

# Insulin-Like Growth Factor -1 (IGF-1) Derived Neuropeptides, a Novel Strategy for the Development of Pharmaceuticals for Managing Ischemic Brain Injury

Jian Guan

Liggins Institute, The University of Auckland, New Zealand

## Keywords

Endogenous neuropeptides; GPE; G2-mPE; Ischemic brain injury in rats.

## Correspondence

Dr. Jian Guan  
Liggins Institute, The University of Auckland,  
Private Bag 92019, Auckland, New Zealand.  
Tel: +(64-9) 923-6134;  
Fax: +(64-9) 308-2385;  
E-mail: j.guan@auckland.ac.nz

doi: 10.1111/j.1755-5949.2009.00128.x

Insulin-Like Growth Factor-1 (IGF-1) is neuroprotective and improves long-term function after brain injury. However, its clinical application to neurological disorders is limited by its large molecular size, poor central uptake, and mitogenic potential. Glycine-proline-glutamate (GPE) is naturally cleaved from the IGF-1 N-terminal and is also neuroprotective after ischemic injury, thus providing a potential novel strategy of drug discovery for management of neurological disorders. GPE is not enzymatically stable, thus intravenous infusion of GPE becomes necessary for stable and potent neuroprotection. The broad effective dose range and treatment window of 3–7 h after the lesion suggest its potential for treating acute brain injuries. The neuroprotective action of GPE is not age selective, is not dependent on cerebral reperfusion, plasma glucose concentrations, and core body temperature. G-2mPE, a GPE analogue designed to be more resistant to enzymatic activity, has a prolonged plasma half-life and is more potent in neuroprotection. Neuroprotection by GPE and its analogue may be involved in modulation of inflammation, promotion of astrocytosis, inhibition of apoptosis, and in vascular remodeling. Small neuropeptides have advantages over growth factors in the treatment of brain injury, and modified neuropeptides, designed to overcome the limitations of their endogenous counterparts, represent a novel strategy of pharmaceutical discovery for neurological disorders.

## Introduction

In the following days hypoxia-ischemia (HI) injury in the developing rat brain, the mRNAs for insulin-like growth factor (IGF)-1, its binding protein and its receptor are expressed with different temporal and spatial characteristics depending on the area of infarction; IGF-1 protein has been shown immunohistochemically to be associated with reactive glia [1–3] in infant and adult [4] rats. These data raised the possibility that IGF-1 may play an important role as a neurotrophic peptide following brain injury [5].

Central administration of IGF-1 prevents neuronal and white matter injury after HI brain injury in the adult rat [6] as well as following ischemia in the fetal sheep [7,8]. The treatment effects are dose-dependent, with a clear bell-shaped dose dependency of neuronal survival

following a 30 min period of cerebral ischemia in fetal sheep [6,7]. Treatment with IGF-1 improves long-term somatomotor-sensory function, examined using the bilateral tactile test, and long-term histological outcome examined 3 weeks after the injury [9]. Although the central uptake of IGF-1 has been reported to be poor [10], neuroprotection by IGF-1 after peripheral administration has been reported on several occasions [11,12]. The potential metabolic and mitogenic effects of IGF-1 are the major concerns for its use in the treatment of neurological disorders.

## N-terminal Tripeptide of IGF-1, GPE

Following the discovery of Des-N-(1-3) insulin-like growth factor-1 (Des-IGF-1), a truncated form of IGF-1 isolated from brain tissue, most scientific interest

focused on establishing the differences between the native form of IGF-1 and its truncated analogue, des-IGF-1. The other product of IGF-1 cleavage, its N-terminal tripeptide glycine-proline-glutamate (GPE), was, until the late 1980s, generally believed to be a nonbioactive byproduct of IGF-1 metabolism. The bioactivity of this tripeptide in cell culture was first reported in 1989 [13]. Synthetic GPE stimulated both dopamine and acetylcholine release in brain slice culture without interacting with IGF-1 receptors [14]. A novel ion-channel-associated receptor was suggested to be involved in the mode of action because GPE showed only partial displacement by an *N*-methyl *D*-aspartate (NMDA) receptor antagonist in cell culture [14]. The discovery of an acid protease in both plasma and brain tissues provided further evidence for the existence of GPE as an endogenous neurobioactive peptide [15–17]. This endogenous cleavage can be enhanced in an acidic environment.

### GPE is Neuroprotective

Using organotypic culture, Saura et al. showed that GPE dose-dependently protected hippocampal neurons from NMDA-induced neuronal toxicity. Neuroprotection by GPE was similar to that by MK801, a noncompetitive NMDA receptor antagonist. Despite the consistent NMDA effects of GPE previously reported by Bourguignon's group [18,19], the authors ruled out the possibility of NMDA-receptor mediated neuroprotection by GPE because of the weak binding of tritiated GPE in the dentate gyrus (DG), where NMDA receptors are strongly expressed [20].

Central administration of GPE 2 h after HI injury, at a dose equimolar to the effective dose of IGF-1, produced a reduction in the extent of cortical infarction [21]. As with IGF-1, the treatment effects were seen in most brain regions examined, but particularly in the lateral cortex, striatum, and some subregions of the hippocampus. Interestingly, a profound treatment effect of GPE was found in the CA1-2 subregions of the hippocampus, where IGF-1 has never been seen to be neuroprotective [21,22]. The most obvious explanation for that lack of effect in this particular brain region would be a relatively lower density of IGF-1 receptors. This spatial difference between IGF-1 and GPE also suggests a unique mode of action for GPE in neuroprotection. With no interactions with the IGF-1 receptors and a small molecular size, GPE appeared to overcome the disadvantages that have prevented the clinical application of IGF-1 for central nervous system (CNS) disorders; however, as an endogenous peptide, GPE is not enzymatically stable.

### Pharmacokinetics of GPE

As determined with a locally developed radioimmunoassay and by HPLC mass spectrometry, the half-life of GPE in plasma is extremely short after single-bolus i.v. (<2 min) or i.p. (<4 min) administration to normal Wistar rats [23,24]. Endogenous proteases appear to have a role in GPE metabolism, as Bestatin, a protease inhibitor, can depress GPE metabolism in both plasma and brain tissues [24]. Protease activity in the CNS *ex vivo* appears to be lower than in plasma, which may provide an explanation for the longer half-life of GPE (>30 min) in cerebrospinal fluid (CSF) and brain tissues [23,24]. Thus to maintain efficacious plasma concentrations of GPE, continuous intravenous (IV) administration would be necessary to achieve consistent neuroprotection after ischemic brain injury.

The central penetration of GPE is injury-dependent despite its small molecular size [25], as the elevation of GPE concentrations in the CSF was observed only in HI injured rats when given intravenously 2 h after HI injury. It is well-known that the blood-brain barrier (BBB) can become functionally and morphologically more permeable as a result of brain injury [26,27]. Given the hydrophilic nature of GPE, the loss of basal lamina due to activation of matrix metalloproteinase (MMP)-2 and -9 [28] would be more associated with GPE central uptake [28,29]. Compared to substances with free access to the CNS, injury-mediated central penetration may allow specificity to injured brain regions while limiting un-wanted effects outside these regions.

### Neuroprotection of GPE after Peripheral Administration

To overcome the extremely short half-life of GPE, continuous IV infusion appears to maintain efficacious blood concentrations and thus stable central uptake. Intravenous infusion of GPE consistently achieved robust neuroprotection in all the brain regions examined, with a broad effective dose range between 0.3 and 30 mg/(kg h) over a 4 h continuous infusion [25]. Although the rapid plasma clearance of GPE suggests only limited application for chronic neurological conditions, this could be favorable for treating acute neurological conditions, because adverse effects associated with drug accumulation can be minimized [25]. A broad window of opportunity also has been demonstrated by showing that a delayed 4 h infusion of GPE, initiated either 3 or 7 h after HI injury, produced a similar degree of neuroprotection compared with earlier administration initiated 1 h after injury [25]. However, i.v. infusion of GPE initiated 1 h after HI injury improved long-term neuronal outcome examined 21

days later [25]. With a broad effective dose range, the extended window of opportunity that GPE provides offers further promise for its clinical development for treating acute brain injury.

While most stroke patients are over 65-year-old, most neuroprotective compounds have been evaluated in animal models of brain injury induced in young adults. More recently, Shapira et al. also compared the neuroprotection of GPE between young adult and aged rats using a microsphere-induced ischemic-nonreperfusion injury [30]. They reported that i.v. infusion of GPE (12 mg/kg) significantly reduced the degree of brain damage in both young and aged rats, however, the treatment effects appeared to be less prominent compared to those after HI induced ischemia–reperfusion injury. The treatment effects appeared not to be dependent on blood glucose concentrations, core temperature, or oxygen saturation. Reestablishing cerebral perfusion is critical for the recovery of stroke patients. Thrombolytic treatment that promotes cerebral reperfusion, remains the only treatment currently available, however, the restrictive 3 h therapeutic window for the use of thrombolysis may leave many patients with very delayed or no cerebral reperfusion [31]. Given that the majority of neuroprotective compounds have been evaluated in animal models with ischemia–reperfusion injury, it is most advantageous that GPE prevents neuronal injury without depending on cerebral reperfusion.

### **Glycine 2-Methyl Proline Glutamate (G-2mPE)**

To overcome the instability of GPE in plasma, a GPE analogue has been generated by adding a methyl group to the proline ring of GPE with the intention of increasing resistance to enzymatic degradation [32]. The half-life of G-2mPE in the plasma was indeed significantly prolonged compared with that of GPE [33]. The neuroprotective effects of this analogue have been reported subsequently in both adult and neonatal rat models of brain injury [33–35]. Treatment with G-2mPE also prevents ischemic-nonreperfusion injury after permanent middle cerebral artery occlusion with a dose-dependent antiseizure activities [36]. Continuous i.v. infusion of G-2mPE at a 10-fold lower dose than GPE significantly reduced the neurological deficit compared with the vehicle-treated rats [33,34]. The authors also found an additive treatment effect between G-2mPE and Caffeinol (a mixture of caffeine and ethanol), but not between GPE and Caffeinol, [34]. Svedin et al. reported that daily treatment with G-2mPE, given i.p. for 7 days after inducing HI injury at postnatal day 7 in rats, improved neuronal outcome when examined 7 days after the injury [35].

### **Mode of Action of GPE**

The mode of action of GPE is still not clear. However, a considerable amount of information for possible modes of action has been reported. It is known that GPE does not interact with the IGF-1 receptor and the literature has focused on NMDA receptors. With glycine and glutamate at opposite ends of the molecule, GPE appears to have the perfect stereochemistry to activate NMDA receptors by binding to both the glutamate and glycine binding sites of the receptor. Both antagonistic and agonistic effects of GPE have been demonstrated [18,19,37]. Potential NMDA antagonistic effects of GPE were examined by comparison with a classic NMDA receptor antagonist (AP-5), its parent peptide (IGF-1) and its sibling peptide (des-IGF-1). The results suggested that both IGF-1 and GPE, but not des-IGF-1, have comparable antagonistic NMDA effects to those of AP-5. Thus, these authors suggested an antagonistic activity of GPE and IGF-1, acting as a prohormone of GPE on NMDA receptors [18,19]. Ikeda et al. observed that MK801, an NMDA receptor antagonist, blocked the effects of GPE on glial proliferation and that there was an additive effect of des-IGF-1 and GPE on glial proliferation, suggesting that the action of des-IGF-1 is mediated via a different mechanism, presumably via IGF-1 receptors. That study provided further evidence that the bioactivity of IGF-1 is mediated via different modes of action through its two cleavage products [37]. However, the neuroprotective effect of GPE appeared not to be comparable with that of MK801, as an effective dose of MK801 given immediately after ischemic injury failed to be effective when given 2 h after HI injury, contrary to what is seen with GPE. It is possible that the NMDA effects observed result from interference with a number of signaling pathways that lead to the activation of NMDA receptors rather than from direct interaction with the receptors. In addition, the neuroprotective effects of several other GPE analogues have been tested *in vitro* [38]. However, the neuroprotective actions of both GPE and its analogues recently have been suggested not to be associated with glutamate receptors [38].

### **Possible Mechanisms of Neuroprotection by GPE**

Inhibiting caspase-3-activated and noncaspase-3-activated apoptotic pathways may be involved in the neuroprotection by GPE after HI injury [25]. Terminal deoxynucleotidyl transferase mediated dUTP nick-end labeling (TUNEL) and caspase-3 immunostaining have been widely used as markers of neurons that undergo apoptosis, in which neurons are committed to die via

a more progressive process [39]. While the increased TUNEL-positive cells were seen mainly in the CA3 subregion of the hippocampus, the majority of caspase-3 positive cells were located in the CA4 subregion after HI injury, an injury where neuronal damage scores are similar in both regions [25]. This HI injury-induced different spatial distribution between caspase-3 activation and TUNEL labeling indicates that the caspase-3 pathway may not necessarily lead to DNA fragmentation indicated by positive TUNEL labeling. This disassociation between TUNEL and caspase-3 immunoreactivity has also been suggested outside of the CNS [40]. GPE treatment inhibited both TUNEL and caspase-3 positive neurons [25], suggesting 2 potentially different mechanisms underlying its neuroprotective action.

An autoradiomicrographic study showed that the tracer signal of  $^3\text{H}$ -GPE is clearly associated with glial fibrillar acidic protein (GFAP) positive astrocytes (unpublished data), suggesting a role for astrocytes in the neuroprotective action of GPE. Treatment with GPE prevents the HI-induced loss of astrocytes. Reactive astrocytes have been suggested to be involved in BBB integrity, angiogenesis, intracellular iron homeostasis, and neurotrophic actions [41,42], findings that can be controversial under pathological conditions. For example, in contrast to receptor mediated excitatory amino acid release in physiological situations, brain injury causes the leakage of excitatory amino acids due to astrocyte swelling [42–44], which can lead to damaged homeostasis and contribute to further neuronal injury [45]. Thus maintaining astrocyte integrity may be critical for neuroprotection by GPE.

Astrocytes have an important role in vascular remodeling [46–48] through angiogenesis, a major pathway for injury-associated vascular remodeling [47]. Using a unilateral brain injury model in neonatal rat, Svedin et al. reported that G-2mPE treatment increased the capillary density in both contralateral and ipsilateral hemispheres. The study did not determine whether the treatment effects on capillary density were due to preservation of blood vessels following HI or to a higher degree of subsequent revascularization in the injured hemisphere. However, there is a clear correlation between increased capillary density and neuroprotection in some brain regions [35]. The increase of vascular density in the contralateral hemisphere in the G-2mPE treated group demonstrated its role in vascular remodeling.

Treatment with G-2mPE also significantly increased GFAP density correlated to the increased vascular density, probably through promoting astrocytic angiogenesis. IGF-1, the parent peptide of GPE, and has been reported to have a critical role in vascular remodeling by increasing vessel growth in the perile-

sional area after injury in adult mice brains [49]. The role of G-2mPE in angiogenesis needs to be further investigated.

In contrast to its parent peptide IGF-1 [50], GPE treatment inhibits HI injury-induced reactive microglial proliferation. Several neuroprotective agents, such as  $\text{TGF}\beta$ -1 [51], have been shown to have antiinflammatory properties, which could be involved in neuroprotection by GPE after HI injury.

Furthermore, GPE treatment completely restored the loss of glutamate decarboxylase (GAD), but not parvalbumin-containing, neurons, both of which are co-expressed in  $\gamma$ -aminobutyric acid (GABA) interneurons after HI injury, suggesting an upregulation of enzymatic immunoreactivity rather than an effect caused solely by preventing the loss of GABA neurons. Given these effects of GAD, an enzyme transferring glutamate to GABA, the upregulation of GAD may help protect neurons from glutamate-induced toxicity [37]. Similarly, treatment with GPE also upregulates nitric oxide synthase (NOS), an enzyme of NO synthesis, which has a critical role in improving cerebral perfusion after brain injury [52].

## Summary

The induction of endogenous IGF-1 after brain injury suggests a role for IGF-1 in moderating brain injury. Indeed, treatment with exogenous IGF-1 after brain injury is protective and leads to improved long-term neurological function. However, the poor central uptake of IGF-1 and its potential mitogenic nature prevent its beneficial clinical application. Intriguingly, the naturally cleaved N-terminal tripeptide of IGF-1, GPE, crosses the BBB and does not interact with IGF receptors. Intravenous infusion of GPE for 4 h prevents brain injury and improves long-term functional recovery. GPE has a broad effective dose range and a 3–7 h window of opportunity for treatment. The neuroprotective effect of GPE is not dependent on the blood glucose concentration or core body temperature. It is not age selective and is independent of cerebral reperfusion. Continuous administration is essential for neuroprotection by GPE because of its enzymatic instability. G-2-meth-PE, an analogue of GPE modified to improve enzymatic stability, has a prolonged plasma half-life and is neuroprotective after ischemic brain injury in both adult and neonatal rats. Promotion of astrocytosis and vascular remodeling while inhibiting apoptosis and microglial activation may be the underlying mechanisms of neuroprotection by GPE and its analogue.

## Conflict of Interest

The authors have no conflict of interest to declare in relation to this review.

## References

1. Beilharz EJ, Bassett NS, Sirimanne ES, Williams CE, Gluckman PD. Insulin-like growth factor II is induced during wound repair following hypoxic-ischemic injury in the developing rat brain. *Mol Brain Res* 1995;**29**:81–91.
2. Gluckman PD, Ambler GR. Therapeutic use of insulin-like growth factor I: Lessons from *in vivo* animal studies. *Acta Paediatr Suppl* 1992;**383**:134–136.
3. Lee WH, Wang GM, Seaman LB, Vannucci SJ. Coordinate IGF-I and IGFBP5 gene expression in perinatal rat brain after hypoxia-ischemia. *J Cerebral Blood Flow Metab* 1996;**16**:227–236.
4. Yamaguchi F, Itano T, Miyamoto O, et al. Increase of extracellular insulin-like growth factor I (IGF-I) concentration following electrolytic lesion in rat hippocampus. *Neurosci Lett* 1991;**128**:273–276.
5. Scheepens A, Williams CE, Breier BH, Guan J, Gluckman PD. A role for the somatotrophic axis in neural development, injury, and disease. *J Pediatr Endocrinol Metab* 2000;**13**(Suppl 6):1483–1491.
6. Guan J, Williams C, Gunning M, Mallard C, Gluckman P. The effects of IGF-1 treatment after hypoxic-ischemic brain injury in adult rats. *J Cereb Blood Flow Metab* 1993;**13**:609–616.
7. Johnston BM, Mallard EC, Williams CE, Gluckman PD. Insulin-like growth factor-1 is a potent neuronal rescue agent after hypoxic-ischemic injury in fetal lambs. *J Clin Invest* 1996;**97**:300–308.
8. Guan J, Bennet L, George S, et al. Insulin-like growth factor-1 reduces postischemic white matter injury in fetal sheep. *J Cereb Blood Flow Metab* 2001;**21**:493–502.
9. Guan J, Miller OT, Waugh KM, McCarthy D, Gluckman PD. Insulin-like growth factor-1 improves somatosensory function and reduces the extent of cortical infarction and ongoing neuronal loss after hypoxia-ischemia in rats. *Neuroscience* 2001;**105**:299–306.
10. Pardridge WM. Drug delivery to the brain. *J Cereb Blood Flow Metab* 1997;**17**:713–731.
11. Liu XF, Fawcett JR, Thorne RG, Frey WH. Noninvasive intranasal insulin-like growth factor-1 reduces infarct volume and improves neurologic function in rats following middle cerebral artery occlusion. *Neurosci Lett* 2001;**308**:91–94.
12. Saatman KE, Contreras PC, Smith DH, et al. Insulin-like growth factor-1 (IGF-1) improves both neurological motor and cognitive outcome following experimental brain injury. *Exp Neurol* 1997;**147**:418–427.
13. Sara VR, Carlsson-Sdwirut C, Bergman T, et al. Identification of Gly-Pre-Glu(GPE), the aminoterminal tripeptide of insulin-like growth factor 1 which is truncated in brain, as a novel neuroaction peptide. *Biochem Biophys Res Commun* 1989;**165**:766–771.
14. Nilsson-Hakansson L, Civalero I, Zhang X, et al. Effects of IGF-1, truncated IGF-1, and the tripeptide Gly-Pro-Glu on acetylcholine release from parietal cortex of rat brain. *Neuroreport* 1993;**4**:1111–1114.
15. Yamamoto H, Maake C, Murphy LJ. Enhanced proteolytic activity directed against the N-terminal of IGF-I in diabetic rats. *J Endocrinol* 1999;**162**:243–250.
16. Yamamoto H, Murphy LJ. Enzymatic conversion of IGF-I to Des(1–3)IGF-I in rat serum and tissues: A further potential site of growth hormone regulation of IGF-I action. *J Endocrinol* 1995;**146**:141–148.
17. Yamamoto H, Murphy LJ. N-terminal truncated insulin-like growth factor-I in human urine. *J Clin Endocrinol Metab* 1995;**80**:1179–1183.
18. Bourguignon JP, Gerard A. Role of insulin-like growth factor binding proteins in limitation of IGF-1 degradation into the N-methyl-D-aspartate receptor antagonist GPE: Evidence from gonadotrophin-releasing hormone secretion *in vitro* at two developmental stages. *Brain Res* 1999;**847**:247–252.
19. Bourguignon JP, Gerard A, Alvarez Gonzalez ML, Franchimont P. Acute suppression of gonadotropin-releasing hormone secretion by insulin-like growth factor I and subproducts: An age-dependent endocrine effect. *Neuroendocrine* 1993;**58**:525–530.
20. Saura J, Curatolo L, Williams CE, et al. Neuroprotective effects of Gly-Pro-Glu, the N-terminal tripeptide of IGF-1, in the hippocampus *in vitro*. *Neuroreport* 1999;**10**:161–164.
21. Guan J, Waldvogel HJ, Faull RL, Gluckman PD, Williams CE. The effects of the N-terminal tripeptide of insulin-like growth factor-1, glycine-proline-glutamate in different regions following hypoxic-ischemic brain injury in adult rats. *Neuroscience* 1999;**89**:649–659.
22. Guan J, Williams CE, Skinner SJ, Mallard EC, Gluckman PD. The effects of insulin-like growth factor (IGF)-1, IGF-2, and des-IGF-1 on neuronal loss after hypoxic-ischemic brain injury in adult rats: Evidence for a role for IGF binding proteins. *Endocrinology* 1996;**137**:893–898.
23. Batchelor DC, Lin H, Wen J-Y, et al. Pharmacokinetics of glycine-proline-glutamate, the N-terminal tripeptide of insulin-like growth factor-1, in rats. *Anal Biochem* 2003;**323**:156–163.
24. Baker AM, Batchelor DC, Thomas GB, et al. Central penetration and stability of N-terminal tripeptide of insulin-like growth factor-1, glycine-proline-glutamate in adult rat. *Neuropeptides* 2005;**39**:81–87.
25. Guan J, Thomas GB, Lin H, et al. Neuroprotective effects of the N-terminal tripeptide of insulin-like growth factor-1, glycine-proline-glutamate (GPE) following

- intravenous infusion in hypoxic-ischemic adult rats. *Neuropharmacology* 2004;**47**:892–903.
26. Betz AL, Keep RF, Beer ME, Ren XD. Blood–brain barrier permeability and brain concentration of sodium, potassium, and chloride during focal ischemia. *J Cereb Blood Flow Metab* 1994;**14**:29–37.
  27. Preston E, Sutherland G, Finsten A. Three openings of the blood–brain barrier produced by forebrain ischemia in the rat. *Neurosci Lett* 1993;**149**:75–78.
  28. Fujimura M, Gasche Y, Morita-Fujimura Y, Massengale J, Kawase M. Early appearance of activated matrix metalloproteinase-9 and blood–brain barrier disruption in mice after focal cerebral ischemia and reperfusion. *Brain Res* 1999;**842**:92–100.
  29. Planas AM, Sole S, Justicia C. Expression and activation of matrix metalloproteinase-2 and -9 in rat brain after transient focal cerebral ischemia. *Neurobiol Dis* 2001;**8**:834–846.
  30. Shapira S, Mathai S, Zhang R, Guan J. Delayed peripheral administration of the N-terminal tripeptide of IGF-1 (GPE) reduces brain damage following microsphere induced embolic damage in young adult and aged rats. *Neurosci Lett* 2009;**454**:53–57.
  31. Lees KR. Management of acute stroke. *Lancet Neurol* 2002;**1**:41–50.
  32. Harris PWR, Brimble MA, Muir VJ, et al. Synthesis of proline-mediated analogues of the neuroprotective agent glucyl-L-prolyl-glutamic acid (GPE). *Tetrahedron* 2005;**61**:10018–10035.
  33. Bickerdike MJ, Thomas GB, Batchelor DC, et al. NNZ-2566: A Gly-Pro-Glu analogue with neuroprotective efficacy in a rat model of acute focal stroke. *J Neurol Sci* 2009;**278**:85–90.
  34. Zhao X, Liu SJ, Zhang J, et al. Combining insulin-like growth factor derivatives plus caffeine produces robust neuroprotection after stroke in rats. *Stroke* 2005;**36**:129–134.
  35. Svedin P, Guan J, Mathai S, et al. Delayed peripheral administration of a GPE analogue induces astrogliosis and angiogenesis and reduces inflammation and brain injury following hypoxia-ischemia in the neonatal rat. *Develop Neurosci* 2007;**29**:393–402.
  36. Lu XC, Si Y, Williams AJ, et al. NNZ-2566, a glypromate analog, attenuates brain ischemia-induced non-convulsive seizures in rats. *J Cereb Blood Flow Metab* 2009;**29**:1924–1932.
  37. Ikeda T, Waldbillig RJ, Puro DG. Truncation of IGF-I yields two mitogens for retinal Muller glial cells. *Brain Res* 1995;**686**:87–92.
  38. Alonso De Diego SA, Gutierrez-Rodriguez M, Perez de Vega MJ, et al. The neuroprotective activity of GPE tripeptide analogues does not correlate with glutamate receptor binding affinity. *Bioorg Med Chem Lett* 2006;**16**:3396–3400.
  39. Dirnagl U, Iadecola C, Moskowitz MA. Pathobiology of ischaemic stroke: An integrated view. *Trends Neurosci* 1999;**22**:391–397.
  40. Blomgren K. Pathological apoptosis in the developing brain. *Apoptosis* 2007;**12**:993–1010.
  41. Persidsky Y, Ramirez SH, Haorah J, Kanmogne GD. Blood–brain barrier: Structural components and function under physiologic and pathologic conditions. *J Neuroimmune Pharmacol* 2006;**1**:223–236.
  42. Kraig RP, Lascola CD, Cagiano A. Glial response to brain ischemia. In: Kettenmann H, Ransom BR, editors. *Neuroglia*. New York: Oxford University Press, 1995; 964–976.
  43. Panicker KS, Norenberg MD. Astrocytes in cerebral ischemic injury: Morphological and general considerations. *Glia* 2005;**50**:287–298.
  44. Ridet JL, Malhotra SK, Privat A, Gage FH. Reactive astrocytes: Cellular and molecular cues to biological function. *Trends Neurosci* 1997;**20**:570–577.
  45. Maragakis NJ, Rothstein JD. Mechanisms of disease: Astrocytes in neurodegenerative disease. *Nat Clin Pract Neurol* 2006;**2**:679–689.
  46. Acker T, Beck H, Plate KH. Cell type specific expression of vascular endothelial growth factor and angiopoietin-1 and -2 suggests an important role of astrocytes in cerebellar vascularization. *Mech Dev* 2001;**108**:45–57.
  47. Frontczak-Baniewicz M, Walski M. Nonsprouting angiogenesis in neurohypophysis after traumatic injury of the cerebral cortex. Electron-microscopic studies. *Neuro Endocrinol Lett* 2002;**23**:396–404.
  48. Salhia B, Angelov L, Roncari L, et al. Expression of vascular endothelial growth factor by reactive astrocytes and associated neoangiogenesis. *Brain Res* 2000;**883**:87–97.
  49. Lopez-Lopez C, LeRoith D, Torres-Aleman I. Insulin-like growth factor I is required for vessel remodeling in the adult brain. *Proc Natl Acad Sci USA* 2004;**101**:9833–9838.
  50. Cao Y, Gunn AJ, Bennet L, et al. Insulin-like growth factor (IGF)-1 suppresses oligodendrocyte caspase-3 activation and increases glial proliferation after ischemia in near-term fetal sheep. *J Cereb Blood Flow Metab* 2003;**23**:739–747.
  51. McNeill H, Williams C, Guan J, et al. Neuronal rescue with transforming growth factor-beta 1 after hypoxic-ischaemic brain injury. *Neuroreport* 1994;**5**:901–904.
  52. Tanaka K, Fukuuchi Y, Gomi S, et al. Inhibition of nitric oxide synthesis impairs autoregulation of local cerebral blood flow in the rat. *Neuroreport* 1993;**4**:267–270.