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Intrahypothalamic injection of insulin decreases firing rate of sympathetic nerves

(hypothalamic lesion/brown adipose tissue/kainic acid/rat)

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ABSTRACT **Injection of picomolar quantities of insulin into the ventromedial hypothalamus of rats significantly reduced the firing rate of sympathetic nerves that supply interscapular brown adipose tissue. The minimal firing rate was reached in 2 min, and the effect was gone within 4 min. The effect of insulin was dose-related and did not occur when comparable volumes of physiological saline were injected into the ventromedial hypothalamus. Destruction of neurons in the ventromedial hypothalamus by injection of kainic acid abolished the inhibitory effects of insulin. These data suggest that insulin may play a role in modulating the sympathetic firing rate to thermogenically important tissues.**

Since the demonstration by Bernard (1) more than 100 years ago that piqûre of the midbrain could produce diabetes in experimental animals, the hypothalamus has been a site of interest for diabetologists. The hypothesis that the hypothalamus might respond directly to insulin is supported by several studies. Infusions of insulin into the cerebral ventricle have been reported to decrease food intake in baboons (2) and in rats (3). Injection of insulin into the cerebral vesicles (4, 5), into the hypothalamus itself (6–8), or into the carotid artery (9) can decrease blood sugar. More directly, Oomura and Kita (10) have shown that iontophoretic application of insulin into the ventromedial hypothalamus (VMH) reduced the discharge rate of glucoreceptor neurons. When glucose or glucose plus insulin were added to the same neurons, the electrical discharge rate increased (10, 11).

The VMH can modulate both the parasympathetic and sympathetic nervous systems (12). Electrical stimulation of the VMH increases the activity of the sympathetic nerves to brown adipose tissue (13, 14). Conversely, destruction of the VMH decreases the electrical firing rate of sympathetic nerves to this tissue (15). These observations suggest that the decrease in electrical activity of neurons in the VMH in the presence of insulin might decrease the sympathetic outflow from this region as reflected in firing rate of sympathetic nerves. To explore this hypothesis we have developed a technique for directly recording from sympathetic nerves supplying brown adipose tissue. Utilizing this technique, we have explored the effect of insulin on the firing rate of sympathetic nerves to interscapular brown adipose tissue.

MATERIALS AND METHODS

Animals. Twenty-two male Wistar rats (250–300 g) were used in these studies. They were fed a standard diet (Oriental Yeast M10, Osaka, Japan) with free access to tap water and were housed individually in a room maintained at 19 ± 3°C.

Hypothalamic Lesions. After anesthesia with pentobarbital (45 mg/kg), animals were mounted in a stereotaxic apparatus. Twenty-two-gauge guide cannulas were aimed at +6.0 mm anteriorly, ±1.0 mm laterally, and −3.5 to 4.0 mm below the zero plane in the VMH, using the coordinates of Pellegrino *et al.* (16). These cannulas were inserted through burr holes and anchored to the skull with dental cement and stainless steel screws. Twelve of the 22 rats received injections of kainic acid through a 28-gauge needle inserted through the 22-gauge guide cannulas. The kainic acid (Sigma) was dissolved in physiological saline (0.15 M NaCl), and 0.8 μl of a 1.0 μM solution of kainic acid was infused into the VMH over 2 1/2 min. The 28-gauge cannula was left in place for an additional 2 min. The remaining 10 animals received saline and served as sham-lesioned controls.

Nerve Recording. Sympathetic nerve recordings were performed 6–9 days after the implantation of the guide cannula and injection of kainic acid or saline. Anesthesia was induced by injection of pethidine hydrochloride (2.5 mg/kg, i.m.) and pentobarbital (45 mg/kg i.p.). The depth of anesthesia was kept constant by subsequent injections of pentobarbital (7.5 mg/kg, i.p.) at 30-min intervals (17). Interscapular brown adipose tissue was exposed through a dorsal incision. The five sympathetic nerve bundles were identified. One of these bundles was randomly sectioned as close as possible to the brown adipose tissue. The proximal segment was then microdissected longitudinally into several fine filaments, and one filament was placed on a pair of silver wire electrodes immersed in a mixture of liquid paraffin and petroleum jelly to prevent dehydration. Spontaneous efferent nerve activity was amplified by means of a condenser-coupled differential amplifier and stored on magnetic tapes. Analysis of nerve activity was performed after converting the spikes to standard pulses through a window discriminator and removing the background noise. A rate meter with reset time of 5 sec was used to observe the time course of nerve activity that was displayed on the storage oscilloscope. Blood samples of 0.5 ml were obtained from a tail cut, 5 min before, just prior to, and 5 min after the hypothalamic injection of insulin.

Histology. Following completion of the study, the position of the cannula and the areas lesioned with kainic acid were determined by preparing serial frozen coronal sections of the brain at 25-μm intervals and staining them with luxol fast blue and cresyl violet. All cannulas were located just above the VMH, and no animals were discarded from the study based on inappropriate location of cannulas or lesions.

Analytical Measurements. Glucose and insulin were measured in the samples of blood obtained before and after the injection of insulin. Glucose was measured by the method of Salomon and Johnson (18), and insulin was measured by radioimmunoassay.

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Abbreviation: VMH, ventromedial hypothalamus.

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Reagents. Insulin was obtained from Novo Pharmaceuticals (Copenhagen) and dissolved in 0.15 M NaCl. It was infused in a volume of 0.25–1.0 μl through the 28-gauge cannulas. Kainic acid was purchased from Sigma and dissolved in 0.15 M NaCl.

Data Analysis. The data from this study were taken from the initial response of the animals to one of the injected solutions and were analyzed by an analysis of variance and Duncan's multiple range test.

RESULTS

Fig. 1 shows an example of the changes in efferent sympathetic activity following the injection of 143 pmol of insulin (0.5 μl, 287 μM insulin; 1 pmol of insulin is ≈ 6 ng) into the VMH of animals with sham-lesions. The insulin injection produced a transient reduction in nerve activity. The fall in sympathetic nerve activity reached its nadir about 2 min after the injection and lasted 3–4 min (Fig. 2). The effect of insulin was dose-related (Fig. 3). The lowest dose of insulin (71.5 pmol, or 429 ng) produced a reduction of almost 40% in firing rate. When 143 pmol of insulin was injected, there was a 50% decrease, and with 287 pmol, there was a 90% reduction in firing rate. Comparable volumes of physiological saline had no effect on the basal firing rate of sympathetic nerves to brown adipose tissue.

The effect of destroying the neurons in the VMH with kainic acid can be seen in Fig. 4. The basal firing rate was depressed by 60% in rats with kainic acid lesions. Following the injection of 287 pmol of insulin, firing rate of intact rats was reduced 94.3 ± 0.1%. In animals with hypothalamic lesions induced by kainic acid, a comparable amount of insulin produced only an 8.9 ± 0.1% reduction in firing rate. These differences were highly significant ($P < 0.001$). Plasma concentrations of glucose 5 min before, just prior to, and 5 min after the injection of insulin were 5.14 mM, 5.13 mM, and 5.12 mM, respectively. The concentration of insulin in serum was also unchanged (111.3 ± 18.2 pM, 119.0 ± 17.9 pM, and 105.6 ± 75.3 pM, respectively).

DISCUSSION

These studies show that injections of insulin into the VMH significantly reduce the efferent firing rate of sympathetic nerves innervating brown adipose tissue and that this intrahypothalamic effect of insulin is abolished when VMH neurons are destroyed by injections of kainic acid (19).

The VMH appears to modulate both the sympathetic and parasympathetic nervous system (12). Electrical stimulation of the VMH increases the activity of the sympathetic nervous system, as indicated by an increase in plasma free fatty acids (20) and a rise in temperature of interscapular brown adipose tissue (14). In contrast to the increased sympathetic activity that follows stimulation of the VMH, there is a reciprocal reduction in the activity of the parasympathetic nervous system (21). These reciprocal relationships are also seen following destructive lesions in the VMH. Destruction of the VMH decreases the sympathetic activity and increases the parasympathetic activity (10).

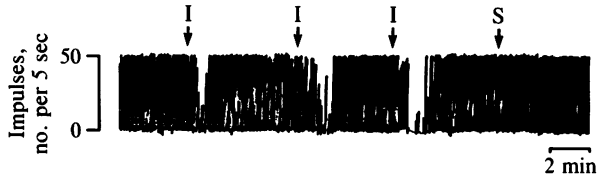

FIG. 1. A typical example of the effect of insulin and saline on the firing rate of sympathetic nerves to brown adipose tissue. Rats were prepared with 22-gauge cannulas aimed at the VMH. Filaments from one of the five nerve bundles supplying brown adipose tissue were placed on silver wire electrodes and the firing rate was recorded. I, times at which 143 pmol of insulin was injected; S, time at which a comparable volume of physiological saline was injected.

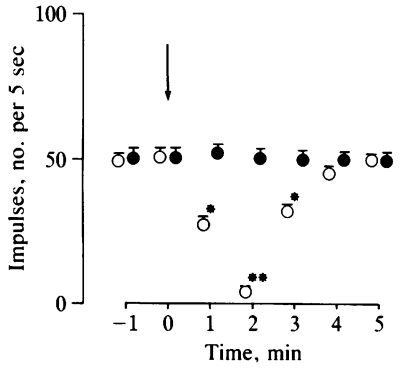

FIG. 2. Time course of response to insulin. Firing rate after injection (arrow) of insulin (287 pmol; $n = 12$) is shown by open circles, and the response to a comparable volume of saline ($n = 12$) is shown by solid circles. Error bars show SEM. *, $P < 0.05$; **, $P < 0.01$ compared to saline.

As a consequence of these changes in function of the autonomic nervous system, there is an increase in insulin secretion and a decrease in thermogenesis. Stimulation of the vagus nerve in dogs (22) and cats (23) increases insulin and glucagon secretion. Conversely, stimulation of the splanchnic nerve reduces insulin secretion (24, 25). The change in insulin secretion results in part from increased vagal activity to the pancreas, and in part from decreased sympathetic tone to this tissue with resultant increased sensitivity to circulating catecholamines (26). When the sympathetic nerves to interscapular brown adipose tissue are stimulated, there is an increase in heat output from this tissue, and when these nerves are cut, the tissue involutes (27).

The present studies suggest that insulin may be one modulator for the hypothalamic control of sympathetic nervous function to brown adipose tissue. Injection of insulin into the VMH decreases the electrical discharge of glucoreceptor neurons (10, 11). Although the mechanism for this effect is unclear, the observations that insulin produces hyperpolarization of the muscle membrane (28) and that it can increase the exchange of sodium (29) may provide one explanation. An increase in polarization of the hypothalamic glucoreceptors might account for their decreased rate of

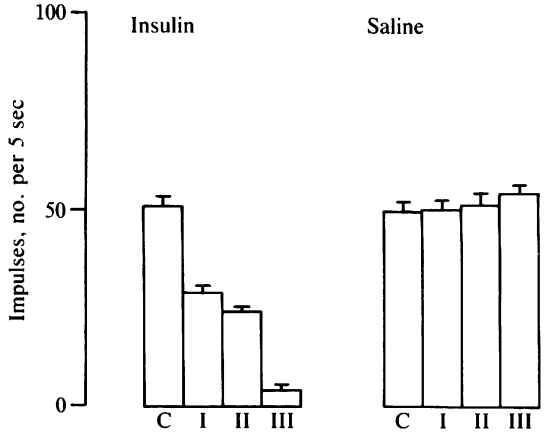

FIG. 3. Dose response to insulin and saline. Volumes of 0 (C), 0.25 (I), 0.50 (II), and 1.0 (III) μl (72, 143, and 287 pmol of insulin) were injected, and the integrated response over 2 min is shown as the spike rate per 5 sec. Each bar represents the mean of 12 rats. For insulin, $P < 0.01$ for I and II vs. C; $P < 0.002$ for III vs. C.

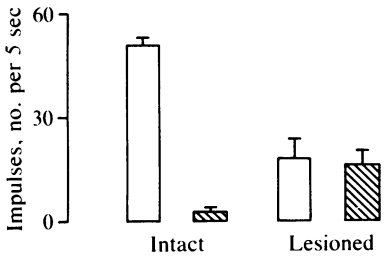

FIG. 4. Kainic acid lesions of the VMH prevent the response to insulin. Basal firing rate is shown by open bars; firing rate 2 min after injection of 287 pmol of insulin is shown by hatched bars. Intact animals show a 94% decrease in firing rate after insulin ($P < 0.001$). Basal firing rate of the lesioned rats was decreased by 40%, but the insulin response was almost completely abolished. Each bar represents the mean of $n = 12$ (lesioned) or $n = 8$ (intact) rats.

discharge and for the reduced firing of sympathetic nerves to brown adipose tissue.

The effects of insulin on the activity of the sympathetic nervous system might be produced in several ways. Insulin might enter the central nervous system by transport across the blood–brain barrier (30). Insulin might also act through the insulin receptors located on the blood–brain capillaries (30). Insulin receptors have been reported to be widely distributed throughout the central nervous system of the rat and have been identified on the blood–brain barrier (31). By whichever mechanism insulin may reach the central nervous system, our data suggest that insulin-responsive neurons in the VMH may modulate the firing rate of sympathetic nerves to brown adipose tissue (32).

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