known about the mechanisms of necrosis. As stated above (Fig. 2), however, it is only accepted that necrosis is caused by energy failure due to loss of cellular ATP (Eguchi et al., 1997; Fujita & Ueda, 2003a,b; Zong & Thompson, 2006). The cellular ATP levels of cortical neurons rapidly decrease immediately after the start of serum-free culture, and this decrease is markedly inhibited by the addition of ProTα (Ueda et al., 2007). As previously reported (Fujita & Ueda, 2003a,b), the rapid decrease and its reversal by ProTα seem to be parallel to the activity of glucose transport, since the [^{3}H]-2-DG uptake was markedly decreased by serum-free culture and reversed by ProTα. Quite similar changes were reproduced in the LOG ischemia–reperfusion model of the culture. Addition of ProTα reversed the rapid decrease in the cellular ATP levels of cortical neurons following LOG-ischemic stress and reperfusion with serum-containing medium (Ueda et al., 2007). We previously reported that the membrane translocation of the glucose transporters GLUT1/4 is largely inhibited in serum-free cultures of cortical neurons, which leads to necrotic cell death (Fujita & Ueda, 2003a,b). In fact, LOG stress decreased GLUT1/4 membrane translocation, as evaluated by immunocytochemistry and western blot analysis, and addition of ProTα reversed these changes. This cell biological change with ProTα-induced GLUT1/4 membrane translocation enabled us to successfully characterize the putative receptor signaling mechanisms (Ueda et al., 2007). The ProTα-induced GLUT1/4 translocation was blocked by the treatments with pertussis toxin, PLC inhibitor and PKCβ inhibitor. More specifically, the action was abolished by PKCβ$_{II}$ antisense oligodeoxynucleotide (AS-ODN), though ProTα activates PKC isoforms, α, β$_I$ and β$_{II}$, but not γ, δ, ε or ζ. Taken together, the pharmacological studies revealed that the ProTα-induced membrane translocation of GLUT1/4, which underlies the mechanisms for necrosis inhibition, is mediated through activation of putative $G_{i/o}$-coupled receptor, PLC and PKCβ$_{II}$ (Fig. 3).

5.2. Prothymosin α-induced apoptosis

The molecular machineries for apoptosis are relatively better characterized than those for necrosis. In terms of the activation of various caspases, caspase-3 is believed to be the final execution molecule for apoptotic cell death linked to DNA breakdown and nuclear fragmentation (Ferri & Kroemer, 2001; Danial & Korsmeyer, 2004). ProTα activates caspase-3 in serum-free and permanent ischemia models, as well as caspase-9, but not caspase-8 or caspase-12, as previously reported (Ueda et al., 2007). As stated above (Fig. 1), it is accepted that the apoptosis through mitochondrial pathway is caspase-9-dependent, while the one through cell death receptor or ER stress is caspase-8 or caspase-12-dependent, respectively. These results suggest that ProTα causes apoptosis through the mitochondrial pathway. This view was clearly confirmed by the findings that ProTα increased the expression of pro-apoptotic Bax and Bim, and slightly decreased the expression of anti-apoptotic Bcl-2 and Bcl-xL, which regulate mitochondrial apoptotic signaling. On the other hand, a PKCβ$_{II}$ AS-ODN reversed the ProTα-induced pro-apoptotic Bax expression. However, it should be noted that the Bax expression was also abolished by treatment with an AS-ODN for PKCβ$_I$, but not an AS-ODN for PKCα. This fact means that the PKC involved in ProTα-induced GLUT1/4 membrane translocation underlying necrosis is more specific for β$_{II}$-isoform. As seen in the case with necrosis, ProTα-induced Bax expression was abolished by PTX. Furthermore, we observed that ProTα causes membrane ruffling of microglia through putative $G_{i/o}$-coupled receptor, PLC, PKCβ$_I$ and β$_{II}$ (Uemura, Fujita and Ueda, unpublished data). All these findings suggest that various cell biological actions of ProTα are mediated by the activation of $G_{i/o}$-coupled receptor, PLC and PKC. It is interesting if we could speculate that PKCβ$_{II}$ exerts the rapidly occurring GLUT1/4 membrane translocation, while PKCβ$_I$ does the lately occurring pro-apoptotic Bax expression as well as β$_{II}$-isoform. Further studies on differential roles of ProTα-activated PKC isoforms including α-isoform should be the next subject.

The second important issue is that ProTα switches the cell death mode by causing apoptosis (Ueda et al., 2007). Since serum-free stress itself does not cause mitochondrial cyto *c* release, this stress is unlikely to drive the apoptosis machinery as well as the necrosis machinery. Furthermore, ProTα-induced apoptosis may not be

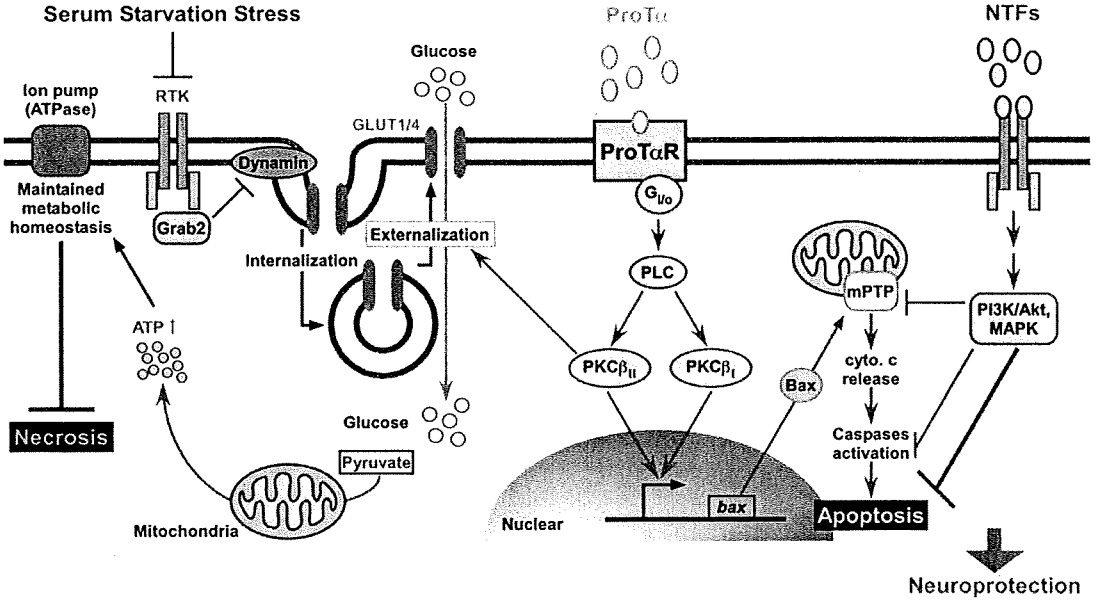

Fig. 3. ProTα-induced cell death mode switch in neuronal cultures. Serum-free or starvation stress leads to endocytosis of the glucose transporters GLUT1/4, which in turn causes bioenergetic catastrophe-mediated necrosis through a rapid loss of glucose supply. Addition of ProTα to ischemia-treated neurons causes translocation of GLUT1/4 to the membrane to allow sufficient glucose supply through activation of $G_{i/o}$, PLC and PKCβ$_{II}$. ProTα-induced apoptosis occurs later at 12 h after the start of serum-free stress. The machinery is mediated by upregulation of Bax, which in turn causes mitochondrial cyto *c* release and subsequent apoptosis. Bax upregulation is also mediated by activation of $G_{i/o}$, PLC and PKC, similar to the case for necrosis. However, both PKCβ$_I$ and PKCβ$_{II}$ upregulations mediate this apoptotic mechanism. Since caspase-3-mediated PARP degradation minimizes the ATP consumption, the apoptosis induction may have a crucial role in inhibiting the rapid necrosis. In addition, since pyruvate, a substrate for ATP production in mitochondria, inhibits necrosis but does not cause apoptosis, the apoptosis machinery seems to be independent of the necrosis inhibition. Neurotrophins, such as BDNF or EPO, which are expected in the ischemic brain and retina, can inhibit the apoptosis machinery at a later stage.

secondary to the inhibition of necrosis. In fact, addition of pyruvate, which increases ATP levels through the TCA cycle, inhibited the necrosis in this culture system, but did not induce apoptosis. ProTα induced upregulation of pro-apoptotic Bax and Bim and down-regulation of anti-apoptotic Bcl-2 and Bcl-xL, and treatments with siRNAs for Bax or Bim abolished the ProTα-induced apoptosis in the LOG-ischemic stress and reperfusion model. The ProTα-induced upregulation of Bax was also abolished by treatments with AS-ODNs for $PKC\beta_I$ and β_{II} activation, consistent with reports that PKCβ activation leads to Bax upregulation through NF-κB (Mattson & Camandola, 2001; Herrmann et al., 2005). Taken together, $PKC\beta_{II}$ is likely to be an important switch molecule for determining the cell death mode. The lack of contribution of $PKC\beta_I$ to the ProTα-induced necrosis inhibition may be related to a deficiency of the membrane-anchoring C-terminal peptide of $PKC\beta_{II}$ (Ono et al., 1986).

5.3. Blockade of prothymosin α-induced apoptosis by neurotrophins

Although addition of ProTα delayed the cell death of cortical neurons in serum-free culture, most of the neurons completely died by apoptosis after 24 h. However, when neurons were treated with ProTα under conditions of ischemia and subsequent reperfusion with serum-containing medium, no significant cell death was observed for at least 48 h (Ueda et al., 2007). These findings indicate that serum factors prevented the ProTα-induced apoptosis. Indeed, further addition of nerve growth factor (NGF), brain-derived growth factor (BDNF), basic fibroblast growth factor (bFGF) or interleukin (IL)-6, comprising representative apoptosis inhibitors (Kaplan & Miller, 2000; Ay et al., 2001; Huang & Reichardt, 2001; Patapoutian & Reichardt, 2001; Sofroniew et al., 2001; Yamashita et al., 2005), rescued the cell survival in serum-free culture for 48 h, while these factors alone had no effects on the survival (Ueda et al., 2007). Similar effects were observed with BIP-V5, which blocks the translocation of Bax to mitochondria (Yoshida et al., 2004). BIP-V5 also selectively inhibited the ProTα-induced apoptosis.

5.4. Inhibition of necrosis by apoptosis induction

It should be noted that concomitant addition of *N*-benzyloxycarbonyl-Val-Ala-Asp (OMe)-fluoromethylketone (zVAD-fmk), a pan-type caspase inhibitor, with ProTα did not lead to long-lasting survival, but caused marked cell death by necrosis at the later stage. Since zVAD-fmk does not affect cyto *c* release, mitochondrial bioenergetic dysfunction may lead to loss of ATP and necrosis induction. Alternatively, the blockade of caspase activity may allow a large PARP-mediated consumption of ATP, as stated above (Fig. 1). The fact that zVAD-fmk inhibits apoptosis, but causes necrosis, is consistent to the previous studies that decreases in intracellular ATP levels changed the type of cell death from apoptosis to necrosis after stimulation of cells by extracellular apoptosis signals, such as Fas ligand and anticancer drugs (Eguchi et al., 1997; Leist et al., 1997). Indeed we observed that the addition of PARP inhibitor 3-aminobenzamide reversed the rapid decrease in the intracellular ATP levels and inhibited necrosis after the start of the low-density and serum-free culture, while z-VAD-fmk significantly reversed the increased ATP levels and necrosis inhibition by ProTα (Fujita and Ueda, unpublished data). In other words, apoptosis induction in the early stage after ischemia may play a defensive role in inhibiting rapid cell death by necrosis (Fig. 3). However, as the late phase of apoptosis is also induced by many other cytotoxic cytokines and nitric oxide through different pathways, we have to consider that the beneficial role of apoptosis in terms of anti-necrosis could be limited in the case with the ProTα-induced one. Furthermore it remains to be determined how potent the apoptosis-induced anti-necrosis effect is, particularly in the in vivo status. This issue is unlikely important, however, since the ProTα-induced apoptosis is effectively blocked by several kinds of neurotrophins.

5.5. Cellular roles of prothymosin α in cell death regulation

With regard to cell death regulation, intracellular ProTα was reported to play a cytoprotective role by inhibiting apoptosome formation in NIH3T3 cells subjected to apoptotic stress (Jiang et al., 2003). This finding is inconsistent with reports that ProTα is released upon necrotic stress and protects against neuronal death (Ueda et al., 2007). ProTα is a highly acidic nuclear protein of the α-thymosin family, and is found in the nuclei of virtually all mammalian cells (Haritos et al., 1985; Clinton et al., 1991). ProTα is generally thought to be an oncoprotein that is correlated with cell proliferation by sequestering anticoactivator factor, a repressor of estrogen receptor activity, in various cells (Martini et al., 2000; Bianco & Montano, 2002). On the other hand, ProTα has been reported to act as an extracellular signaling molecule, as observed in the activation of macrophages, natural killer cells and lymphokine-activated killer cells, and in the production of IL-2 and TNFα (Pineiro et al., 2000). Therefore, ProTα has multiple functions inside and outside of the cell, particularly in terms of cell survival and proliferation (Gomez-Marquez, 2007). Furthermore, the extracellular actions of ProTα seem to be cell type-specific even among brain cells, since ProTα binding is observed in neurons and microglia, but not in astrocytes. Although the identification of ProTα-binding proteins remains to be carried out (Pineiro et al., 2001; Salgado et al., 2005), a pharmacological study demonstrated that the mechanisms for ProTα-induced GLUT1 or 4 translocation or Bax expression involve the activation of $G_{i/o}$-coupled receptors followed by PLC and $PKC\beta_{II}$ and/or β_I activation, as mentioned above (Ueda et al., 2007).

6. Prothymosin α-induced inhibition of cerebral ischemia-induced necrosis and apoptosis

Clinical availability is the most important issue to be discussed. Although ProTα inhibits the rapid cell death of neurons following serum-free ischemic stress by inhibiting necrosis, the neurons soon completely die by apoptosis. Consequently, it is likely that a pathophysiological/physiological role of ProTα will be involved in the cessation of expanding necrosis in focal ischemia, and in the possible convergence of cell death by apoptosis induction. However, it should be noted that the cell death in cultured experiments was completely blocked by the combination of ProTα and anti-apoptotic growth factors. Since such growth factors are supposed to be secreted in brain ischemia, the neuroprotective potency of ProTα would be more significant for ischemia stress in vivo than in cell cultures.

6.1. Transient focal ischemia model

As a focal cerebral ischemia model, middle cerebral artery occlusion (MCAO) in rats is often performed by using monofilament nylon surgical sutures (3-0 in size). In the protocol, the monofilament is removed at 60 min after the occlusion to restore the blood flow. In the focal ischemic model of MCAO for 1 h followed by reperfusion in rats, a marked loss of triphenyltetrazolium chloride staining, which detects the presence of mitochondrial enzymes, was specifically observed in the ipsilateral regions of the cerebral cortex, hippocampus and striatum at 24 h after reperfusion. Ischemia-induced motor dysfunction evaluated by a so-called clinical score started immediately after the occlusion, attained the maximum value at 2–4 days and lasted for more than several weeks. Approximately half of the treated rats died by 2 days after the ischemia–reperfusion. Systemic administration of recombinant rat ProTα (rrProTα; 100 μg/kg, intraperitoneally) at 30 min and 3 h after reperfusion largely reversed this brain damage and suppressed ischemia-induced motor dysfunction and lethality (Fujita and Ueda, 2007). A similar degree of ProTα-induced prevention of ischemic damage was observed when rrProTα was administered as a single injection at 30 min or 3 h after reperfusion or in a pair of later injections at 3 h and 6 h after reperfusion. Since Myc-tagged

recombinant ProTα administered intraperitoneally was detected in the cortex at 3 h after MCAO stress (Fujita and Ueda, 2007), the neuroprotective actions of ProTα administered through systemic routes are likely to be due to transient disruption of the blood–brain barrier (BBB) in the ischemic brain (Paul et al., 2001). It is noteworthy that the neuroprotective actions of ProTα were observed with systemic injection at 3 h after the ischemia, a time point within the therapeutic window for treatment of stroke with tPA. In preliminary studies, we observed that delayed treatment with ProTα was still effective at 6–7 h after permanent MCAO (Fujita, Ueda et al., unpublished data). A previous paper described that added ProTα is incorporated into the cell and nuclei and causes inflammatory actions through enhancement of cytokine gene transcription, while a ProTα mutant lacking the NLS inhibits these effects (Shiau et al., 2007). However, since the Myc-ProTα incorporated into the brain is full size and a ProTα mutant lacking the C-terminal NLS retains the survival activity of the full-length ProTα, regulation of cytokine gene transcription is unlikely to be involved in the neuroprotection.

6.2. Blockade of prothymosin α-induced apoptosis by neurotrophins in vivo

By cytochemical analysis, both necrosis and apoptosis were observed in damaged brain regions at 24 h after MCAO, when cell death was evaluated by in vivo PI and activated-caspase-3 staining. Systemic ProTα injection markedly inhibited both necrotic and apoptotic cell death (Fujita & Ueda, 2007), in contrast with the findings that ProTα inhibits necrosis and causes apoptosis in serum-free culture (Ueda et al., 2007). However, complete inhibition of cell death in culture was observed when anti-apoptotic neurotrophins were coadministered with ProTα. These findings suggest that the apoptosis caused by ProTα may also be inhibited by brain neurotrophins. In fact, administration of anti-BDNF or anti-erythropoietin (EPO) IgG (1 μg each) into the subarachnoid space through a parietal bone at 30 min prior to MCAO stress reversed the ProTα-inhibited apoptosis, but not necrosis (Fujita & Ueda, 2007). Therefore, ProTα is a unique cell death regulatory molecule, in that it converts irretrievable necrotic cell death into controllable apoptosis. Since this apoptosis can be inhibited by growth factors secreted upon ischemic stress, it is expected that ProTα may have an overall neuroprotective role in the treatment of stroke.

6.3. Prothymosin α-induced prevention of memory and learning dysfunction

Bilateral common carotid artery occlusion (BCCAO) is often used as a model for global ischemia. In a sublethal global ischemic model using BCCAO for 30 min, there were marked losses of neurons in the pyramidal cell layers and dentate gyri of the surviving mice at 28 days after occlusion (Fujita & Ueda, 2007). A single systemic (intraperitoneal) injection of recombinant mouse ProTα (rmProTα; 100 μg/kg) at 24 h after occlusion completely prevented brain damage, learning and memory deficits in a step-through passive avoidance task and lethality. Significant prevention was also observed with rmProTα at a very low dose (1 μg/kg, intraperitoneally).

7. Prothymosin α-induced inhibition of retinal ischemia-induced damage

A transient global retinal ischemia model resulting from acute vascular occlusion may provide information regarding retinal dysfunction in terms of cellular damage and visual loss, as observed in cases of acute glaucoma, diabetic retinopathy, retinal arterial occlusion and ischemic optic neuropathy. This model has the additional advantages that various kinds of treatments with drugs, AS-ODNs or antibodies are very easy and semi-quantitative analyses can be carried out. A successful example of ProTα-induced neuroprotection using a retinal ischemia model has been reported (Fujita et al., 2009) and is described below.

7.1. Prothymosin α-induced prevention of histological and functional retinal ischemic damage

In the model, mouse eyes were subjected to hydrostatic pressure (130 mm Hg) for 45 min followed by reperfusion (Adachi et al., 1998; Ueda et al., 2004). Decreases in the thicknesses of retinal cell layers, namely the ganglion cell layer (GCL), inner nuclear layer (INL) and outer nuclear layer (ONL), were observed in a time-dependent manner (Fujita et al., 2009). The maximal decrease was attained on day 7, but no further damage was observed on day 14 after ischemia and reperfusion. By TEM analysis, INL and ONL cells died by necrosis, which was characterized by a loss of electron-density in the cytosol and swollen mitochondria without nuclear condensation as early as day 1 after ischemic stress. At 3 days after the stress, necrotic cells disappeared, but apoptotic cells appeared instead. Apoptosis was characterized by nuclear fragmentation or condensation, but mitochondrial swelling was not observed. PI staining has been used as a marker for necrosis due to plasma membrane damage. To avoid artificial PI staining during the sample preparation, PI (2 ng/eye) was intravitreously administered at 1 h prior to isolation of the retina. Marked PI staining was observed in the ONL as early as 6 h after the ischemia, and in the INL and GCL at 12 h and 1 h, respectively. However, the PI signals substantially disappeared thereafter until day 3, possibly owing to lysis of the dead cells. Regarding apoptosis induction, there was significant activation of caspase-3-like protease activity and immunoreactivity at days 1 and 3. At day 5, most of the apoptosis activity had returned to the control level. Ischemia-induced functional damage was evaluated by electroretinography (ERG). In this method, the amplitudes called a-waves represent the function of photoreceptor cells, while b-waves represent the functions of bipolar and Muller cells. On day 7 after the ischemia, the a- and b-wave amplitudes were markedly decreased to approximately 25% and 15%, respectively, relative to those in vehicle-treated control animals.

Intravitreous application of rmProTα at 30 min prior to the ischemia or at 3 or 24 h after the ischemia prevented the decreases in the thicknesses of the layers. Systemic administration of rmProTα for 3 h after the ischemia also showed complete protection. Similar ProTα-induced retinal protection was observed when PI staining, caspase-3/TUNEL staining and TEM analysis were performed. Furthermore, the functional damage evaluated by ERG was effectively abolished by systemic administration of rmProTα. Similar to the cerebral ischemia model, ischemia-specific retinal transport of biotinylated ProTα administered through a systemic route (intravenously) was observed.

7.2. Prothymosin α-induced cell death mode switch

By analogy with cerebral ischemia (Fujita & Ueda, 2007), in vivo neurotrophins are expected to inhibit ProTα-induced apoptosis. In immunohistochemical studies, weak BDNF immunoreactivities were observed throughout the retina, and in the INL, inner plexiform layer, ONL and ONL optic disk. Ischemic stress selectively elevated the BDNF level in the GCL at 24 h after the stress, while administration of ProTα (100 μg/kg, intravenously) further enhanced the BDNF expression throughout the retinal layers (GCL, INL and ONL optic disk). Since ProTα treatment alone without ischemia had no effect, the combination of ischemia and ProTα seems to potentiate BDNF expression through unknown mechanisms. On the other hand, EPO expression was specifically elevated in the GCL after ischemic stress. ProTα administration did not change the EPO levels in the GCL or INL, but increased the level in the ONL optic disc. Western blot analysis confirmed that ProTα plus ischemic stress caused significant elevations of BDNF and EPO, but not NGF or bFGF, in the retina.

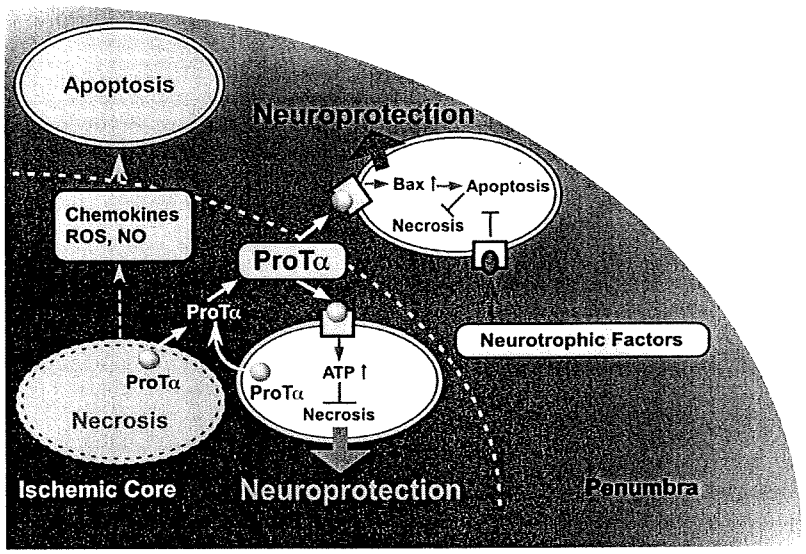

Fig. 4. In vivo neuroprotective role of ProTα in the ischemic brain and retina. In the ischemic core, rapid necrosis of neurons occurs, which in turn causes secondary necrosis by cytotoxic molecules released from the dead cells. ProTα release precedes the necrotic cell damage and prevents the secondary necrosis through re-translocation of the glucose transporters GLUT1/4, which allows the supply of sufficient amounts of glucose for subsequent production of ATP. ProTα also induces caspase-3 activation, which in turn inhibits or delays necrosis by a reduction of ATP consumption owing to PARP degradation. This delay of cell death seems to be important, since ischemia induces de novo expression of neurotrophins, which inhibit the induced apoptosis. Although dead cell-derived cytotoxic molecules also cause late apoptosis in the penumbra, the initial blockade of necrosis may minimize the occurrence of late apoptosis. This view may be consistent with the rationale for therapies for acute ischemic stroke.

When anti-BDNF or anti-EPO IgG (1 μg/eye) was intravitreously administered at 30 min prior to ischemic stress, ProTα-induced TUNEL staining reappeared more intensely compared with the vehicle control, but no significant change in the intensity of PI staining was observed (Fujita et al., 2009). However, these IgG treatments alone had no effect on the levels of either necrosis or apoptosis. These findings strongly suggest that ProTα inhibits the necrosis of retinal cells, but causes apoptosis. Furthermore, the apoptosis caused is inhibited by neurotrophins, such as BDNF or EPO, which are upregulated by ProTα under ischemic conditions (Fig. 4).

7.3. In vivo neuroprotective role of prothymosin α in a retinal ischemia model

To examine the in vivo role of ProTα, intravitreous pretreatment with an AS-ODN against ProTα was carried out. This AS-ODN, but not a mismatched scrambled oligodeoxynucleotide (MS-ODN), significantly worsened the histological damage at day 4 after ischemic stress. Similar results were observed for intravitreous pretreatment (1 μg/eye, 30 min prior to ischemic stress) of anti-ProTα IgG, which absorbs ProTα (Fujita et al., 2009). Functional damage was also deteriorated by this antibody treatment, as evaluated by ERG. Since ProTα-like immunoreactivities completely disappeared without exception at 3 h after the stress, it is evident that ProTα released upon ischemic stress plays in vivo neuroprotective roles (Fig. 4).

8. Comparison of neurotrophin and thrombolytic therapies for stroke

Stroke is the third leading cause of death in developed countries and the leading cause of major disability in adults. During the last decade, there has been significant progress in the development of thrombolytic therapies for acute ischemic stroke. tPA was first approved by the Food and Drug Administration in 1996. Although tPA therapy has significant benefits ("Tissue plasminogen activator for acute ischemic stroke. The National Institute of Neurological Disorders and Stroke rt-PA Stroke Study Group", 1995), it has a restrictive time window of 3 h, which allows only 1–2% of patients with acute ischemic stroke to receive tPA therapy. Furthermore, thrombolytic therapies have frequent risks of cerebral hemorrhage, which restrict their use in certain patients. Therefore, there is a requirement for the development of neuroprotective therapies as sole regimens or in combination with tPA or thrombolytic therapies.

Most of the ~30 neurotrophins have been shown to exhibit neuroprotective effects in brain ischemia, injury or neurodegenerative diseases. In animal studies, NGF, BDNF and bFGF were reported to show significant neuroprotective actions (Hefti, 1986; Williams et al., 1986; Henderson et al., 1993; Tsukahara et al., 1994; Ay et al., 1999). However, clinical trials with these neurotrophins as treatments for the above diseases have failed, possibly due to their poor BBB permeability or unexpected side effects (Olson et al., 1992; "A controlled trial of recombinant methionyl human BDNF in ALS: the BDNF Study Group (Phase III)", 1999; Turner et al., 2001; Bogousslavsky et al., 2002). Chimeric peptide approaches for targeting transfer receptors on the BBB are now being evaluated as a new type of approach (Wu, 2005). In light of the present situation, ProTα, which penetrates the BBB and shows potent neuroprotection in the ischemic brain even after delayed and systemic administration, would be a strong candidate as a sole regimen as well as in combination with tPA and thrombolytic therapies for acute ischemic stroke. Future subjects for research into ProTα would be more practical studies regarding the therapeutic window, toxicology, pharmacokinetics and development of the molecule with better pharmacodynamics.

The study of ProTα-induced anti-ischemic actions has just started since the discovery in 2007. Therefore the concerns of ProTα-induced mechanisms in this review are limited to the neuroprotection based on neuronal cell death mode switch. It is also interesting to examine other possible mechanisms, such as anti-oxidation, anti-neuroinflammation or direct prevention of ischemia-induced cerebrovascular damages.

9. Conclusions

The discovery of ProTα initiated investigations into what happens in the event of neuronal necrosis, followed by searches for compounds that inhibit necrosis, based on the detection of its presence in conditioned medium from neuronal cultures. The hypothesis that ProTα

acts as a cell death mode switch from uncontrollable necrosis to neurotrophin-reversible apoptosis may provide a promising new strategy for preventing serious damage in stroke. However, the complete clarification of mechanisms underlying potent neuroprotective actions of ProTα in cerebral and retinal ischemia should wait for the identification of the specific receptor. From this point of view, the involvement of unknown mechanisms independent of $G_{i/o}$-coupled receptors may not be excluded at present. Regarding clinical issues, it is evident that recombinant ProTα by itself has unique and potent therapeutic potentials against acute stroke.

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