The Influence of Intravenous Glucose on Blood-Insulin Activity in the Rat

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Changes in the glucose uptake of the isolated rat diaphragm were first applied by Groen, Kamminga, Willebrands & Blickman (1952) to the quantitative assessment of the insulin activity of blood plasma. Since that time this method, or variants of it, have been widely used for the detection and assay of plasma-insulin activity (Randle, 1954, 1956; Vallance-Owen & Hurlock, 1954). With such a method Perlmutter, Weisenfeld & Mufson (1952) failed to find evidence for a rise in serum-insulin activity when glucose was given intravenously to human beings, though Candela, Rovira & Candela (1954, 1955) did find a rise in the plasma-insulin activity of normal dogs receiving glucose intravenously. We have applied this method to blood plasma from normal rats at various times after the intravenous administration of glucose, and in addition have simultaneously measured the glucose uptake in buffer of the diaphragms from these animals *in vitro*.

METHODS

Animals. Normal female albino rats of an inbred laboratory strain were used. The rats were maintained on a stock laboratory diet (Short & Parkes, 1949) and were used in groups of 12 when they weighed 90–130 g. The rats had free access to water but were without food for 18–20 hr. before the experiment.

Procedure. In one series of experiments six of the 12 rats used in each experiment were anaesthetized with Nembutal (6 mg./100 g. body wt.). Each of three of the anaesthetized rats was given 200 mg. of glucose in 1 ml. of water intravenously. Three control animals were given 1 ml. of 0·9% NaCl. At timed intervals, 5–225 min. after the glucose administration, the animals were bled from the dorsal aorta into syringes containing a small amount of heparin, and the blood from each group was pooled separately for the subsequent preparation of the plasma.

Immediately after the blood was drawn, the diaphragm from each animal was excised, cut into halves, and placed in buffer containing 250 mg. of glucose/100 ml. for a preliminary soaking of approximately 10 min. The buffer used (Gey & Gey, 1936) is a balanced salt solution of electrolyte composition similar to that of extracellular fluid, and buffered with bicarbonate. Each hemidiaphragm was gently blotted after soaking and transferred to a beaker containing 1 ml. of the glucose-containing buffer. The hemidiaphragms were incubated in a Dubnoff metabolic shaker at 37° for the next 90 min. The gas phase was $O_2 + CO_2$ (93:7).

Plasma was prepared from the blood of both the glucose-injected and the control animals, and the glucose concentration was determined. Samples of plasma were then diluted with 3 vol. of Gey & Gey (1936) buffer, and the final glucose concentration of each sample was adjusted to 250 mg./100 ml. by the addition of glucose.

The six unanaesthetized rats were killed by decapitation, the diaphragms removed, soaked for approximately 10 min. in the glucose-containing buffer and transferred to beakers containing 1 ml. of diluted plasma. These hemidiaphragms were incubated for 90 min. as above. At the end of the incubation period each hemidiaphragm was thoroughly blotted and weighed on a torsion balance.

In another series of experiments a group of 11 rats was anaesthetized with Nembutal and eight of them were injected intravenously with glucose (200 mg./rat). Groups of two animals each were bled at intervals of 5, 10, 15 and 20 min. after the injection. Three rats served as controls. The blood was drawn and the diaphragms were prepared as described above. Diaphragms from the injected animals were incubated in buffer immediately. The plasma from these animals was prepared and stored at 2° overnight. The following day it was diluted with 3 vol. of buffer. Diaphragms from 12 normal rats were then incubated in the samples of plasma from these animals. This same procedure was repeated for time intervals of 20, 60, 90 and 120 min.

The initial glucose concentration of the diluted plasma, and the final glucose content of each beaker, were determined by the Somogyi (1952) modification of the Nelson method.

The results, calculated as glucose uptake in plasma or buffer, in terms of mg. of glucose/g. of wet diaphragm/hr., were expressed as a percentage of the value for control diaphragm tissue in plasma or buffer respectively. The statistical significance of the change in glucose uptake at any given time was calculated by analysis of variance. A P value of 0·05 or less was taken as indicating a significant change.

RESULTS

Glucose uptake of diaphragms from glucose-injected rats incubated in buffer

From Fig. 1 (*a*) it may be seen that the glucose uptake of diaphragms from glucose-injected rats is significantly higher than that of control diaphragms

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15, 25, 30 and 35 min. after the injection of glucose. There is no significant difference at 45 or 60 min. At 90 and 120 min. after the injection of glucose, the glucose uptake of diaphragms from the injected rats is lower than that from control diaphragms but is not significantly different from control values at 145 and 225 min.

From Fig. 2 (*a*) the glucose uptake of diaphragms from the glucose-injected rats is higher than that of control diaphragms 10, 15 and 20 min. after the injection of glucose. The utilization of glucose is the same at 90 min., and at 120 min. is lower than that of diaphragms from the control rats.

Glucose uptake of diaphragms from normal rats incubated in plasma from glucose-injected rats

It may be seen from Fig. 1 (*b*) that plasma from glucose-injected rats is less effective in promoting the glucose uptake of normal rat diaphragm than plasma from normal rats 5, 15, 20, 25 and 30 min. after the administration of glucose. At 120, 145 and 225 min. after the administration of glucose, plasma from glucose-injected rats raises the glucose uptake of normal rat diaphragm above that seen in the presence of the control serum. From Fig. 2 (*b*) it is seen that the glucose utilization of normal rat diaphragm is greater than that of control plasma, 60, 90 and 120 min. after the treatment with glucose.

It must be emphasized that the changes in the glucose uptake of isolated normal rat diaphragm in the presence of plasma from the glucose-injected animals were not entirely consistent. At each time interval where a significant change is indicated in Fig. 1 (*b*), one experiment considered by itself would not have revealed a significant difference. The source(s) of the variations cannot be ascertained at present.

Comparison of Fig. 1 (*b*) with Fig. 1 (*c*), and of Fig. 2 (*b*) with Fig. 2 (*c*), shows that a greater glucose uptake of normal diaphragm in the presence of plasma from the glucose-injected animals occurs only after the blood sugar of the glucose-injected rat has returned to about control values.

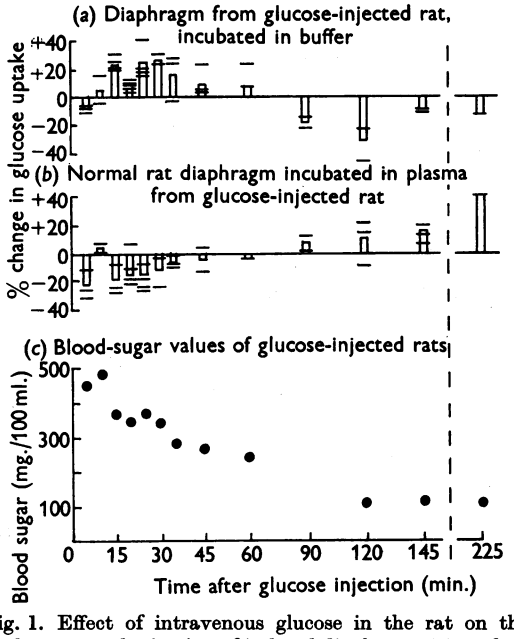

Fig. 1. Effect of intravenous glucose in the rat on the glucose uptake *in vitro* of isolated diaphragm (*a*), and on blood-insulin activity (*b*). The glucose-injected rats had the mean blood-sugar values indicated in (*c*), at the time when the blood was drawn and the diaphragm excised. In these experiments at each period of time the result for each experimental observation is compared with that for a control observation at the same period of time. Each column in (*a*) and (*b*) represents the percentage change in the average glucose uptake of six hemidiaphragms compared with that of an equal number of controls. Each bar represents the average change for all the experimental points at each time. The uptake of glucose by normal rat diaphragm *in vitro* in our Laboratory is 3·0–3·5 mg./g. of diaphragm/hr.

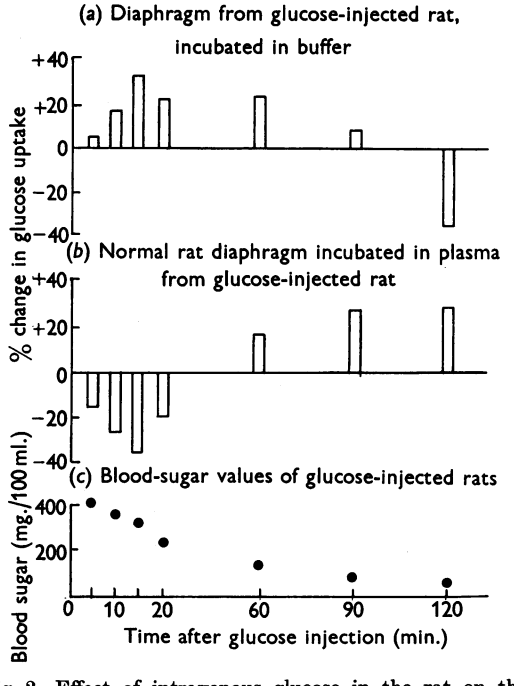

Fig. 2. Effect of intravenous glucose in the rat on the glucose uptake *in vitro* of isolated diaphragm (*a*), and on blood-insulin activity (*b*). The glucose-injected rats had the mean blood-sugar values indicated in (*c*), at the time when the blood was withdrawn and the diaphragm excised. In these experiments the results from experimental observations at a series of times are compared with three for a pooled sample of plasma taken from control animals.

DISCUSSION

The high glucose uptake of diaphragm which is removed from the glucose-injected rat and incubated in buffer may be most readily explained by assuming that insulin secreted by the pancreas is bound to the muscle tissue under these conditions and continues to exert its customary stimulating effect on glucose uptake during the incubation. Stadie, Haugaard & Vaughan (1952) have shown that isolated rat diaphragm will bind insulin *in vitro* and that the bound insulin is not removed by rinsing the muscle in successive changes of buffer. In our experiments the elevated glucose uptake was observed after a preliminary soaking in buffer for 10 min., a process which should almost completely remove free insulin. The reduced glucose uptake, in buffer, of diaphragm from the glucose-injected animals at 90 min. (Fig. 1*a*) and 120 min. (Figs. 1*a* and 2*a*) is difficult to explain. Temporary saturation of one or more of the mechanisms involved in the uptake, utilization and storage of glucose in the diaphragm is one possibility. It is interesting, although it may be only fortuitous, that the diminution in glucose utilization, in buffer, of diaphragm from the glucose-injected rat coincides approximately with the rise in glucose uptake of the normal rat diaphragm in the presence of plasma from the glucose-injected animal.

It is generally accepted that an elevation of the blood glucose results in a rise in the secretion of insulin by the pancreas. Anderson & Long (1947), using the adrenodemedullated-diabetic-hypophysectomized rat for the assessment of insulin activity (Anderson, Lindner & Sutton, 1947), obtained evidence for the secretion of insulin when glucose was added to the perfusate of an isolated rat pancreas. With the adenalectomized-diabetic-hypophysectomized rat, Bornstein (1950) found a substantial increase in plasma insulin 2·5 hr. after ingestion of glucose by man, and with the isolated rat diaphragm for the detection of plasma-insulin activity in man Vallance-Owen & Hurlock (1954) also usually found an increase 1 hr. after the ingestion of glucose. On the other hand, Perlmutter *et al.* (1952) failed to find evidence for a rise in serum-insulin activity under similar conditions in human subjects. In fact, their experimental results suggest a fall rather than a rise. Candela *et al.* (1954) gave glucose intravenously to a dog and found that when the blood sugar had returned to a normal value the insulin activity of the plasma, measured by the isolated diaphragm, was greatly increased. They believed that this must result from an elevated rate of insulin secretion. Subsequently Candela *et al.* (1955) observed that although the blood-insulin activity was raised 5 min. after the intravenous injection of glucose into the dog, this rise disappeared at 10 min. but reappeared 15 min. after the treatment with glucose. They concluded that insulin disappeared from the plasma at 10 min. because the hormone was taken up by the tissues in exerting its effect on the metabolism of glucose.

If, in our experiments, the rise in glucose uptake of the normal rat diaphragm in the presence of plasma from the glucose-injected animal over that in control plasma be taken as indicating a rise in insulin content of the plasma, it is clear that a greater insulin activity appears in the plasma only after the blood sugar has returned to about a normal level. The reduction in glucose uptake of normal diaphragm in the presence of plasma from glucose-injected rats taken shortly after the administration of glucose, as compared with the uptake in normal plasma, may indicate for the rat a fall in plasma-insulin activity below normal in response to a rise of blood-sugar level. If this be so, then the simplest interpretation of the observations is that, under the stimulus of a rise in blood-sugar level, insulin leaves the plasma and becomes bound to the tissues of the diaphragm of the rat at a site where it can exert a physiological effect. But other interpretations cannot at present be excluded. It is clear that the time at which blood is taken for insulin assay after the administration of glucose is of great importance, and neglect of this may account for some of the mutual discrepancies in the observations of other investigators.

SUMMARY

1. The isolated rat diaphragm has been used to measure the insulin-like activity of plasma from rats given glucose intravenously. The activity is lower than normal for 30 min. after the injection of glucose, and then rises above normal.

2. The glucose uptake of diaphragm obtained from the glucose-injected rats has been simultaneously examined by incubation in glucose-containing buffer. The glucose uptake first rises and then falls below normal.

3. A possible interpretation of these results is that when the blood glucose rises insulin leaves the plasma and becomes bound to the tissues of the diaphragm, where it exerts a physiological effect.

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Some Hormonal Influences on the Glucose Uptake of Normal Rat Diaphragm *in vitro*

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It has been reported that serum from alloxan-diabetic rats inhibits the glucose uptake *in vitro* of diaphragm from normal rats (Tuerkischer & Wertheimer, 1948). Bornstein & Park (1953) confirmed and extended this observation, showing that hypophysectomy of the alloxan-diabetic animal is followed by a loss of the inhibitory properties of the serum. They have further shown that the simultaneous administration of growth hormone and cortisone to the hypophysectomized alloxan-diabetic rat results in the reappearance of the inhibitor of glucose uptake, although the administration of either hormone alone, or the addition of either or both hormones to the incubation medium, is without effect on the glucose utilization *in vitro* of the isolated rat diaphragm.

The present investigation confirms and extends these observations of Bornstein & Park (1953).

METHODS

Animals. Serum was prepared from the blood of albino rats of an inbred strain weighing 200–300 g. and maintained on a stock laboratory diet (Short & Parkes, 1949). Diabetes was induced in rats fasting for 24–48 hr. by a single intravenous injection of alloxan (45 mg./kg.). The alloxan was synthesized in this Laboratory by the method of Hartman & Sheppard (1943). Only those animals which had a 24 hr. fasting blood-glucose value of at least 250 mg./100 ml. at least 3 weeks after the injection of alloxan were used for these studies. One group of diabetic rats was subsequently hypophysectomized by the parapharyngeal approach and used 10 days later for experiment.

All serum-donor animals, whether operated, treated or control, had free access to water but were without food for 18–20 hr. before they were used. Each rat was lightly anaesthetized with Nembutal, the blood was drawn into a syringe from the dorsal aorta, and serum was prepared. The glucose content of the serum was determined, and each sample was diluted with an equal vol. of buffer (Gey & Gey, 1936). The final glucose concentration of the diluted serum was adjusted to 250 mg./100 ml. by the addition of glucose. In what follows the term serum will refer to serum diluted in this manner.

Hormone preparations. Growth hormone was prepared by a modification of the method of Wilhelmi, Fishman & Russell (1948). For injection the hormone was dissolved in 0·9 % NaCl at pH 8·5–9·0.

Procedure. Hypophysectomized alloxan-diabetic rats were treated with growth hormone by intraperitoneal injection at the rate of 200 μg./rat/day. Cortisone acetate was administered as a suspension in 0·9 % NaCl by intraperitoneal injection at a rate of 100 μg./rat/day. Normal rats were treated in one of three different ways. In the first experiment growth hormone was given, where indicated, at the rate of 2 mg./rat/day and cortisone at the rate of 1 mg./rat/day, for 3 days. In the second experiment 3 mg. of growth hormone and 5 mg. of cortisone/rat/day were administered for 3 days. In the third experiment the latter dosages of growth hormone and of cortisone were administered for 14 days. In all instances the treated serum-donors remained without food for 18 hr. after treatment and before serum was prepared. Control animals received appropriate treatment with 0·9 % NaCl.

Diaphragm tissue was obtained from normal rats weighing 100–130 g. which had fasted for 18–20 hr. The rats were decapitated, the diaphragms quickly and gently excised, divided into halves and allowed to soak for 15 min. in buffer (Gey & Gey, 1936) containing 250 mg. of glucose/100 ml. Each hemidiaphragm was then blotted and transferred to a beaker containing 1 ml. of the incubation medium (either buffer or the previously prepared serum). The tissues were incubated in a Dubnoff metabolic shaker for 90 min. at 37°, with a gas phase of $O_2 + CO_2$ (93:7). At the end of the incubation the hemidiaphragms were blotted and weighed, and the glucose content of each beaker was estimated

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