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Azelastine protects against CA1 traumatic neuronal injury in the hippocampal slice

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Abstract

Activation of NMDA receptors appears to play a important role in traumatic neuronal injury. Additionally, *N*-methyl-D-aspartate (NMDA) excitotoxicity may involve leukotriene production. Therefore, we investigated whether azelastine, an anti-allergic agent inhibiting the synthesis and release of leukotrienes, could protect against CA1 traumatic neuronal injury in the hippocampal slice. Fluid percussion trauma produced evidence of severe neuronal injury with CA1 antidromic population spike amplitude recovering after 95 min to only a mean 16 ± 1% S.E. of initial amplitude. With 15 μM azelastine treatment given after trauma for 35 min this recovery improved to 112 ± 17%. The azelastine EC_{50} for this protection was 10 μM. Significant protection was also seen with azelastine application begun 15 min after trauma. Azelastine also protected the ability to induce long-term potentiation after trauma. The specific leukotriene inhibitors, MK-571 and MK-886, similarly provided significant neuroprotection. These findings suggest that CA1 traumatic neuronal injury may be mediated by leukotriene production.

Keywords: Azelastine; Traumatic brain injury; Leukotriene; NMDA (*N*-methyl-D-aspartate); Hippocampus; (In vitro)

1. Introduction

Recent evidence indicates that cytotoxic processes triggered by head trauma may greatly augment neuronal damage from traumatic neuronal injury (Faden et al., 1989). This damage appears to be mediated in large part by NMDA receptor stimulation (Bullock and Fujisawa, 1982). Activation of NMDA receptors induces many cellular effects, including the release of arachidonic acid (Pellerin and Wolfe, 1991). Arachidonic acid in turn may be metabolized to leukotrienes through the action of the enzyme 5-lipoxygenase (Lewis et al., 1990). Additional data suggest that leukotrienes may be involved in the generation of excitotoxic damage, since azelastine, an inhibitor of leukotriene C_4 synthesis and release (Achterrath-Tuckermann et al., 1988), has been found to be protective against *N*-methyl-D-aspartate (NMDA)-induced injury (Wallis and Panizzon, 1993).

Azelastine, ((±)-4-[(4-chlorophenyl)methyl]-2-(hexahydro-1-methyl-1*H*-azepin-4-yl)-1(2*H*)-phthalazinone monohydrochloride) (Zechel et al., 1981), is a new anti-allergic agent, which has been shown in clinical trials to be effective for the treatment of rhinitis and eczema (Achterrath-Tuckermann et al., 1988). To examine the potential neuroprotective effects of azelastine against trauma, we assessed the ability of this compound to prevent CA1 traumatic neuronal injury in the hippocampal slice.

2. Materials and methods

Male Sprague-Dawley rats, 200–400 g were briefly anesthetized with halothane and decapitated, as previously described (Wallis et al., 1992). The brain was removed within 1 min and placed in cold artificial cerebrospinal fluid for 1 min. Artificial cerebral spinal fluid was composed of (in mM) NaCl, 126; KCl, 4.0; KH_2PO_4, 1.4; $MgSO_4$, 1.3; $CaCl_2$, 2.4; $NaHCO_3$, 26; and glucose, 4.0. This fluid had a pH of 7.4 and was saturated with 95% O_2-5% CO_2. Hippocampi were dissected and transverse slices, 475 μm thick, were cut using a McIlwain tissue chopper. Slices were placed in a recording well with the temperature of the surrounding bath thermostatically controlled to 34 ± 0.5°C. Azelastine was supplied by Carter-

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Wallace; MK-571 and MK-886 were supplied by Merck & Co.

One hour after slice placement, the orthodromic CA1 population spike of each slice was assessed. This potential which serves as an indicator of neuronal function, was elicited by stimulation given via a twisted, bipolar electrode placed over the CA3 Schaffer collaterals. Responses were recorded in the pyramidal layer of CA1 using a tungsten electrode, and population spike amplitudes were measured (Schurr et al., 1984). Current strengths and recording electrode depth were adjusted to obtain maximal CA1 population spike amplitude. Only slices having an orthodromic CA1 population spike of 3 mV or greater on initial assessment were used for further testing. Stimulation of CA3 Schaffer collaterals also elicited CA3 fiber volley and CA1 field e.p.s.p. (excitatory post-synaptic potential) responses. These potentials are reflective of axonal and dendritic function, respectively, and were also monitored throughout testing. CA1 antidromic population spike was elicited by stimulation over the alveus, while responses were monitored from the same recording electrode within the CA1 pyramidal layer. Generation of CA1 antidromic population spike response does not require synaptic activation. Therefore, loss of CA1 antidromic response reflects neuronal cell body dysfunction, as would be expected in severe neuronal injury, and helps to exclude synaptic depression as a cause of orthodromic population spike loss. CA1 orthodromic evoked response was assessed every 30 s while slices were held within recording wells. Antidromic CA1 population spike was assessed at the beginning and end of each experiment.

To induce trauma, slices were transferred to a 7 ml specialized chamber filled with artificial cerebral spinal fluid and sealed with a rubber piston. A 1 kg weight was then dropped upon the piston from a height of 61 cm producing percussion of the fluid surrounding the slice. After trauma, each slice was returned to the recording chamber and electrodes positioned within 1 min to continue monitoring of CA1 orthodromic evoked response.

Pharmacological treatments were given after trauma in all cases for 35 min after trauma. Final recoveries after trauma were assessed 60 min after cessation of pharmacological treatment. To induce long-term potentiation, tetanic stimulation with a high frequency train at 100 Hz for 1 s, was delivered via the orthodromic stimulating electrode. Responses were then monitored for an additional 60 min. In all cases, n equalled at least four trials. Percentage recovery of evoked responses was calculated by dividing final value by initial value, multiplied by 100. Four slices of both the azelastine-treated and the unmedicated slice groups were given extended monitoring after trauma for 4 h. Initial electrophysiological responses of azelastine-treated and unmedicated slices were compared by one-way analysis of variance. Results of paired trials were compared with Student's correlated t-test. Other comparisons utilized Wilcoxon rank-sum test.

3. Results

Treatment with azelastine afforded significant protection from fluid percussion against CA1 traumatic neuronal injury in the hippocampal slice (Fig. 1). In paired slices treated with 15 μM azelastine beginning within 1 min following trauma and continued for 35 min, CA1 orthodromic population spike recovered to a mean 116 $\pm$ 25% S.E. of initial amplitude at 95 min after trauma (Table 1). In contrast, paired unmedicated slices exhibited a mean recovery of only 21 $\pm$ 7%. Recovery of CA1 antidromic population spike paralleled the azelastine protection seen for CA1 orthodromic responses. With azelastine post-traumatic treatment CA1 antidromic population spike recovered to 112 $\pm$ 7% of initial amplitude. This compared to paired unmedicated, traumatized slices which recovered to only 16 $\pm$ 1%. The marked improvement in CA1 evoked response with azelastine treatment did not appear to be the result of differing initial population spike amplitudes, since no significant difference in initial evoked responses was observed between slice groups (Table 1). Azelastine treatment afforded similar protection against traumatic injury for CA1 e.p.s.p. Slices treated with azelastine showed a mean recovery of 99 $\pm$ 12% of original e.p.s.p. slope, while unmedicated slices recovered to only 11 $\pm$ 11% of original e.p.s.p. slope (Table 1). Azelastine also provided protection for CA3 fiber volley amplitude after trauma. Azelastine-treated slices recovered 100 $\pm$ 0% of initial CA3 fiber volley amplitude, while untreated slices showed a recovery of 36 $\pm$ 7% (Table 1).

The recovery with azelastine occurred with a rapid time course (Fig. 2). Slices treated with 15 μM azelastine demonstrated virtually full recovery of CA1 orthodromic population spike amplitude within a few minutes after trauma. At 5 min following trauma, the mean percentage population spike recovery for the azelastine-treated slices was 125 $\pm$ 16%, significantly exceeding the 28 $\pm$ 23% recovery seen in unmedicated slices.

In slices given extended monitoring after trauma, recovery in azelastine-treated slices appeared to be stable (Fig. 2), with CA1 orthodromic population spike averaging 112 $\pm$ 10% of initial amplitude after 4 h. Paired traumatized

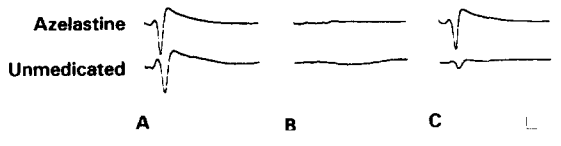

Fig. 1. Azelastine protection of CA1 orthodromic evoked responses in hippocampal slices against traumatic injury. (A) Initial orthodromic evoked responses in paired slices prior to trauma; scale = 2 ms, 2 mV. (B) Both slices received trauma 1 min before tracings were obtained. Azelastine (15 μM) was begun within 1 min following trauma and continued for 35 min thereafter. (C) Final assessment at 95 min after trauma demonstrates nearly full recovery of CA1 orthodromic population spike amplitude in slice treated with azelastine, while unmedicated slice shows only minimal recovery.

Table 1
Mean CA1 evoked response in paired hippocampal slices receiving trauma, with and without azelastine (15 μM) treatment

Response	Initial potential	Percentage of initial potential	
		5 min after trauma	95 min after trauma
Orthodromic CA1 population spike			
Trauma	6.5 ± 0.7 mV	28 ± 23%	21 ± 7%
Trauma, azelastine	7.5 ± 1.2	125 ± 16 *	116 ± 25 *
CA3 fiber volley			
Trauma	0.9 ± 0.1 mV	55 ± 24	36 ± 7
Trauma, azelastine	0.9 ± 0.1	58 ± 22	100 ± 0 *
CA1 e.p.s.p. slope			
Trauma	0.6 ± 0.1 mV/ms	17 ± 17	11 ± 11
Trauma, azelastine	1.0 ± 0.5	109 ± 16 *	99 ± 12 *
Antidromic CA1 population spike			
Trauma	6.2 ± 0.9 mV	–	16 ± 1
Trauma, azelastine	6.1 ± 1.3	–	112 ± 17 *

Azelastine-treated and paired, unmedicated slices were given trauma. Each mean represents a minimum of four paired trials. Azelastine treatment was begun within 1 min following trauma and continued for 35 min. Values represent mean ± S.E. * $P < 0.05$ for azelastine-treated slices versus unmedicated slices, Student's correlated t-test.

slices which were unmedicated demonstrated no further recovery of evoked response beyond that which had been gained in the first hour after trauma, and after 4 h demonstrated only a mean 11 ± 4% of initial CA1 orthodromic population spike amplitude.

Azelastine treatment after trauma also preserved long-term potentiation. One hour after completion of post-traumatic azelastine treatment, tetanic stimulation with no change in current from supramaximal threshold levels set at the beginning of the experiment, induced a mean increase in CA1 orthodromic population spike amplitude of 129 ± 6% (Fig. 3). This increase was maintained throughout 1 h of post-tetanus monitoring, consistent with long-term potentiation. This response was similar to the response of unmedicated, non-traumatized slices which showed an increase of 131 ± 4% in this paradigm utilizing supramaximal threshold stimulation (Fig. 3). Brief treatment with azelastine alone for 35 min without trauma produced similar results. One hour after the completion of azelastine treatment, tetanic stimulation was given to these slices, and induced an increase of CA1 orthodromic population spike amplitude of 130 ± 6% which was sustained in 60 min of monitoring. Sham slices (unmedicated slices which were transferred but were not subjected to fluid percussion trauma) showed responses which were similar to those of unmedicated non-traumatized slices, gaining a CA1 orthodromic population spike amplitude of 132 ± 8% after tetanus. In contrast, traumatized slices not given

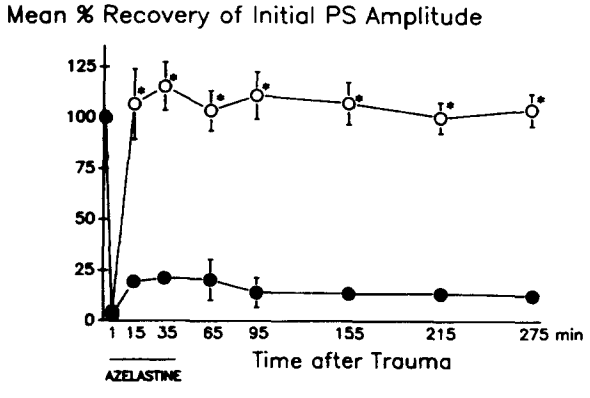

Fig. 2. Recovery of CA1 orthodromic population spike amplitude after fluid percussion trauma is sustained over 4 h of electrophysiological monitoring. Open circles indicate mean percentage population spike recovery of slices treated with azelastine. Azelastine treatment was initiated within 1 min after trauma, and continued for 35 min. Filled circles indicate of unmedicated traumatized slices. Error bars reflect standard error. In all cases, n equalled four or more paired trials; * $P < 0.05$.

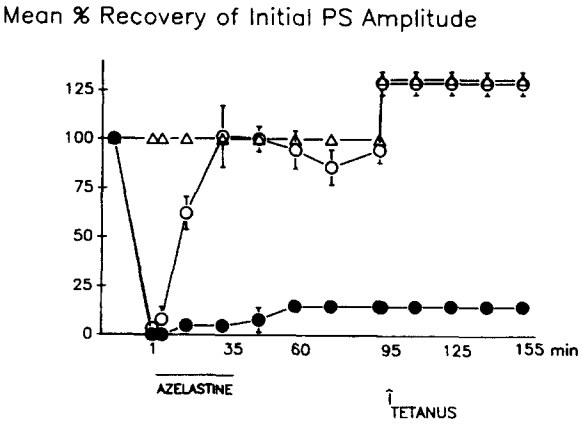

Fig. 3. Brief treatment with azelastine after trauma preserves the ability to induce long-term potentiation in traumatized slices. Azelastine treatment was begun within 1 min after trauma and was continued for 35 min. Tetanic stimulation (one train at 100 Hz for 1 s) was given 95 min after trauma. Responses were monitored thereafter for an additional 60 min. Circles indicate mean population spike amplitude in azelastine-treated slices. Filled circles show responses of unmedicated traumatized slices. Open triangles demonstrate response of slices not given trauma or azelastine treatment. Arrow indicates initiation of tetanus. Error bars reflect standard error. In all cases, n equalled four hippocampal slices; * $P < 0.05$.

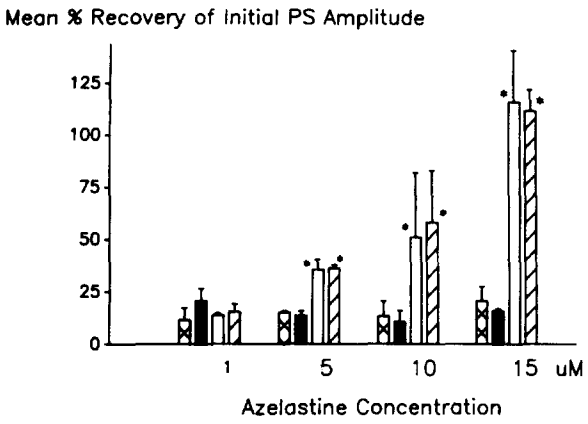

Fig. 4. Azelastine neuroprotection against CA1 traumatic neuronal injury is concentration-dependent. Azelastine treatment was given for a total of 35 min. Final recovery was assessed 1 h after conclusion of azelastine treatment. Vertical bars show mean ± S.E. Cross-hatched bars indicate CA1 orthodromic population spike amplitude in slices receiving trauma without azelastine. Filled bars indicate CA1 antidromic population spike amplitude in unmedicated traumatized slices. Open bars indicate CA1 orthodromic population spike amplitude in traumatized slices treated with azelastine. Diagonal-hatching indicates CA1 antidromic population spike amplitude in traumatized slices treated with azelastine. In all cases n equalled four or more paired trials; $^{*}P < 0.05$.

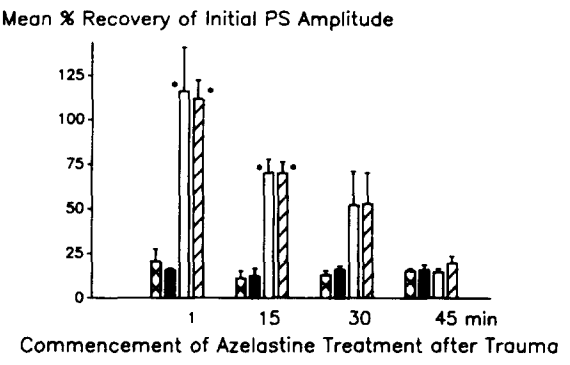

Fig. 5. Initiation of azelastine treatment as late as 15 min following trauma significantly protects against loss of CA1 population spike. Azelastine 15 μM treatment was given for a total of 35 min. Final recovery was assessed 1 h after conclusion of azelastine treatment. Vertical bars show means ± S.E. Cross-hatched bars indicate CA1 orthodromic population spike amplitude in slices receiving trauma without azelastine. Filled bars indicate CA1 antidromic population spike amplitude in unmedicated traumatized slices. Open bars indicate CA1 orthodromic population spike amplitude in traumatized slices treated with azelastine. Diagonal-hatching indicates CA1 antidromic population spike amplitude in traumatized slices treated with azelastine. In all cases n equalled four or more paired trials; $^{*}P < 0.05$.

azelastine treatment showed no response to tetanus (Fig. 3), consistent with the loss of long-term potentiation.

The neuroprotection afforded by azelastine against CA1 traumatic injury was concentration-dependent with a dose-response as illustrated in Fig. 4. The azelastine EC_{50} for protection against traumatic neuronal injury was 10 μM both for CA1 orthodromic and antidromic population spike amplitude. Treatment with 15 μM azelastine initiated at various times after trauma (Fig. 5), demonstrated that significant protection could still be produced when azelastine was initiated as late as 15 min following trauma.

Neuroprotection against CA1 traumatic neuronal injury was also seen with other leukotriene inhibitors. When the highly specific leukotriene D_4 receptor antagonist, MK-571 (Young, 1991) 15 μM, was given after trauma, CA1 orthodromic and antidromic population spike amplitude recovered to 85 ± 10% and 82 ± 10% (Fig. 6). In comparison, traumatized slices recovered to only 18 ± 1% and 9 ± 2%, respectively. The EC_{50} for this MK-571 protection was approximately 8 μM for preservation of CA1 orthodromic and 8.5 μM for antidromic population spike amplitude (Fig. 7). As with azelastine, MK-571 preserved the ability to induce long-term potentiation after trauma. Slices previously traumatized and treated with 15 μM MK-571, demonstrated an increase of 122 ± 8% when compared to the pre-tetanus baseline (taken after 1 h of recovery from trauma). In comparison, slices which were traumatized but not treated with MK-571, showed no response to tetanus. Significant neuroprotection was also seen with MK-886 which provides inhibition of 5-lipoxygenase by inactivating the 5-lipoxygenase activating protein (Young, 1991) (Fig. 6).

Because azelastine has been shown to block the histamine H_1 receptor (Casale, 1989), the effects of diphenhydramine, a histamine H_1 receptor antagonist, were also examined following CA1 traumatic injury. Diphenhydramine, 50 μM, was not protective against traumatic neuronal injury (Fig. 6). Following fluid percussion trauma, mean CA1 orthodromic population spike recovered to a

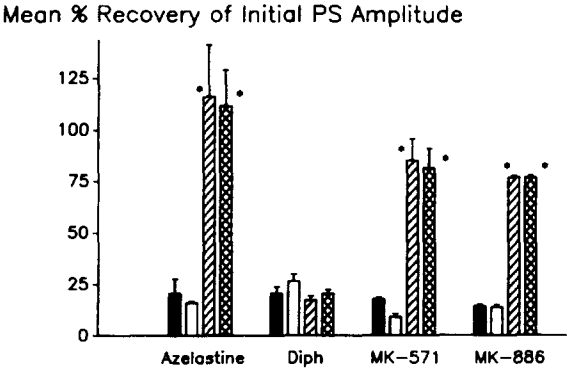

Fig. 6. Leukotriene inhibitors afford significant protection against traumatic loss of CA1 population spike response. Pharmacological treatments were initiated within 1 min after fluid percussion trauma and were continued for a total of 35 min. Pharmacological treatments were administered at the following concentrations: azelastine, 15 μM; diphenhydramine, 50 μM; MK-571, 15 μM; and MK-886, 30 μM. Final recovery was assessed 1 h after conclusion of pharmacological treatments. Vertical bars show means ± S.E. Filled bars indicate CA1 orthodromic population spike amplitude in traumatized unmedicated slices. Open bars indicate CA1 antidromic population spike amplitude in traumatized unmedicated slices. Diagonal-hatched bars indicate CA1 orthodromic population spike amplitude in traumatized slices given pharmacological treatment. Cross-hatched bars indicate CA1 antidromic population spike amplitude in traumatized slices treated with azelastine. In all cases n equalled four or more paired trials; $^{*}P < 0.05$.

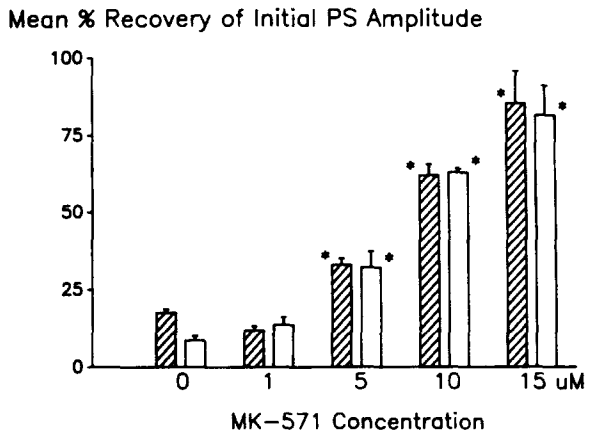

Fig. 7. Protection of the CA1 population spike response with MK-571 against traumatic injury in hippocampal slices is concentration-dependent. Treatment with MK-571 was begun within 1 min after trauma and was continued for 35 min. Final recovery was assessed 1 h after conclusion of azelastine treatment. Vertical bars show means ± S.E. Diagonal-hatched bars indicate CA1 orthodromic population spike amplitude. Open bars indicate CA1 antidromic population spike amplitude. In all cases n equalled four or more paired trials; * $P < 0.05$.

mean 21 ± 3% in slices treated with this histamine blocker, similar to the 17 ± 2% seen in paired unmedicated slices.

4. Discussion

The results of these studies indicate that azelastine, an inhibitor of leukotriene C_4 synthesis and release (Achterrath-Tuckermann et al., 1988), is protective against CA1 traumatic neuronal injury in the hippocampal slice. Protection against trauma was also found with other leukotriene inhibitors suggesting that leukotriene synthesis is at least one mechanism by which CA1 traumatic neuronal injury is mediated.

In these studies, we utilized the hippocampal slice, which possesses several attributes useful for the study of traumatic neuronal injury. Most importantly, this preparation allows the effect of fluid percussion trauma upon CA1 electrophysiological responses to be assessed in an in vitro setting. The neuronal injury induced by trauma in this model produces rapid loss of CA1 evoked response through mechanical deformation from fluid pressure waves. This model shows similarity to concussive neuronal injury where mechanical forces transmitted through CSF flow waves, produce neuronal unresponsiveness resulting in loss of consciousness. The hippocampal slice preparation also allows monitoring of CA1 neurons, a neuronal population known to be among the most vulnerable to traumatic injury (Schmidt-Kastner and Freund, 1991). Additionally, these neurons are involved in processes of learning and memory (Auer et al., 1989), and memory and learning impairment is one of the most disabling aspects of traumatic brain injury (Gronwall and Wrightson, 1974; Rimel et al., 1982).

The CA1 injury produced by trauma in our study appeared to be long-lasting, and involved both CA1 orthodromic and antidromic population spikes, as would be expected with severe cell body dysfunction. Azelastine protected against this injury, and also preserved the ability to induce long-term potentiation at the CA3-CA1 synapse. Long-term potentiation is an indicator of synaptic plasticity which has proved to be a useful model for the investigation of memory and learning in vertebrate animals (Bliss and Collingridge, 1993). Traumatic brain injury induces memory loss and loss of long-term potentiation in vivo (Miyazaki et al., 1992). Therefore, the preservation of long-term potentiation in the present study suggests that azelastine could be neuroprotective for some aspects of memory dysfunction from in vivo head trauma.

Azelastine neuroprotection against trauma was robust, with significant protection seen with post-traumatic application as late as 15 min after trauma. The azelastine concentrations needed for CA1 protection against trauma were consistent with a mechanism of action occurring through leukotriene inhibition. The EC_{50} for azelastine protection against trauma in this study was 10 μM, which is comparable to the azelastine IC_{50} of 10 μM needed for the inhibition of leukotriene C4 synthesis in murine peritoneal cells (Nishihira et al., 1989). The EC_{50} for azelastine protection against trauma was also similar to the azelastine EC_{50} of 9.8 μM needed to protect against CA1 hypoxic injury (Wallis and Panizzon, 1993), indicating that azelastine protection for hypoxia and trauma could occur through the same mechanism.

The neuroprotection against CA1 traumatic neuronal injury was seen with other leukotriene inhibitors. The leukotriene D_4 receptor antagonist, MK-571 afforded protection against traumatic loss evoked response and loss of the ability to induce long-term potentiation. Significant neuroprotection was also seen with MK-886, a 5-lipoxygenase activating protein inhibitor. The protection seen with these agents is consistent with an azelastine neuroprotective mechanism of action occurring through inhibition of leukotriene production.

The mechanism of azelastine neuroprotection is not fully understood. However, it may lie in the excitotoxic effects of arachidonic acid and its metabolites. During cerebral ischemia, liberation of arachidonic acid occurs (Yavin et al., 1992). This long chain polyunsaturated fatty acid serves as a precursor to leukotriene production (Lewis et al., 1990). Elevations of leukotriene C_4 have also been documented during cerebral ischemia both in animals and humans (Moskowitz et al., 1984; Aktan et al., 1991a, b). One deleterious action of leukotriene C_4 during cerebral ischemia has been demonstrated to be vasoconstriction and increased vascular permeability during this injury (Baba et al., 1991; Aktan et al., 1991a, b).

Data from in vivo studies suggest that leukotrienes synthesis may also be a mechanism of injury with head trauma. With concussive injury, leukotriene concentrations

in gerbil forebrain have been shown to increase, reaching peak concentrations 15–30 min following trauma (Kiwak et al., 1985). In addition, application of leukotrienes has been shown to increase blood brain barrier permeability (Black and Hoff, 1985) and such increases have been demonstrated to occur in traumatic brain injury (Baba et al., 1991). However, these mechanisms are not likely to be active in the hippocampal slice preparation. Therefore, given the neuroprotection seen in the present study, azelastine likely provides some aspect of neuroprotection against trauma through intrinsic neural processes. Multiple possibilities exist to explain this neuroprotection. One possible mechanism of leukotriene-induced neuronal injury may occur through NMDA receptor activation, since azelastine provides CA1 neuronal protection against direct NMDA application (Wallis and Panizzon, 1993), and NMDA receptor activation has been shown to play an important role in traumatic brain injury (Faden et al., 1989). Also, NMDA receptor activation results in arachidonic acid release (Dummuis et al., 1988), which further contributes to leukotriene production. Leukotrienes are additionally involved with free radical generation and subsequent injury from free radicals. In this regard azelastine has been shown to decrease superoxide generation in human neutrophils (Umeki, 1992).

In addition to their possible pathophysiological role leukotrienes may also play an integral role in neuronal synaptic plasticity. Previous studies have shown that leukotrienes may act as a retrograde messenger in the expression of synaptic long-term potentiation (Williams and Bliss, 1989). In addition, azelastine protects against the effects of NMDA excitotoxicity (Wallis and Panizzon, 1993), and NMDA receptor activation has been shown to be required for long-term potentiation production (Collingridge, 1992). Thus, a concern might exist that the ability to elicit long-term potentiation would be adversely affected by azelastine treatment. However, our study indicates that brief administration of leukotriene inhibitors may not adversely affect the ability to later produce long-term potentiation in CA1 neurons.

In conclusion, these studies demonstrate that azelastine can protect mature CA1 neurons in the hippocampal slice from the effects of traumatic neuronal injury. These findings further suggest that inhibition of leukotrienes during traumatic neuronal injury may be neuroprotective and that this protection extends to the ability to generate long-term potentiation after trauma. These findings suggest that the arachidonic acid cascade and leukotriene production may mediate NMDA-induced neuronal injury, and that leukotriene inhibitors, therefore, may be beneficial for protection against neuronal injury from trauma.

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References

Achterrath-Tuckermann, U., T. Simmet, W. Luck, I. Szelenyi and B.A. Peskar, 1988, Inhibition of cysteinyl-leukotriene production by azelastine and its biological significance, Agents Actions 24, 217.

Aktan, S., C. Aykut and S. Ercan, 1991a, Leukotriene C_4 and prostaglandin E_2 activities in the serum and cerebrospinal fluid during acute cerebral ischemia, Prostagl. Leukotr. Essent. Fatty Acids 43, 247.

Aktan, S., C. Aykut, S. Oktay, B. Yegen, E. Keles, I. Aykack and S. Ercan, 1991b, The alterations of leukotriene C_4 and prostaglandin E_2 levels following differing ischemic periods in rat brain tissue, Prostagl. Leukotr. Essent. Fatty Acids 42, 67.

Auer, R.N., M.L. Jenson and I.Q. Wishaw, 1989, Neurobehavioral deficit due to ischemic brain damage limited to half of the CA1 section of the hippocampus, J. Neurosci. 9, 1641.

Baba, T., K.L. Black, K. Ikezaki, K. Chen and D.P. Becker, 1991, Intracarotid infusion of leukotriene C_4 selectively increases blood-brain barrier permeability after focal ischemia in rats, J. Cereb. Blood Flow Metab. 11, 638.

Black, K.L. and J.T. Hoff, 1985, Leukotrienes increase blood-brain barrier permeability following intraparenchymal injections in rats, Ann. Neurol. 18, 349.

Bliss, T.V.P. and G.L. Collingridge, 1993, A synaptic model of memory: long-term potentiation in the hippocampus, Nature 361, 31.

Bullock, R. and H. Fujisawa, 1982, The role of glutamate antagonists for the treatment of CNS injury, J. Neurotrauma 9, Suppl. 2, S443.

Casale, T.B., 1989, the interaction of azelastine with human lung histamine H1, beta, and muscarinic receptor-binding sites, J. Allergy Clin. Immunol. 83, 771.

Collingridge, G.L., 1992, The mechanisms of induction of NMDA receptor-dependent long-term potentiation in the hippocampus, Exp. Physiol. 77, 771.

Dummuis, A., M. Sebben, L. Haynes, J.P. Pin and J. Bockart, 1988, NMDA receptors activate the arachidonic acid cascade system in striatal neurons, Nature 336, 68.

Faden, A.I., P. Demediuk, S.S. Panter and R. Vink, 1989, The role of excitatory amino acids and NMDA receptors in traumatic brain injury, Science 244, 798.

Gronwall, D. and P. Wrightson, 1974, Delayed recovery of intellectual function after minor head injury, Lancet 2, 605.

Kiwak, J.J., M.A. Moskowitz and L. Levine, 1985, Leukotriene production in gerbil brain after ischemic insult, subarachnoid hemorrhage, and concussive injury, J. Neurosurg. 62, 865.

Lewis, R.A., K.F. Austen and R.J. Soberman, 1990, Leukotrienes and other products of the 5-lipoxygenase pathway, New Engl. J. Med. 10, 645.

Miyazaki, S., Y. Katayama, B.G. Lyeth, L.W. Jenkins et al., 1992, Enduring suppression of hippocampal long-term potentiation following traumatic brain injury in rat, Brain Res. 585, 335.

Moskowitz, M.A., K.J. Kiwak, K. Hekimian and L. Levine, 1984, Synthesis of compounds with properties of leukotriene C_4 and D_4 in gerbil brains after ischemia and reperfusion, Science 224, 886.

Nishihira, J., T. Hayakawa, K. Suzuki, K.L. Katao and T. Ishibashi, 1989, Effect of azelastine on leukotriene synthesis in murine peritoneal cells and on thromboxane synthesis in human platelets, Int. Arch. Allergy Appl. Immunol. 90, 285.

Pellerin, L. and L.S. Wolfe, 1991, Release of Arachidonic Acid by NMDA-Receptor Activation in the Rat Hippocampus, Neurochem. Res. 16, 983.

Rimel, R.W., B. Giordani, J.T. Barth and J.A. Jane, 1982, Moderate head injury: completing the clinical spectrum of brain injury, Neurosurgery 11, 344.

Schmidt-Kastner, R. and T.F. Freund, 1991, Selective vulnerability in brain ischemia, Neuroscience 40, 599.

Schurr, A., K. Reid, M.T. Tseng, H.L. Edmonds, 1984, The stability of the hippocampal slice preparation, an electrophysiological and ultrastructural analysis, Brain Res. 297, 357.

Umeki, S., 1992, Effects of anti-allergic drugs on human neutrophil superoxide-generating NADPH oxidase, Biochem. Pharmacol. 43, 1109.

Wallis, R.A. and K.L. Panizzon, 1993, Protection from hypoxic and N-methyl-D-aspartate injury with azelastine, a leukotriene inhibitor, Eur. J. Pharmacol. 238, 165.

Wallis, R.A., K.L. Panizzon and E.G. Wasterlain, 1992, Protective effects of felbamate against hypoxia in the rat hippocampal slice, Stroke 23, 546.

Williams, J.H. and T.V.P. Bliss, 1989, An in vitro study of the effect of lipoxygenase and cyclo-oxygenase inhibitors of arachidonic acid on the induction and maintenance of long-term potentiation in the hippocampus, Neurosci. Lett. 107, 301.

Yavin, E., B. Kunievsky, N.G. Bazan and S. Havel, 1992, Regulation of arachidonic acid metabolism in the perinatal brain during development and under ischemic stress, Adv. Exp. Med. Biol. 318, 314.

Young, R.N., 1991, Development of novel leukotriene-based anti-asthma drugs: MK-886 and MK-571, Agents Actions 34, Suppl., 179.

Zechel, H.J., N. Brock, D. Lenke and U. Achterrath-Tuckermann, 1981, Pharmacological and toxicological properties of azelastine, a novel antiallergic agent, Arzneim.-Forsch. 31, 1184.