RESEARCH ARTICLE | Obesity, Diabetes and Energy Homeostasis

Thrombin action on astrocytes in the hindbrain of the rat disrupts glycemic and respiratory control

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INTRODUCTION

Critical injuries, including head trauma, surgery-related bleeding, gunshot wounds, and burns can produce a posttraumatic “metabolic self-destruction” (26). This phenomenon is characterized by a catabolic profile including persistent hyperglycemia, functional insulin resistance, and greatly elevated metabolic fuel use. The association between severe trauma and hyperglycemia is clinically axiomatic (9, 26). The severity of the hyperglycemia is highly correlated with posttraumatic morbidity and mortality. Although no mechanism has been posited to connect severe trauma with a loss of autonomic control over metabolism, traumatic injury causes other failures of autonomic function, notably, gastric stasis and ulceration (“Cushing’s ulcer”), which has been connected with the generation of thrombin. Our previous studies established that proteinase-activated receptors (PAR1; “thrombin receptors”) located on astrocytes in the autonomically critical nucleus of the solitary tract (NST) can modulate gastric control circuit neurons to cause gastric stasis. Hindbrain astrocytes have also been implicated as important detectors of low glucose or glucose utilization. When activated, these astrocytes communicate with hindbrain catecholamine neurons that, in turn, trigger counter-regulatory responses (CRR). There may be a convergence between the effects of thrombin to derange hindbrain gastrointestinal control and the hindbrain circuitry that initiates CRR to increase glycemia in reaction to critical hypoglycemia. Our results suggest that thrombin acts within the NST to increase glycemia through an astrocyte-dependent mechanism. Blockade of purinergic gliotransmission pathways interrupted the effect of thrombin to increase glycemia. Our studies also revealed that thrombin, acting in the NST, produced a rapid, dramatic, and potentially lethal suppression of respiratory rhythm that was also a function of purinergic gliotransmission. These results suggest that the critical connection between traumatic injury and a general collapse of autonomic regulation involves thrombin action on astrocytes.

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ROGERS RC, HASSE EM, HERMANN GE. Thrombin action on astrocytes in the hindbrain of the rat disrupts glycemic and respiratory control. Am J Physiol Regul Integr Comp Physiol 318: R1068–R1077, 2020. First published April 22, 2020; doi:10.1152/ajpregu.00033.2020.—Severe trauma can produce a postinjury “metabolic self-destruction” characterized by catabolic metabolism and hyperglycemia. The severity of the hyperglycemia is highly correlated with posttrauma morbidity and mortality. Although no mechanism has been posited to connect severe trauma with a loss of autonomic control over metabolism, traumatic injury causes other failures of autonomic function, notably, gastric stasis and ulceration (“Cushing’s ulcer”), which has been connected with the generation of thrombin. Our previous studies established that proteinase-activated receptors (PAR1; “thrombin receptors”) located on astrocytes in the autonomically critical nucleus of the solitary tract (NST) can modulate gastric control circuit neurons to cause gastric stasis. Hindbrain astrocytes have also been implicated as important detectors of low glucose or glucose utilization. When activated, these astrocytes communicate with hindbrain catecholamine neurons that, in turn, trigger counter-regulatory responses (CRR). There may be a convergence between the effects of thrombin to derange hindbrain gastrointestinal control and the hindbrain circuitry that initiates CRR to increase glycemia in reaction to critical hypoglycemia. Our results suggest that thrombin acts within the NST to increase glycemia through an astrocyte-dependent mechanism. Blockade of purinergic gliotransmission pathways interrupted the effect of thrombin to increase glycemia. Our studies also revealed that thrombin, acting in the NST, produced a rapid, dramatic, and potentially lethal suppression of respiratory rhythm that was also a function of purinergic gliotransmission. These results suggest that the critical connection between traumatic injury and a general collapse of autonomic regulation involves thrombin action on astrocytes.

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Under circumstances of “survivable” injury, this relationship between trauma and a stress-related increase in fuel availability assists in surmounting the traumatic event and subsequent healing (26). However, the extreme and prolonged hyperglycemia associated with the global metabolic collapse of severe trauma is not adaptive or helpful. Rather, it is better described as a pathophysiology in those individuals who would not have survived the initial insult had there not been timely clinical intervention. Despite the recognition of the association between trauma and metabolic pathophysiology, no detailed mechanism has been posited to connect severe trauma with metabolic control aside from a common citation of “CNS stress-related sympathetic activation” (16, 41).

However, traumatic injury also causes other failures of autonomic function. One of those, gastric stasis and ulceration (“Cushing’s ulcer”), has recently been connected with the generation of thrombin (31). As a protease, thrombin (produced as a consequence of bleeding or burn injury) acts on a unique class of G protein-coupled receptor, the protease-activated receptor (PAR). Four subtypes of receptors have been cloned (PAR1–4) (65); in the CNS, the PAR1 subtype is dominant (35). PARs possess their own tethered peptide ligand; a five-amino acid peptide. Serine proteases (e.g., thrombin) cleave a blocking peptide from the tethered ligand, allowing the short peptide to interact with the receptor within the extracellular loop to affect transmembrane signaling.

Several years ago, we hypothesized that the gastric stasis of traumatic injury occurred as a consequence of the activation by thrombin of PARs located on neurons in the autonomically critical nucleus of the solitary tract (NST) in the dorsal hindbrain. The NST is at the center of a homeostatic control nexus that receives vast quantities of visceral afferent data from cranial nerve afferents including the vagus. This region of the hindbrain is also outside the blood-brain barrier and is accessible to large molecules from the circulation. The NST parses this neural and chemical information to higher-order autonomic control networks to regulate gastrointestinal, metabolic, cardiorespiratory, endocrine, and behavioral functions (8, 30, 61, 63). To our initial surprise, we found that the PARs in the
NST that regulate gastric function are located on astrocytes and not neurons (31). Our subsequent studies confirmed that activation of PARs on the NST astrocytes initiated glutamate “gliotransmission” onto NST neurons involved in autonomic control (74). There may be a convergence between the effects of thrombin to derange hindbrain gastrointestinal control and the hindbrain circuitry that initiates counterregulatory responses (CRRs) to increase glycemia in reaction to critical hypoglycemia. Astrocytes in the hindbrain have been implicated as important detectors of low glucose or glucose utilization, and when activated, these astrocytes trigger CRR (37, 44, 45, 62, 64).

We tested the hypothesis that administration of thrombin to the floor of the fourth ventricle or directly into the NST could trigger increases in glycemia through the mediation of astrocytes. Preexposure of the fourth ventricle to SCH97979 (SCH; PAR1 antagonist) was used to evaluate whether thrombin effects were selective at PAR1 to evoke hyperglycemia. Fluorocitrate (FC; selective blocker of astrocyte metabolite signaling) was used to evaluate astrocyte involvement in thrombin-triggered changes in glycemia. Pharmacological intervention in known gliotransmission pathways involving glutamate, purinergics, and likely transient receptor potential vanilloid (TRPV) channels was then performed. Preliminary studies revealed that thrombin not only caused increases in glycemia but also produced a rapid, dramatic, and potentially lethal suppression of respiratory rhythm. Both the glycemic and respiratory effects were mediated by purinergic gliotransmission.

MATERIALS AND METHODS

All experimental procedures were conducted with the approval of either the Pennington Biomedical Research Center or the University of Missouri Institutional Animal Care and Use Committee and were performed according to the guidelines set forth by the National Institutes of Health. Long-Evans rats of either sex (31 females, 45 males; obtained from the Pennington Biomedical Research breeding colony) were used at Pennington in the first three experimental designs of this project. Male Sprague-Dawley rats (n = 24; obtained from Envigo, Indianapolis, IN) were used at the University of Missouri for the phrenic nerve activity experiments. Animal body weights were between 250 and 450 g, and ages ranged from 2 to 11 mo. At both centers, animals were housed in a temperature-controlled room under a 12:12-h light-dark cycle and provided water and food ad libitum.

Fourth Ventricle Applications (Studies Conducted at Pennington Biomedical Research Center)

Animals were deeply anesthetized with thiothetobarbital [Inactin; 150 mg/kg ip, Sigma-Aldrich; long-term anesthesia with minimal interference on autonomic reflexes (10)]. With the use of an aseptic technique, a tracheal cannula (PE-240) was implanted, and the prepared animal was then secured in a stereotaxic frame. The floor of the fourth ventricle (4V) was exposed by removing the occipital skull plate and opening the foramen magnum; dura and arachnoid layers were retracted. Once all preparatory surgery was complete, systemic glucose levels were monitored via blood samples obtained by tail vein punctures. Glucose concentrations of the 3- to 4-μL blood droplets were determined with Freestyle Lite glucose test strips and glucometer (Abbott Diabetes Care, Alameda, CA). To ensure that baseline blood glucose levels were stable, samples were taken at 0, 30, and 60 min after all preparatory surgery was complete. At the 60-min point, each animal was exposed to one of the following 4V experimental conditions: 1) control (sterile physiological saline, 10 μL; Henry Schein) followed 30 min later by thrombin from human plasma (4 U in 4 μL; cat. no. T4393, cell culture tested; Sigma-Aldrich, St. Louis, MO), “saline + thrombin”, n = 6 rats; 2) SCH [potent and selective nonpeptide thrombin receptor antagonist; 1 nmol in 10 μL; Tocris Bio-Technie, Minneapolis, MN (1)] followed 30 min later by 4 U of thrombin, “SCH + thrombin”, n = 3 rats; 3) fluorocitrate [FC; astrocytic metabolic signal blocker, 5 nmol in 10 μL; Sigma-Aldrich (17, 27, 43, 64, 70)] followed 30 min later by 4 U of thrombin, “FC + thrombin”, n = 7 rats; 4) caffeine [nonselective receptor antagonist of thrombin receptor, 130 nmol in 6 μL; Sigma-Aldrich (59)] followed 30 min later by 4 U of thrombin; “caffeine + thrombin”, n = 6 rats; 5) DPCPX [A1-specific adenosine antagonist; 8-cyclopentyl-1,3-dipropylxanthine; 2 nmol in 2 μL; Sigma Aldrich (40)] followed 30 min later by 4 U of thrombin; “DPCPX + thrombin”, n = 5 rats; 6) dizocilpine (MK801; nonspecific N-methyl-D-aspartate receptor antagonist (NMDA), 18 nmol in 10 μL; Sigma Aldrich (12)] followed 30 min later by 4 U of thrombin; “MK801 + thrombin”, n = 5 rats; 7) [iodoresiniferatoxin (I-RTX); highly selective and extremely potent TRPV1 antagonist, previously identified as a potential gliotransmission agonist, 4 pmol in 4 μL; Tocris Bio-Technie (68)] followed 30 min later by 4 U of thrombin; “I-RTX + thrombin”, n = 5 rats.

The 4-U thrombin dose used for the 4V challenge is at the low end of the dose range (4–20 U) used to model hemorrhagic stroke in the rat (32).

Tail vein blood glucose sampling occurred 30 min after blocker/antagonist pretreatment as well as 15, 30, 60, and 90 min after thrombin delivery. Baseline and peak levels of blood glucose were determined over this time course. Peak changes in blood glucose levels were expressed as percent changes relative to baseline for each individual animal; thus, each animal served as its own control. These normalized values of percent change in blood glucose levels for each group were averaged and subjected to a one-way analysis of variance followed by Dunnett’s post hoc tests for statistical significance (P < 0.05).

Preliminary studies suggested that thrombin applied to the 4V also produced immediate, dramatic, and long-lasting respiratory depression. To better quantify this effect, a microthermocouple probe (Omega MTSS, 0.25 mm diameter) was inserted into the opening of the tracheal cannula. Temperature differences in inspired versus expired air recorded by the probe were analyzed using a LabChart 7-PC system (AD Instruments). The first derivative of the raw temperature records was continuously plotted to provide a convenient record of respiratory rate. This was done using a rate meter in the LabChart software. Respiratory rates were monitored continuously throughout the experiment and charted on a minute-by-minute basis. Changes in breaths per minute were expressed as percent changes relative to baseline for each individual animal; thus, each animal served as its own control. These normalized values of percent change in respiration rate for each group were analyzed at 1, 5, 10, 15, and 30 min after 4V application of thrombin. Across these experimental groups, each of these time points was averaged and subjected to a one-way analysis of variance followed by Dunnett’s post hoc tests for statistical significance (P < 0.05).

Artificial Respiration (Studies Conducted at Pennington Biomedical Research Center)

Respiratory depression and hypoxia have been reported to trigger hyperglycemia (38, 51, 57, 69, 72). With our observation of immediate and dramatic depression of respiration in the preliminary studies, we needed to determine if the hyperglycemia that ventricular thrombin provoked could be attributed solely to hypoxia. To address this question, a subgroup of rats supported by mechanical ventilation was added. Ventilatory rate was matched to the rat’s natural respiratory frequency, which varied from 50 to 60 breaths/min at a tidal volume of 6 mL/kg. That rate was then set on a Harvard model 683 rodent
ventilator, where peak inspiratory pressure (PIP) was set to 15 cmH₂O and positive end-expiratory pressure (PEEP) to 5 cmH₂O (75).

A subset of 10 animals was mechanically ventilated (683 Harvard Apparatus) with O₂-administration of drugs and routine measurement of arterial pressure. Arterial blood gases were measured (Osmetech OPTIenriched room air. Rats underwent bilateral section of the cervical medulla using a Michigan microsurgical dissector (28).

Preliminary results suggested these thrombin injections produced an immediate and lethal suppression of respiratory rhythm. This observation and previous work suggesting that acute hypoxia can produce hyperglycemia (38, 69) required that we use artificial ventilation to maintain the animals for these nanoinjection studies. Therefore, mechanical ventilation was provided by the Harvard model 683 rodent ventilator, as described above.

Additional experimental groups of direct injections into the NST included the following: 1) sterile physiological saline volume controls (50 nL), n = 5 rats; 2) 4V saline (10 μL) followed 30 min later by nanoinjections of thrombin (0.05 U in 50 nL), n = 6 rats; 3) 4V SCH (1 nmol in 10 μL) followed 30 min later by nanoinjections of thrombin, n = 4 rats; 4) 4V FC (5 nmol in 10 μL) followed 30 min later by nanoinjections of thrombin, n = 6 rats; and 5) 4V DPCPX (2 nmol in 2 μL) followed 30 min later by nanoinjections of thrombin, n = 8 rats. The dose used in direct NST nanoinjection while monitoring blood glucose levels was 0.05 U of thrombin in a 50-nL volume of sterile physiological saline.

To evaluate the tissue spread of injections, 50-nL volume injections of a solution of inert 0.04-μm red fluorescent plastic beads (Fluo-Spheres Invitrogen) was injected into the NST of two anesthetized rats. These rats were transected with saline and 4% paraformaldehyde. Hindbrains were removed to a 30% sucrose solution overnight and then sectioned on a freezing microtome at 50 μm. Sections were then viewed on an upright epifluorescence microscope, and the fluorosphere injections sites were mapped.

**Data and Statistical Analysis**

**Blood glucose levels.** Tail vein blood glucose sampling occurred 30 min after blocker/antagonist pretreatment as well as 15, 30, 60, and 90 min after thrombin delivery. Baseline and peak levels of blood glucose were determined over this time course. Peak changes in blood
glucose levels were expressed as percent changes relative to baseline for each individual animal; thus, each animal served as its own control. These normalized values of percent change in respiration rate for each group were analyzed at 1, 5, 10, 15, and 30 min after 4V application of thrombin. Across these experimental groups, each of these time points were averaged and subjected to a one-way analysis of variance followed by Dunnett’s post hoc tests for statistical significance (\( P < 0.05 \)).

Phrenic nerve activity (PhrNA). PhrFreq and apnea duration data in response to different doses of thrombin nanoinjected directly into the NST were subjected to a one-way analysis of variance followed by Dunnett’s post hoc tests for statistical significance (\( P < 0.05 \)). Pretreatment with FC to block the effects of thrombin on PhrFreq were compared with pretreatment with aCSF by use of a paired \( t \) test; \( P < 0.05 \) for statistical significance.

RESULTS

4V Exposure to Thrombin and Effects on Blood Glucose

4V applications of saline or the antagonists SCH, FC, caffeine, DPCPX, MK801, or I-RTX alone had no effect on blood glucose (Fig. 1A). 4V administration of thrombin following saline produced a significant increase in blood glucose, typically within 15–30 min of application compared with control saline applications. This thrombin effect on glycemia was blocked by pretreatment with FC (astrocyte signaling blocker), SCH (PAR1 receptor antagonist), caffeine (nonselective adenosine antagonist), and DPCPX (selective adenosine A1 antagonist). In contrast, neither the NMDA antagonist (MK801) nor the TRPV1 antagonist (I-RTX) blocked the thrombin effect to increase glycemia (ANOVA, \( F_{6,30} = 7.2, P < 0.0001 \); Dunnett’s posttest against saline thrombin, *\( P < 0.05 \)).
Respiratory Depression After 4V Exposure to Thrombin

We were surprised to see that thrombin applied to 4V produced a dramatic and nearly immediate suppression of respiration that persisted for ~30 min after application (Fig. 2). Pretreatment of the hindbrain with FC markedly altered the dynamics of this respiratory response to thrombin, in that the depression was not as large nor lasted as long (Figs. 2 and 3). Indeed, the effects of thrombin on respiration appeared to mirror the effects seen on blood glucose levels; that is, pretreatment with SCH, FC, caffeine, and DPCPX blocked the respiratory depression effects of thrombin. Again, neither the NMDA antagonist MK801 nor the TRPV1 antagonist I-RTX blocked the thrombin effect on respiration (Fig. 4; ANOVA, $F_{6,30} = 7.3, P < 0.0001$; Dunnett’s posttest against saline + thrombin, *$P < 0.05$).

Effects of Artificial Respiration on Thrombin-Induced Hyperglycemia

Our observations of the effect of thrombin on both blood glucose and respiration, together with reports in the literature that respiratory depression and hypoxia appear to trigger hyperglycemia (38, 51, 57, 69, 72), required us to examine whether overriding the hypoxia effects would also suppress the hyperglycemia evoked by hindbrain thrombin. This subset of animals received artificial ventilation, as detailed above, throughout the duration of the experiment. Blood glucose levels in artificially respirated animals after either 4V saline or thrombin were not significantly different from those we observed in the nonventilated animals (Fig. 5; ANOVA, $F_{3,27} = 19.35, P < 0.0001$; Bonferroni selected pairs posttest comparisons, not significant).

Nano injection of Thrombin into the NST: Effects on Glycemia

Fluorescent nanosphere injections revealed that 50-nL injection volumes filled the NST (Fig. 6). Mechanical ventilation combined with 50 nL of saline injections into the NST had no effect on blood glucose (Fig. 7). Unilateral injections of thrombin (0.05 U in 50 nL) into the NST produced an increase in blood glucose similar to that observed with the larger (i.e., 4-U) 4V applications. Likewise, the effects of NST thrombin...
bin on blood glucose levels were blocked by 4V pretreatment with SCH, FC, or DPCPX (ANOVA, $F_{4,25} = 12.32$, $P < 0.0001$; Dunnett’s posttest comparisons against saline 50 nL NST, *$P < 0.05$).

Nanoinjection of Thrombin into the NST: Effects on Phrenic Nerve Activity

To verify that the respiratory depressant effects of thrombin were neurally mediated by actions in the NST, we measured the phrenic nerve activity response to unilateral nanoinjections of thrombin into the NST. A representative example demon-

strates that nanoinjection of aCSF had minimal effects on PhrNA (Fig. 8A). In contrast, NST thrombin nanoinjection (Fig. 8B) produced an abrupt decrease in PhrNA, due primarily to a decrease in PhrFreq with little effect on PhrAmp. Group data indicate that NST nanoinjection of aCSF had little effect (Fig. 9), whereas thrombin injections into the NST induced a concentration-related decrease in PhrFreq (ANOVA, $F_{5,28} = 29.0$, $P < 0.0001$; Dunnett’s posttest comparisons against aCSF, *$P < 0.05$). In addition, the duration of the neural apnea in response to thrombin was concentration related. Nanoinjection of similar concentrations of thrombin outside the NST also had minimal effects that were not different from responses to NST aCSF, consistent with the concept that thrombin acts specifically within the NST to inhibit respiration.

Fig. 5. Effects of artificial respiration on thrombin induced hyperglycemia. This subset of experiments was designed to examine whether overriding hypoxia effects would also suppress hyperglycemia evoked by hindbrain thrombin. A subset of animals received artificial ventilation throughout the duration of the experiment. Blood glucose levels in artificially respirated animals after either fourth ventricular (4V) saline or thrombin were not significantly different from those we observed in nonventilated animals (ANOVA, $F_{3,27} = 19.35$, $P < 0.0001$; Bonferroni selected pairs posttest comparisons; n.s., not significant).

Fig. 6. Histological verification of injection site and spread of injectate volume. Unilateral injections (50 nL) of fluorescent nanospheres demonstrated that this volume could fill the nucleus of the solitary tract (NST). AP, area postrema; CC, central canal; DMN, dorsal motor nucleus of the vagus; Scale bar, 1 mm.

Fig. 7. Effects of direct, unilateral injections (50 nL) into the nucleus of the solitary tract (NST) on blood glucose levels of artificially respirated rats. Mechanical ventilation combined with 50 nL of saline injections into the NST had no effect on blood glucose. Unilateral injections of thrombin (0.05 U in 50 nL) into the NST produced an increase in blood glucose similar to that observed with larger (i.e., 4 units) fourth ventricular (4V) applications (see Fig. 1). Similarly, the effects of NST thrombin on blood glucose levels were blocked by 4V pretreatment (pre-TX) with SCH79797 (SCH), flurocitrinate (FC), or 8-cyclopentyl-1,3-dipropylxanthine (DPCPX) (ANOVA, $F_{4,25} = 12.32$, $P < 0.0001$; Dunnett’s posttest comparisons against 50 nL saline + NST, *$P < 0.05$).

Fig. 8. Representative examples of phrenic nerve activity (PhrNA) from two rats. A: unilateral nucleus of the solitary tract (NST) nanoinjection of artificial cerebrospinal fluid (aCSF; 30 nL) had little effect on neural respiration. B: in contrast, unilateral NST nanoinjection of thrombin (0.02 U/μL, 30 nL) inhibited PhrNA, due primarily to an effect on phrenic frequency.
To evaluate the role of astrocytes in the respiratory depressant effects of thrombin, we nanoinjected thrombin (0.02 U/µL, 30 nL) into the NST under control conditions and after astrocyte signaling blockade with FC (45 nL, 1 mM; n = 11005). FC blunted the response to thrombin (Fig. 10A). Group data indicate that FC significantly reduced the thrombin effect on PhrFreq (P < 0.033, paired t test; Fig. 10B). The duration of phrenic apnea (Fig. 10C) was also significantly reduced by prior injection of FC (P = 0.033, paired t test), whereas prior aCSF administration had no effect (Ctrl: 20.8 ± 3.9 s vs. aCSF: 15.8 ± 3.5 s, P = 0.2, paired t test).

DISCUSSION

Data from our studies demonstrate that the injections of thrombin in the hindbrain can provoke systemic hyperglycemia. Hindbrain thrombin also produces a dramatic depression of respiratory rhythm. Whereas acute hypoxia can produce hyperglycemia (38, 69), the current study demonstrated that artificial ventilation did not prevent the hyperglycemic response to thrombin. Therefore, hypoxia per se was not responsible for the increase in blood glucose levels observed after 4V exposure to thrombin. Pretreatment of the hindbrain with SCH (thrombin receptor antagonist), FC (astrocyte calcium signaling blocker), or adenosine receptor antagonists (caffeine and DPCPX) suppressed thrombin’s effects on hyperglycemia and respiratory depression. These results suggest that these physiological responses to thrombin in the hindbrain involve thrombin receptors, astrocytes, and purinergic gliotransmitters.

Astrocytes and Neuronal Control

Long thought to only have a passive role in maintaining neuronal networks, it is now clear that astrocytes directly regulate neuronal excitability and synaptic plasticity. A single astrocyte may contact tens to hundreds of thousands of synapses and, along with presynaptic terminals and postsynaptic neurons, will form what has been termed the “tripartite synapse” (5, 11, 24, 25, 55). Astrocytes can be activated by neurotransmitters released from neuronal presynaptic terminals or gliotransmitters released by other astrocytes. Furthermore, astrocytes are sensitive to local, homeostatically regulated parameters such as blood gas tension and glucose availability (18, 20, 22, 23, 62, 64). Astrocytes are also stimulated by markers of immune activation such as cytokines (29, 52) and markers of traumatic injury such as thrombin (35, 74). All of these agents can act to increase astrocytic calcium levels (4, 7, 29, 31, 36, 39, 54, 56, 67). This increase in astrocytic calcium is coupled to a release of gliotransmitters (3, 24, 25, 55), such...
as glutamate, ATP, adenosine, and d-serine, among others (2, 7, 14, 19, 53). Astrocyte gliotransmission can potently affect the excitability of adjacent NST neuronal circuitry (18, 62, 74).

**Thrombin, Astrocytes, and Purinergic Control of Glycemia**

Our earlier work on thrombin effects on NST function principally addressed the regulation of gastrointestinal function (31). The NST is the locus of vagal-vagal circuit control of gastric motility and acid secretion. Traumatic injuries involving the production of thrombin are associated with clinical gastroparesis and erosive ulcers (Cushing’s ulcer) (42, 76). Thrombin or PAR1 agonists applied to this circuitry also causes gastroparesis (31). Follow-up neurophysiological and imaging studies show that these effects are, in turn, due to thrombin-induced glial release of glutamate, which modulates the activity of vagal afferents and NST neurons controlling gastric function (50, 74).

Thrombin-generating traumatic injury is also associated with a chronic hyperglycemia, whose magnitude is inversely correlated with injury survival (6, 9, 16, 26, 66). Counterregulatory hyperglycemic response to dangerously low plasma glucose is also controlled by circuitry in the hindbrain; specifically the NST and ventrolateral medulla (60). Our present studies demonstrated that thrombin applied to the floor of the 4V or injected directly into the NST causes a significant increase in blood glucose. This effect is clearly mediated by activation of PAR1 receptors on astrocytes, since 4V pretreatment with the specific PAR1 antagonist SCH blocks the effect, as does the astrocyte calcium-signaling inhibitor FC.

PAR1 activation of astrocytes by thrombin produces downstream effects to control glyemia through the release of adenosine. Caffeine, a nonselective adenosine antagonist, blocked the effects of thrombin to increase glyemia and depress respiration. The thrombin effect is probably mediated through the A1 adenosine receptor, since DPCPX, a selective A1 antagonist, completely blocked both the 4V and direct NST injection effects of thrombin. Surprisingly, blockade of NMDA or TRPV1 gliotransmission had no effect to inhibit thrombin-induced increases in plasma glucose. This is in contrast to the thrombin-astrocyte-glutamate gliotransmission responsible for gastroparesis (31, 74) and earlier reports concerning the involvement of TRPV1 channels in PAR suppression of respiratory control circuits (33). The involvement of purinergic gliotransmitter release in the thrombin induction of hyperglycemia is paralleled by our recent work showing that astrocytes activated by low-glucose challenge trigger counterregulatory responses through the release of purinergic agonists (62, 64).

While we were conducting studies of the effects of thrombin to alter glyemia, it was impossible to ignore the profound effect that thrombin had on respiratory rhythogenesis. Even 4V application of thrombin (4 U) provoked an almost instantaneous suppression of respiration that required nearly 30 min to recover to near-normal patterns. Unilateral, direct injections of as little as 0.0003U (i.e., 30 nL of 0.01 U/μL) of thrombin into the NST significantly depressed PhrFreq activity and increased apnea duration. Pretreatment with unilateral, direct injections of FC suppressed this effect of thrombin. These data confirm that the respiratory depression is neurally mediated and support the concept that it involves astrocytes with the NST.

**Perspectives and Significance**

Hindbrain astrocytes are emerging as important sources of chemosensory control over autonomic and autonomous functions such as glucose homeostasis and gastrointestinal, cardiovascular, and respiratory control (18, 20, 22, 23, 62, 64). Often, these functions are regulated by astrocyte release of the purines ATP and adenosine (18, 23, 58, 62, 64, 73). In particular, adenosine has been identified as a potent inhibitor of hindbrain respiratory rhythmogenesis (34). Suppression of purinergic gliotransmission seems to block these pathological outcomes of thrombin exposure, and this pharmacological approach might prove useful in the immediate management of the effects of trauma-produced thrombin effects on the brain.

Our observations of the effects of thrombin in the dorsal hindbrain to provoke pathological changes [first in gastrointestinal function (31), now in glyemic and respiratory control], reveal the profound and damaging effects that thrombin formation secondary to severe trauma can have on critical autonomic control circuitry. Although insulin therapy is commonly used to control whole body glyemia after trauma, the attendant CNS hypoglycemia that often results makes the aggressive use of insulin controversial, especially in the brain-injured patient. Insulin receptors on glial cells are likely to exert an important modulating influence on glucoregulatory circuits in the brain (21). It is not clear how insulin effects, hypoglycemia, and thrombin would, together, act on CRRs regulating astrocytes in the hindbrain. But each effect, individually, produces strong increases in intracellular calcium in target cells (15, 48, 49, 62). It remains to be seen whether these agents act on the same astrocytes controlling CRRs or on different astrocytes whose outputs converge on CRR-regulatory neurons.

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**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the authors.

**AUTHOR CONTRIBUTIONS**

R.C.R., E.M.H., and G.E.H. conceived and designed research; R.C.R., E.M.H., and G.E.H. performed experiments; R.C.R., E.M.H., and G.E.H. analyzed data; R.C.R., E.M.H., and G.E.H. interpreted results of experiments; R.C.R., E.M.H., and G.E.H. prepared figures; R.C.R., E.M.H., and G.E.H. drafted manuscript; R.C.R., E.M.H., and G.E.H. edited and revised manuscript; R.C.R., E.M.H., and G.E.H. approved final version of manuscript.

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