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COMMENTARY

THYROTROPIN-RELEASING HORMONE (TRH): INSULIN-LIKE ACTION ON GLUCOREGULATION

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Normoglycemia is maintained through a complex interplay between the glucoregulatory hormones, glucagon, epinephrine, the glucocorticoids and insulin [1, 2], and it is under the controlling influence of various neurotransmitters and peptides acting upon the endocrine pancreas, the liver or at the level of the central nervous system (CNS) [3–6]. Most of the hormones and neural factors implicated in glucoregulation are called into play in response to hypoglycemia resulting from diminished supply or increased expenditure of metabolic fuel, and they act to normalize glucose levels by directly or indirectly promoting sugar production [7]. For example, glucagon is released from alpha-cells in the pancreas whenever plasma glucose levels fall below normal, and it stimulates glucose production in the liver through direct action [1, 8] or, additionally, by acting within the CNS [9]. Similarly, epinephrine is released from the adrenal medulla in response to hypoglycemia, and it promotes glucose production through direct action on muscle or liver [1, 10] as well as indirectly, through CNS action [11]. Other factors, such as cholecystokinin, bombesin, the opioid peptides and corticotropin-releasing factor (CRF), influence glucoregulation in part by modulating pancreatic function and in part by indirectly stimulating glucose production through CNS action [4, 12–15].

In contrast to the multitude of apparently redundant counterregulatory mechanisms set in motion by hypoglycemia, only one peptide hormone, insulin, serves to counteract hyperglycemia. Insulin is released from pancreatic beta-cells in response to high glucose, and it normalizes sugar levels partly by stimulating glucose utilization in muscle and adipose tissues and partly by inhibiting glucose output from the liver [16, 17]. In addition, insulin can lower circulating glucose levels by indirectly modulating the production and utilization of sugar through action in the CNS [18–21].

Thyrotropin-releasing hormone (TRH) is a hypothalamic tripeptide (pGlu-His-Pro-NH_2) implicated in the regulation of pituitary thyrotropin and prolactin synthesis and secretion [22, 23]. In addition, it is known for its many extrapituitary actions. Most prominent among these actions are alterations in cardiorespiratory, thermoregulatory, gastrointestinal, motor and appetitive functions; these result, almost exclusively, from the effect of the peptide in the CNS [24, 25]. Furthermore, TRH has been one of several peptides investigated for its action on glucoregulation. However, the findings concerning its glucoregulatory action have been inconsistent; variable effects, ranging from a mild increase in plasma glucose to no change in sugar concentrations, have been observed following central or systemic TRH administration [26–34].

In this commentary, new data on the glucoregulatory action of TRH are reviewed. The most important finding is that TRH, by acting in the CNS, is capable of mimicking, in a highly specific and unique fashion, the action of insulin in eliciting hypoglycemia in normoglycemic animals and in normalizing glucose levels in hyperglycemic animals. The experimental work characterizing this novel "insulin-like" action of TRH and the research on the neural and endocrine mechanisms mediating this effect are described in some detail in order to present a comprehensive account of the existing evidence and to suggest directions for future research. In addition, hypotheses as to the possible physiological role of TRH in glucoregulation, stimulated by the findings described here as well as by previous studies and some preliminary data, are presented.

TRH action in normoglycemia

Previous studies on the glucoregulatory action of TRH have revealed that pharmacological doses of the peptide may cause a moderate increase in circulating glucose, secondary to increments in epinephrine and glucagon concentrations [27–29, 32, 33]. Our initial studies revealed that, when microinjected into the lateral cerebroventricular system in normally-fed mice, TRH (0.1 to 10 μg) rapidly and dose dependently lowered blood glucose concentrations, producing powerful, sustained hypoglycemia that resembled, both in magnitude as well as in duration, the decrease in plasma glucose observed after systemic insulin challenge [34–36]. Systemic injection of a similar dose of TRH had no effect on circulating glucose levels, indicating that the hypoglycemia after central TRH injection was due exclusively to local action of the peptide and not to a peripheral effect of peptide escaping from the

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CNS into the systemic circulation. TRH, given systemically at exceedingly high doses (>100 μg), was effective in reducing plasma glucose concentrations to hypoglycemic levels. Similar high doses of TRH had no effect on either glucose transport or oxidation in isolated muscle or adipose tissues *in vitro* (unpublished observation), suggesting that the hypoglycemia after systemic TRH injection resulted from penetration of the peptide into the CNS and not from a local insulin-like stimulatory action on glucose metabolism.

TRH action in hyperglycemia

A subsequent series of studies examined the central effect of TRH in hyperglycemic mice. In these experiments, TRH was administered centrally immediately before or at various times after animals were treated with one of the following hyperglycemic agents: epinephrine, clonidine, glucagon, morphine, compound 48/80, glucose, 2-deoxyglucose and lipopolysaccharide endotoxin. Other hyperglycemic stimuli used were immobilization and electric footshock. It was found that TRH, given centrally immediately before or after induction of hyperglycemia, potently and dose dependently prevented the rise in plasma glucose otherwise noted in the drug-treated or stressed control mice [37–42]. Systemic injection of TRH at doses similar to those given centrally (0.1 to 10 μg) had no effect (0.1 to 1 μg) or only a mild effect (10 μg). Higher doses (>100 μg) given systemically completely prevented the hyperglycemic response. In other studies, TRH injection was delayed by up to 30 min after hyperglycemic drug treatment in order to allow for a significant hyperglycemia to develop prior to the peptide challenge. Invariably, central TRH rapidly reversed the hyperglycemia, lowering plasma glucose concentrations to normoglycemic or, in some cases, hypoglycemic levels. Various other neuroactive peptides tested in this experimental paradigm, including somatostatin, substance P, CRF, ACTH, enkephalin, melanocyte-stimulating hormone release-inhibiting factor (MIF-1), and alpha-melanocyte-stimulating hormone (alpha-MSH), failed to mimic this TRH action [39]. Thus, in addition to eliciting hypoglycemia in normoglycemic mice, central TRH, in a unique manner, blocked the development of, as well as rapidly reversed, experimentally-induced hyperglycemia. The ability of TRH to exert these actions was independent of the stimulus used to increase glucose levels; TRH prevented hyperglycemia due to peripherally or centrally acting agents, or to stress, suggesting that the anti-hyperglycemic effect did not result from an interaction at the site of action of the hyperglycemic agents tested but, rather, was due to action on a common pathway residing at a level distal to the site(s) mediating the hyperglycemic effect.

In other experiments, we examined the influence of several variables on the antihyperglycemic action of TRH, including strain, sex, age, nutritional status, diurnal rhythm and environmental temperature. TRH was found to be equipotent in lowering plasma glucose in all mice strains tested, including ICR, SJL, C57BL/6, BALB/C, DBA/2, and AKR. Furthermore, TRH was active in female mice, in food-deprived mice, and in mice of different ages, including very young (10-days-old), young adult (20- to 30-days-old) and mature animals (120-days-old). Finally, the effect of TRH was independent of diurnal cycle or environmental temperature. Thus, the action of central TRH in lowering glucose levels is a general and robust phenomenon, independent of strain, sex, age, nutritional status or environmental conditions [39].

Role of insulin

Studies on the central hypoglycemic action of exogenous insulin have suggested that this effect depends, in part, on stimulation of insulin release from the pancreas [18, 43]. In our studies, we examined the role of endogenous insulin in mediating the central hypoglycemic effect of TRH by measuring changes in circulating insulin after central TRH treatment, and by studying the effect of TRH in insulin-depleted mice. In the first series of experiments we found that, in normoglycemic mice, central TRH elicited a significant, transient increase in plasma insulin levels; this increase in circulating insulin paralleled the decreases in circulating glucose concentrations observed in these animals [35]. Moreover, in clonidine-treated mice, central TRH reversed the drug-induced suppression of plasma insulin, coincidently blocking the rise in circulating glucose levels [37]. In the second series of studies, we tested the effect of TRH in alloxan- or streptozotocin-treated, insulin-deficient mice. In the first series of experiments, mice treated with a single large dose of the beta-cell toxin alloxan exhibited powerful hyperglycemia; central TRH at all doses tested had no effect on plasma glucose, although other effects typically seen after central TRH injection, such as shivering, were observed [36]. Similarly, TRH had no effect on plasma glucose in alloxan- or streptozotocin-treated epinephrine-challenged hyperglycemic mice [39]. Thus, the action of central TRH in lowering plasma glucose depends on the structural integrity of beta-cells in the pancreas, and it is accompanied by an increase in circulating insulin, suggesting insulin as the peripheral agent mediating this effect.

Central mechanism of action

The first step of TRH action in the pituitary is binding to membrane receptors, and specific TRH receptors that resemble peptide binding sites in the pituitary have been identified in the mammalian CNS [44–47]. To test the hypothesis that TRH initiates its central antihyperglycemic effect by activating specific CNS receptors, we compared the central effect of native TRH on plasma glucose to that of many TRH structural analogs and fragments exhibiting diminished binding affinity to TRH receptors. We found that peptides differing from TRH by a single amino acid residue (e.g. pGlu-His, His-Pro, Glu-His-Pro-NH_2, pGlu-Phe-Pro-NH_2, pGlu-His-Gly-NH_2) are inactive in lowering plasma glucose in epinephrine-treated, hyperglycemic mice. Furthermore, we observed that some TRH analogs that resemble TRH in structure but possess an exceedingly low affinity to TRH receptors as compared to the native peptide are as active as TRH (i.e. pGlu-His-Pro-OH) or even

more so (i.e. pGlu-His-Pro-Gly-NH_2) in reversing hyperglycemia [37–39]. These results suggest that the antihyperglycemic activity of TRH depends on the structural integrity of the peptide, yet it is dissociated from interaction with the "classic" TRH receptor.

To further examine the central mechanism involved in the initiation of TRH action in hyperglycemia, we considered the role of the calcium- and phospholipid-dependent second messenger, protein kinase C. Earlier studies have implicated the involvement of protein kinase C in the mechanism of TRH stimulation of prolactin release from the pituitary. In these experiments, direct activation of protein kinase C by the tumor promoter 12-tetradecanoylphorbol-13-acetate (TPA) mimicked the action of TRH in stimulating prolactin secretion from clonal pituitary cells, whereas TRH was found to activate protein kinase C by increasing cytosolic free calcium and diacylglycerol concentrations in these cells [48–51]. Furthermore, protein kinase C has been implicated in the mechanism of central hypoglycemic action of insulin. Insulin can activate protein kinase C in the CNS [52], and it has been shown that TPA enhances, whereas the protein kinase C blocker polymyxin B inhibits, the central hypoglycemic effect of the hormone [53]. To examine the involvement of protein kinase C in central TRH action on glucoregulation, experiments were conducted in which the effect of direct activation of protein kinase C on the hypoglycemic effect of TRH was evaluated. It was found that central injection of TPA (0.1 to 1 μg) strongly enhanced the hypoglycemic effect of TRH, whereas central treatment with 4-alpha-phorbol, an inactive TPA analog, had no effect on central TRH action [54]. TPA also potentiated the action of some active TRH analogs (i.e. CG 5309, CG 3703, DN 1417, RX 77368, KPC-TRH, pGlu-N-Val-Pro-NH_2, pGlu-His-Pro-Gly-NH_2) but had no effect on plasma glucose when given either alone or together with inactive TRH analogs (i.e. Glu-His-Pro-NH_2, pGlu-Phe-Pro-NH_2, pGlu-His-Gly-NH_2) [54]. The finding that TPA can enhance the effect of TRH yet is unable to mimic it when given alone or together with inactive TRH analogs may argue against a direct role for protein kinase C in mediating the central effect of the peptide on glucose. Alternatively, these results suggest that a protein kinase C-regulated mechanism is involved in modulating the effect of TRH. Such modulation may occur at the level of the TRH binding site or further downstream, at the level of the neural substrate activated by TRH after it binds to the receptor. Accordingly, activation of this protein kinase C-regulated mechanism by TPA (or by other factors, e.g. a neurotransmitter or hormone) would have the effect of enhancing the action of TRH at the binding site or of facilitating the neural events triggered by action at the receptor, which lead to hypoglycemia. In contrast, direct activation of this protein kinase C-regulated mechanism by TPA in the absence of the triggering stimulus produced by TRH at the level of the binding site would be ineffective in setting into motion the neural events leading to hypoglycemia. Interestingly, we have observed in a preliminary study that insulin, injected into the CNS at doses that are ineffective at modifying circulating glucose levels (<0.1 μg), can enhance the hypoglycemic response to central TRH (unpublished observation). Because insulin may be capable of activating protein kinase C in the CNS [52, 53], it is tempting to speculate that the effect of insulin in enhancing the central glucoregulatory action of TRH, like that of TPA, occurs via a protein kinase C-dependent modulatory mechanism.

Role of the pituitary

Because an important action of TRH is to stimulate hormone release from the pituitary, and because a number of pituitary hormones purportedly responsive to TRH, such as ACTH, have been shown to influence insulin secretion [54–57], it was of interest to study whether pituitary factors may be involved in mediating the central effect of TRH in hyperglycemia. To test this possibility we compared the central effect of native TRH to that of several TRH analogs having TRH-like CNS activity profiles but devoid of hypophysiotropic influences. In addition, we tested the effect of central TRH in blocking hyperglycemia in hypophysectomized mice. It was found that centrally-acting TRH analogs, such as pGlu-N-Val-Pro-NH_2, CG 3509, and DN 1417, are as effective as, or even more active than, the native peptide at blocking glucagon-induced hyperglycemia. Moreover, TRH was fully active in blocking glucagon-induced hyperglycemia in hypophysectomized mice [58]. These results indicate that the central effect of TRH in blocking hyperglycemia is dissociated from action of the peptide on the pituitary or, alternatively, that pituitary factors do not play a role in mediating this effect of TRH.

Role of the autonomic nervous system

One of the effects of centrally-administered TRH in experimental animals is stimulation of the sympathetic and parasympathetic pathways descending from the CNS to the adrenals and gut. Activation of these autonomic pathways by central TRH has been shown to be associated with various effects, including changes in cardiorespiratory [59, 60] and gastrointestinal functions [61]. Moreover, autonomic mechanisms play a central role in the regulation of pancreatic hormone secretion [62, 63], and activation of the autonomic efferent pathways secondary to central TRH administration has been shown to trigger the release of pancreatic hormones, including insulin [29, 31, 32]. To study the autonomic system as a possible mediator for central TRH action in blocking hyperglycemia, we examined the effect on TRH action of pharmacological perturbations of the sympathetic and parasympathetic outflows. In the first series of experiments it was found that pharmacological blockade of the parasympathetic outflow by treatment with the muscarinic cholinergic antagonist atropine methyl nitrate nearly completely prevented the hypoglycemic effect of central TRH in normoglycemic mice. In contrast, treatment with atropine only partially blocked the central effect of the peptide in lowering glucose levels in hyperglycemic mice. When atropine was coadministered with hemicholinium, a choline uptake blocker, a more pronounced, albeit not complete, blockade of

TRH antihyperglycemic action was observed. Furthermore, it was found that selective blockade of the sympathetic system with chlorisondamine chloride, 6-hydroxydopamine or surgical denervation of the adrenal glands had no effect on TRH action in normoglycemic or hyperglycemic mice. However, sympathetic blockade with chlorisondamine enhanced the inhibitory action of atropine. Nonetheless, even the concomitant blockade of the sympathetic and parasympathetic outflows failed to abolish completely the central effect of TRH in blocking hyperglycemia [35–39]. These findings indicate that autonomic mechanisms play a variable role in mediating the central effect of TRH in lowering glucose levels. Specifically, they suggest that parasympathetic mechanisms are responsible for TRH action in normoglycemic mice and that both parasympathetic as well as sympathetic mechanisms are involved in mediating TRH action in hyperglycemic animals. However, additional non-neuronal factors seem to contribute to TRH action in hyperglycemia, because even complete blockade of these systems failed to prevent completely TRH action in hyperglycemic mice. As pituitary peptides, some of which have been shown to be insulin secretagogues, are not involved, other peptides capable of stimulating insulin release, such as those demonstrated in the hypothalamus [62–67], may be implicated. Indeed, preliminary experiments suggest the hypothalamus as possible site of action of TRH in blocking hyperglycemia, because chemical hypothalamic damage induced by neonatal treatment with monosodium glutamate or goldthioglucose administration can diminish the effect of TRH in lowering plasma glucose in hyperglycemic mice (unpublished observation). Nonetheless, the possibility that TRH acts in the hypothalamus to mobilize these insulin secretagogues remains to be established.

Possible physiological role of TRH in glucoregulation

In contrast to insulin, TRH is unable to directly stimulate glucose utilization in muscle and adipose tissues. However, as suggested by the work described above, TRH can influence glucose metabolism indirectly, by stimulating insulin release via action in the CNS. As a first step towards examining the possibility that the observed pharmacological effect of TRH in lowering glucose levels represents a physiological function of central TRH neurons, we studied the effect of immunoneutralization of TRH in the CNS by means of central anti-TRH serum injection, upon recovery from hyperglycemia, induced by treatment with 2-deoxyglucose. It was argued that, if TRH neurons in the CNS play a role in normalizing glucose levels during hyperglycemia, then perturbation of the CNS TRH system by means of antibody treatment should impair insulin responses to, and delay recovery from, experimental hyperglycemia. Measurements of glucose levels obtained in two preliminary experiments revealed that immunoneutralization of central TRH, while having no effect on the development of hyperglycemia following 2-deoxyglucose treatment, significantly prolongs the time required for reversal of hyperglycemia and reinstatement of normoglycemia (see Ref. 39). This preliminary finding is consistent with the view that reversal of hyperglycemia may depend, at least in part, on the functional integrity of TRH mechanisms in the CNS.

If, as proposed above, TRH neurons in the CNS play a physiological role in correcting hyperglycemia, then it might be expected that these neurons would be sensitive to changes in circulating glucose levels or, alternatively, that high glucose would be capable of activating these neurons and/or enhancing TRH action at those sites mediating the hypoglycemic action. Consistent with these expectations, previous studies have shown that experimental manipulations of the circulating glucose levels are capable of modifying TRH turnover rates in the hypothalamus [68–72]. Furthermore, glucose has been shown to influence TRH action at the pituitary [73], and we have observed in preliminary experiments that central microinjection of glucose together with TRH significantly enhances the hypoglycemic effect of the peptide in normoglycemic animals (unpublished observation). On a more speculative level, the possibility that TRH neurons in the CNS are sensitive to glucose may be in line with previous findings as to the presence of TRH in the pancreas [74]. Accordingly, studies have shown that TRH is co-localized with insulin in secretory granules within the beta-cells of the islets of Langerhans and can be released from these cells together with insulin in response to high glucose. Peak concentrations of pancreatic TRH occur in the late fetal and early neonatal periods; at this developmental stage hypothalamic TRH concentrations are exceedingly low. Subsequently, there is a steady decrease in pancreatic TRH levels and concomitant increase in hypothalamic concentrations of TRH with peak levels occurring in adulthood [75–83]. The redistribution of TRH during ontogenesis coincides with an increase in the number of TRH receptors in the CNS [84]. These developmental events suggest that, at least on a functional level, TRH neurons in the CNS may be descendants of or, alternatively, capable of acquiring some characteristics expressed by, the glucose-sensitive pancreatic beta-cells. This postulated pathway for the development of glucose sensitivity may be pertinent to the proposed role of central TRH as a modulator of hyperglycemia.

Summary, conclusions, and future directions

This review describes new research on the pharmacological action of TRH on glucoregulation. The most significant finding is that TRH has a unique insulin-like effect on circulating glucose not shared by any other neuropeptide previously implicated in glucoregulation, i.e. reversal of hyperglycemia. TRH elicits this glucoregulatory action by stimulating pancreatic insulin secretion through an effect in the CNS. Insulin release secondary to central TRH action is mediated in part by autonomic mechanisms and in part by other, as yet unidentified, factors. It is suggested that TRH may be capable of stimulating the secretion of insulin secretagogues produced in the CNS. Such insulin-releasing factors have been identified within the hypothalamus, the anatomical site most likely involved in mediating TRH action on glucose.

Furthermore, the studies described here suggest

that the central effect of TRH on glucose is not the result of a conventional peptide–TRH receptor interaction. This is implied by the fact that TRH analogs exhibiting exceedingly low binding affinity to the "classic" TRH receptor are as active as is TRH in reversing hyperglycemia. Moreover, preliminary studies on the molecular mechanism involved in TRH action suggest a role for the second messenger, protein kinase C. Earlier studies have implicated the involvement of protein kinase C in the receptor-mediated action of TRH on pituitary hormone secretion, because direct activation of the enzyme with TPA could mimic the effect of TRH in this system. In contrast, the experiments with TPA described here suggest that a protein kinase C-regulated mechanism may function merely as a modulator of TRH action on glucoregulation but may not be directly involved in mediating the effect of the peptide at the level of the binding site. This conclusion is consistent with the finding that TPA greatly enhances the effect on glucose triggered by TRH but is unable to elicit the response in the absence of the stimulus provided by the peptide.

Finally, it is proposed that TRH may play a physiological role in glucoregulation. Preliminary studies, demonstrating impaired glucose tolerance in 2-deoxyglucose-treated hyperglycemic mice after immunoneutralization of central TRH, suggest the involvement of TRH mechanisms in the regulation of insulin secretion and normalization of glucose levels. Possibly, TRH neurons in the CNS may be activated in hyperglycemia and, in turn, enhance glucose utilization by stimulating pancreatic insulin secretion. The possibility that TRH neurons in the CNS are responsive to glucose may be related to the presence of TRH in pancreatic beta-cells during development. TRH-containing cells in the pancreas are sensitive to glucose, and TRH neurons in the hypothalamus may retain this sensitivity following the redistribution of TRH from the pancreas to the CNS. Interestingly, glucose-sensitive neurons [85–88] as well as high concentrations of TRH [89, 90] have been demonstrated in the hypothalamus, and glucose has been shown to influence TRH turnover rates in this anatomical site [68–71].

In summary, a novel central action of TRH is described, which produces in the mouse an effect similar to that observed after insulin challenge. It is necessary to extend the findings reviewed here to other animal species, such as the rat, and to design more definitive studies on the receptor and on neural and humoral mechanisms mediating this action. Moreover, it is of interest to examine further the hypothesis that TRH may play a physiological role in glucoregulation. Particular attention should be directed towards elucidating the involvement of central TRH neurons in the regulation of insulin release in response to hyperglycemia. In this field of investigation, evaluating the effect of central TRH after changes in local glucose, and measuring glucose and insulin kinetics after selective immunoneutralization of TRH in specific CNS sites in hyperglycemic animals, should be most fruitful. Finally, it is of interest to examine further the anatomical and functional relationship between pancreatic and hypothalamic TRH. It is proposed here that the central TRH neurons exhibit glucose sensitivity which is linked in some ways to the production of TRH in the insulin-containing beta-cells in the pancreas. Assessing the responses to central TRH and glucose after selective perturbations of the pancreatic TRH system at different pre- and postnatal periods, by means of anti-TRH antibody treatment or beta-cell toxin administration, may prove to be a beneficial approach.

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