NAP Enhances Neurodevelopment of Newborn Apolipoprotein E-Deficient Mice Subjected to Hypoxia

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ABSTRACT

Perinatal hypoxic injury is associated with significant neonatal morbidity and long-term neurodevelopmental complications. NAP, a peptide derived from ADNP (activity-dependent neuroprotective protein), has previously shown neuroprotective abilities in various animal models. To evaluate its neuroprotective role in neonatal hypoxic-ischemic injury, we evaluated the neurodevelopmental outcome in apolipoprotein E (ApoE)-deficient (knockout) mice (a breed prone to brain damage during hypoxic insult) exposed to postnatal global hypoxic damage with and without treatment with NAP. ApoE-deficient (n = 80) and control (C57B6) mice pups (n = 81) were exposed to postnatal global hypoxia (35 min of 8% O2 within 24 h of birth) or room air with or without subsequent subcutaneous NAP treatment during postnatal days 1 to 14. Pups were then evaluated for neonatal motor reflex attainment, spatial learning ability in the Morris water maze, and locomotor open-field activity. The C57B6 and ApoE-deficient anoxic groups showed significantly slower achievement of neonatal reflexes, diminished locomotor activity, and diminished spatial learning ability compared with their control groups. This was more pronounced in the anoxic ApoE-deficient pups. NAP treatment had a pronounced effect on neurodevelopmental outcome in both breeds, particularly in the ApoE-deficient mice. ApoE-deficient and control mouse pups exposed to postnatal hypoxia and treated with NAP showed improvement in neurodevelopmental outcome compared with nontreated mice pups. ApoE-deficient mice show a greater susceptibility to hypoxic damage and better response to NAP treatment.

Neonatal encephalopathy is a major predictor of neurodevelopmental disability in term infants and occurs in 0.1 to 0.6% of live term births. Fifteen to 20% of affected infants die during the newborn period, and an additional 25% have permanent neurologic deficits (Ferriero, 2004). A major etiologic factor for neonatal encephalopathy is hypoxic-ischemic injury to the developing brain due to perinatal asphyxia, and encephalopathy ensuing during immediate postanoxic period in neonates has been termed hypoxic-ischemic encephalopathy (HIE). The degree of HIE correlates well with subsequent neurodevelopmental outcome (Ferriero, 2004).

Neuronal injury due to brain hypoxia involves various mechanisms. Anaerobic metabolism, release of excitatory amino acids, nitric oxide production, and an increase in free radical levels contribute to neuronal damage (Ferriero, 2004). Neurons in the penumbra are apt to respond to neuroprotective treatment during 1 to 2-h postdamage and could be salvaged by neuroprotectants (Volpe, 2001).

Here, the neuroprotective properties of a femtomolar-acting peptide derived from activity-dependent neuroprotective protein (ADNP) (Bassan et al., 1999) were investigated. ADNP is regulated by vasoactive intestinal peptide (VIP) (Bassan et al., 1999). Discovered in the intestine (Said and Mutt, 1970), VIP was later found in abundance in central and peripheral nervous system. Here, we have demonstrated that anoxic-ischemic injury in neonatal mouse pups is ameliorated by treatment with a femtomolar concentration of ADNP (Bassan et al., 1999). This work was supported by the United States-Israel Bi-National Science Foundation; the Israel Science Foundation; the Lily and Avraham Gildor Chair for the Investigation of Growth Factors; the Dr. Diana and Zelman Elton (Elbaum) Laboratory for Molecular Neuroendocrinology; NIA, National Institutes of Health; NICHD (intramural), National Institutes of Health; Institute for the Study of Aging; and Allon Therapeutics Inc. Professor Illana Gozes serves as the Chief Scientific Officer of Allon Therapeutics Inc., Vancouver, BC, Canada.

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ABBREVIATIONS: HIE, hypoxic ischemic encephalopathy; ADNP, activity-dependent neuroprotective protein; ADNF, activity-dependent neurotrophic factor; ApoE, apolipoprotein E; VIP, vasoactive intestinal peptide; NMDA, N-methyl-D-aspartate; MK-801, (5R,10S)-(+-)5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine.

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peripheral neurons, providing neuromodulator, neurotransmitter, and growth factor/neuroprotective functions (Gozes and Brenneman, 1989, 1993; Gozes et al., 1999). Systemically injected VIP analogs effectively protected developing mice against hypoxia and cholinergic blockade (Gozes et al., 1998), particularly the developing white matter, against excitotoxic lesions in a mouse model mimicking periventricular leucomalacia in human premature newborns (Gressens et al., 1999). VIP treatment of astrocytes produced an increase in ADN mRNAs (Bassan et al., 1999) and the secretion of growth factors (e.g., ADNF, activity-dependent neurotrophic factor) (Brenneman and Gozes, 1996). ADNP and ADNF share a short peptide motif: NAPVSIIPQ (NAP) in ADNP and SALLRSIPA (ADNP-9) in ADNF, which exhibit immunological similarity and fentomolar neuroprotection in cell culture (Gozes et al., 1997b; Brenneman et al., 1998; Bassan et al., 1999). NAP rescued rat cerebral cortical neurons from death associated with a wide range of neurotoxic agents, including the β-amyloid peptide and N-methyl-D-aspartate, NMDA (Bassan et al., 1999). Furthermore, NAP was shown to be neuroprotective in a mouse model of closed head injury and a rat model of stroke (Beni-Adani et al., 2001; Leker et al., 2002).

The secondary structure of NAP (determined by circular dichroism) is a random coil in both aqueous and organic solutions (Gozes et al., 2005). NAP is highly soluble and readily bioavailable. After intranasal administration of [3H]NAP to rats, radioactivity was detected in the blood and in the various organs of the body (Gozes et al., 2000). Intact peptide was identified in the rat cerebral cortex 30 and 60 min after intranasal administration. In rat model of stroke, intravenous administration of [3H]NAP resulted in measurable levels in the cerebellum and cortex 15 min after injection and was maintained for at least 30 min in the ischemic tissue (Leker et al., 2002). Liquid chromatography mass spectrometry assays in rats and dogs extended these results. A pharmacokinetic study in rat suggested a correlation between plasma and cerebrospinal fluid levels of NAP (Gozes et al., 2005). Furthermore, in a Phase Ia clinical trial (Gozes et al., 2005), a single intranasal dose of NAP gave rise to detectable plasma levels. Therefore, it is likely that, after intranasal or systemic administration, NAP reaches the brain.

Apolipoprotein E (ApoE) has been associated with Alzheimer’s disease in recent years (Poirier, 2005). ApoE-deficient (knockout) mice and control groups, respectively. Animals from each breed were divided into three study groups postpartum: 1) anoxia group, subjected to perinatal hypoxic-ischemic insult with no concomitant treatment; 2) NAP group, subjected to perinatal hypoxic-ischemic insult with concomitant NAP treatment; 3) control group with no perinatal hypoxic-ischemic insult or treatment. Female mice were naturally inseminated and kept in standard laboratory conditions until birth. All newborn pups were used from each litter. The number of dams and newborn pups in each group is portrayed in Table 1.

**Materials and Methods**

**Experimental Animals.** ApoE-deficient (knockout) mice and C57B6 inbred mice were used as study and control groups, respectively. Animals from each breed were divided into three study groups postpartum: 1) anoxia group, subjected to perinatal hypoxic-ischemic insult with no concomitant treatment; 2) NAP group, subjected to perinatal hypoxic-ischemic insult with concomitant NAP treatment; 3) control group with no perinatal hypoxic-ischemic insult or treatment. Female mice were naturally inseminated and kept in standard laboratory conditions until birth. All newborn pups were used from each litter. The number of dams and newborn pups in each group is portrayed in Table 1.

**Anoxic Insult.** Newborn pups were subjected to hypoxic insult within 24 h of birth. The pups were placed in a sealed glass chamber and exposed to a gas mixture containing 8% oxygen and 92% nitrogen for 35 min. Air flushing was achieved by inserting the gas mixture in 3 l/min for 1 min and subsequently sealing the chamber and reducing gas flow to a minimum. After 35 min, pups were taken out of the chamber, and resuscitation by chest compression and limb stretching was performed as necessary to restore activity and breathing. After the pups recovered and showed a good breathing pattern, they were returned to their mothers. The control groups underwent the same procedure using no gas mixture exposure. Previous studies have shown that exposure of neonatal rodents to the same conditions (8% oxygen and 92% nitrogen) resulted in pO2, pCO2, and brain temperature decreases during the hypoxic stress; whereas the arterial pH did not change, recovery was noted at later time points (Ikeda et al., 1999).

**Administration of NAP.** After hypoxia, mice were injected daily with a solution containing 25 μg/ml NAP (synthesized by Peptide Technologies, Bethesda, MD) or with saline (control groups). Subcutaneous injections were performed daily on postnatal days 1 to 14, 1 h prior to neurobehavioral studies. NAP was injected at increasing dose over the 14 day treatment period as the animals grew in size; protective treatment has shown behavioral improvement in adult ApoE-deficient mice (Gozes et al., 1997a). Furthermore, intrathecal delivery of an ApoE-derived peptide prevented posts ischemic brain necrosis following neonatal hypoxic-ischemic injury (McAdoo et al., 2005). Previous studies suggested that, during early brain development, the blood-brain barrier is open and that systemically applied (subcutaneously) compounds (including NAP) reach the mouse brain and affect neurodevelopment (e.g., Hill et al., 1991; Gozes et al., 1997a; Bassan et al., 1999).

Therefore, we sought to determine the neuroprotective abilities of NAP in neonatal hypoxic injury. Our study subjected ApoE-deficient mice and a control breed to perinatal hypoxic-ischemic insult. Neurodevelopmental outcome was assessed to ascertain evidence for NAP-neuroprotective abilities in this setting.

### Table 1

<table>
<thead>
<tr>
<th>Strain</th>
<th>Dams No.</th>
<th>Range</th>
<th>Mean ± S.D.</th>
<th>Pups Total No. Day</th>
<th>Pups Total No. Day</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1</td>
<td>45</td>
</tr>
<tr>
<td>C57B6</td>
<td>Control</td>
<td>3</td>
<td>4-15</td>
<td>8 ± 4.96</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>Anoxia</td>
<td>4</td>
<td>4-9</td>
<td>7.5 ± 2.06</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>Anoxia+NAP</td>
<td>3</td>
<td>8-10</td>
<td>9 ± 0.82</td>
<td>27</td>
</tr>
<tr>
<td>ApoE-deficient</td>
<td>Control</td>
<td>3</td>
<td>7-9</td>
<td>8 ± 0.82</td>
<td>24</td>
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<tr>
<td></td>
<td>Anoxia</td>
<td>3</td>
<td>7-11</td>
<td>9.33 ± 1.70</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td>Anoxia+NAP</td>
<td>3</td>
<td>7-11</td>
<td>9.33 ± 1.70</td>
<td>28</td>
</tr>
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</table>

*Pups per dam (ANOVA) NS.*
20, 40, and 80 μl were injected on days 1 to 4, 5 to 10, and 11 to 14, respectively (Bassan et al., 1999).

Motor and Cognitive Evaluation. Evaluation was performed daily between 12:00 AM and 4:00 PM, approximately 1 h after subcutaneous injections were performed. The following tests were conducted on the pups.

Neonatal Reflexes. Attainment of neonatal reflexes was tested daily on postnatal days 1 to 14 (Fox, 1965). Reflexes tested included the righting reflex (rat placed on back on flat surface turns onto ventral surface), cliff avoidance (rat put on edge of board with nose and feet forced just over the edge shows withdrawal of head and both forefeet from edge), negative geotaxis (rat placed head downwards on a 30° slope turns to face up the slope), and placing reflex (rat held near edge of bench with dorsal part of forelegs touching the side extends forelegs and places them on the bench). Scoring was given near edge of bench with dorsal part of forelegs touching the side extends forelegs and places them on the bench). Scoring was given according to the time taken to achieve full reflex (0, no response; 1, achievement < 15 s; 2, achievement < 10 s; 3, achievement < 5 s).

Short-Term Spatial Memory Using the Morris Water Maze. Five consecutive swimming days were performed from day 21. Two consecutive tests were performed daily, giving the pups a 20-s rest on the platform before and between tests. Hidden platform diameter was 15 cm, and pool diameter was 90 cm. Maximal latency time was set at 120 s. No cues were given, although the examination room was not sterile. Platform placement was changed daily. The time required to reach the platform during the first daily test (indicative of learning and intact reference memory) and the time required to reach the platform in the second trial (indicative of short-term working memory) were measured and recorded separately. Tracking was done using the HVS IMAGE-computerized system (HVS Image, Buckinghamshire, UK) (Morris, 1984; Bassan, 1999).

Locomotor Activity Test. Open-field activity was measured in a round white chamber 30 cm in diameter on postnatal days 16, 25, 35, and 45 for 5 min. Tracking was done by the modified water maze HVS IMAGE-computerized system. Total distance was measured as an indication for pup activity (Speiser et al., 1983; Bassan et al., 2005).

Statistical Analysis. Differences in subgroups within each mouse breed were detected using ANOVA analysis. Post hoc Student-Newman-Keuls multiple comparison of means test was implemented to define ANOVA differences within each breed, with α = 0.05. In addition to ANOVA analysis, Morris water maze learning ability was further measured using unpaired t test for detecting significant differences between test days 1 and 5. Chi square test was used when required and is stated in the text.

Data Calculations. The differences between ApoE-deficient and C57B6 mouse breeds regarding the effect of hypoxia and NAP treatment on neurodevelopment in comparison with baseline were detected as follows. Daily achievement values of hypoxia- and NAP-treated groups were divided by (for Morris water maze and activity modalities) or subtracted by (for motor reflexes) the average score of the baseline achievement for every day, yielding percentage of change values and score difference, respectively. ANOVA was used to detect significant changes between different breed anoxia groups and between different breed anoxia + NAP-treated groups.

Results

C57B6 Mice Subject to Hypoxia Show Improved Outcome When Treated with NAP

Survival. On day 45, the survival rate was 16/30 (50%) in the anoxia group (with the majority of deaths occurring during the first postnatal week) and 19/24 (79%) in the control group, indicating a significant reduction in survival after anoxia (Chi square test). Anoxia + NAP treatment resulted in survival rate that is similar to the hypoxia values (15/27, 55%).

Weight Gain. No significant difference was observed between groups on the first postnatal day. On day 10, the anoxia group showed a marked decrease in body weight compared with control (4.91 ± 0.7 g versus 5.5 ± 0.8 g, p = 0.0012), whereas the NAP-treated group showed preservation of weight similar to the control mice (5.6 ± 0.3 g) (Fig. 1).

Neonatal Reflex Attainment. The anoxia group showed significantly slower attainment of the cliff avoidance and placing reflexes, significantly lower negative geotaxis scores on days 11 and 12, and significantly lower righting scores on days 1, 2, 8, 9, and 10 compared with the control group. The NAP-treated anoxic group showed significant improvement in cliff avoidance and placing reflexes compared with the anoxia group and a higher righting score on days 8 to 10 (Fig. 2, only cliff avoidance and placing results are shown).

Open-Field Activity. The anoxic and NAP-treated groups showed a significantly lower activity score on day 55 compared with the control group. A notable yet nonsignificant difference was observed between the hypoxia and NAP groups (Fig. 3).

Spatial Learning and Memory in the Morris Water Maze. Tests were performed on 21-day-old mice before the onset of potential activity deficits. The anoxia group showed a slow learning curve with no significant changes in the latency time on either test (first or second swim) noted between swimming days 1 and 5. A significant positive learning

![Fig. 1](https://via.placeholder.com/150) Effects of anoxia on body weights. NAP-treated C57B6 mice (A) and ApoE-deficient mice (B) show preservation of weight on day 10, whereas anoxia-exposed groups show marked decreases in body weight compared with control. ApoE-deficient (knockout) mice and C57B6 inbred mice were used. The anoxia groups were exposed on postnatal day 1 to a gas mixture containing 8% oxygen and 92% nitrogen for 35 min. The anoxia + NAP groups were subjected to hypoxic insult and subcutaneously injected daily on postnatal days 1 to 14 with gradually increased doses of NAPVSIPQ (Bassan et al., 1999). Pups were weighed on postnatal days 1 and 10. ***, p = 0.0012 anoxia group is significantly different from NAP-treated and control groups; ***, p = 0.000087 anoxia group is significantly different from NAP-treated and control groups; #, p = 0.000002 significant difference among all groups.
A curve was noted in the control and NAP groups, both in the learning and intact reference memory test (first swim) and the short-term working memory test (second swim). No significant differences were noted between groups on any test on any of the swimming days (Fig. 4).

ApoE-Deficient Mice Subject to Hypoxia Show Improved Outcome When Treated with NAP

Survival. On day 45, survival rate was 22/28 (78.5%) in the anoxia group (with majority of death occurring during the second month of life). No death was noted in the control group. NAP treatment had no effect on survival (23/28, 82.1%), although in the NAP-treated group, most of the death occurrence was during the first 3 weeks of life.

Weight Gain. A significant difference was noted among all groups on the first postnatal day (control group, 1.42 ± 0.1 g; anoxia group, 1.29 ± 0.1 g; NAP group, 1.49 ± 0.2 g; p = 0.000002). On day 10, the anoxia group showed marked decrease in body weight compared with the control group (4.63 ± 1.0 g versus 5.68 ± 0.6 g; p = 0.000087), whereas the NAP group showed similar weight compared with the control group (5.36 ± 0.9 g) (Fig. 1).

Neonatal Reflex Attainment. The anoxia group showed significantly slower attainment of all neonatal reflexes tested.
scores compared with control in cliff avoidance (days 4 and 5) and placing (days 2–4) reflexes (Fig. 2, only cliff avoidance and placing results are shown).

**Open-Field Activity.** The anoxia group showed notable yet nonsignificant lower activity on day 25 and a significantly lower activity score on day 35 compared with the control group. NAP ameliorated the anoxic adverse effects on open-field activities, and the hypoxia + NAP treatment group showed higher activity compared with the anoxia group on days 25 and 35 (Fig. 3).

**Short-term Spatial Memory in the Morris Water Maze.** Similar patterns were noted regarding the learning and intact reference memory test (first swim) and short-term working memory test (second swim). The anoxia group did not exhibit a learning curve; i.e., no significant changes in latency were noted between swimming days 1 and 5. In contrast, a significant learning curve was noted in the control and NAP groups. The anoxia group showed significantly increased latency on days 3, 4, and 5 compared with control. The NAP group showed a slower learning curve compared with control and reached comparable latency patterns mostly noted on day 5 (Fig. 4).

**ApoE-Deficient Mice Show Increased Susceptibility to Hypoxia and a Greater Response to NAP Treatment Compared with C57B6 Mice in Short-Term Memory, Open-Field Activity, and Neonatal Reflex Attainment**

**Survival.** When analyzing the data in Table 1, the normal C57BL6 mice seemed to be more susceptible to hypoxia. Seventy-eight percent of the ApoE-deficient mice survived hypoxia, whereas only 53% C57BL6 group survived. However, ApoE mice (82%) do respond better than C57BL mice (55%) when treated with NAP.

**Short-Term Spatial Memory in the Morris Water Maze.** Previous studies have indicated that ApoE-deficient mice are cognitively impaired compared with control mice (e.g., Bassan et al., 1999). Here, Fig. 4 shows a similar trend with the C57BL6 group of mice performing better than ApoE group and exhibiting shorter latency times to find the hidden platform. In terms of hypoxia, the ApoE-deficient mice anoxia group had significantly increased percentage of change values for latency change compared with the C57B6 mice anoxia group on days 3, 4, and 5 for the 1st swim (p < 0.01) and on days 3 through 4 for the 2nd swim (p < 0.001). NAP treatment ameliorated, in part, hypoxia-associated deficiencies.

**Open-Field Activity.** A significant (p < 0.001) difference was noted between the percentages of change values of ApoE-deficient mice anoxia group compared with the C57B6 mice anoxia group on day 45, with ApoE-deficient mice being hypoxia compared with baseline and C57B6 mice being hyperactive. The change from baseline of ApoE-deficient mice treated with NAP was significantly different on days 15, 35, and 45 compared with the C57B6 mice NAP-treated group (p < 0.001).

**Neonatal Reflex Attainment.** No specific trend was noted for percentage of change values between the anoxia groups in any of the reflexes tested. The change from baseline of the ApoE-deficient mice treated with NAP was significantly different from the C57B6 mice treated with NAP during the early postanoxic days for all reflexes tested [days 1–4 for placing (p < 0.001); days 2, 4, and 5 for righting (p <
Discussion

Different strategies for treating and preventing HIE and its damage have been explored to minimize the damage occurring in the penumbra during and after the ischemic insult. These include antioxidants, calcium channel blockers, nitric oxide inhibitors, and free radical scavengers (i.e., allopurinol and indomethacin), with no obvious favorable results in humans to date (Volpe, 2001). Inhibitors of excitatory amino acids, such as NMDA antagonists and MK-801, showed protection from ischemic damage up to 1 h postanoxia. Antisense oligodeoxynucleotides to a component of the NMDA receptor lowered expression of the receptor and diminished the extent of ischemic damage (Wahlestedt et al., 1993). These agents for neuroprotection have yet to be studied in humans. A substantive amount of research has been conducted concerning the neuroprotective abilities of mild hypothermia as an option for protection from ischemic damage with no established guidelines for actual treatment (Ferriero, 2004). Neuropeptides have an important regulatory role in rehabilitative processes after neuronal damage, thus having a putative role in minimizing postanoxic damage. Our research demonstrates how NAP, a peptide with neuroprotective features shown in previous work, has a positive effect on postnatal cognitive and motor abilities in two mouse breeds exposed to postnatal hypoxic-ischemic damage.

Neuroprotection can be maintained by various methods aimed at reducing cell death. Nerve growth factor, which maintains target-neuron interactions (Levi-Montalcini et al., 1969), was first described in 1949. Many other peptides and proteins have since been described, including neurotrophins and their receptors, cytokines, antioxidants, protease inhib-
NAP is an eight-amino acid peptide derived from ADNP. NAP has the ability to reach the rat brain after being administered intranasally and systemically, as has been demonstrated in previous reports (Gozes et al., 2000; Leker et al., 2002). The ability of NAP to reach the rat brain is further facilitated in newborns because of the relative permeability of the newborn blood-brain barrier. (Hill et al., 1991; Gozes et al., 2000). The neuroprotective abilities of NAP have been demonstrated by improved behavioral abilities, coupled with concomitant histopathologic and radiologic changes (Beni-Adani et al., 2001; Leker et al., 2002). We have demonstrated improvement in biometric and neurodevelopmental outcomes in mouse pups subjected to hypoxic ischemic damage and subsequent neurodevelopmental delay. Although both breeds reacted to NAP treatment, a differential improvement was noted between mouse breeds, with ApoE-deficient mice responding better to NAP treatment in certain modalities. ApoE-deficient mice demonstrated a significantly increased improvement in motor ability compared with C57B6 mice, as noted in motor reflex attainment during the first postanoxic days of life. ApoE-deficient mice exposed to hypoxia and NAP treatment resulted in an improvement in spatial memory abilities in the hypoxia-treated group, comparable with the control nonhypoxic group. The differential positive effect of NAP on neurodevelopmental abilities in ApoE-deficient versus C57B6 mice may further demonstrate an apparent vulnerability of the ApoE-deficient brain to anoxic insult and highlights the possible neuroprotective effect of NAP on the anoxic brain. Further evidence for the positive effect of NAP on the ApoE-deficient mice is the improvement of cliff avoidance and placing reflex attainment in the NAP-treated group compared with the nonanoxic control group. The apparent neuroprotective abilities of NAP on the ApoE-deficient mouse should be further investigated.

Some restraints should be considered in interpretation of the results. The outcome measures were behavioral neurodevelopmental abilities with no histopathologic or biochemical measurements. During postnatal development, ApoE-deficient mice exhibited a significant (25%) decrease in brain choline acetyltransferase activity compared with age-matched (21-day-old) C57B6 mice (Gozes et al., 1997a). Daily subcutaneous injections of ApoE-deficient mice with NAP resulted in brain choline acetyltransferase activity (at 21 days of age) that was not significantly different from C57B6 mice and was significantly improved compared with untreated ApoE-deficient mice (Bassan et al., 1999). Further research will need to define the effects of NAP treatment on anoxic neurons. ApoE-deficient pup survival rate was higher than C57B6 pups in all subgroups, with NAP treatment having no effect on survival on day 45. ApoE and the ApoE genotype have been associated before as a risk factor for Alzheimer’s disease (Poirier, 2005), brain development, and arteriosclerosis (e.g., Gozes et al., 1997a). Here, a lack of ApoE expression in the pups may potentially offer some protective benefit against death associated with ischemia compared with the C57B6 genotype. This research adds to the body of knowledge regarding the in vivo neuroprotective abilities of NAP (Bassan et al., 1999; Gozes et al., 2000; Beni-Adani et al., 2001, Leker et al., 2002). Because perinatal hypoxic injury is associated with high morbidity and mortal-
ity in the neonate, this is an important issue. The initial evidence of a possible neuroprotective ability of a peptide able to reach significant levels in the brain during the develop-
mental window with a relatively permeable blood-brain
barrier may have possibilities for future research. NAP is in
clinical development at Allon Therapeutics Inc. (Vancouver,
BC, Canada); Phase I clinical trials in healthy adults showed
the treatment to be very well tolerated by either intravenous
or intranasal routes of administration.

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