Effects of epinephrine, stress, and exercise on insulin secretion by the rat

WRIGHT, PETER H., AND WILLY J. MALAISSE. Effects of epinephrine, stress, and exercise on insulin secretion by the rat. Am. J. Physiol. 214(5) : 1031-1034. 1968.—Rats were injected with guinea pig anti-insulin serum and 125I-labeled human serum albumin. After 45 min, intermittent electrical shocks were applied; epinephrine was injected subcutaneously; or the animals swam in tepid water. All animals were killed at 75 min. Compared with endogenous insulin secreted in control animals between 45 and 75 min, significantly less secretion was observed in the three groups of treated rats. It is suggested that endogenous epinephrine released during stress or exercise is sufficient to suppress insulin secretion even under conditions of hyperglycemia.

In view of recent reports that insulin secretion is inhibited by epinephrine in vivo (9, 13, 14, 21) and in vitro (4, 18), the present experiments were carried out to confirm this observation in rats and to see whether similar inhibition is induced by exercise and stress. The technique used to measure insulin secretion in vivo involves the intravenous injection of guinea pig anti-insulin serum and the induction of insulin deficiency (16, 29).

Materials and Methods

Male albino rats (Holtzman, Wis.) were fed on a standard diet (Lab-blox, Allied Mills, Chicago, Ill.) and had free access to both food and water up to the time of use. Animals of similar weight (mean = 196 ± 1.6 g; N = 23) were each injected intravenously (tail vein) at zero time with the same volume of solution (1.5 ml) containing guinea pig anti-insulin serum (0.8 or 0.9 ml; capable of neutralizing 2.4 to 2.6 units bovine insulin) and 125I-labeled human serum albumin (ca. 2.5 μc; RISA, Abbott Laboratories, North Chicago, Ill.) and saline (0.9%; w/v). Blood (0.4-0.5 ml) was drawn from the severed end of the tail immediately before and at intervals of 2 and 45 min after this injection. A fourth sample was obtained from the severed neck when the animal was killed by decapitation at 75 min. The volumes and hematocrits of all samples of blood were measured and the plasma separated as rapidly as possible. Concentration of sugar in the plasma was determined by an automated microtechnique (AutoAnalyzer; Technicon Instruments, Chauncey, N. Y.) using a method based on that described by Hoffman (10). Gamma radiation emitted by 125I-labeled albumin in aliquots (0.1 ml) of each sample of plasma was measured in an automatic well-type scintillation counter (Packard Instrument, LaGrange, Ill.). Residual reactive insulin antibodies which had not been neutralized by endogenously secreted insulin in vivo were then estimated in the same samples of plasma (0.1 ml) using a mixture of unlabeled and a trace of 125I-labeled bovine insulin; the method has been described in detail elsewhere (27, 28). It has been shown elsewhere (16, 29) that the volume of distribution of 125I-labeled albumin is initially almost the same as that of the injected antibodies and, in the alloxan-diabetic rat which secretes little or no endogenous insulin, increases at the same rate for the first 3 hr. From the volumes of distribution of the labeled albumin and simultaneously measured concentrations of reactive (unneutralized) antibodies in the plasma, the total amount of unneutralized antibody in each rat was determined at each time of bleeding. Knowing the total amount of antibody injected and taking into account losses encountered at each bleeding, the rate of neutralization of the injected antibodies between successive bleedings was calculated and is equated here to the rate of endogenous insulin secretion for that period. Since the ability of guinea pig anti-insulin serum to bind bovine insulin falls linearly during progressive neutralization with mammalian insulins but fails to do so once more than about half of the antibodies have been neutralized (28), sufficient antibody was injected initially to insure that more than half remained unneutralized at the end of each experiment.

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RESULTS

As shown in Table 1, the mean value for the hematocrit fell from 41 to 37% during the initial period. Thereafter it fell significantly further in the control animals than in those submitted to shocks or swimming; but did not fall in the rats injected with epinephrine. The volume of distribution of labeled albumin rose from 9.84 to 12.57 ml during the initial period and then continued to rise to almost the same extent in the control animals and those which were shocked or swam; after epinephrine, no such rise was seen. Plasma sugar concentration rose from a normal mean value for fed animals (148 mg/100 ml) before injection to about 300 mg/100 ml at 45 min. Comparable further increases to about 400 mg/100 ml were seen in the control rats and those which were shocked or swam; but the animals injected with epinephrine became more hyperglycemic (309 mg/100 ml).

TABLE 1. Insulin secretion in rats submitted to stresses or injections of epinephrine

<table>
<thead>
<tr>
<th></th>
<th>Initial</th>
<th>Experimental</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time, min</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Treatment</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hematocrit, %</td>
<td>40.8±0.49</td>
<td>37.0±0.16</td>
</tr>
<tr>
<td>Albumin volume, ml</td>
<td>9.84±0.18</td>
<td>12.57±0.23</td>
</tr>
<tr>
<td>Plasma sugar, mg/100 ml</td>
<td>148±2.3</td>
<td>201±22</td>
</tr>
<tr>
<td>Insulin secreted, milliunits</td>
<td>291±22</td>
<td>477±52</td>
</tr>
<tr>
<td>Observations, N</td>
<td>23</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>30.8±0.83</td>
<td>36.2±1.24*</td>
</tr>
<tr>
<td>Epinephrine</td>
<td>39.3±1.54*</td>
<td>39.3±1.54*</td>
</tr>
<tr>
<td>Shocks</td>
<td>36.2±1.24*</td>
<td>39.3±1.54*</td>
</tr>
<tr>
<td>Swimming</td>
<td>33.8±0.40†</td>
<td>33.8±0.40†</td>
</tr>
</tbody>
</table>

Mean values (±SE) for hematocrit, volume of distribution of labeled albumin, and plasma sugar concentration are stated at four times of bleeding before (0 min) and after (2, 45, and 75 min) injection of anti-insulin serum. Mean amounts of endogenously secreted insulin are stated for the whole series of animals (N = 23) during the initial period (2-45 min); and for the smaller groups (N = 5 or 6) during the experimental period (45-75 min) when rats remained untreated (controls) or were submitted to injections of epinephrine, electrical shocks, or swimming. The significance of differences from the mean values observed in control animals during the experimental period are also shown. * P < 0.005, † P < 0.02.

For at least 30 min before and for 45 min after the single intravenous injection, all rats were kept in warm cages with free access to water and none were handled except at times of bleeding or injection. After the bleeding at 45 min, rats were divided into four groups in a total of three experiments:

 Controls. Two animals in each experiment were replaced in the warm cage and were not handled until they were killed at 75 min.

 Epinephrine. In one experiment, six rats were each injected subcutaneously with a solution of epinephrine (200 µg, 0.2 ml, 1:1,000) at 46, 55, and 65 min. When killed at 75 min, these animals all had noticeably cold feet, pale ears, and warm bodies. None showed evidence of systemic ill effects but all appeared slow in their movements just before they were killed.

 Electrical shocks. The feet of six rats in a second experiment were smeared with electrode paste (Burdick Corp., Milton, Wis.) and the animals placed on a metal grill. Between 45 and 75 min, intermittent electrical impulses (12-15 v at 10/sec) were passed for 5 sec every 30 sec. Only one animal did not receive stimulations for the full period and was discarded. No animal suffered obvious ill effects but all remained highly excitable and jumped about the cage during each period of stimulation.

 Swimming. In the third experiment, six rats were placed in tanks of deep (18 inches) tepid (30-32°C) water. For the first 10-15 min they were very active, but as they learned how to conserve energy and float they moved more slowly. To maintain activity, a second rat was placed with the first after 15-20 min. After swimming for 30 min, none of the animals showed any sign of exhaustion or distress.

Mean values (±SE) for all observations are quoted for the entire group of animals (N = 23) during the initial period up to 45 min after the initial injection; and for the four individual groups of animals (N = 5 or 6) during the experimental period between 45 and 75 min.

DISCUSSION

During muscular exercise or stress, the levels of circulating epinephrine and norepinephrine rise in man (8, 25) and both catecholamines are excreted in the urine in increased amounts (1, 26). In the rat, exercise induces a fall in the epinephrine content of the adrenal gland, a fall which can be accentuated if synthesis of this hormone is prevented by administration of a-methyl tyrosine (7). Such increased sympathetic activity appears to be essential for the efficient performance of work since complete blockade (19) or adequate surgical removal (22) of the sympathetic system greatly reduces an animal's...
ability to tolerate exercise. After blockade of the rat’s sympathetic system, exercise induces a fall instead of a rise in blood sugar concentration, an abnormally small increase in the level of circulating free fatty acids and no increase in body temperature (19). Adrenalectomy has a similar effect on the response to exercise but in this case can be reversed by administration of cortisone (19, 22). It is therefore agreed that adequate adrenocortical and sympathetic function is necessary for rapid adaptation of the normal animal to increased needs for fuel (2).

On the other hand, insulin does not appear to be essential since pancreatectomized rats (12) and dogs (20) are as well able to perform work as normal animals. In fact, insulin could antagonize the lipolytic and glycogenolytic actions of the catecholamines, growth hormone (11, 23), and possibly cortisol (5) released endogenously during exercise or stress.

Infusion of epinephrine into man (13, 21), the pig (9), or the monkey (14) induces hyperglycemia but no increase in plasma insulin concentration until after the infusion is stopped. Epinephrine will also inhibit release of insulin induced by glucose in vitro from pieces of pancreatic tissue of the rabbit (4) and rat (18). One of the functions of epinephrine released during exercise or stress could therefore be the suppression of insulin secretion. Evidence for this hypothesis includes the observations that levels of circulating insulin-like substances (6) and of immunoreactive insulin (3, 24) either do not alter or actually fall in man during muscular exercise. The present results provide more convincing evidence for two reasons. First, hyperglycemia is not initially in marked evidence during exercise or stress but in the present experiments all animals had plasma sugar concentrations above 300 mg/100 ml during the experimental period; glucose provided a potent stimulus to the β-cells in all animals. Secondly, an acute change in plasma insulin concentration could be due to altered utilization or altered secretion of the hormone, whereas in the present experiments all insulin secreted from the pancreas during the initial and experimental periods was trapped by circulating injected insulin antibodies. The present results therefore show more conclusively that even when a potent stimulus to insulin secretion is applied in vivo, exercise and stress do have an inhibitory effect.

It cannot be concluded solely from the results of the present experiments that endogenously secreted epinephrine is the only factor responsible. Apart from epinephrine, however, no other hormone, including cortisol (15) and growth hormone (Malaisse, Malaisse-Lagae, King, and Wright; unpublished data), has been shown to have a direct inhibitory effect on insulin secretion in vitro; norepinephrine has a weak inhibitory effect (18). It seems most likely, therefore, that epinephrine released during stress or exercise does, inter alia, inhibit insulin secretion and so prevent the antilipolytic and antiglycogenolytic effects of this hormone. In doing so it is also able to overcome the potent stimulant effect of glucose on β-cells which, under the present experimental conditions, have been shown to be excessively sensitive to glucose in vitro (17).

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REFERENCES


