Neuroprotection by Alpha 2-Adrenergic Agonists in Cerebral Ischemia

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Abstract: Ischemic brain injury is implicated in the pathophysiology of stroke and brain trauma, which are among the top killers worldwide, and intensive studies have been performed to reduce neural cell death after cerebral ischemia. Alpha 2-adrenergic agonists have been shown to improve the histomorphological and neurological outcome after cerebral ischemic injury when administered during ischemia, and recent studies have provided considerable evidence that alpha 2-adrenergic agonists can protect the brain from ischemia/reperfusion injury. Thus, alpha 2-adrenergic agonists are promising potential drugs in preventing cerebral ischemic injury, but the mechanisms by which alpha 2-adrenergic agonists exert their neuroprotective effect are unclear. Activation of both the alpha 2-adrenergic receptor and imidazoline receptor may be involved. This mini review examines the recent progress in alpha 2-adrenergic agonists - induced neuroprotection and its proposed mechanisms in cerebral ischemic injury.

Key Words: Alpha 2-adrenergic agonist, adrenoceptor, imidazoline receptor, cerebral ischemia, neuroprotection, excitatory amino acids, sympathoinhibition, stroke.

INTRODUCTION

Ischemic brain injury is implicated in the pathophysiology of stroke and brain trauma, which are among the top killers worldwide and intensive studies have been performed to reduce neural cell death after cerebral ischemia. Beside stroke and brain trauma there exist several other causes of cerebral ischemia, like vasospasm after subarachnoid hemorrhage, and occlusion of cerebral vessels during operation. The initiating event for neural cell death is considered to be the rapid release of excitatory amino acids (EAAs) and their accumulation in the extracellular space due to diminished energy supplies to maintain transmembrane ion gradients, and also associated acidosis. Increased EAAs leads to cellular Ca^{++} influx, increased free radicals and oxidative damage [63,70]. High intracellular Ca^{++} concentrations are detrimental to neurons. They trigger catabolic intracellular processes by activation of lipid peroxidases, proteases, and phospholipases which in turn increase the intracellular concentration of free fatty acids (FFA) and free radicals. These events lead to membrane degeneration of cellular structures and consecutive lyses of the cell. The same depolarizing and increased Ca^{++} influx *via* voltage gated Ca^{++} channels will also lead to release of other neurotransmitters. Recently, alpha 2-adrenergic agonists have been shown to improve the histomorphological and neurological outcome after cerebral ischemia when administered during and before ischemia, suggesting that epinephrine release could also contribute to post-ischemic damage [3,27,28,32,33,45,52,53,65,81]. Since the 1970s, alpha 2-adrenergic agonists have been applied successfully to treat patients with hypertension and to decrease afterload in congestive heart failure and aortic surgery [38]. They produce diverse responses, including analgesia, anxiolysis,

sedation, and sympatholysis [15]. Thus they can be clinically well-tolerated. Since clinically effective neuroprotective drugs to improve the outcome after stroke have not yet been established, investigation of mechanisms by which alpha 2-adrenergic agonists induce neuroprotection could reveal new targets or strategies for treating ischemic brain injury. This mini review will examine recent progress in alpha 2-adrenergic agonist-induced neuroprotection and its proposed mechanisms in cerebral ischemic injury.

PHARMACOLOGY [see table 1]

Alpha 2-adrenergic agonists bind to alpha 2-adrenoceptors and imidazoline receptors that are widely distributed among the different mammalian tissues. Based on their pharmacology, there are three subtypes alpha 2A, alpha 2B and alpha 2C adrenoceptors to which alpha 2-adrenergic agonists bind and produce effects. In addition to their broad distribution in peripheral system, alpha 2-adrenoceptors are also found throughout the central nervous system. Presynaptic alpha 2-adrenoceptor agonists are known to suppress the release of norepinephrine and other neurotransmitters [59,73]. Alpha 2-adrenoceptors also induce the phosphorylation of mitogen-activated protein kinase (MAPK) and inhibit the cyclic AMP-dependent phosphorylation of the cAMP response element-binding protein (CREB) [1,22]. There is growing evidence in favor of a neuroprotective role for alpha 2A receptors by modulating depolarization induced noradrenalin release in the locus coeruleus, whereas alpha 2C receptor agonists do not [9]. At lower doses alpha 2-adrenergic agonists produce hypotension by inhibition of the firing of the locus coeruleus in the brain stem and decreasing norepinephrine release at synapse. At higher doses hypertension can be induced *via* the activation of alpha 2B adrenoceptors located on smooth muscle cells in the resistance vessels. In the cardiovascular system the predominant action is block of the cardioaccelerator nerve and therefore a decrease in tachycardia. Both a vasodilatory action *via* sympatholysis and vasoconstriction mediated through the smooth muscle receptors are observed in

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Table 1. Alpha 2-Adrenergic Agonist Receptor Types and Subtypes in the Central Nervous System

Type	Subtype	Location	Function
Alpha 2 adrenoceptor	Alpha 2A	Throughout brain, spinal cord, locus coeruleus. (pre- and postsynaptically, mostly on neuron)	Produces anesthetic, sympatholytic responses
	Alpha 2B	Thalamus, vascular smooth muscle cells. (pre- or postsynaptically?)	Induces hypertensive response (short-term)
	Alpha 2C	Locus coeruleus, especially in basal ganglia (pre- and postsynaptically)	Modulates hypnotic-sedative, analgesic, anxiolytic actions
Imidazoline Receptor	I ₁	Lateral reticular nucleus, locus coeruleus. (most presynaptically)	Modulates blood pressure, stimulates neuronal firing
	I ₂	Throughout brain, such as interpeduncular nucleus, arcuate and pineal gland. (most presynaptically)	Increase glial fibrillary acid protein expression, monoamine turnover

peripheral vasculature [38]. Alpha 2B/2C can be most easily distinguished from alpha 2A on the basis of the selective alpha 1-adrenoceptor antagonist prazosin [7,8].

In addition, because all alpha 2-adrenergic agonists have an imidazoline ring in their structure, these compounds also interact with the imidazoline receptors. At least two distinct populations of imidazoline binding sites exist [56]. Imidazoline receptors are distinguished from alpha 2-adrenoceptors on the basis of anatomical distribution [12,39], signal transduction mechanisms [57], binding profiles [18,19] and insensitivity to catecholamines and GTP analogues [5,6,80]. I₁-site selective drugs have been used in treating hypertension, whereas I₂-site receptors play a role in monoamine turnover. Therefore I₂ receptor ligands may potentially affect a wide range of brain functions.

NEUROPROTECTION [see Fig. (1)]

Alpha 2-adrenergic agonists have been shown to be neuroprotective in cerebral ischemia. The exact mechanisms by which alpha 2-adrenergic agonists exert their neuroprotective effect may include their mediations on both a supracellular level (reduction of excitatory neurotransmitter release) and a cellular level (inhibition of adenylate and guanylate cyclases).

Attenuation of Excitatory Transmitter Release

It is well-documented that one of the initial responses in the central nervous system after cerebral ischemia is the massive release of norepinephrine, among many other neurotransmitters, in the hippocampus and striatum [24,25, 55,79]. High concentrations of norepinephrine may trigger

Fig. (1). Scheme of possible mechanisms for neuroprotection by alpha 2 adrenergic agonists. IR = imidazoline receptor; GFAP = glial fibrillary acid protein; GLU = glutamate;

ASP = aspartate; NE = norepinephrine; R = receptor; $\ominus$ = inhibition.

neuronal damage by inducing imbalances between cerebral oxygen demand and oxygen supply, increasing the sensitivity of pyramidal neurons to excitatory neurotransmitters such as glutamate and decreasing perfusion in the ischemic area. However, the importance of increased noradrenergic activity remains unclear because norepinephrine has been suggested to play both a protective and damaging role in ischemia-induced neural injury. It has been demonstrated that alpha 2-adrenoceptor agonists decrease the release and turnover of norepinephrine in the brain [48,50]. If the increased norepinephrine release is a component of the total injury, alpha 2-adrenergic agonists may provide protection against the damaging effects of cerebral ischemia by preventing the release of norepinephrine induced by ischemia. In support of this idea, Hoffman *et al.* [32,33] showed that clonidine and dexmedetomidine prevented increased adrenaline and noradrenaline in the plasma, which may in turn aggravate excitotoxicity after cerebral ischemia. Globus *et al.* found that dexmedetomidine may attenuate the excessive release of noradrenaline induced by ischemia through the activation of presynaptic alpha 2-adrenoceptors [25]. Dexmedetomidine and clonidine, when administered immediately before the ischemia, have been reported to reduce the degree of ischemic injury in the rat forebrain [32,33] and decrease neuronal damage in a rabbit focal model of ischemia [52]. Similar to neuroprotection induced by anesthetic preconditioning in Kapinya *et al.*'s study [40], transient hypotension induced by clonidine and dexmedetomidine may precondition the rat against the ischemia.

There is little doubt that EAAs are involved in neural tissue damage in cerebral ischemia. In addition to the effects on catecholamine release, alpha 2-adrenoceptor agonists have been demonstrated to reduce release of EAAs neurotransmitters in several experimental models of cerebral ischemia [4,69,77]. Tizanidine, a centrally acting muscle relaxant that has an effect and structure similar to those of the selective alpha 2-adrenoreceptor agonist clonidine, has been shown to stimulate the presynaptic alpha 2-adrenoceptors in the central EAA system and to inhibit aspartate and glutamate release [44,51,54]. The newly developed alpha 2-adrenergic agonist dexmedetomidine suppressed kainic acid-induced convulsions and prevented hippocampal cell death in rats [29]. In the hippocampus slices, dexmedetomidine inhibited hypoxia-induced glutamate release [77]. Consistent with this, Maier *et al.* [52] have suggested that alpha 2-adrenergic agonists prevent ischemia-induced brain damage by inhibiting glutamate accumulation. However, the molecular mechanism by which alpha 2-adrenergic agonists inhibit ischemia-induced release of EAAs is unclear. Huang *et al.* [34] showed that dexmedetomidine enhances glutamine disposal by oxidative metabolism in astrocytes and thereby reduces the availability of glutamine as a precursor of glutamate. MK-801, an antagonist of N-methyl-D-aspartate (NMDA) glutamate receptor, has been shown to prevent neuronal degeneration in stroke and CNS trauma and suppress neuropathic pain, but with severe side effects limiting its clinical use [60]. Interestingly, alpha 2-adrenergic agonists have been reported to prevent these adverse effects of NMDA antagonists [20,35]. Nonetheless, Engelhard *et al.*'s data [17] showed that the increase of circulating catecholamine concentrations during cerebral ischemia was suppressed with

dexmedetomidine, whereas brain norepinephrine and glutamate concentration associated with cerebral ischemia was not suppressed. It was therefore concluded that the neuroprotective effects of dexmedetomidine are not related to inhibition of presynaptic norepinephrine or glutamate release in the brain. Consistent with the findings by Engelhard *et al.* [17], Kim *et al.* showed that dexmedetomidine does not reduce EAA after transient global ischemia in rabbits [41]. Therefore, there is controversy regarding whether or not the neuroprotective effects induced by alpha 2-adrenergic agonists are due to the inhibition of neurotransmitter releases. *In vivo* ischemic model studies [17,41] have concluded that the protection was not caused by decreasing the concentrations of excitatory neurotransmitters, whereas *in vitro* studies [4,69,77] reported that the reduction of these excitatory transmitters plays an important role in producing neuroprotection. Discrepancies emerge when *in vitro* results are extrapolated to an intact animal ischemic model. The use of *in vitro* preparations as models for understanding the pathology of cerebral ischemia has limitations. These include a variable injury layer in the preparations and uncertainty concerning the degree to which biochemical alterations in these preparations during hypoxia/ischemia mimic those *in vivo* [11].

Inhibition of Calcium Channels and Activation of G Protein-Linked Inward Rectifying K⁺ Channels

Alpha 2-adrenoceptor agonists can induce inhibition of voltage-operated calcium channels, which are activated by NMDA-evoked depolarization and contributes to the cytotoxic calcium overload [61,75]. Alpha 2-adrenergic agonists have been demonstrated to induce activation of inward rectifying G-protein-coupled K⁺ channels and block voltage-gated calcium channels [14]. Therefore, activated alpha 2-adrenergic receptors will hyperpolarize neurons and inhibit the presynaptic release of glutamate, aspartate, and norepinephrine [46], and thus contribute to the neuroprotection from cerebral ischemic injury. [see Fig. (1)]

Inhibition of Adenylate and Guanylate Cyclases

All the subtypes of alpha 2-adrenergic receptor activate G-proteins. Neurotransmitters that stimulate the synthesis of cAMP exert their intracellular effects primarily by activating protein kinase A (PKA). The stimulation (*via* G_s) or inhibition (*via* G_i) of adenylate cyclase will activate or inactivate PKA, respectively. A broad range of important functions are regulated by PKA such as gene expression *via* effects on CREB. This was supported by Engelhard *et al.* showing that the neuroprotective properties of dexmedetomidine and S(+)-ketamine seen in previous studies involves an early modulation of the balance between pro- and anti-apoptotic proteins [16]. PKA also regulates other important functions includes phosphorylase kinase, cell morphology (MAP-2), postsynaptic sensitivity (AMPA receptors), and membrane conductance (L-type Ca²⁺ channel). Different G-protein subtypes may couple in different locations, such as locus coeruleus and vasculature, to produce various effects. Thus the alpha 2-adrenergic receptors typically couple to G_i-proteins, which can lead to inhibition of adenylate cyclase and to changes in the open probability of K⁺ channels and voltage-dependent Ca²⁺ channels [13,26,31,74]. In Zhang's

[81] study on pretreatment with clonidine, clonidine was given at different time points before ischemia. The half-life of clonidine is 8-12 hours and therefore a significant amount of clonidine may exist in rat tissues at 24 hours after the injection, the longest time point with neuroprotection after clonidine administration observed in his study [81]. However, the hypotensive effects lasted for only 90 minutes and other physiological parameters (such as increased glucose levels observed by Jolkonen *et al.* [37]), were already normal at 6 hours (the earliest time point that Zhang measured in his study) after the administration. These results suggest that the physiological effects and, therefore the activation of alpha 2-adrenergic receptors, may not last through 24 hours after the injection, and long-lasting intracellular changes after the temporary activation of α_2 -adrenergic receptors by clonidine may play an important role in the neuroprotection observed in this study. However, it has not been established that the above signaling pathways play an important role in the neuroprotection observed in the neuroprotective effects caused by alpha 2-adrenergic agonists during ischemic insult.

In addition, alpha-2 adrenergic agonists were shown to decrease cerebellar cyclic guanosine 3',5'-monophosphate (cGMP), an effect that correlates with their anesthetic and anticonvulsant effects. The nitric oxide (NO)-cGMP pathway has emerged as a neuroprotective signaling system involved in communication between neurons and glia, the reduction of cGMP may be involved in producing neuroprotective effects by alpha 2-adrenergic agonists *via* the NO-cGMP pathway. In the central nervous system, neurotransmitters, drugs or conditions that cause increased cGMP levels, do so *via* the neuromodulator NO, whereas those that cause sedation decrease cGMP [21]. It was also reported that nitric oxide synthase (NOS) antagonists prevent the alpha 2 adrenoceptor-mediated analgesic effect of clonidine [30,36,62]. Inhibition of the noradrenergic input from the locus coeruleus to the cerebellum may modulate cerebellar cGMP concentrations, Purkinje cell activity, and motor activity. Alpha-2 adrenergic receptors are coupled to the NO-cGMP effector pathway and the role of the NO-cGMP pathway in the action of the alpha-2 adrenoceptor agonists may be crucial to induce neuroprotection by decreasing cGMP [78].

Effects on Neurons or Astrocytes?

Alpha 2-adrenergic agonists are known to suppress the release of norepinephrine and other neurotransmitters such as aspartate and glutamate *via* alpha-2 adrenoceptor located at presynaptic neuronal terminal. In addition to neurons, astrocytes may also play an important role in the neuroprotection induced by alpha 2-adrenergic agonists. Astrocytes are as numerous as neurons and closely surround blood vessels in the brain; many of their properties are similar to those seen in neurons, such as they have receptors for various brain chemical transmitters. Astrocytes occupy 20~30% of the brain volume, depending on the region, and are found to undergo rapid swelling after a number of acute pathological states, such as ischemia and traumatic brain injury [2,42,43,58,72]. Increased calcium influx into astrocytes was found to be critical in modulating elevated levels of calcium in nervous tissue following cerebral

ischemia; excess extracellular calcium is detrimental to neurons [71].

Although its function is not fully understood, glial fibrillary acidic protein (GFAP) protein is probably involved in controlling the shape and movement of astrocytes and in the interactions of astrocytes with other cells, which are required for the formation and maintenance of the insulating layer (myelin) that covers nerve cells. Additionally, GFAP may assist in maintaining the protective barrier that allows only certain substances to pass between blood vessels and the brain (blood-brain barrier). Therefore, changes in GFAP can be used to document the existence and location of cerebral ischemic injury. In cultured rat cerebral cortical astrocytes, imidazoline I₂-site activation leads to an increase in levels of mRNA for (GFAP) [68]. Garcia-Sevilla's group has also noted that I₂-sites regulate levels of GFAP, and chronic treatment of rats with an I₂-site selective compound increased GFAP immunoreactivity in cerebral cortex [23]. This association with GFAP is of interest since idazoxan's neuroprotective effects following brain ischemia are proposed to be mediated *via* I₂-sites [27].

Recently it has been suggested that astrocytes contribute to synaptogenesis and synaptic function [64]. The neuroprotective effects induced by dexmedetomidine and clonidine on isolated neurons suggest the involvement of somatodendritic, rather than presynaptic alpha 2-adrenoceptors [49]. Within the cerebral cortex, neuronal imidazoline receptors are absent [39], whereas cortical astrocytes express the imidazoline I₂ subtype on the outer mitochondrial membrane [80]. Ozog *et al.* [65] concluded that rilmenidine *in vivo* may enhance uptake of calcium from extracellular fluid by astrocytes, a process that may contribute to the neuroprotective effects of this agent. Rilmenidine stimulates both Ca²⁺ release from intracellular stores and Ca²⁺ influx by a mechanism independent of alpha 2-adrenergic receptors. Furthermore, there appears to be a strong link between I₂-sites and monoaminergic oxidase (MAO), and several I₂-site selective compounds have been found to inhibit amine oxidation in liver cells and adipocytes [10,47,66]. Therefore, both neuron and astrocyte may play roles in neuroprotection caused by alpha 2-adrenergic agonists, and these effects may differ according to the different types of alpha 2-adrenergic agonists used because of their varied pharmacologic character.

The Alpha 2-Adrenoceptor and Imidazoline Receptor Hypotheses [see Table 2]

There is controversy regarding the mode of action of alpha 2 agonist drugs. Two hypotheses have been developed [76]: the alpha 2-adrenoceptor hypothesis and the imidazoline hypothesis. Table 2 lists these two hypotheses and their support from several different studies. However, studies supporting one hypothesis may not be inconsistent with the other occurring as well, since both may be important in producing protective effects. The first indication that imidazoline receptors are involved in neuroprotection was from Gustafson *et al.* [27,28], who showed that idazoxan, an imidazoline receptor ligand which is also an alpha 2-adrenergic receptor antagonist, reduces the infarct size in rats following occlusion of the middle cerebral artery. Idazoxan's

Table 2. Two Hypotheses Regarding Alpha 2 Agonist - Induced Neuroprotection in Cerebral Ischemia

Hypothesis	Finding	Reference
Alpha 2 adrenoceptor hypothesis	Clonidine decrease plasma catecholamines and improves outcome from incomplete ischemia	Hoffman <i>et al.</i> 1991
	Dexmedetomidine improves neurologic outcome from incomplete ischemia which is reversed by atipamezole	Hoffman <i>et al.</i> 1991
	Neuroprotection by the alpha 2-adrenoceptor agonist dexmedetomidine in a focal model of cerebral ischemia	Maier <i>et al.</i> 1993
	Neuroprotective effects of dexmedetomidine in the gerbil hippocampus after transient global ischemia	Kuhmonen <i>et al.</i> 1997
	Tizanidine pretreatment significantly reduces ischemic damage.	Berkman <i>et al.</i> 1998
	Neuroprotection exerted <i>via</i> alpha 2-adrenergic receptors and not imidazoline-preferring sites	Huang <i>et al.</i> 2000
	Clonidine pretreatment induces neuroprotective effects	Zhang 2004
Imidazoline receptor hypothesis	Idazoxan decreases neuron damage in rat brain	Gustafson <i>et al.</i> 1989
	Idazoxan protects by several mechanisms	Gustafson <i>et al.</i> 1990
	Rilmenidine and idazoxan reduce focal ischemic infarction	Maiese <i>et al.</i> 1992
	Rilmenidine interactions with central imidazoline receptors can be neuroprotective	Reis <i>et al.</i> 1994
	Rilmenidine stimulates both Ca ²⁺ release from intracellular stores and Ca ²⁺ influx by a mechanism independent of alpha 2-adrenergic receptor	Ozog <i>et al.</i> 1998

neuroprotective effects following brain ischemia appeared to be mediated *via* I₂-sites, but the mechanism of this activation of the imidazoline receptor is unclear. If it results from a direct action within the “ischemic penumbra” (the term generally used to define ischemic but still viable cerebral tissue in comparison to the term of “ischemic core” which is an area of severe ischemia, the loss of inadequate supply of oxygen and glucose), it must be mediated through mitochondrial imidazoline receptors on astrocytes, since cortical neurons are devoid of imidazoline receptors. Reis *et al.* [67] concluded that the rilmenidine-induced neuroprotection might occur by selectively stimulating Ca²⁺ uptake into astrocytes, and thereby reducing Ca²⁺ uptake into neurons. It is also possible that rilmenidine may act indirectly to activate other pathways in the brain that are neuroprotective [68]. In contrast, Huang *et al.* showed that dexmedetomidine, the latest developed alpha 2-adrenergic agonist, enhanced glutamine disposal by oxidative metabolism in astrocytes *via* effects on alpha 2-adrenergic receptors but not *via* imidazoline-preferring sites [34]. Thus, depending on the substance used, both adrenoceptor and imidazoline receptors might be important in mediating the neuroprotection effects induced by alpha 2-adrenergic agonists.

In conclusion, it is suggested that alpha 2-adrenergic agonists, a widely used group of agents for clinical treatment of hypertension, analgesia and sedation, improves neurological deficit scores and reduces infarct sizes in cerebral ischemia. Thus some alpha 2-adrenergic agonists may become part of future standard care for the treatment of cerebral ischemia. However, there are still many unsolved problems that must be addressed before these agents are considered safe for clinical use. These problems include: 1) the exact mechanisms by which alpha 2-adrenergic agonists induce neuroprotection against cerebral ischemic injury and

the duration of the effects. 2) Whether adverse effects such as hypotension and bradycardia will restrict the prophylactic use of alpha 2-adrenergic agonists. Answers to these questions will not only advance our knowledge of intracellular signal transduction pathway in neuroprotection by alpha 2-adrenergic agonists, but also identify sites through which alpha 2-adrenergic agonists may protect brain from ischemic injury. Given the known beneficial effects of alpha 2-adrenergic agonists in surgical patients (e.g., sedation and attenuation of sympathetic and cardiovascular responses) [54] along with their anti-ischemic effect, adjuvant use of alpha 2-adrenergic agonists with anesthetics may greatly benefit patients at high risk of cerebral ischemia.

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