Effect of Insulin, Glucose, and 2-Deoxy-glucose Infusion into the Third Cerebral Ventricle of Conscious Dogs on Plasma Insulin, Glucose, and Free Fatty Acids

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SUMMARY

Previous studies have demonstrated that exogenous insulin, injected into central cerebrospinal fluid cavities of dogs, stimulates the release of endogenous insulin from the pancreas. To determine whether this response was elicited by (1) insulin per se, (2) an effect of insulin on glucose transport, or (3) glucopenia in the cerebrospinal fluid, we measured plasma insulin, glucose, and free fatty acids during the infusion of insulin, glucose, and 2-deoxy-glucose (2DG), individually or in combination, into the third cerebral ventricle of conscious dogs. As expected, the third ventricular infusion of insulin alone elicited a small, but significant, rise of plasma insulin. Surprisingly, infusion of insulin with glucose produced a smaller increase of plasma insulin ($P < 0.05$) and the infusion of insulin with 2DG produced a much larger increase of plasma insulin ($P < 0.05$) than did the third ventricular infusion of insulin alone. The third ventricular infusion of either glucose alone or 2DG alone had no effect on the plasma levels of insulin. These data suggest that administration of insulin into the cerebral ventricles stimulates pancreatic insulin secretion but not by accelerating the transport of glucose into a chemosensitive area of the brain. DIABETES 29:278–283, April 1980.

The autonomic nervous system exerts a dual control over insulin secretion. The parasympathetic nervous system can augment insulin secretion: electrical stimulation of the vagus enhances glucose-induced insulin release.[1] The sympathetic nervous system can produce a net impairment of insulin release: electrical stimulation of the mixed pancreatic nerve during the infusion of atropine inhibits both basal[2] and glucose-induced insulin release.[2,3] The parasympathetic pathway may be under the central influence of the ventrolateral hypothalamus, since electrical stimulation in this area also augments insulin secretion.[4] The sympathetic pathway appears to be under the central influence of the ventromedial hypothalamus, since electrical stimulation of this area can inhibit insulin secretion.[5] The demonstration of chemoreceptors in these areas of the brain raises the possibility that the hypothalamus may do more than simply integrate signals from higher centers and peripheral afferents. For example, Oomura[6] has found hypothalamic neurons which alter their rate of firing in response to iontophoretic application of insulin, glucose, and 3-o-methylglucose. In addition, Havrankova et al.[7] have recently demonstrated the specific binding of insulin to hypothalamic as well as other brain tissue. These putative receptors appear to influence the neural input which the beta-cells receive, since Chen, Woods, and Porte[8,9] have shown that injection of insulin into the cerebrospinal fluid (CSF) of dogs stimulates the secretion of insulin from the pancreas. In these experiments, the injection of insulin decreased the glucose concentration of the CSF, leading Woods and Porte[9] to hypothesize that the secretion of insulin observed was stimulated by an increase of glucose uptake into some area of the brain. If this hypothesis were correct, the addition of glucose to the centrally administered insulin should augment the neural stimulation of insulin release. Conversely, the addition of 2-deoxy-glucose (2DG), a competitive inhibitor of glucose transport and phosphorylation,[10] to the centrally administered insulin should diminish the neural stimulation of insulin release. Therefore, we examined the effect of insulin, glucose, and 2-deoxy-glucose (2DG) infused individually or in combination into the third cerebral ventricle of conscious dogs on plasma insulin, glucose, and free fatty acids.

METHODS

Thirteen adult dogs of mixed German shepherd breed, weighing from 26 to 34 kg, were used in the 26 experiments performed. At least 3 days before the experiments, a blood-

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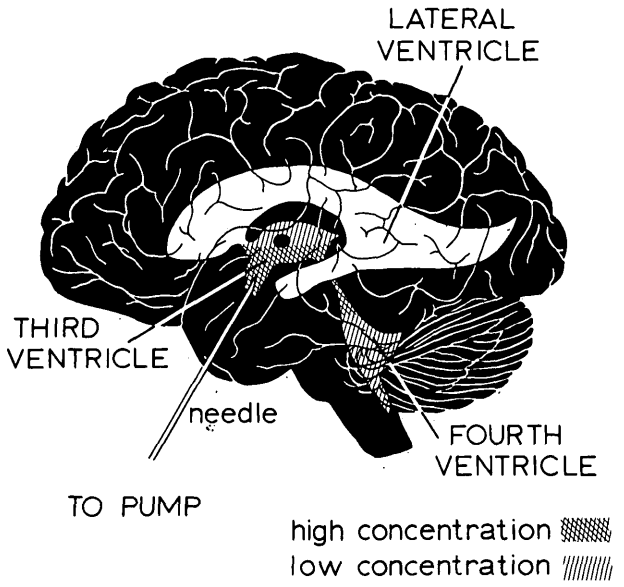
sampling catheter was implanted in the right atrium of the heart, and an infusion catheter was implanted in the third ventricle of the brain. A 12-gauge polyvinyl chloride catheter was inserted into the right jugular vein and advanced to the right atrium of the heart. The catheter was tied into position, and the free end was passed beneath the skin of the back of the neck where it exited through a midline incision. The catheter was flushed with 20 ml of sterile saline, filled with 3 ml of saline containing 100 U/ml heparin, and capped. The third cerebral ventricle was catheterized by the technique of Maran and Yates, as described elsewhere.[11]

Before the experimental series was begun, the dogs were trained to lie quietly on a warm table for extended periods. Dogs were fasted for 18 h before the start of all experiments, which were performed with the dogs in a fully conscious, relaxed state. At the end of each experimental series, the dogs were killed and the placement of both catheters was verified as follows. One hundred microliters of fluorescein dye was infused into the brain via the cerebral catheter. The brain was removed, dissected along the midline, and examined under ultraviolet illumination. Ventricular catheterization was judged successful if the fluorescein dye stained both medial walls of the third ventricle. The catheterization was judged unsuccessful if the fluorescein dye was restricted to a "pocket" in brain tissue on either side of the third ventricle. With successful catheterization, the dye appeared to be most concentrated on the basal part of the third ventricular walls, directly adjacent to the ventromedial hypothalamus and progressively less concentrated downstream in the fourth ventricle (Figure 1). Dye was never observed upstream in the lateral ventricle. Thus, contact with high concentrations of infusate was presumably limited to medial brain areas at the level of the thalamus and below. After assessing the placement of the cerebral catheter, the chest was opened, the pericardium incised, and the right atrium palpated for the tip of the blood-sampling catheter.

Each experiment was divided into three periods—an adjustment period, a control period, and a test period. During the adjustment period (30 min), the dog was allowed to relax; no blood samples were drawn. During the control period (25 min), the dog received a third ventricular infusion of mock cerebrospinal fluid (MCSF) at a rate of 5 or 50 μl/min. MCSF has the following composition: NaCl, 125 mM; $NaHCO_3$, 25 mM; KCl, 3.5 mM; $CaCl_2$, 1.3 mM; $MgCl_2$, 1.14 mM; NaH_2PO_4, 0.51 mM; urea, 1.67 mM; and glucose, 3.38 mM.[12] During the test period (30–90 min), the dog received one of five cerebroventricular infusates, depending on the experiment: (1) insulin (200 mU/min); (2) insulin (200 mU/min) with glucose (0.225 mg/min); (3) insulin (200 mU/min) with 2DG (0.225 mg/min); (4) glucose (5.5 mg/min); or (5) 2DG (5.5 mg/min). Infusates 1, 2, and 3 were delivered at 5 μl/min; 4 and 5 were infused at 50 μl/min; all were dissolved in MCSF. Insulin (regular) was obtained from Eli Lilly (Indianapolis); glucose and 2DG were obtained from Sigma Chemicals (St. Louis).

Blood samples of 2.5 ml were drawn at 5-min intervals during the control and test periods. Samples were placed in tubes containing heparin and NaF and then centrifuged; plasma was separated and frozen for later analysis. A maximum of 50 ml of blood (20 samples) was drawn during an experiment. No change of hematocrit was observed. Insulin was measured by radioimmunoassay using the dextran-charcoal separation method of Herbert et al.[13] Glucose was measured using a glucose analyzer (Beckman Instruments, Inc., Fullerton, California) by an automated modification of the glucose oxidase technique. Plasma free fatty acids (FFA) were measured by the colorimetric assay of Smith.[14] Mean values within a treatment group were compared using paired Student's t tests. Comparisons between treatment groups were performed using the two-sample Student's t test or the Welch t test[15] if the variances were significantly different.

FIGURE 1. Schematic illustration of the brain showing the position of the third ventricular catheter (needle) and the spread of infused dye (hatched area) observed after brain dissection.



RESULTS

During the infusion of MCSF into the third cerebral ventricle, plasma insulin averaged 32 ± 7 μU/ml ($\bar{x}$ ± SEM, N = 8, see Figure 2, Table 1). During the first half-hour of insulin infusion into the third ventricle (200 mU/min), the level of plasma insulin averaged 64 ± 9 μU/ml; during the second half-hour of insulin infusion into the third ventricle, plasma insulin averaged 51 ± 7 μU/ml. Both averages were significantly greater than those observed during the preceding intraventricular infusion of MCSF (both $P < 0.05$, see Figure 2, Table 1). Plasma glucose averaged 89 ± 3 mg/dl during the infusion of MCSF and did not change significantly during the intraventricular infusion of insulin (see Figure 2). Plasma FFA averaged 779 ± 74 μeq/l during the infusion of MCSF and decreased significantly ($P < 0.01$) to 641 ± 77 μeq/l during the second half-hour of the intraventricular insulin infusion (see Figure 2).

When glucose was infused into the third ventricle (0.225 mg/min) in combination with insulin (200 mU/min), a different pattern of plasma insulin concentration was observed: no significant change was noted during the first half-hour of infusion (see Table 1 and Figure 3). Neither plasma glucose nor plasma free fatty acids changed significantly during the third ventricular infusion of insulin plus glucose (see Figure 3). Infusions of glucose alone into the third cerebral ventricle at 5.5 mg/min, a rate over 20 times the previous one, had no effect on plasma insulin (see Tables 1 and 2). Likewise,

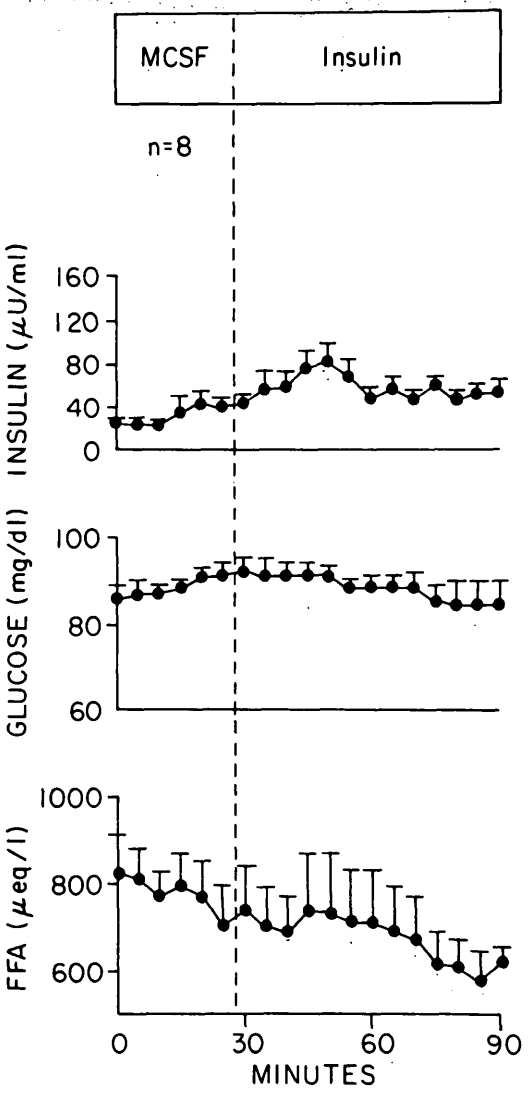


FIGURE 2. The effect of third ventricular infusions of mock cerebrospinal fluid (MCSF) (0–25 min) or insulin (200 mU/min, 30–90 min) on plasma insulin, glucose, and FFA ($\bar{x}$ ± SEM, N = 5).

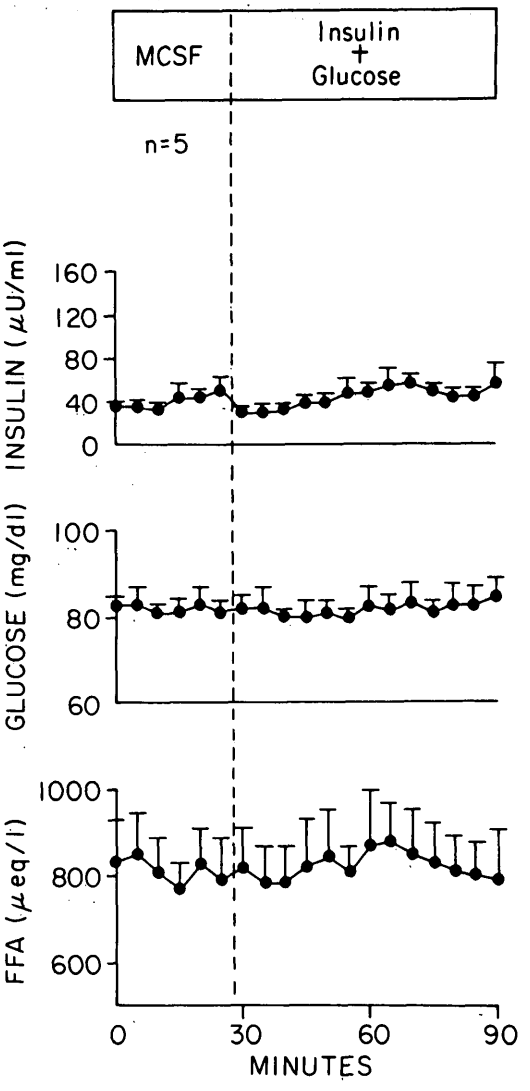


FIGURE 3. The effect of third ventricular infusions of MCSF (0–25 min) or insulin (200 mU/min) plus glucose (0.225 mg/min, 30–90 min) on plasma insulin, glucose, and FFA ($\bar{x}$ ± SEM, N = 5).

plasma glucose, which averaged 82 ± 2 mg/dl, and plasma FFA, which averaged 668 ± 38 μeq/L during the infusion of MCSF, did not change significantly during the hour-long third ventricular infusion of glucose alone (see Table 2).

TABLE 1
Average concentration of plasma insulin (μU/ml, mean ± SEM) during infusion of mock cerebrospinal fluid (MCSF) or combinations of insulin, glucose, and 2-deoxy-D-glucose (2DG) into the third cerebral ventricle of conscious dogs

	Treatment after MCSF				
	Insulin alone (N = 8)	Insulin + glucose (N = 5)	Insulin + 2DG (N = 5)	2 DG alone (N = 4)	Glucose alone (N = 4)
MCSF	32 ± 7	41 ± 5	34 ± 6	29 ± 6	28 ± 6
Treatment (1st ½ h)	64 ± 9	37 ± 6*	46 ± 6	28 ± 9*	28 ± 4*
Treatment (2nd ½ h)	51 ± 7	51 ± 10	150 ± 48*	26 ± 7*	27 ± 5*

* Significantly different from average value during treatment with insulin alone ($0.01 < P < 0.05$).

Third ventricular infusions of 2-deoxy-glucose (2DG) at 0.225 mg/min with insulin (200 mU/min) produced a large increase of plasma insulin during the second half-hour of infusion (see Figure 4), which was significantly greater than that produced by the infusion of insulin alone ($P < 0.05$, see Table 1). This rise of plasma insulin was accompanied by a progressive decrease of plasma glucose ($P < 0.025$) that averaged 18 ± 6 mg/dl below control during the second half-hour of infusion (see Figure 4). The apparent

TABLE 2
Average concentration ($\bar{x}$ ± SEM) of plasma insulin, glucose, and FFA during infusion of mock cerebrospinal fluid (MCSF) on glucose into the third cerebral ventricle of conscious dogs

	Plasma concentrations of:		
	Insulin (μU/ml)	Glucose (mg/dl)	FFA (μeq/L)
MCSF	28 ± 6	82 ± 2	668 ± 38
Glucose alone (1st ½ h)	28 ± 4	83 ± 2	667 ± 72
Glucose alone (2nd ½ h)	27 ± 5	85 ± 2	594 ± 87

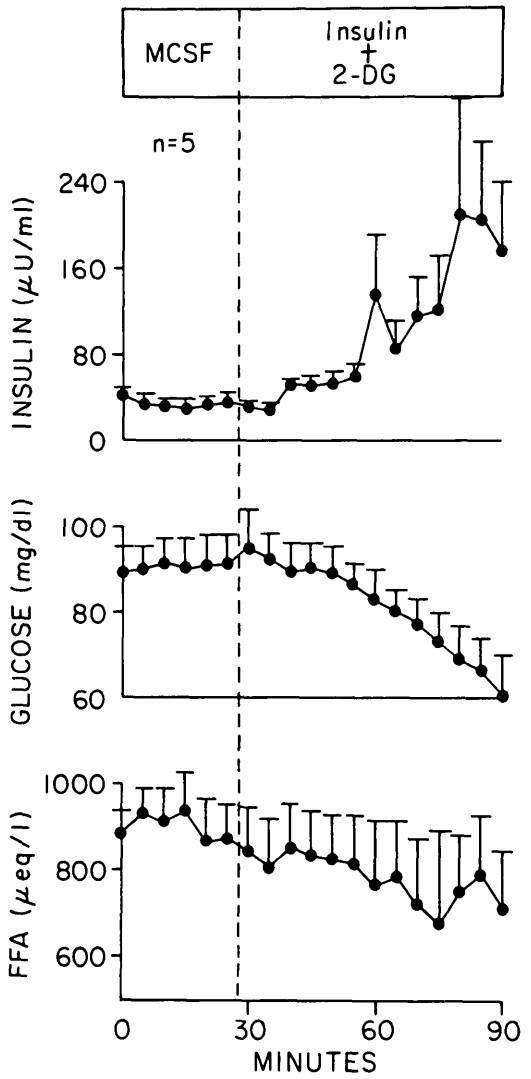


FIGURE 4. The effect of third ventricular infusions of MCSF (0–25 min) or insulin (200 mU/min) plus 2DG (0.225 mg/min, 30–90 min) on plasma insulin, glucose, and FFA ($\bar{x}$ ± SEM, N = 5).

decrease of plasma free fatty acids during the second half-hour of infusion did not reach statistical significance (Δ = −159 ± 96 μeq/L, P = 0.09). Infusions of 2DG alone into the third cerebral ventricle at 5.5 mg/min, over 20 times the previous dose, had no significant effect on plasma insulin (see Tables 1 and 3). Likewise, plasma glucose, which averaged 85 ± 2 mg/dl, and plasma FFA, which averaged 813 ± 100 μeq/L during the infusion of MCSF, did not change significantly during the hour-long third ventricular infusion of 2DG alone (see Table 3).

TABLE 3
Average concentration ($\bar{x}$ ± SEM) of plasma insulin, glucose, and FFA during infusion of mock cerebrospinal fluid (MCSF) or 2-deoxy-D-glucose (2DG) into the third cerebral ventricle of conscious dogs

	Plasma concentrations of:		
	Insulin (μU/ml)	Glucose (mg/dl)	FFA (μeq/L)
MCSF	29 ± 6	85 ± 2	813 ± 100
2DG alone (1st ½ h)	28 ± 9	85 ± 2	735 ± 97
2DG alone (2nd ½ h)	26 ± 7	85 ± 2	752 ± 128

DISCUSSION

These data on plasma insulin levels from conscious dogs are consistent with those from anesthetized dogs in which Chen, Woods, and Porte[8,9] showed that injection of insulin into the cerebrospinal fluid (CSF) stimulated the secretion of insulin via a vagal pathway. However, the present experiments were designed to test a separate hypothesis of Woods and Porte[9] that centrally administered insulin stimulates such a response by increasing the transport of glucose into a glucoregulatory area of the brain. If this latter hypothesis is correct, one might expect that the addition of glucose to an insulin infusate should increase glucose uptake further and, thus, produce a larger rise of plasma insulin than a third ventricular infusion of insulin alone. In contrast to expectations, the combination of insulin and glucose produced a significantly smaller increase in plasma insulin than did the third ventricular infusion of insulin alone. In accord with this paradoxic result, the combination of insulin and 2-deoxy-D-glucose, a competitive inhibitor of glucose transport and phosphorylation,[10] elicited a much larger rise of plasma insulin than did the third ventricular infusion of insulin alone. Thus, neither set of data supports the hypothesis that increased glucose uptake is the stimulus for neurally mediated insulin secretion. Rather, these data suggest that insulin has a direct effect on some population of central neurons and that glucose metabolism in these neurons can alter the effectiveness of insulin.

This effect of insulin may be on ionic flux. Insulin appears to increase potassium uptake[16] in various tissues, including skeletal muscle,[17] adipose tissue,[18] liver,[19] and perhaps brain.[20] Insulin also alters sodium flux in muscle[21] and phosphate flux in liver.[22] As might be expected from these observations, insulin is known to hyperpolarize the membrane potential of cells from both muscle[23] and adipose[24] tissues. In addition, either systemic[25] or local[6] administration of insulin alters the endogenous firing rate of certain hypothalamic neurons. Oomura has identified a group of hypothalamic neurons in which the insulin effect is accentuated by the simultaneous application of 2DG[6] and in which it is inhibited by the simultaneous application of glucose.[6] Thus, the known effect of insulin to alter the rate of ionic flux across the cell membranes of other tissue may also explain its ability to influence the central neurons that modulate insulin release. It should be pointed out, however, that alternative mechanisms, such as the acceleration of amino acid transport by insulin,[26] could mediate this central action.

Woods and Porte[9] reported that the intracisternal injection of insulin produced an acute hypoglycemia, which was presumably the result of the neurally mediated release of insulin. We observed a smaller increase of plasma insulin during the third ventricular infusion of insulin alone with no change of plasma glucose. Chen et al.,[8] in a companion study, also failed to observe acute hypoglycemia despite the substantial insulin release stimulated by the injection of insulin into the lateral ventricle. The reason for these discrepancies in glycemia is not clear. However, since Woods and Porte[9] have implicated the vagus in the neurogenic release of insulin, it is possible that a vagally mediated re-

lease of glucagon[27] also occurs. The ratio of insulin to glucagon secreted might determine the net effect on plasma glucose.

Recent work has convincingly demonstrated the presence of endogenous insulin in cerebrospinal fluid[28] as well as in brain tissue.[29] Others have recently reported the specific binding of insulin to various brain areas,[7,30] suggesting the presence of central receptors for insulin. Earlier, Oomura[6] had demonstrated a local effect of insulin by showing that iontophoretic application of minute amounts of insulin to the surface of certain hypothalamic neurons can change their rate of firing. Chen, Woods, and Porte[8,9] demonstrated that centrally administered insulin can influence the function of cells outside the brain by showing that insulin infused into the CSF activates a neural pathway, which in turn stimulates the release of insulin from the pancreas. Taken together, these studies outline a central system influencing insulin secretion. The data from the present study, however, would suggest that this system is activated more effectively by the combination of central insulin and glucopenia, which calls into question its potential physiologic significance. Thus, despite the presence of insulin and insulin-specific receptors in the brain, and despite the demonstration of both local and peripheral effects of centrally administered insulin, the physiologic role of central insulin in the regulation of the endocrine pancreas remains obscure.

The precise location of the central sites of insulin action has not been determined in the present studies. However, the results of our dye infusions would indicate that the infusate is restricted to the level of the third ventricle and below (see Figure 1). While penetration of hydrophilic protein molecules the size of insulin into brain tissue is thought to be minimal, the specific binding of insulin to circumventricular organs and the medial basal hypothalamus, which is adjacent to the third ventricle, has recently been demonstrated.[31] These areas represent possible sites for the central action of insulin.

In our experiments, glucose was infused into the third cerebral ventricle of conscious dogs and had no significant effect on the plasma level of glucose, confirming the findings of Sloviter et al.[32] and Coimbra et al.[33] In addition, this glucose infusion was without effect on the plasma level of insulin and FFA. Coimbra et al.[33] also reported no change of plasma insulin after the injection of glucose into the lateral cerebral ventricles of fasted rats; they did, however, observe a significant decrease of plasma FFA. Differences between their study and ours, either in the site of infusion (lateral ventricle versus third ventricle), the species of animal studied (rat versus dog), or the variability of FFA levels, may account for the differences in the observed effect of central glucose on plasma FFA.

In the present study, third ventricular infusion of 2DG alone had no significant effect on the plasma levels of insulin or FFA. Coimbra et al.[33] also reported no change of plasma insulin after the injection of 2DG into the lateral ventricle. They did, however, observe a significant increase of plasma FFA in fed rats. Difference in the observed effect of central 2DG on plasma FFA may be due to the experimental differences listed above as well as differences in the duration of fasting. Both Müller et al.[34] and Coimbra et al.[33] observed an increase of plasma glucose following the injection of 2DG into the lateral ventricle of rats. Frohman and Nagai[35] have observed a similar hyperglycemia following the infusion of 2DG into the lateral cerebral ventricle of conscious dogs. It is important to note, however, that the doses used in the present study are well below the minimum effective dose necessary to elicit this hyperglycemic response in the dog.[35] This comparison underscores the potency of these relatively small doses of 2DG and glucose to modulate the effects of intraventricular insulin on plasma insulin concentration.

In summary, infusions of insulin into the third cerebral ventricle of conscious dogs produce a small rise of plasma insulin, which is inhibited by the addition of glucose to the intraventricular infusate and enhanced by the addition of 2DG to the insulin infusate. These data suggest that glucopenia of certain brain cells potentiates the neurally mediated release of insulin after the administration of exogenous insulin to the CSF. It is suggested that central insulin may change the firing rate of those neurons that modulate insulin release by altering the flux of ions across their cell membranes, although other possible mediators, such as amino acids, cannot be excluded.

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