A Method for Studying Acute Insulin Secretion and Glucose Tolerance in Unanesthetized and Unrestrained Rats

The Effect of Mild Stress on Carbohydrate Metabolism

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SUMMARY

A technique is described for glucose infusions and for frequent sampling of small quantities of blood in unrestrained and unanesthetized small laboratory animals. Under pentobarbital anesthesia, polyethylene catheters were implanted into the jugular vein and the aorta, and distal ends were exteriorized on the back of the neck of 250-gm. rats. Five to seven days following surgery the rats regained weight and were in a normal anabolic state, despite indwelling catheters. On the day of the intravenous glucose tolerance test (ivGTT), the exterior ends of the indwelling jugular and aortic catheters were connected to specially prepared extension catheters, through which a glucose pulse was given and frequent blood samples in small quantities were collected, respectively. During the entire procedure, the animals were resting quietly, unrestrained and unanesthetized. In another group of similar rats with indwelling catheters, ivGTT was performed after they were restrained in plastic restrainers. During the ivGTT, serum glucose levels were significantly higher in the restrained rats than those observed in the control rats. The mean glucose disposal rate (K) of 2.2 ± 0.2 was significantly slower in restrained rats than the K of 3.0 ± 0.3 in unrestrained rats. Following the glucose pulse, insulin secretion was significantly lower in restrained rats than that observed in the unrestrained rats. These observations emphasize the importance of controlling the modifying effects of mild stress on glucose tolerance and insulin secretion. DIABETES *26*:1-6, January, 1977.

During the past several years considerable interest has developed in the dynamics of insulin secretion in response to various stimuli. It is now well established that a biphasic insulin release[1,2] occurs in response to a constant glucose infusion. The acute phase of insulin secretion occurs promptly between the two and the five minutes following the glycemic stimulus. The necessity of frequent and early sequential sampling of blood in order to detect the acute insulin secretion has precluded in-vivo studies of phasic insulin secretion in small laboratory animals. Furthermore, conventional techniques (e.g., orbital sinus punctures[3] or heating and milking of the tail vein[4]) used to collect blood cause excitement and trauma to small laboratory

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animals, the stress of which may induce marked changes in various hormonal levels.[5,6] Similarly, the blood samples obtained in an anesthetized animal by cardiac puncture[7] or jugular vein catheterization may affect the carbohydrate metabolism and cause wide variation in glucose and insulin levels.

The present study describes a small laboratory animal model for infusion of glucose and other stimulatory agents, which also allows frequent and sequential blood sampling to study biphasic insulin secretion in unanesthetized, undisturbed, and unrestrained rats. The study also examines the effect of mild stress on glucose tolerance and insulin secretion.

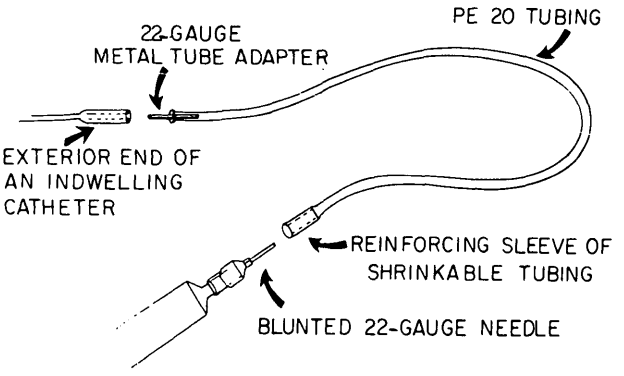

FIG. 1. Assembly of the extension catheter, fabricated from a polyethylene tubing no. 20 (PE 20, I.D. 0.015 inches). Only exterior end of an indwelling catheter is shown, which is connected to extension catheter via metal tube adapter at the time of the procedure.

MATERIALS AND METHODS

Preparation of Catheters

Fabrication of the indwelling catheters for the jugular vein and the aorta was adopted with slight modification from the techniques previously described.[8,9]

The extension catheters were made by utilizing 25-cm. polyethylene tubing (PE 20, I.D. 0.015 inches). As shown in figure 1, one end of this tubing was reinforced with a 1-cm.-long sleeve of shrinkable tubing by gently warming on a hot-air jet. The openings of the tube at both ends were flared in order to accept 22-gauge blunted needles. The presence of sleeve and flared ends facilitates inserting a needle to infuse glucose or withdraw blood via syringe. A 2-cm. metal tubing adapter (made by cutting a 22-gauge needle, both ends being blunted) was attached to the end of PE 20 tubing not reinforced with the sleeve (figure 1). These extension catheters are used only at the time of the study for infusion of various agents through the jugular vein catheter or for collecting blood samples through the aortic catheter, allowing the animal to be awake, unrestrained, and in the relaxed state throughout the entire procedure.

Implantation of the Indwelling Catheters

The surgical procedure for implanting the aortic and venous catheters was adapted with slight modifications from the methods previously described by Still et al.[10] and Weeks et al.[8] A brief description of the surgical technique is as follows:

Male Sprague-Dawley 250-gm. rats were anesthetized with intraperitoneal injection of sodium pentobarbital (5 mg./100 gm. body weight). The skin over the anterior abdominal wall and anterior and posterior neck region was shaved and prepared with mild detergent solution. Aortic catheterization was done first. The abdominal cavity was opened with a 4-cm. incision and the intestines were deflected laterally and covered with a warm, moist saline sponge to visualize the aorta and the inferior vena cava. With the help of a trochar, the distal end of the aortic catheter was passed through the left psoas muscle at the level of the bifurcation of the aorta, then through the subcutaneous tissue of the back (just beneath the skin) and exteriorized in the region of the posterior neck of the animal. After blood flow was obliterated in the aorta with the pressure of the index finger at the level of renal arteries, a small opening was made in the aorta just above the bifurcation with a 25-gauge sharp needle. Through this opening the proximal end of the aortic catheter was directed cephalad and was inserted 2 cm. into the aorta. The index-finger pressure was released and a free blood flow through the catheter was established. The catheter was then flushed and filled with normal saline; the exterior end was then plugged with a 22-gauge and 1-cm.-long blunted metal wire. The intestines were replaced in the peritoneal cavity and the abdominal wound was closed in layers.

The neck tissues were exposed with a 2-cm. incision on the right side of the neck and extending to the right clavicle. The right jugular vein was identified and isolated. The distal end of the venous catheter was exteriorized with a trochar through subcutaneous tissue to the posterior neck of the animal. A small opening was made in the jugular vein with a 25-gauge sharp needle, and the proximal end of the catheter was advanced into the jugular vein for 2 cm. Free blood flow in the catheter was established, and after the catheter was flushed and filled with normal saline, the exterior end of the venous catheter was closed with a 22-gauge 1-cm. metal-wire plug. The neck wound was closed in layers. The exterior ends of the aortic

and the jugular catheters were secured to the posterior neck skin with silk sutures.

Postoperatively, the animals were isolated and allowed to recuperate individually in metabolic cages. Most of the animals regained weight and were in a normal anabolic state by the fifth postoperative day, despite the indwelling catheters. The infusion studies were carried out only after the animal gained weight and appeared to be in a normal metabolic state.

Glucose Tolerance Tests

Intravenous glucose tolerance tests (ivGTT) were done in seven fasted rats. The metal-wire plugs were removed from the exterior ends of the indwelling catheters, and metal tube adapters of the extension catheters were connected (figure 1). The distal ends of the extension catheters were used to infuse glucose or withdraw blood samples while the animal was awake in the resting condition and was free to move about in its cage. Following a 30-minute rest period after attaching the extension catheters, a baseline blood sample was collected. A glucose pulse of 150 mg. (1.5 ml. of 10 per cent dextrose in water solution) was administered via the jugular-vein-extension catheter in 30 seconds. The glucose solution in the catheter dead space was flushed with 0.2 ml. of normal saline. Blood samples (0.3 ml.) were collected via the aortic-extension catheter at 2, 3, 5, 15, 30, 45, and 60 minutes for serum glucose and insulin determinations. Prior to collecting each blood sample, the saline in the catheter lumen was withdrawn and discarded. Following each blood collection the aortic-extension catheter was flushed and filled with 0.1 ml. of normal saline to prevent clotting within the catheter.

In order to determine the effect of mild stress on glucose tolerance and insulin secretion, ivGTT was performed in a separate group of seven similar rats with indwelling catheters, except that these rats were restrained in a plastic restrainer for one-half hour prior to and during the ivGTT. Although restrained from moving freely, these animals were not in pain, their respiratory movements were not impaired, and they remained quiet, without any struggle, during the tests. Blood samples were collected at the identical time intervals as described above.

Serum glucose was measured immediately on a glucose analyzer (glucose oxidase method utilizing 10 μl. of the serum) and remaining sera were frozen at $-20°$ C. for future determination of immunoreactive insulin (IRI) by a micromodification of a radioimmunoassay technique[11] utilizing rat insulin standards. Glucose disappearance rate (K value) was calculated by the method of least squares[12] using natural logarithms of the actual glucose levels. The areas above baseline under glucose and IRI time curves were calculated by a modification of the trapezoidal rule and were expressed in arbitrary units. Insulinogenic index[13] was expressed as a ratio of area IRI to area glucose above baseline. All calculations were performed on a programable calculator, and statistical analyses were done by applying the Student t-test to group differences between restrained and unrestrained rats.

RESULTS

Surgical Technique and the Patency of the Catheters

Under the technique described above, the jugular vein catheter remained patent in greater than 95 per cent of the animals for six weeks. The aortic catheter remained patent in 70 per cent of the animals for six weeks. Since both catheters remained functional for several weeks, it was possible to perform several ivGTTs in the same animal. Figure 2 shows reproducible responses in serum glucose and IRI during the two different ivGTTs done 10 days apart in a representative animal.

The Effect of Mild Stress on Glucose Tolerance and IRI Secretion

The fasting glucose levels in the restrained rats were slightly but not significantly higher than those of the unrestrained rats. However, following the glucose pulse the mean glucose levels reached and remained significantly high ($p < 0.001$ to < 0.02) throughout the procedure in the restrained rats (figure 3). The mean K value of 2.2 ± 0.2 in the restrained rats was significantly lower ($p < 0.05$) than the mean K value of 3.0 ± 0.3 observed in the unrestrained rats (figure 4). Baseline IRI levels were similar in the restrained and unrestrained groups of the rats. However, following the glucose pulse, serum IRI levels were significantly lower ($p < 0.02$ to < 0.05) in the restrained rats throughout the observation period (figure 5). As shown in table 1, the total insulin output (calculated as area above baseline under the insulin time curve) in restrained rats was also significantly less ($p < 0.01$) than that observed in the unrestrained rats. Similarly, insulinogenic indexes in the restrained rats were significantly less ($p < 0.005$) than in the unrestrained rats, further suggesting that the insulin secretion in response to the level of the glycemic stimulus was less in the restrained rats (table 1).

DISCUSSION

Several studies have documented the adverse effect

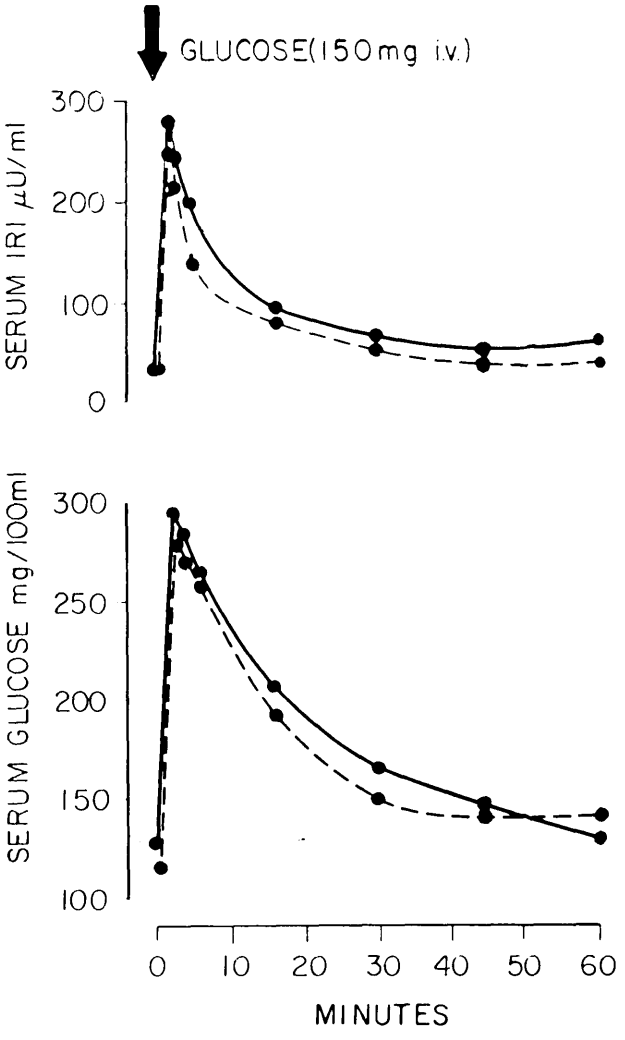

FIG. 2. Serum glucose and IRI levels during the two ivGTTs done 10 days apart in a representative rat.

of various stresses on carbohydrate metabolism and glucose tolerance.[7,14-16] Curtailed insulin secretion and the glucose intolerance observed in the restrained animals in the present study support these findings. Even the mild stress employed in this report (confinement to the plastic restrainer without struggle), which profoundly affected carbohydrate metabolism, has important implications. It has been presumed that simultaneously performed control experiments using similar techniques would solve the problem of variation caused by the stress of handling and of collecting blood employed in the small animals. However, the stress-induced alterations in the carbohydrate metabolism may preclude any further alterations expected during the experimental studies.

TABLE 1

Comparison of IRI and glucose areas above baseline and insulinogenic indexes between the stressed and unstressed rats

Rats	Area ΔIRI	Area ΔGlucose	Insulinogenic Index $\frac{\text{Area } \Delta \text{ IRI}}{\text{Area } \Delta \text{ glucose}}$
Stressed (n=7)	1,199 ± 194	4,454 ± 508	0.31 ± 0.08
Unstressed (n=7)	2,340 ± 308	2,753 ± 349	0.93 ± 0.15
P	< 0.01	< 0.02	< 0.005

Results are expressed as mean ± standard error.

As with all chronic indwelling intravascular catheters, infection and clotting within the catheter are potential problems. Appropriate sterile precautions prevent infections. A dilute solution of heparin can be utilized to fill the indwelling catheter to prevent clotting. However, in our experience careful flushing and filling of catheters with normal saline alone prevents clotting within the catheter, and it remains patent for several weeks in most of the animals.

As portrayed in figure 2, reproducible results are

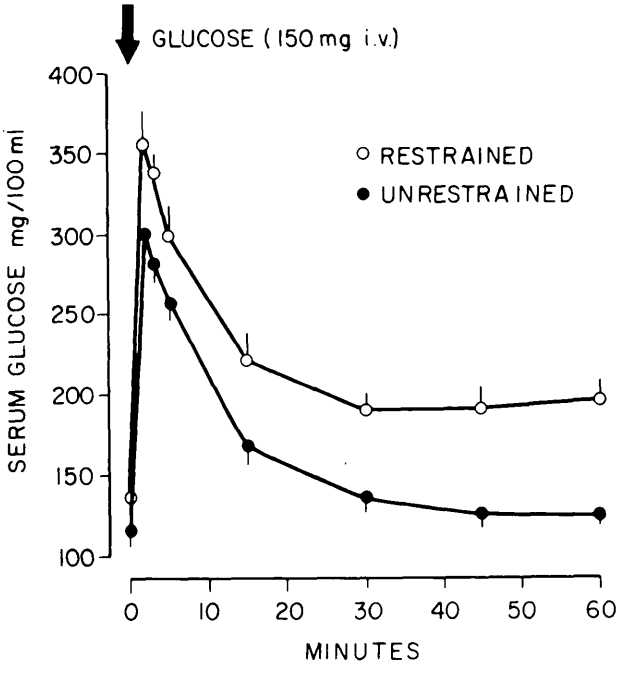

FIG. 3. Mean serum glucose following the glucose pulse (150 mg., i.v.) in restrained (n=7) and unrestrained (n=7) rats. Serum glucose values between two and 60 minutes were significantly higher in the restrained than in the unrestrained rats ($p < 0.001$ to < 0.02). Baseline serum glucose was not significantly different between the restrained and the unrestrained groups. The vertical line represents standard error.

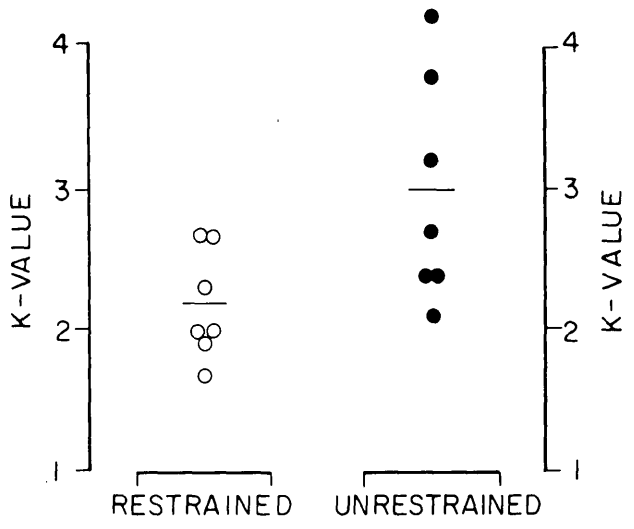

FIG. 4. Comparison of the glucose disappearance rate (K value) of the individual rats in the restrained and unrestrained groups. The mean K value (represented by the transverse line) in the restrained animals was significantly less ($p < 0.05$) than that in the unrestrained animals.

obtainable when repeat ivGTTs are carried out 10 days apart in these rats. This model also lends itself to numerous other applications, such as chronic infusions of glucose and other stimulatory agents to study physiologic and pathophysiologic responses in insulin and other hormone secretion.

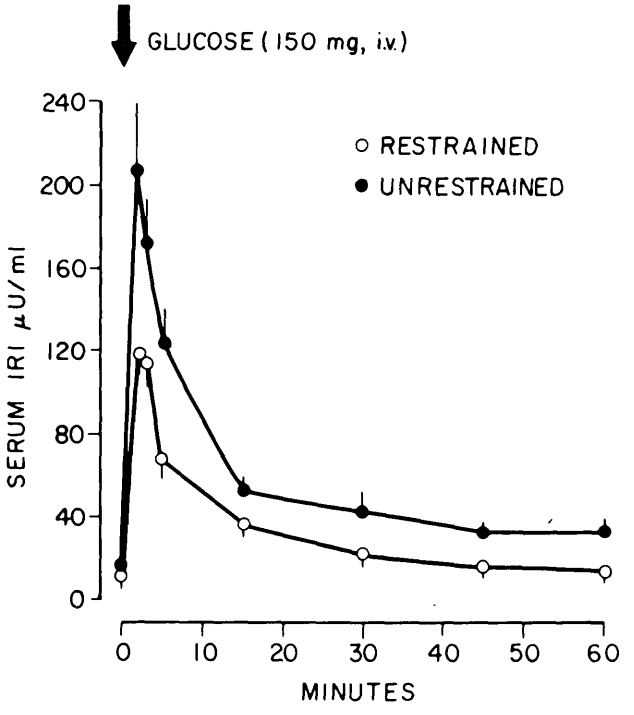

FIG. 5. Mean serum IRI responses following the glucose pulse (150 mg., i.v.) in restrained (n=7) and unrestrained (n=7) rats. The serum IRI values between two and 60 minutes were significantly lower in the restrained rats than those in the unrestrained rats ($p < 0.02$ to < 0.05). The vertical line represents standard error.

Thus, the technique described here is relatively easy, simple to perform, and reproducible where the jugular and aortic catheters can remain patent in place for several weeks without interfering with the growth and metabolic balance of the small animals. This technique is also convenient, yields valid and reproducible results, and eliminates undue manipulation and excitation of the animals, which remain unanesthetized, undisturbed, and unrestrained during the glucose infusion and serial sampling of blood. From this model, the observations presented in this study emphasize the importance of controlling the modifying effect of the mild stress of experimental procedure on carbohydrate metabolism.

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