rich in carbohydrates is administered directly into the stomach, bypassing the mouth, the resulting insulin and glucose levels are higher than those measured when the same meal is spontaneously ingested (1). These studies further showed that one difference between the ingestion and the intragastric administration of the meal was the presence, after the orally ingested meal, of a cephalic phase of insulin release. This neurally mediated insulin secretion occurs before the appearance of any ingested glucose in the blood and is thought to represent a reflex with an afferent limb consisting of olfactory, visual, gustatory, and oropharyngeal receptors; central integrating units in the central nervous system; and an efferent limb involving the vagus nerve, whose fibers modulate the overall endocrine pancreas secretory activity (2,3). Thus, it has been postulated but not demonstrated that insulin released reflexly before the start of absorption improves glucose tolerance by facilitating and accelerating postabsorptive glucose uptake (4,5). In partial agreement with such a concept was the observation that the lack of a cephalic-phase insulin response resulted in a glucose tolerance that was significantly impaired when compared with that of animals with cephalic-phase insulin output (6).

Although these latter studies seemed to offer evidence that cephalic-phase insulin release elicited by the presence of food in the oral cavity could improve glucose tolerance, the mechanism(s) responsible for such improvement remained unknown. Furthermore, it is known that reflexes other than the cephalic-phase insulin secretion, such as those bearing on glucagon, gastric acid, or pancreatic polypeptide release (7,8), are also present at the start of food intake and could possibly play a role in the handling of the ingested nutrients.

The aim of our study was to determine the underlying mechanism(s) by which glucose taken orally results in an optimal glucose tolerance. For this purpose, the total rate of glucose appearance, the gut-derived rate of glucose appearance, hepatic glucose production, and the metabolic clearance rate of glucose were measured in normal conscious rats after either the spontaneous ingestion or intragastric administration (via preimplanted gastric catheter bypassing oral cavity) of an identical glucose load. This was made possible by a technique described in detail elsewhere (9–11) that permits (with two glucose tracers, [^{3}H]glucose and [^{14}C]glucose) determination of those parameters in non-

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Received for publication 24 July 1986 and accepted in revised form 26 January 1987.

steady-state conditions corresponding to those of the tests mentioned above.

The data presented here indicate that a series of reflexes, triggered by spontaneous glucose ingestion within the oropharynx, optimize glucose tolerance by improving peripheral glucose clearance.

MATERIALS AND METHODS

Animals. Fifteen male monozygotic lean (*FA/FA*) rats aged 13–15 wk and weighing 299 ± 5 g were used. The animals were bred in our laboratory and were fed throughout with a standard laboratory chow (12% water, 17% protein, 3% lipid, 59% carbohydrate, 4% cellulose, and 5% mineral; UAR laboratory chow, Epinay, Villemoisson, France). They were maintained at a constant temperature (22°C) with a fixed (12 h) artificial light cycle. At 13 wk of age, under Nembutal anesthesia (70 mg/kg i.p.), the rats were implanted with one gastric and two jugular catheters. The right jugular catheter was threaded to the heart and was used for blood sampling; the left jugular catheter was shorter and was used for infusion of tracer. Both catheters were made of Silastic. The gastric catheter was of polyethylene and was introduced via a laparotomy into the greater curvature of the stomach. This catheter was secured with a purse-string suture and tethered to the abdominal muscles via a second suture. All catheters were threaded subcutaneously to the back of the neck and fixed to the skull with acrylic cement as previously described (12,13). The jugular catheters were rinsed daily with 1 ml saline solution containing 25 U heparin and 20,000 U penicillin. The gastric catheters were rinsed with distilled water. Before being used for the experiments, the rats were allowed to recover from surgery for 10 days, at which time they had a normal food intake and were gaining weight. All experiments were carried out on 6- to 7-h food- and water-deprived animals. The food and water were removed in the morning (0800 h), and the experiments started between 1400 and 1500 h.

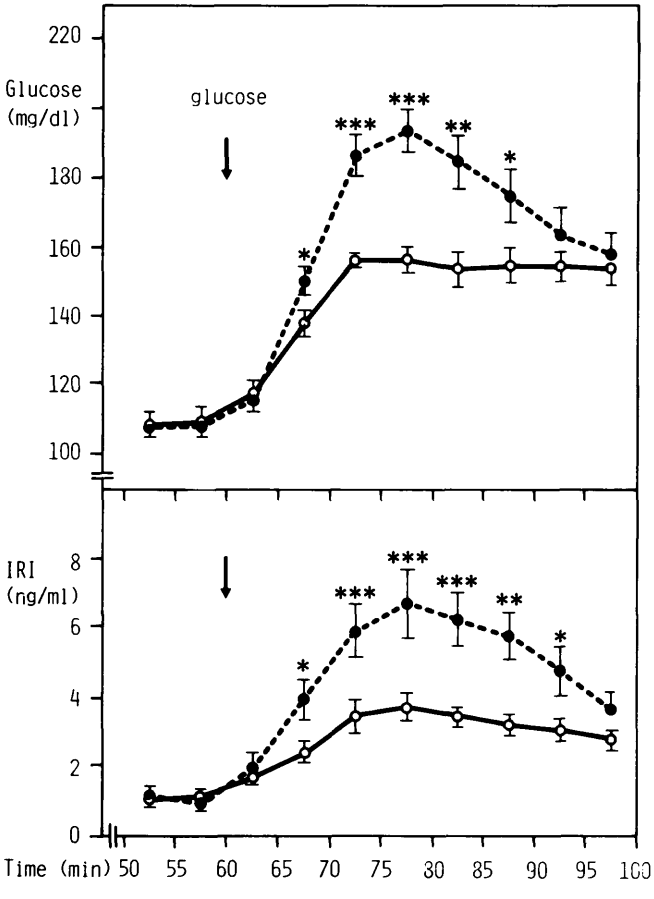

FIG. 1. Plasma glucose and immunoreactive insulin (IRI) levels before and after either spontaneous ingestion (○) or intragastric administration (via preimplanted catheter; ●) of 2 g/kg glucose (*arrow*) to normal freely moving rats. Ingested and intragastrically administered glucose was standardized with regard to both amount and delivery rate, as described in MATERIALS AND METHODS. Glucose and insulin values are means for each 5-min interval, because rates of glucose appearance or disappearance are measured over such time intervals. Statistical significance: *$P < .05$; **$P < .01$; ***$P < .001$.

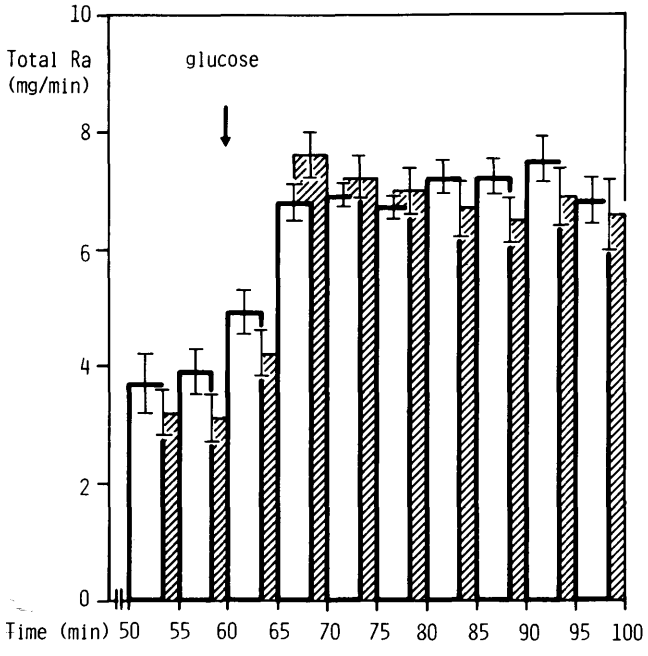

FIG. 2. Total rate of glucose appearance (total R_a) after either spontaneous ingestion (*open bars*) or intragastric administration (*hatched bars*) (via preimplanted catheter) of glucose (*arrow*) to normal freely moving rats.

Experimental design. After taking a baseline blood sample (210 μl), a primed constant infusion of [3-^{3}H]glucose (New England Nuclear, Boston, MA) was commenced at 15 μCi/h by a peristaltic pump (Gilson Minipulse 2, Villiers Le-Bel, France). The priming dose was given over the first 5 min; the infusion rate was 5 times the constant infusion rate for the first 2 min, 4 times for the second 2 min, and 3 times for the 5th min. This allowed tracer equilibrium to be reached at 50 min, after which the infusion continued to the end of the test (100 min). Beginning at 50 min, 210-μl blood samples were withdrawn every 5 min for measurement of glucose specific activity and plasma glucose and insulin levels. At 60 min, rats in one group were presented with a dish containing 1 ml of 60% glucose labeled with [1-^{14}C]glucose (Amersham, Amersham, UK), i.e., 2 g/kg containing 30 μCi of [1-^{14}C]glucose, which they spontaneously drank within 1 min after 2 days of initial training. The other group of animals received the same volume of labeled glucose injected within

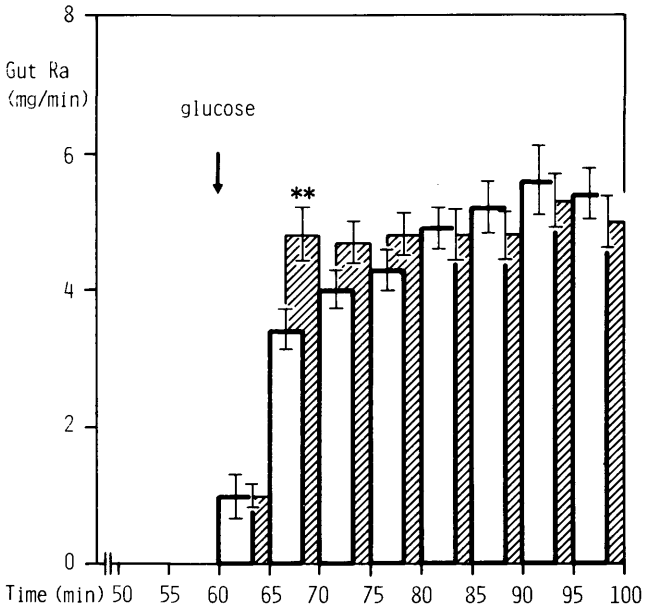

FIG. 3. Rate of gut glucose absorption (gut R_a) after either spontaneous ingestion (*open bars*) or intragastric administration (*hatched bars*) (via preimplanted catheter) of glucose (*arrow*) to normal freely moving rats. All intergroup differences were not significant except $^{**}P < .01$.

1 min into the stomach via the chronically implanted gastric catheter. Rats were chosen at random to have the glucose orally or intragastrically. One week later (when there was no longer any detectable radioactivity in the plasma), the experiment was repeated, with each rat receiving the glucose via the alternate route. Of the 15 rats studied, 9 underwent both studies. The other 6 underwent only one experiment; technical problems with the catheters precluded a second experiment. Overall, there were 12 experiments in which glucose was ingested spontaneously and 12 experiments in which glucose was injected intragastrically. After glucose ingestion or administration, blood samples were withdrawn at 5-min intervals for measurement of insulin and glucose levels, up to 100 min. For each experiment the infusion rate of [3-^{3}H]glucose was precisely measured, and the specific activity of the 60% [1-^{14}C]glucose in the ingested glucose was determined. The total amount of blood taken was 2.5 ml over 100 min, which has been shown to alter neither the basal glycemia nor the hematocrit.

Glucose was measured with a Beckman glucose analyzer 2 (Palo Alto, CA). Plasma insulin concentrations were measured by radioimmunoassay with dextran-coated charcoal separation of the bound and free fractions (14). For determination of [3-^{3}H]glucose and [1-^{14}C]glucose specific activities, 30 μl of plasma were deproteinized in duplicate by 60 μl $ZnSO_4$ (0.3 M) and 60 μl $Ba(OH)_2$ (0.3 M). One hundred microliters of the supernatant were passed through an ion-exchange resin (Ag-2X8, Bio-Rad, Richmond, CA) to remove ^{14}C-labeled charged metabolites of glucose. The columns were washed with distilled water, and the eluant was collected in scintillation vials, which were dried in an oven to remove tritiated water. Distilled water was then added to redissolve the glucose, and the samples were separated into two aliquots. The first one was used after Lumagel (Lumac/3M, Schaesberg, The Netherlands) addition to count ^{3}H and ^{14}C with a two-channel liquid scintillation counter (1217 Rackbeta, LKB Wallac); a dual-label counting program was used to correct for spillover. The second aliquot was used to determine the rate of randomization of the ^{14}C atom from the first position to the other positions (measurement of Cori cycle) by isolating the ^{14}C atom on the sixth position and multiplying the counts obtained by a factor of 4, as previously described (15,16).

Calculations. The Steele equation (9) was used to calculate the total rate of glucose appearance (total R_a) and disappearance (R_d) from [3-^{3}H]glucose specific activity. The pool fraction used (fraction of total glucose pool in rapidly mixing compartment) was 0.5. Indeed, we have recently validated the Steele equation for use in the rat and found that 0.5 gave the smallest error (10). The [1-^{14}C]glucose specific activity allowed, by transposition of the Steele equation, for the measurement of the rate of gut glucose absorption (gut R_a). The rate of hepatic glucose production (HGP) was calculated as the difference between total R_a and gut R_a (11). The metabolic clearance rate of glucose (MCR_g) was calculated as R_d divided by the glycemia. Statistical analysis was performed with the two-tailed Student's *t* test for unpaired data. An unpaired *t* test was used because 3 studies in each group of 12 were not paired.

RESULTS

Figure 1 shows the plasma glucose and insulin levels basally and after the glucose load. There was no difference in basal glycemia or insulinemia between the two groups. After the glucose load, both glycemia and insulinemia were statistically lower when glucose was ingested (oral group) than when it was administered directly intragastrically (intragastric group). Due to the heavy protocol mentioned above and to the amount of blood that would have been needed (4 additional 1-min samples between 60 and 65 min), the cephalic phase of insulin release per se could not be mea-

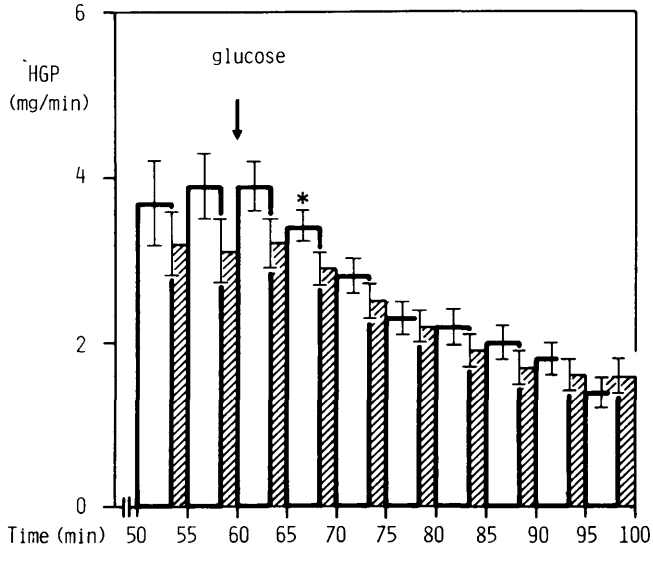

FIG. 4. Hepatic glucose production (HGP) after either spontaneous ingestion (*open bars*) or intragastric administration (*hatched bars*) (via preimplanted catheter) of glucose (*arrow*) to normal freely moving rats. All intergroup differences were not significant except $^{*}P < .05$.

sured. Figure 2 shows that there was no difference, at any time point, in total R_a either basally (there was a trend for the oral group to be higher than the intragastric group, but it did not reach statistical significance) or after spontaneous ingestion or intragastric administration of the glucose load. As shown in Fig. 3, gut R_a was initially (65–70 min) significantly smaller in the rats that ingested glucose spontaneously than in those that were given the substrate directly into the stomach. However, at all further time points, gut R_a was equivalent in the two groups. HGP (Fig. 4) was suppressed after glucose administration, regardless of the route of entry. However, there was an initial delay in the group that drank the glucose spontaneously: their HGP was significantly higher than that of the intragastric group 5–10 min after ingestion (65–70 min).

Total glucose disappearance and MCR_g are shown in Fig. 5. The initial R_d was increased significantly after the spontaneous ingestion of glucose, resulting in MCR_g values that were higher for 20 min (60–80 min) in this group than in the intragastric group. Subsequent R_d and MCR_g values were equivalent in the two groups, despite the higher glucose and insulin levels prevailing in the intragastric group.

Finally, Fig. 6 illustrates the data as total areas under the curves for the first 15 min after the glucose load (60–75 min). It can be seen that there was no difference between the two groups in total R_a. Note, however, R_d was higher in the oral group than in the intragastric group. Furthermore, because the difference between R_a and R_d (Fig. 6) represents glucose that has remained in the plasma over the first 15 min, it can be seen that initially less glucose remained in the blood in the oral group than in the intragastric group.

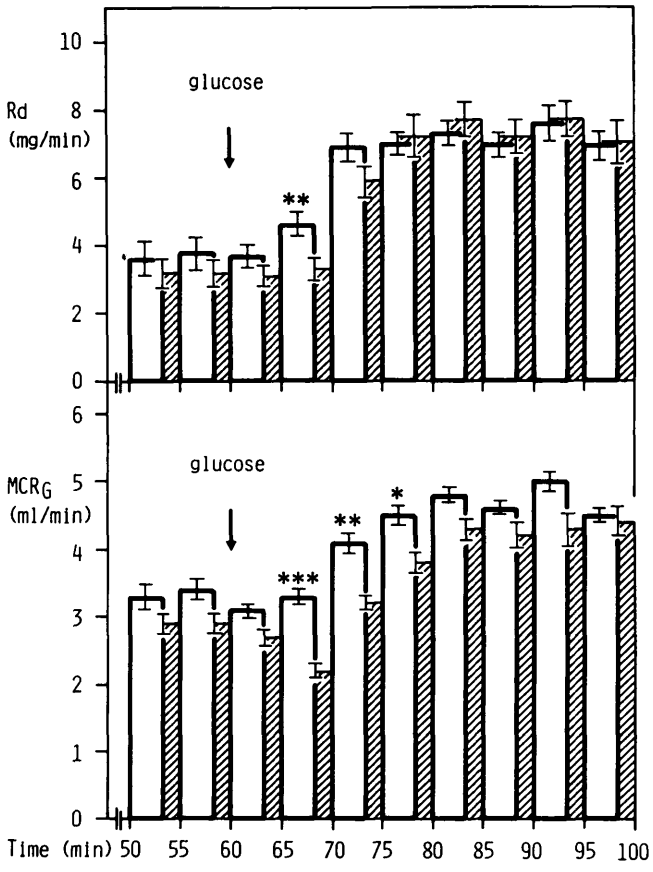

FIG. 5. Rate of glucose disappearance (R_d) and metabolic clearance rate of glucose (MCR_g) after either spontaneous ingestion (*open bars*) or intragastric administration (*hatched bars*) (via preimplanted catheter) of glucose (*arrows*) to normal freely moving rats. Statistical significance: *$P < .05$; **$P < .01$; ***$P < .001$.

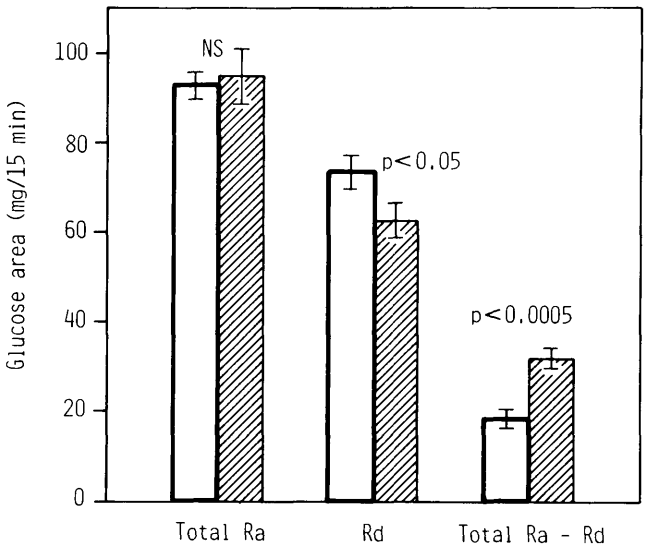

FIG. 6. Total rate of glucose appearance (total R_a) and glucose disappearance (R_d) calculated as 15-min integrated areas after spontaneous ingestion (*open bars*) or intragastric administration (*hatched bars*) (via preimplanted catheter) of glucose (*arrow*) to normal freely moving rats. Total R_a – R_d represents amount of glucose that remained in plasma over initial 15 min.

Because the basal values of total R_a and R_d are lower in the intragastric group than in the oral group (although difference did not reach statistical significance), the areas over baseline during the first 15 min (60–75 min) are represented in Table 1. The same conclusions drawn from the data in Fig. 6 can be reached when considering the values displayed in Table 1.

DISCUSSION

The results presented in this study show that when glucose is spontaneously ingested by normal freely moving rats (oral group), the resulting hyperglycemia and hyperinsulinemia are considerably less marked than those obtained by administering (via preimplanted gastric catheter) the same amount of glucose at the same speed directly into the stomach of the same rats (intragastric group) (Fig. 1). This is in agreement with the previous data of others (1) and further supports the view that various sensors present in the oro-

TABLE 1
Areas over baselines of total glucose appearance (total R_a) and glucose disappearance (R_d) during first 15 min after glucose ingestion or intragastric glucose administration

	Total R_a (mg/15 min)	R_d* (mg/15 min)
Oral (n = 12)	36.2 ± 5.0	18.2 ± 3.4
Intragastric (n = 12)	40.8 ± 3.1	9.2 ± 2.6

Glucose load, 2 g/kg body wt.
*$P < .05$.

pharynx must have an influence on the occurrence of a normal glucose tolerance and, as a likely consequence, on insulin release. However, previous studies describing the above-mentioned findings have not included measurements of glucose turnover (1,4,5), such that no mechanistic explanations have been proposed for these observations.

The results of these investigations showed that the lower glycemia observed in the oral group compared with the intragastric group was due to an initial increased R_d, as measured during the first 15 min after glucose ingestion (Fig. 6; Table 1). Note that there was indeed no intergroup difference with regard to total R_a (Fig. 6; Table 1), despite the lower gut-derived R_a in the oral group during the time interval 65–70 min (Fig. 3). This was due to the fact that during the same time interval, the HGP of the oral group was higher than that of the intragastric group (Fig. 4).

These results suggest that when glucose is ingested orally, the body gets "prepared" to receive it. This "priming" effect of a series of cephalic reflexes has a significant impact on glucose tolerance. Reflex insulin secretion (which could not be measured with our protocol) could be one of the candidates for this priming action. Although the final effect of the reflexes elicited by the ingestive process bears on the rate of glucose utilization, which is increased, this study was unable to distinguish into which tissues the glucose actually disappears. In the oral group, the increases in R_d and MCR_g [a parameter that partly compensates for expected increase in R_d in response to hyperglycemia (17)] may be due to increased hepatic glucose uptake and storage, because there is evidence that insulin can increase the rate of glucose utilization by the liver (18). A second possibility is that reflexes originating in the mouth and/or esophagus cause the release of humoral factor(s), e.g., from the gut, which may act to increase glucose entry into cells. Finally, it is conceivable that these oral reflexes act directly on tissues to facilitate glucose transport. It is now well substantiated that insulin is not the only factor capable of modifying glucose transport. Muscle contraction or increased muscle work load have been shown to stimulate glucose transport and the glucose transporter system (19). These phenomena could partly play a role during the arousal phase of actual glucose ingestion, a phase that would be missing in the intragastric route.

In conclusion, this study demonstrates for the first time that the well-known effect of the cephalic reflexes (including cephalic-phase insulin release) on glucose tolerance is mainly mediated through their capacity to accelerate the rate of disappearance of glucose from the plasma and not through an effect on total glucose appearance. The nature of this action remains to be elucidated.

ACKNOWLEDGMENTS

We acknowledge the expert technical assistance of Catherine Camenzind and thank Clarisse Deferne for typing the manuscript.

This work was supported by Swiss National Science Foundation Grants 3.851.083 and 3.822.086 and by a grant-in-aid from Nestlé S. A.

J.P. holds a National Health and Medical Research Council (Australia) Neil Hamilton Fairley Fellowship.

REFERENCES

1. Steffens AB: Influence of the oral cavity on insulin release in the rat. *Am J Physiol* 230:1411–15, 1976
2. Powley T: The ventromedial hypothalamic syndrome, satiety and a cephalic phase hypothesis. *Psychol Rev* 84:89–126, 1977
3. Berthoud HR, Bereiter DA, Trimble ER, Siegel EG, Jeanrenaud B: Cephalic phase, reflex insulin secretion: neuroanatomical and physiological characterization. *Diabetologia* 20:393–401, 1981
4. Louis-Sylvestre J: Relationship between two stages of prandial insulin release in rats. *Am J Physiol* 4:E103–11, 1978
5. Steffens AB: The regulatory role of the central nervous system on insulin and glucagon release during food intake in the rat. In *Hormones and Cell Regulation*. Amsterdam, Elsevier/North-Holland, 1981, p. 185
6. Trimble ER, Siegel EG, Berthoud HR, Renold AE: Intraportal islet transplantation: functional assessment in conscious unrestrained rats. *Endocrinology* 106:791–97, 1980
7. Nilsson G, Uvnäs-Wallensten K: Effect of teasing and sham feeding on plasma glucagon concentration in dogs. *Acta Physiol Scand* 100:298–302, 1977
8. Taylor IL, Feldman M, Richardson CT, Walsh JH: Gastric and cephalic stimulation of human pancreatic polypeptide release. *Gastroenterology* 75:432–37, 1978
9. Steele R: Influences of glucose loading and of injected insulin on hepatic glucose output. *Ann NY Acad Sci* 82:420–30, 1959
10. Proietto J, Rohner-Jeanrenaud F, Ionescu E, Terrettaz J, Sauter JF, Jeanrenaud B: Nonsteady state measurement of glucose turnover in rats by using a one-compartment model. *Am J Physiol* 252:E77–84, 1987
11. Steele R, Bjerknes C, Isbel Rathgeb BA, Altszuler N: Glucose uptake and production during the oral glucose tolerance test. *Diabetes* 17:415–21, 1968
12. Steffens AB: A method for frequent sampling of blood and continuous infusion of fluids in the rat without disturbing the animal. *Physiol Behav* 4:833–36, 1969
13. Berthoud HR, Trimble ER, Siegel EG, Bereiter DA, Jeanrenaud B: Cephalic-phase insulin secretion in normal and pancreatic islet transplanted rats. *Am J Physiol* 238:E336–40, 1980
14. Herbert V, Lau KS, Gottlieb, CW, Bleicher SJ: Coated charcoal immunoassay of insulin. *J Clin Endocrinol Metab* 25:1375–84, 1965
15. Dunn DF, Friedmann B, Maass AR, Reichard GA, Weinhouse S: Effects of insulin on blood glucose entry and removal rates in normal dogs. *J Biol Chem* 225:225–37, 1957
16. Reichard GA Jr, Moury NF Jr, Hochella NJ, Patterson AL, Weinhouse S: Quantitative estimation of the Cori cycle in the human. *J Biol Chem* 238:495–501, 1963
17. Proietto J, Harewood M, Aitken P, Nankervis A, Caruso G, Alford FP: Validation of a practical in vivo insulin dose-response curve in man. *Metabolism* 31:354–58, 1982
18. Terrettaz J, Assimacopoulos-Jeannet F, Jeanrenaud B: Inhibition of hepatic glucose production by insulin in vivo in rats: contribution of glycolysis. *Am J Physiol* 250:E346–51, 1986
19. Zaninetti D, Crettaz M, Jeanrenaud B: Dysregulation of glucose transport in hearts of genetically obese (*fa/fa*) rats. *Diabetologia* 25:525–29, 1983