Long-Term Infusion of Norepinephrine Plus Serotonin Into the Ventromedial Hypothalamus Impairs Pancreatic Islet Function

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To examine the possibility of a cause-effect relationship between enhanced monoamine content in the ventromedial hypothalamus ([VMH] a characteristic of hyperinsulinemic and insulin-resistant animals) and islet dysfunction, we infused norepinephrine ([NE] 25 nmol/h) and/or serotonin ([5-HT] 2.5 nmol/h) into the VMH of normal hamsters for 5 weeks and then examined insulin release from the isolated pancreatic islets. VMH infusion of NE + 5-HT, but not of either neurotransmitter alone, produced a marked leftward shift in the dose-response curve of glucose-induced insulin release (twofold to sixfold increase at 5 to 7.5 mmol/L glucose *v* vehicle-treated animals). In addition, the islet responsiveness to 1 μmol/L NE and 10 μmol/L acetylcholine was abolished in these NE + 5-HT VMH-infused hamsters. These findings indicate that an increase of NE and 5-HT content in the VMH can induce dysregulation of islet insulin release in response to glucose and neurotransmitters. Inasmuch as VMH NE and 5-HT levels are elevated in hyperinsulinemic and insulin-resistant animals, the present findings suggest that an endogenous increase in these hypothalamic monoamines may contribute to islet dysfunction, which is one of the characteristics of type 2 diabetes.

ABNORMAL islet glucose sensitivity, characterized by an increased insulin response to basal glucose, contributes significantly to hyperinsulinemia and the consequent insulin resistance.[1] However, the cause(s) of this islet dysfunction is still obscure. The central nervous system, especially the hypothalamus, plays a crucial role in the overall regulation of glucose metabolism and insulin release. Recently, we reported that (1) the insulin-resistant glucose-intolerant state is associated with increases in ventromedial hypothalamus (VMH) noradrenergic and serotonergic activity and (2) pharmacological intervention to reduce these elevations in VMH monoamine activity also normalizes the insulin resistance and glucose intolerance.[2] Similarly, other laboratories have observed a positive correlation between an increase in the VMH noradrenaline level and the insulin-resistant state.[3,4] Short-term administration of noradrenaline to the VMH causes an immediate increase in plasma glucose, insulin, and glucagon,[5] and these hypothalamic noradrenergic activities can be potentiated by serotonin (5-HT).[6,7]

However, the effects of long-term noradrenaline and 5-HT infusion into the VMH of normal animals (to mimic the VMH environment of hyperinsulinemic and insulin-resistant animals) on islet β-cell function have not been investigated. The aim of the present study was to examine the effect of such chronic increases in VMH monoamine content on insulin release from pancreatic β cells in normal hamsters.

MATERIALS AND METHODS

Male Syrian hamsters (160 to 190 g; Simmonsen Laboratories, Gilroy, CA) were randomized to one of four groups (n = 10 to 15 per group) and infused continuously (0.5 μL/h) for 5 weeks with (1) vehicle (10 mmol/L sodium bisulfate saline solution), (2) norepinephrine ([NE] 25 nmol/h), (3) 5-HT (2.5 nmol/h), or (4) NE (25 nmol/h) + 5-HT (2.5 nmol/h) into the right VMH via osmotic minipumps placed subcutaneously. Animals with a cannula placed outside the VMH were excluded from the analyses. Following this treatment, pancreatic islets were isolated by collagenase digestion, and the insulin response to various concentrations of glucose or neurotransmitters was studied using islet static incubation (10 islets per incubation tube) at 37°C for a 60-minute period as described previously.[8] The biphasic insulin response stimulated by 15 mmol/L glucose was tested by islet perifusion (50 islets per perfusion chamber) for a 90-minute period as described previously.[9] Insulin content in the incubation buffer or in the perifusate was determined by radioimmunoassay (Linco Research, St. Charles, MO). The DNA content in isolated islets was determined by a fluorometric method using a DyNA Quant 200 fluorometer (Hoefer Pharmacia Biotechnology, San Francisco, CA).

RESULTS

The dose-response curve for glucose-induced insulin release was shifted leftward by NE + 5-HT infusion into the VMH (but not by NE or 5-HT alone) relative to vehicle controls. When islets from hamsters receiving NE + 5-HT infusion into the VMH were incubated with medium containing 5 or 7.5 mmol/L glucose, insulin release was sixfold or twofold higher, respectively, versus vehicle-infused hamsters ($P < .01$; Fig 1A). Insulin release at higher glucose concentrations (10 to 15 mmol/L) showed no significant differences among islets from the four hamster groups. The biphasic insulin response induced by 15 mmol/L glucose did not differ in the first or second phase of insulin release among the four treatment groups (Fig 1B).

In vehicle-infused controls, 1 μmol/L NE markedly inhibited the insulin release stimulated by 15 mmol/L glucose (by 52%, $P < .05$). However, in islets from hamsters receiving VMH infusion of NE, 5-HT, or NE + 5-HT, 1 μmol/L NE had no inhibitory effect on glucose-stimulated insulin release. Also, the stimulatory effect of 10 μmol/L acetylcholine on glucose-induced insulin release was abolished in islets from hamsters infused with 5-HT alone or NE + 5-HT (Table 1).

DISCUSSION

We recently found that a long-term infusion of NE + 5-HT into the VMH of normal hamsters induces hyperinsulinemia and glucose intolerance without causing hyperphagia (food intake, 14.3 ± 0.8, 11.1 ± 0.8, 12.3 ± 0.7, and 13.0 ± 1.1 g/d for vehicle, NE, 5-HT, and NE + 5-HT groups, respectively) or

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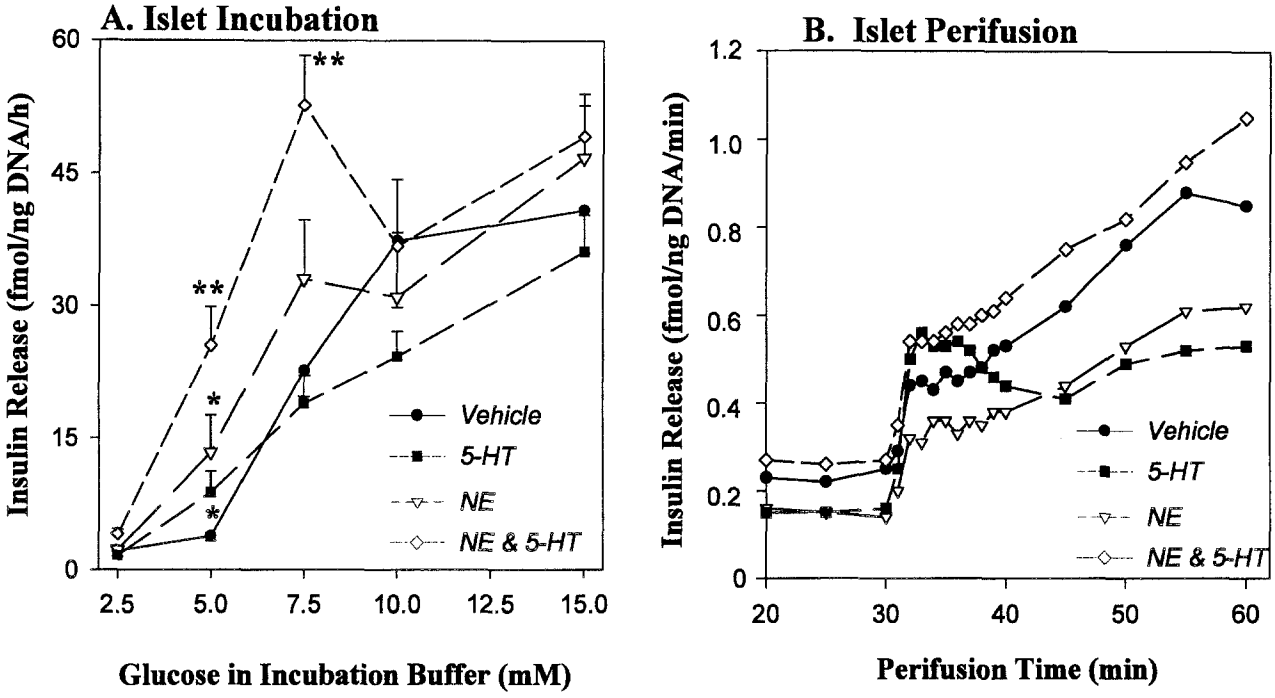

Fig 1. Glucose-induced insulin release from islets isolated from hamsters receiving a 5-week infusion of vehicle, NE, 5-HT, or NE + 5-HT into the VMH. (A) Islets incubated in the buffer containing different glucose concentrations (n = 6-12 experiments per point). (B) Islets perifused with 5 mmol/L glucose for 30 minutes and then with 15 mmol/L glucose for 60 minutes. *$P < .05$, **$P < .01$ *v* vehicle-infused hamsters (2-way ANOVA).

body weight gain (changes from baseline, 25, 19, 21, and 25 g for vehicle, NE, 5-HT, and NE + 5-HT groups, respectively).[10] Moreover, hyperinsulinemia and glucose intolerance could not be induced if the infusion cannula was located just outside of the VMH. Similarly, others have found that long-term infusion of NE into the VMH, but not the lateral hypothalamus or paraventricular hypothalamic nucleus, of normal rats induces hyperinsulinemia.[3] The present study demonstrates that long-term VMH infusion of NE + 5-HT induces a marked leftward shift in the dose-response curve for glucose-induced insulin release from isolated islets. These results indicate a cause-effect relationship between increased VMH noradrenergic/serotonergic extracellular concentrations and dysregulation of insulin release from pancreatic β cells, which may potentiate the hyperinsulinemia observed in response to this treatment.

Table 1. Effect of NE and Acetylcholine on Insulin Release Induced by 15 mmol/L Glucose (fmol/ng DNA/h, mean ± SE, n = 6-10)

Group	5 mmol/L Glucose	15 mmol/L Glucose	15 mmol/L Glucose + 1 μmol/L NE	15 mmol/L Glucose + 10 μmol/L Acetylcholine
Vehicle	4.3 ± 1.0	39.7 ± 5.0‡	19.0 ± 5.7*	73.2 ± 10.7*
NE	12.3 ± 3.4§	48.5 ± 6.5†	41.2 ± 7.2	71.0 ± 5.8*
5-HT	10.1 ± 2.1§	44.5 ± 6.8†	32.0 ± 6.3	55.8 ± 8.2
NE + 5-HT	19.9 ± 3.7‖	47.2 ± 5.5†	35.2 ± 10.5	53.3 ± 8.5

*$P < .05$ *v* islets incubated in medium containing 15 mmol/L glucose in the absence of neurotransmitters in the same group (*t* test).

†$P < .05$, ‡$P < .01$ *v* islets incubated at 5 mmol/L glucose in the same group (*t* test).

§$P < .05$, ‖$P < .01$ *v* islets incubated in medium containing 5 mmol/L glucose from the vehicle group (*t* test).

Although long-term NE and 5-HT infusions into the VMH each increased basal (5 mmol/L glucose) insulin release, only NE + 5-HT infusion produced a leftward shift in the dose-response curve for glucose-induced insulin release. The mechanism responsible for this interactive effect of VMH NE + 5-HT is unknown. However, centrally acting 5-HT_2 and 5-HT_{1A} agonists have been demonstrated to modulate and potentiate central (hypothalamic) noradrenaline release and activity.[6,7,11,12] This monoamine interaction may lead to overstimulation of the sympathetic nervous system and subsequent desensitization of the NE inhibition of insulin release (description follows). Interestingly, we have previously observed increases in VMH extracellular metabolite levels of NE and 5-HT in hyperinsulinemic glucose-intolerant hamsters versus normal hamsters.[2]

The neuroendocrine events mediating the VMH NE/5-HT effect on islet function remain to be delineated, but are likely to involve alterations in sympathetic and parasympathetic nervous system activities. VMH stimulatory effects on hepatic glucose production, adrenal epinephrine secretion, and glucagon secretion via activation of the sympathetic nervous system[13,14] may be expected to impact insulin secretion and β-cell function. In this regard, overactivation of autonomic parasympathetic and sympathetic neurons, in certain instances, can lead to target-organ desensitization to the respective neurotransmitters. Specifically regarding the autonomic innervation of pancreatic β cells, it has been demonstrated that sympathetic hyperinnervation induces β-cell desensitization to α-adrenergic stimuli.[15] Likewise, overstimulation of parasympathetic vagal input to the islet via a VMH lesion causes desensitization of β-cell responsiveness to acetylcholine.[16] Noradrenergic receptor binding

within the VMH (potentiated by 5-HT) may result in physiologic consequences similar to the above-mentioned situations regarding overactivation of the autonomic nervous system, in that it inhibits neuronal activity therein[17] (as does a VMH lesion leading to increased parasympathetic vagal tone) but also activates the sympathetic nervous system.[13,18] Consequently, it is interesting that long-term noradrenergic and serotonergic infusion into the VMH also results in the desensitization of islet insulin secretory responses to NE and acetylcholine as demonstrated in the present study. The net effect of this dual overactivation of the autonomic nervous system may be a dose-dependent differential desensitization to NE and acetylcholine at the β cell favoring an increased responsiveness to glucose as demonstrated herein. The in vivo physiologic consequences of this desensitization and its possible mechanism(s) are under further investigation.

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