Incretin mimetics as pharmacologic tools to elucidate and as a new drug strategy to treat traumatic brain injury

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Abstract

Traumatic brain injury (TBI), either as an isolated injury or in conjunction with other injuries, is an increasingly common event. An estimated 1.7 million injuries occur within the USA each year and 10 million people are affected annually worldwide. Indeed, nearly one third (30.5%) of all injury-related deaths in the USA are associated with TBI, which will soon outpace many common diseases as the major cause of death and disability. Associated with a high morbidity and mortality and no specific therapeutic treatment, TBI has become a pressing public health and medical problem. The highest incidence of TBI occurs in young adults (15–24 years age) and in the elderly (≥75 years of age). Older individuals are particularly vulnerable to these types of injury, often associated with falls, and have shown increased mortality and worse functional outcome after lower initial injury severity. In addition, a new and growing form of TBI, blast injury, associated with the detonation of improvised explosive devices in the war theaters of Iraq and Afghanistan, are inflicting a wave of unique casualties of immediate impact to both military personnel and civilians, for which long-term consequences remain unknown and may potentially be catastrophic. The neuropathology underpinning head injury is becoming increasingly better understood. Depending on severity, TBI induces immediate neuropathologic effects that, for the mildest form, may be transient; however, with increasing severity, these injuries cause cumulative neural damage and degeneration. Even with mild TBI, which represents the majority of cases, a broad spectrum of neurologic deficits, including cognitive impairments, can manifest that may significantly influence quality of life. Further, TBI can act as a conduit to longer term neurodegenerative disorders. Prior studies of glucagon-like peptide-1 (GLP-1) and long-acting GLP-1 receptor agonists have demonstrated neurotrophic/neuroprotective activities across a broad spectrum of cellular and animal models of chronic neurodegenerative (Alzheimer’s and Parkinson’s diseases) and acute cerebrovascular (stroke) disorders. In view of the mechanisms...
1. Introduction

Traumatic brain injury (TBI) is a significant cause of disability and death worldwide, particularly in industrialized countries. Beyond any ensuing physical disabilities and the signature cognitive deficits, predominantly in attention, learning and memory, and higher order executive functions, TBI is a major conduit for the development of chronic neurodegenerative disorders, especially Alzheimer’s disease (AD), Parkinson’s disease (PD), amyotrophic lateral sclerosis (ALS), and chronic traumatic encephalopathy (CTE) [1]. Within the USA alone it is estimated that at least 1.7 million people have a TBI event annually, which results in some 235,000 hospitalizations and an excess of 50,000 deaths. Indeed, at least 5.3 million Americans are currently living with a long-term disability associated with a TBI incident [2]. In this regard, the elderly are particularly vulnerable to TBI. Often associated with falls in this increasingly large segment of the population, TBI carries a higher mortality and worse functional outcome after lower initial injury severity, as compared with younger adults [3]. Worldwide, the incidence of TBI is approximately 0.5% per year.

TBI is also a much too common occurrence among military forces serving in modern combat operations. Military operations ongoing in Iraq and Afghanistan (“Operation Enduring Freedom” for the war in Afghanistan, and “Operation Iraqi Freedom” together with “Operation New Dawn” for military operations in Iraq after August 2010) have spanned more than a decade from their initiation in 2001 and have involved >2.4 million American and coalition personnel. Bomb explosions, primarily caused by improvised explosive devices, have become increasingly common in these war theaters, impacting military personnel and increasingly civilians as a consequence of urban terrorist attacks and sectarian violence. Estimates range from approximately 15% [4] to 19.5%–22.8% of all returning deployed U.S. troops [5] as suffering from blast exposure TBI, with the total number of such injuries estimated as high as 320,000 [4,6]. The vast majority of TBIs experienced by military personnel have been classified as mild injuries, based on clinical severity [7]. However, many involved likely have repeated injury, where cumulative long-term effects could have particularly serious implications. Emerging evidence suggests possible dose- and frequency-dependent associations between TBI and a risk of neurodegenerative disease; however, a threshold for clinical manifestation remains to be determined and more than likely could be affected by genetic predisposition and environmental factors [8,9].

Some 10% of all concussive TBIs result in significant cognitive and emotional dysfunction, requiring around-the-clock care. The more prevalent mild injuries frequently result in significant long-term effects on an individual’s health. TBI can cause meaningful deficiencies across a broad range of brain functions, but mild and moderate TBIs characteristically induce headaches; impairments in sleep, memory, attention, and cognitive processes; stress; as well as affective disorders [10]. Such clinical problems may persist long after injury occurrence, or even permanently. There is a range of similarities and differences in relation to blast exposure TBI, where symptoms include headache, nausea, vomiting, dizziness/balance problems, fatigue, and sleep disturbances, together with concentration and cognitive impairments [7,11]. Such symptoms are reported in >70% of cases, occur in the absence of overt histologic or standard clinical radiologic evidence of damage, and are often accompanied by ringing in the ears and sensitivity to light and/or sound. Clearly acutely disabling, particularly in a battlefield environment, such symptoms may resolve or persist for years after initial injury, degrading the subjects’ quality of life and placing an enormous burden on military, veteran, and civilian healthcare systems. Although there are symptomatic treatments to address some of the aforementioned aspects of mild TBI, the development of effective pharmacologic treatments to protect against the injury-induced secondary neurodegeneration that primarily contributes to and largely underpins cognitive dysfunction has been slow and only relatively recently has been evaluated under true battlefield conditions [7].

In recent years, advances in our knowledge of the molecular mechanisms that regulate the health and survival of neurons, together with an understanding of key pathways induced by TBI that lead to neuronal dysfunction and death [12–14], have been applied to the development of experimental drugs with features potentially beneficial in mild TBI treatment—whether concussive or blast-related. Primary brain injury is induced by immediate insult to the head, likely as a consequence of mechanical forces inducing shearing and compression of neuronal and vascular tissue at the time of impact, and rotational head acceleration. A cascade of pathologic events may then follow leading to further secondary brain injury that takes place from minutes to days after the trauma [15]. Most of the damage apparent in mild injury patients derives from the secondary events of the trauma, such as brain edema, ischemia, inflammatory responses, free radical generation, elevated excitatory neurotransmitters (e.g., glutamate excitotoxicity), loss/disruption of synaptic connections, or altered synaptic physiology and DNA damage [12–16]. This leads to neuronal dysfunction, as manifested by changes in long-term potentiation [17] and dendritic and synaptic loss (personal communication—Dr. Ronald F. Mervis, Center for ...
Aging and Brain Repair, University of South Florida College of Medicine, Tampa, FL) and, when cellular damage is sufficiently profound, the pro-apoptotic protein p53 will initiate the process of apoptosis [18–25], which may then exacerbate inflammatory processes and instigate development of a self-propagating adverse cycle of events [26–32]. Clinical and experimental research indicates that the hippocampus is particularly vulnerable to secondary damage, underpinning the manifestations of deficits in the hippocampus-dependent functions of learning and memory [33–37]. Such ensuing secondary injury may be amenable to intervention and is worsened by secondary physiologic insults. A clear conundrum is that the resulting impairments render the TBI victim less capable of avoiding further potential head injury, thereby increasing the likelihood of repetitive mild TBI to which the brain is even more vulnerable. Specific risk factors for poor outcome after TBI have been established, some of which can be recognized at the time of injury, including age, gender, mechanism of injury, and presenting signs, whereas others, such as hyperglycemia, hypoxia, and hypotension, are possible areas for medical intervention [38]. Although prompt and specialist neurocritical care is associated with improved outcome [7,39], no successful drug treatments have been approved for improving patient outcome [38].

2. Animal models of TBI

Across the clinical spectrum of all TBIs there is significant heterogeneity and thus numerous TBI models have been developed that mimic, to a lesser or greater degree, select aspects of human injury [40]. The classification of human TBI is conventionally based on the presenting symptoms and level of consciousness, with routine use of the Glasgow Coma Scale (GCS). Although patients can have a similar GCS score, they may have remarkably different radiologic appearances (such as contusions, skull fractures, blood–brain barrier disruption, hematoma, axonal injury, etc.—or, indeed, none of these), reiterating that TBI is not a single disorder. Mimicking all aspects of TBI in a single animal model is clearly not feasible, and thus for translational purposes, when assessing treatment strategies, the use of multiple models appears prudent. Recent comprehensive reviews of TBI animal models include those by Marklund and Hillered [40] and Xiong et al. [41].

In humans, the form of concussive mild TBI that can occur in traffic accidents, sports injuries, and falls can, to varying degrees, be mirrored in a classic weight-drop rodent model [42]. In this closed-head model, injury is diffuse and occurs throughout the brain, both ipsi- and contralateral to the side of impact. In some TBIs, a focal lesion ensues, with the most common in human TBI being a cortical contusion, in which destruction of brain tissue with microhemorrhages can occur [40]. Such contusions frequently arise in frontal and temporal regions, although a contrecoup contusion may occasionally occur within a brain region opposite to the initial injury, and such contusions can noticeably expand during the early days post-TBI. A widely used model in this context of moderate TBI involves fluid percussion injury, where damage is inflicted by a pendulum striking a piston associated with a fluid reservoir in contact with the brain surface via a craniotomy. This generates a fluid pressure pulse to the brain that, depending on craniotomy placement (whether lateral or midline), can result in a local focal cortical contusion and diffuse subcortical neuronal injury to the hippocampus and thalamus [43]. This model can also reproduce intracranial hemorrhage, brain swelling, and progressive gray matter damage, all classic features of human moderate TBI [43]. A further commonly used, more severe contusion TBI model that mimics focal human TBI is the controlled cortical impact model, which generates extensive cortical tissue loss, together with hippocampal and thalamic damage [44]. In addition, in recent years, interest has grown in the development of blast TBI models, and several have been established in rodents [45–49]. In general, these models of blast injury involve either the use of a compression-driven shock tube to simulate blast effects in a confined laboratory setting or the exposure of animals to a controlled detonation, providing a “real-world” model of an improvised explosive device [49].

Potential limitations to currently available animal models are that, although there are significant similarities in the neurophysiology between nonhuman mammal (particularly rodents) and human brains, there are also clear differences in brain size, complexity, and white-to-gray matter ratio. It is unknown whether such dissimilarities translate into differences in behavior, neurochemistry, and drug responses after TBI, and thus the importance of assessing more than a single animal model for translational drug studies is reiterated.

3. Counterbalance between neuronal cell death and survival as a target for TBI therapy

The most widely studied type of programmed cell death in the nervous system, apoptosis [18,24,28,50], is a process regulated by specific cysteine proteases—caspases. Numerous triggers of neuronal apoptosis exist, such as oxidative stress, cell surface receptor engagement (e.g., activation of glutamate receptors or tumor necrosis factor-α receptor engagement), trophic factor insufficiency, DNA damage, and accumulation of damaged proteins—all of which have been reported after TBI [51,52]. Several families of proteins and specific biochemical signal-transduction pathways regulate cell death. Cell death signaling can involve plasma membrane death receptors, mitochondrial death proteins, proteases, kinases, and transcription factors. Players in the cell death and cell survival orchestra include the Fas receptor Bel-2 and Bax (and their homologs), cytochrome c, caspases, and extracellular signal–regulated protein kinases [18,24,28,50,53]. Other forms require gene activation, RNA synthesis, and protein synthesis. Irrespective of this, many of these share
biochemical cascades that regulate ensuing cell death processes, and thereby provide targets for intervention.

Counterbalancing this, biochemical cascades can be triggered that activate specific proteins associated with cell survival. For example, numerous growth factors and G-protein–coupled receptors activate PI3 kinase, a well-known upstream regulator of cell survival processes such as Bad and caspase-9, to inhibit their ability to induce cell death [54]. It is apparent that Akt promotes cellular survival via a series of distinct pathways that involve the Forkhead family of transcription factors, GSK-3β, β-catenin, eIF2β, c-Jun, CREB, Bad, IKK, p53, and JIPs [55]. As apoptosis and opposing cellular survival pathways are finely tuned both within neurons and glial cells and are activated in neurodegenerative diseases and TBI, pharmacologic approaches to up- or downregulate them provide the opportunity to utilize them as “pharmacologic tools” to elucidate the relevance of specific biochemical cascades in TBI and neurodegenerative disorders as well as to test investigational drugs for treatment.

It is also clear that, after any form of brain injury, particularly TBI, new neurons can be generated from stem cells within the subgranular layer of the dentate gyrus and the subventricular zone throughout life [56–61], and some of them are capable of migrating into the granule cell layer of the dentate gyrus [57]. There they develop granule cell morphology and neuronal markers [62], connect to their target area [63], and become functionally integrated into local circuitry [64], similar to mature cells [65–67]. During TBI, however, there is a loss of neural precursor cells from these neurogenic areas with an accompanying reduction in newly generated neurons [58]. This is associated with learning impairment [68] and an increase in new glial cells [58]. Neural stem cells are exquisitely sensitive to trauma and inflammatory cytokines [69,70], which are known to be elevated in TBI, and to impact their differentiation and survival [58]. The neurogenesis/regenerative process provides an additional intervention strategy to potentially maximize neural stem cell survival and optimize differentiation toward neurons versus glial cells.

4. Incretin mimetics as a mechanism-based mild TBI treatment strategy to promote neuronal survival

Counterbalancing apoptotic pathways leading to cell death are biochemical cascades that promote cell survival [50,53]. To this end, we and others have been assessing cell survival/neuroprotection consequent to G-protein–coupled receptor (GPCR) activation, focusing on the glucagon-like peptide-1 (GLP-1) receptor (R), which is of clinical relevance in type 2 diabetes mellitus (T2DM) and neurodegenerative disorders [71–79]. The incretin GLP-1 (Fig. 1) is an endogenous 30-amino-acid insulinotropic peptide that controls blood glucose levels via activation of the GLP-1R on pancreatic β-cells [80,81]. GLP-1 derives from the posttranslational modification of proglucagon and is secreted from enteroendocrine L cells present throughout the small and large intestine, where its basal secretion is rapidly and substantially elevated in response to food intake [81–84]. Interestingly, such cells display a molecular profile that largely overlaps with other gut endocrine cell types and coexpress multiple peptide hormones that, depending on their posttranslational modification by activities such as prohormone convertase 1/3, can generate a diversity of essential physiologic peptides along the length of the gastrointestinal tract [85]. GLP-1 has an array of physiologic actions, with its insulinotropic ones being the most notable and well studied (Fig. 2). These are exerted through a

Fig. 1. Amino acid sequence of GLP-1 and that of the long-acting GLP-1 analogs, exendin-4 (Ex-4), lixisenatide, and liraglutide. Ex-4 is known clinically as Byetta and Bydureon for subcutaneous (SC) twice-a-day dosing and extended-release dosing (once weekly), respectively. Lixisenatide (trade name Lyxumia) and liraglutide (trade name Victoza) are administered SC once daily. Amino acid homology (blue circles) and differences (pink circles), in a comparison with GLP-1, are highlighted. The peptidase cleavage of GLP-1 by DPP-IV can be seen. Ex-4 and lixisenatide are close analogs that differ in their tail region. GLP-1 and liraglutide are likewise close analogs, with the latter possessing a C-16 fatty acid (palmitic acid) with a glutamic acid spacer attached to the lysine residue at position 26, permitting its binding to albumin to augment its half-life. By contrast, exendin (9–39) (bottom) is a widely used pharmacologic agent that acts as a GLP-1R antagonist.
distinct GPCR, the GLP-1R that is highly expressed on pancreatic islet β-cells. The insulinotropic actions of GLP-1 include glucose-dependent stimulation of insulin secretion and inhibition of glucagon secretion [82]. GLP-1 also confers glucose sensitivity to glucose-resistant β-cells and acts as a trophic agent, inducing pancreatic β-cell proliferation and neogenesis, as well as inhibiting β-cell apoptosis [80–82]. Hence, it is a key regulator of β-cell mass that, with its other physiologic actions, spurred the development of GLP-1R agonists for treatment of T2DM [80–84]. The biochemical cascades and cellular repertoire responsible for effective GLP-1R signaling within the β-cell to promote GLP-1–mediated trophic actions have been an area of intense study and recently, together with a detailed analysis of the gut–brain GLP-1 axis, have been comprehensively reviewed by Campbell and Drucker [82]. As the insulinotropic

Fig. 2. Direct (upper) and indirect (lower) pharmacologic actions of GLP-1R agonists. Top: GLP-1 agonists act directly by stimulating the GLP-1R to induce multiple coordinated actions at the level of pancreatic islet cells, within the heart, gastrointestinal tract, on subsets of immune cells, and within the brain. Bottom: Within the brain, GLP-1R agonists appear to enter freely; activation of select GLP-1R signaling pathways instigates biologic actions within the gastrointestinal tract, liver, and adipose tissue. Activation of GLP-1R signaling cascades within the central and peripheral nervous systems appear to underpin the benefits of GLP-1R agonists described in preclinical animal models of AD, PD, stroke, ALS, Huntington’s disease, and, more recently, TBI (adapted from Campbell and Drucker [82] and Salcedo et al. [75]).
actions of GLP-1 and agonists are glucose-dependent, advantageously and unlike other current agents for diabetes treatment, their pharmacologic actions are not associated with a high risk of hypoglycemia [80,83,84]. As illustrated in Figure 2, GLP-1R is also widely expressed in non-islet cells where its activation also exerts indirect metabolic actions. Thus, there is considerable interest in identifying extrapancreatic actions of GLP-1R activation [86]. GLP-1 is also generated within the central nervous system, chiefly in the caudal part of the nucleus of the solitary tract within the brainstem [87–91], from where it can diffuse within brain to induce assorted metabolic, vascular, and neuroprotective actions (Fig. 2).

Like most endogenous peptides, however, GLP-1 is short-lived in the circulation after its release [92–94]. It is rapidly degraded by the effective protease dipeptidyl peptidase IV (DPP-IV), rendering it inert at the GLP-1R, and then cleared renally. Elegant studies by Eng and colleagues [95,96] resulted in the discovery and characterization of a naturally occurring DPP-IV–resistant GLP-1R agonist, exendin-4 (Ex-4), present within the saliva of a large and heavy-bodied venomous lizard (i.e., the Gila monster, Heloderma suspectum), which reaches up to 1.25 feet in length and is native to the western and southern foothills of Arizona through to the Mexican state of Sonora. Purely synthetic forms of Ex-4, as well as more recent GLP-1 and Ex-4 analogs, have been generated (Fig. 1). These possess substantially longer half-lives than GLP-1, and exenatide (synthetic Ex-4 as a twice-daily [Byetta] or once-weekly [Bydureon] formulation), liraglutide (Victoza, once daily), and lixisenatide (Lyxumia, once daily, but not yet approved in the USA) are now in clinical use as agents for type 2 diabetes mellitus [80–84].

Although chiefly localized to pancreatic islets [81,82], GLP-1R expression also occurs on neurons throughout the brain and nervous system (primarily localized to dendrites [97]), which GLP-1 and analogs can readily enter after systemic administration [98–101]. Expanding upon our prior interest in GLP-1 and Ex-4 in T2DM, their insulinotropic and anti-apoptotic actions [98,99,102,103], the structure–activity relation of their amino acid sequence [104–106], and their translation to the clinic setting [75,107], we hypothesized that GLP-1R stimulation in brain would provide neurotrophic/protective activity as: (i) GLP-1 has trophic action on β-cells [108–113]; and (ii) the GLP-1R is coupled to the cAMP second messenger pathway, increases in which have been well documented to be associated with neuroprotection [114,115]—a function very different from its previously known role in brain in the regulation of food intake and satiety [116,117]. Hence, we characterized the action of GLP-1 analogs on neuronal cells, both in cell culture and animal studies [118–127], and demonstrated potent neurotrophic and neuroprotective actions that have been widely confirmed [75–79,128–132].

Our cell culture studies with both immortal PC12 and rat primary hippocampal cells established that GLP-1R expression and activation stimulated adenylyl cyclase to elevate intracellular cAMP in a manner similar to pancreatic β-cells [118]. GLP-1 and analogs induced differentiation in PC12 cells in a manner similar to nerve growth factor (NGF), inducing dendrite extension that was reversed by coincubation with selective GLP-1R antagonist Ex 9–39 (Fig. 1). Across a variety of neuronal cell types, coincubation of neurons with GLP-1 and/or Ex-4 enhanced the robustness of their neuronal phenotype, as exemplified by cholinergic cells expressing elevated levels of cholinergic markers (e.g., choline acetyltransferase) [127], and dopaminergic neurons heightened tyrosine hydroxylase activity [123]. GLP-1 analogs enhanced NGF-initiated differentiation and rescued degenerating cells from NGF-mediated withdrawal in the absence of cellular dysfunction or toxicity [118]. This indicates that this incretin can provide trophic support in the absence of growth factors, which has been confirmed in other neuronal cell types [127]. The binding affinity of GLP-1 for receptors on hippocampal neurons (EC50 = 14 nmol/L—a concentration apparently achievable [125]) is in the range of its binding to pancreatic β-cells [99,100], and GLP-1 analogs provided complete protection against apoptotic cell death induced by glutamate, amyloid-β (Aβ1–42), hypoxia and a variety of oxidative stressors (e.g., Fe2+, H2O2, and 6-hydroxydopamine) [118–120,123,125–127].

Our translational studies and the elegant work of others across numerous independent laboratories have demonstrated that GLP-1R agonists are effective in animal models of: (i) AD—reducing levels of amyloid precursor protein (APP), Aβ, and tau hyperphosphorylation; augmenting long-term potentiation (LTP); inducing synapogenesis; augmenting neurogenesis; suppressing neuroinflammation; reversing brain insulin resistance; and improving memory performance [119,120,123,132–145], (ii) Peripheral neuropathy—promoting neurite outgrowth and inhibiting neuron dysfunction and loss [122,146–149], (iii) Stroke—reducing stroke volume and neuroinflammation, and augmenting neuronal survival and neurogenesis [123,150–154], (iv) PD—augmenting dopaminergic cell survival, reducing neuroinflammation, increasing neurogenesis, and improving motor coordination [123,155–157], which has recently translated to motor and cognitive improvements in moderately affected PD patients in an open-label clinical trial [158]. (v) ALS—promoting motor neuron survival [127,159,160], (vi) Huntington’s disease [124]. (vii) Various excitotoxic lesion models [119,160,161]. In defining cellular pathways mediating these neurotrophic/protective actions, our studies thus far have implicated participation of PI3-kinase and ERK/MAPK-dependent pathways, with additional involvement of protein kinase A (PKA) signaling [72,126] to divert signaling away from apoptosis toward cell survival. In light of the numerous commonalities existing across these disorders and their occurrence in TBI (neuronal cell death, glutamate excitotoxicity, neuroinflammation, synaptic loss, etc.), we hypothesized that...
GLP-1R agonists would be useful to mitigate TBI on two complementary levels: (i) as neuroprotective/neurotrophic agents; and (ii) as anti-hyperglycemic agents—because TBI-induced hyperglycemia is associated with increased mortality and morbidity in humans with moderate to severe injury [38,162–164].

5. Traumatic brain injury and GLP-1 receptor agonists

Our studies have focused on three complementary rodent models. In one, experimental concussive (weight drop) mild TBI was induced in anesthetized mice using the closed-scalp, head trauma device described previously [21,22, 25,36,37,42] in which a defined weight (up to 30 g) was dropped through a metal tube of fixed length (80 cm). The opening of the tube was positioned directly over the cushioned animal’s head just anterior to the right ear, and the animal held in a manner such that the force of impact to the skull generated anterolateral movements without any rotational movements, analogous to that occurring in a closed-head injury from a car accident. This induced a diffuse injury within the brain in which apoptotic neurons appear throughout the cerebral cortex and hippocampus both ipsi- and contralateral to the injury site [21,22]. Pro- and anti-apoptotic markers (epitomized by p53 and Bcl-2, respectively) are upregulated by as little as a 5-g insult when evaluated at 72 hours [21,22], with degeneration of neurons and their processes becoming apparent at insults of 15 g within cortical regions and 25–30 g in hippocampus and dentate gyrus, as evaluated by silver staining with 15 g within cortical regions and 25–30 g in hippocampus and dentate gyrus, as evaluated by silver staining with verification of apoptosis by terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) staining [21,22] as well as by Fluoro-Jade B and NeuN immunohistochemistry [25]. Likewise, markers associated with mitochondrial dysfunction and failure, cytochrome c, and Bax, together with a marker of inflammation, tumor necrosis factor-α (TNF-α), are found to be elevated within 8 to 72 hours [24,25,165]. Interestingly, when evaluated with a broad neurologic battery at 1 or 24 hours postinjury, it is not possible to differentiate mild TBI from control (uninjured) mice. However, with the use of selected behavioral paradigms initiated as early as 72 hours (forced swim/Porsolt test [24]), or at 7 and 30 days for learning and memory tests (such as Y-maze and novel object recognition paradigms [166–169]), mild TBI and uninjured animals can be readily differentiated, and such cognitive deficits in spatial and nonspatial tasks have been shown to persist for as long as 90 days in the absence of gross neurologic sequelae [42], such as those seen in humans [1].

The use of the very same mouse strain in a “real-life” blast injury mild TBI model [49] allows one to not only assess the potential of experimental treatment strategies but, importantly, to also compare deficits and associated mechanisms induced by blast and concussive mild TBI. Our blast mild TBI model involves a controlled explosion (500 g TNT) detonated at 4 meters and 7 meters from anes-thetized mice to expose them to blast pressures of 5.5 and 2.5 psi, respectively, in line with human exposure to an improvised explosive device. Similar to our concussive (weight drop) mild TBI model, a diffuse neuronal injury occurs, with axonal and myelin abnormalities, and the development of persistent select cognitive deficits in the absence of alterations in basic neurologic assessment or brain gross pathology [49]. Interestingly, whereas both forms of mild TBI are distinctly different regarding the mechanism of trauma induction (blast vs. concussive injury), there are striking similarities in the cognitive and affective status of exposed animals and, similar to humans, the examined indices of cognition and anxiety-like behavior in mice subjected to both forms of TBI were quite similar [170]. Within contrast to these similar mouse behavioral outcomes after either concussive or blast mild TBI, we observed marked differences in gene expression profiles by RNA extracted from intact TBI hippocampal tissue. Although there was a degree of overlap in genes coregulated by the two distinctly different mechanisms of injury, there was a larger number of genes uniquely expressed in an injury-specific manner. Consequently, molecular pathways, particularly those associated with ribosomal proteins, the electron transport chain, oxidative stress, inflammation, and neurogenesis, displayed very similar patterns of injury-dependent regulation. However, although these data suggest involvement of common cascades initiated in response to different types of head injury, there was a divergence in individual gene expression within these pathways, suggesting that the two types of injury (blast vs. concussive mild TBI) are different at the molecular level [170]. Interestingly, pathways associated with AD displayed a markedly different form of regulation, depending on the type of TBI [170]. Furthermore, particularly in the blast mild TBI model, changes were evident in other pathways associated with neurologic disorders, including the “Parkin pathway,” involving genes associated with PD, as well as genes found to be reduced in major depressive disorders and schizophrenia [170]. This is in line with multiple studies linking TBI to long-term neuropsychiatric/neurodegenerative disorders, as recently reviewed by Walker and Tesco [171].

Remarkably, administration of a clinically translatable dose of Ex-4 to mice with either form of mild TBI (blast or concussive), whether administered before or shortly after insult, fully ameliorated the cognitive deficits [166,167] (Fig. 3). Importantly, the mild TBI-induced gene expression changes, including genes associated with AD, were largely prevented by Ex-4 both at the pathway analysis level as well as at the individual gene level, as illustrated in Figure 4 for mice exposed to concussive (weight drop) mild TBI. As described, blast mild TBI primarily impacted the very same gene pathways as those shown changed for concussive mild TBI, albeit that, in large part, different individual genes were up- and downregulated by blast mild TBI within these pathways [170]. Our studies indicate that Ex-4 likewise ameliorated the majority of these at the individual
and pathway levels. Hence, taken together, these data suggest a strong beneficial action of Ex-4 in managing secondary events induced by these two very different forms of mild TBI.

In light of the fact that no single animal TBI model perfectly mimics the human condition [40], additional studies to verify the activity of Ex-4 in moderate TBI were undertaken—specifically in rats after fluid percussion injury. As the most commonly used rodent model of moderate TBI, fluid percussion injury replicates many of the key cognitive and histologic changes evident in human head injury [172,173]. Of the two variations of the model developed, central (i.e., midline) fluid percussion injury was induced in anesthetized animals that produces both focal and diffuse pathology. This generates both primary (a local contusion, edema, and hemorrhage at the gray/white matter interface) and secondary damage (gliosis, inflammation, axonal injury, and delayed cell death) [171,172] and, similar to human TBI, this model adversely impacts hippocampal function and produces long-term cognitive and memory deficits that can last for weeks or months after injury [174,175]. Administration of Ex-4 post-injury at a dose comparable to that of the aforementioned mild TBI mouse studies dramatically mitigated fluid percussion injury–induced cognitive impairments, as assessed in the classical Morris water maze paradigm [176].

Further supporting evidence for GLP-1R activation as a therapeutic strategy for TBI derives from elegant studies by Heile and Brinker [177,178], who, together with colleagues, developed an approach in which encapsulated human mesenchymal stem cells generate and secrete steady-state levels of GLP-1. The implantation of these cells in the lateral ventricle of rats subjected to controlled cortical impact–induced moderate TBI significantly attenuated hippocampal neuronal cell loss as well as cortical glial and neuronal cytoskeletal abnormalities [177].

In conclusion, activation of the GLP-1R–mediated incretin pathway has been demonstrated to be both neurotrophic and neuroprotective across a broad number of cellular and animal neurologic models, and it has also been shown to provide functional and behavioral benefits over a wide range of pathophysiologic paradigms. These have been seen in independent laboratories, and verified across different animal models of the same disease in the fields of AD, PD, stroke, peripheral neuropathy, and, more recently, ALS and TBI [71–79]. In large part, commonalities in fundamental biochemical cascades appear to be triggered across diverse acute and chronic neurologic disorders that cause neuronal cell populations associated with defined disease to become dysfunctional and undergo apoptosis. The GLP-1 signaling pathway can clearly counterbalance these and, as has been successfully undertaken for GLP-1R activation in pancreatic

Fig. 3. Ex-4 posttreatment protects against concussive mild TBI (mTBI)-induced cognitive loss, as assessed by novel object recognition at 7-day and 30-day testing post-mTBI in two separate groups of mice (a similar protection was provided in a blast TBI model that closely mimics military personnel trauma). Concussive (30-g weight drop) mTBI induces an impairment in visual memory (red column), as assessed by the novel object recognition paradigm, which was fully ameliorated when Ex-4 was administered (green column): pretrauma, (A) 7 days and (B) 30 days; and posttrauma, (C) 7 days and (D) 30 days. Mice undergoing cognitive testing initiated on day 7 were euthanized on day 14, and their hippocampus was subjected to gene expression analyses. The mouse Ex-4 dose was 3.5 pmol/kg/min (subcutaneously administered as a steady-state dose), which equals; 21 µg/kg/day and equivalent to a dose of 1.7 µg/kg/day in a human after normalization of body surface area between mouse and human. This dose compares favorably to once-weekly exenatide LAR: that is, 2 mg/week, which provides a 60-kg human subject 4.8 µg/kg/day. Significantly impaired behavior animals vs. sham (uninjured) controls: Fisher’s least-significant-difference post hoc test, *P < .05, **P < .01. Performance of mice was quantitatively assessed 7 and 30 days post-mTBI as a preference index, calculated as: (time near the new object – time near the old object) / (time near the new object + time near the old object). Values are expressed as mean ± SEM (adapted from Rachmany et al. [166]).
-cells, these pathways are now being mapped for the GLP-1R on neurons [72,125]. GLP-1R appears to be present across a number of neuronal cell types (e.g., motor neurons, neural stem cells, etc.) that underlie its diverse actions to provide neurotrophic/protective actions, augment long-term potentiation and synaptic plasticity, and favorably impact neurogenesis. In addition, its appearance in other cell types, such as in multiple immune cell subpopulations [179–181] and glial cells [182], accounts for other positive actions, such as suppression of proinflammatory cytokines and reduced microglial activation within the brain. Most likely it is the combination of these actions, rather than any specific and separate one, that supports the current promise of GLP-1R activation as a therapeutic strategy across neurologic disorders, including TBI. Maximizing the translational potential of the strategy is clearly crucial, although three GLP-1R agonists have been approved and are well tolerated in type 2 diabetes mellitus [80–84], and are promising clinical studies in PD [158] support repositioning for neurologic disorders. Careful and hypothesis-driven preclinical and clinical studies are warranted [183] to define the optimal neurologic use of this promising drug class to ensure that its promise is not lost in translation [184].

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