Insulin acts on the hypothalamic glucose-facilitated neurons to induce hyperglycemia and hyperinsulinemia in the rat[1]

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Abstract. Microinjection of insulin (0.04–0.12 IU/μl) into the anterior hypothalamus or the lateral hypothalamus, but not the vertromedial hypothalamus of the rat brain, caused a dose-dependent rise in blood glucose and in serum insulin. The majority (71.5%) of the glucose-facilitated neurons recorded in the lateral hypothalamic area were excited by intracerebral injection of insulin. The data indicate that insulin acts on the hypothalamic glucose-facilitated neurons to induce hyperglycemia and hyperinsulinemia. It is unknown whether insulin normally reaches the hypothalamic area, or how it might do so.
Key words. Hypothalamus; insulin; hyperglycemia; hyperinsulinemia; neuronal activity.

Although it is unknown whether insulin can reach the brain under physiological conditions, considerable evidence has accumulated indicating a direct effect of insulin on neurones located in specific nuclei of the central nervous system[2,3]. For example, insulin administered to dogs intracisternally into the cerebrospinal fluid caused a fall of plasma glucose which was blocked by vagotomy[4]. In addition, insulin has been demonstrated in the brain, with the highest levels in the hypothalamus[5]. The hypothalamus has also been shown to contain insulin-receptors[6]. These observations suggest the exciting possibility that the hypothalamic insulin may play a role in glucoregulation. The intention of the current study was to assess the effects of insulin administration into the ventricle or hypothalamus on blood glucose or serum insulin, as well as on hypothalamic neurons classified as glucose-responsive or glucose-unresponsive, in order to assess the role of insulin in glucoregulation.

Materials and methods

Experiments were performed on male Sprague-Dawley rats weighing between 250 and 300 g. The animals were fed with a dry powder chow. They were housed in groups in wire mesh cages in a room maintained at 24 ± 2.0 °C with natural light-dark cycles. Each animal was anesthetized with pentobarbital sodium (60 mg/kg, i.p.), and a burr hole was drilled in the calvaria above the hypothalamus. The cannula guide tube with trocars was implanted using the stereotaxic atlas and coordinates of Paxinos and Watson[7]. The following coordinates were used: lateral hypothalamus (LH): A 7.2, L 1.6–2.3, and H 1.3–1.9 mm; ventromedial hypothalamus (VMH): A 6.7, L 0.2–1.0, and H 0.7–1.0 mm; and anterior hypothalamus (AH): A 7.2, L 0.3–0.8, and H 0.7–1.2 mm[7]. At the time of injection, the cannula insert was connected to a 10-μl Hamilton microsyringe by PE 10 polyethylene tubing. The volume of the injection down each cannula was 1.0 μl. The animals were injected intraventricularly or intrahypothalamically with either Isophane insulin (NPH, 0.04–0.12 IU/ml at pH 7.2; CWLTH Serum Laboratories, Melbourne, Australia) or vehicle solution.

Continuous blood glucose assays were carried out by connecting the catheterized femoral veins to a Biostator glucose monitor (Miles Laboratories, Elkhart, Ind., USA). The instrument is capable of monitoring whole-blood glucose readings over the useful range of 17–700 mg/dl. Once the instrument's glucose analyzer had been properly set up and calibrated, it could be attached to the blood vessels of the animals. Plasma immunoreactive insulin was measured by a double-antibody radioimmunoassay technique[8].

For the recording of unit activity, each animal with an intracerebroventricular cannula was maintained between 36.6–37.5 °C using a water-perfused pad under the animal. The animals were mounted stereotaxically with the heads fixed according to the coordination system of Paxinos and Watson[7]. A piece of bone was removed from the right half of the skull, and the underlying dura was removed. Recordings of single-unit discharges were made from the right half of the LH area. Single-barrel micropipets were filled with 3 M NaCl saturated with fast green dye and used for extracellular recording. The overall tip diameter of the micropipets was 2–5 μm. It generally had an impedance of 7–8 mΩ. After the micropipet had been lowered into the desired location in the LH area, a hydraulic microdrive was used to slowly advance the micropipets. Single-unit activity was processed using standard cathode follower and amplification circuitry for extracellular spike potential[9]. Impulses were counted in intervals of 1 s and displayed on a Grass polygraph recorder. At the end of each experiment, the vertical location of the micropipet was recorded and 25 μA of negative current was passed through the micropipet for 10 min to deposit fast green dye at the site. The locations of the fast green spots were used to verify the locations of the recording sites.

Results and discussion

Histological examination revealed that 18 out of 26 injection sites were accurately positioned within the desired hypothalamic sites. The results from rats with correct

Table 1. The effects of administration of normal saline or insulin into different hypothalamic nuclei on blood glucose or insulin in rats

Treatment	Δ Blood glucose (mg/dl)	Δ Plasma insulin (μU/ml)
Lateral hypothalamus		
0.9% Saline	7 ± 4	9 ± 5
insulin 0.04 IU	41 ± 4*	19 ± 6*
insulin 0.08 IU	100 ± 8*	40 ± 19*
insulin 0.12 IU	157 ± 19*	73 ± 28*
Anterior hypothalamus		
0.9% Saline	11 ± 6	10 ± 6
insulin 0.04 IU	29 ± 5*	21 ± 5*
insulin 0.08 IU	76 ± 7*	34 ± 11*
insulin 0.12 IU	132 ± 20*	59 ± 18*
Ventromedial hypothalamus		
0.9% Saline	9 ± 5	8 ± 4
insulin 0.04 IU	12 ± 7	7 ± 5
insulin 0.08 IU	13 ± 8	11 ± 6
insulin 0.12 IU	10 ± 6	13 ± 6

The values are means ± SEM of 6 animals for each group. * Significantly different from corresponding control rats, at $p < 0.05$ (Student's t-test).

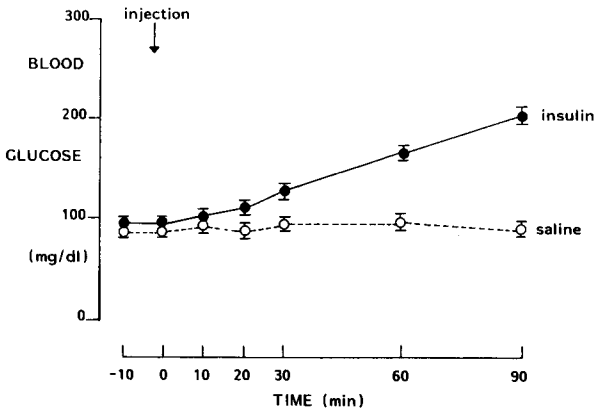

Figure 1. Hyperglycemic responses produced by administration of insulin (0.08 IU) into the lateral hypothalamus in 6 rats. The points represent the mean elevation in blood glucose (mg/dl), and the vertical bars denote means values ± SEM.

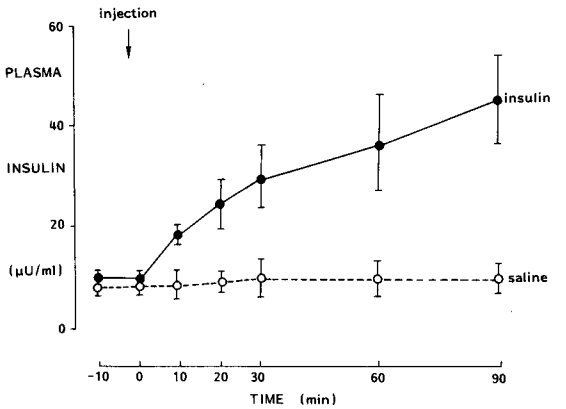

Figure 2. Hyperinsulinemic responses produced by administration of insulin (0.08 IU) into the lateral hypothalamus in 6 rats. The points represent the mean elevation in plasma insulin (μU/ml), and the vertical bars denote means values ± SEM.

positioning of the implanted cannulae were used for the data analysis. The results from rats with incorrect positioned cannulae were all negative. Table 1 shows the effects of intrahypothalamic administration of normal saline or insulin (0.04–0.12 IU/μl) on the blood glucose or serum insulin in rats. Administration of 0.9% saline into VMH, LH or AH caused an insignificant change of blood glucose or serum insulin. A single, unilateral injection of insulin into the AH or LH, but not into the VMH, caused a dose-dependent increase in blood glucose or serum insulin.

Figure 1 and Figure 2 illustrate the hyperglycemic and the hyperinsulin responses, respectively, produced by intra-LH administration of insulin (0.08 IU/μl) in rats. The increases of blood glucose or serum insulin began almost immediately after drug injection and reached a maximal level at about 90 min. By 300 min both the blood glucose and the serum insulin had returned to normal. Thirty-seven single units in the LH area were examined in 37 rats under urethane anesthesia. Each unit was subjected to intracerebroventricular injection of either 0.9% saline, 50% glucose or 0.08 IU insulin. Injection volume was always 10 μl for all intracerebroventricular injections. Table 2 shows that the proportions of glucose-facilitated, glucose-depressed and glucose-unresponsive units were 38, 16 and 46%, respectively, of the total units recorded in the LH area. Although the study of 37 cells is not an extensive sample, each cell was from a different rat.

Table 2 also contains the responses of the 37 hypothalamic units, classified by their reaction to glucose, to the test application of insulin. It can be seen that intracerebroventricularly applied insulin displays differential effects for glucose-responsive and glucose-unresponsive hypothalamic neurons. As indicated in the table, most of the glucose-facilitated (85.7%) and glucose-depressed (66.6%) neurons, but only 17.6% of glucose unrespon-

Table 2. Effects of insulin on 37 hypothalamic units in LH area classified as glucose-facilitated, glucose-depressed, or glucose-unresponsive

Unit type	Number of units tested	Responses to intraventricular insulin		
		Excitation	Inhibition	No effect
Glucose-facilitated	14	10 (71.5%)	2 (14.2%)	2 (14.3%)
Glucose-depressed	6	2 (33.3%)	2 (33.3%)	2 (33.4%)
Glucose-unresponsive	17	1 (8.8%)	2 (8.8%)	14 (82.4%)
Total	37	13 (35.0%)	6 (16.4%)	18 (48.6%)

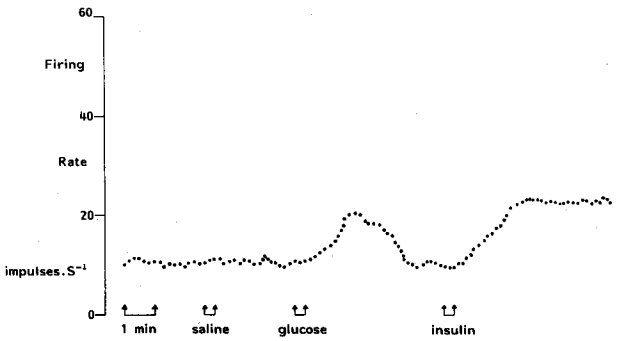

Figure 3. Excitation of a glucose-facilitated unit in the lateral hypothalamic area produced by intracerebroventricular application of insulin. Control injection of 0.9% saline was without effect.

sive neurons, were affected by insulin. Statistical analysis indicates that the differences between the insulin responses of either glucose-facilitated or glucose-depressed neurons and glucose-unresponsive neurons are significant ($p < 0.05$). Furthermore, there are significant differences ($p < 0.05$) between insulin responses for glucose-facilitated and glucose-depressed neurons recorded in the LH area. As shown in table 2, the majority (71.5%) of glucose-facilitated units were excited by insulin (fig. 3). However, insulin displays no differential effects for glucose-depressed units.

The current results are consistent in part with the findings of other investigators[3, 10–13]. It was shown that the activity as recorded from the LH was decreased in the presence of hyperglycemia in streptozocin-diabetic monkeys[12, 13]. Electrical stimulation of LH resulted in an increase in blood glucose, a rise in serum insulin, and an increase in food intake in monkeys[3]. In contrast, stimulation of VMH or AH was associated with a decrease in circulating insulin, a fall in blood glucose, and a reduction in food intake. These observations demonstrated that glucose and insulin rose concomitantly in the peripheral blood, as shown in the present results.

The VMH and the LH are important in the regulation of glucose/appetite. Oomura[11] demonstrated that, although glucoreceptor neurons occurred in the VMH and had receptor sites for glucose, the neurons located in the LH area were glucosensitive with only insulin receptor sites. The present results indicate that insulin, when administered intrahypothalamically, may activate the insulin receptor sites located in the glucose-sensitive neurons of the LH area or AH area and lead to hyperglycermia and hyperinsulinemia in rats. Recently, it was found that insulin administration resulted in marked acute, time-dependent, bidirectional changes in the hypothalamic neuronal activities of norepinephrine and 5-hydroxytryptamine[14]. Iontophoretic application of the endogenous organic acid, 3-hydroxybutyric acid (an anorectic agent), affected the glucose-sensitive neurons in the LH area[15]. The changes in the activities of the hypothalamic monoamines or 3-hydroxybutyric acid, may in turn influence the plasma levels of glucose or insulin. Of course, this needs further verification in future studies.

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