Original Paper

Hypocapnia Induces Caspase-3 Activation and Increases Aβ Production

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Abstract

Background: At least half of all cases of early onset (<60) familial Alzheimer's disease (FAD) are caused by any of over 150 mutations in three genes: the amyloid precursor protein (APP), presenilin 1 (PS1), and presenilin 2 (PS2). Mutant forms of PS1 have been shown to sensitize cells to apoptotic cell death. *Objective:* We investigated the effects of hypocapnia, a risk factor for both cognitive and neurodevelopment deficits, on caspase-3 activation, apoptosis, and amyloid β-protein (Aβ) production, and assessed the influence of the PS1Δ9 FAD mutation on these effects. *Method:* For this purpose, we exposed stably transfected H4 human neuroglioma cells to conditions consistent with hypocapnia ($PCO_2 < 40$ mm Hg) and hypocapnia plus hypoxia ($PO_2 < 21\%$). *Results:* Hypocapnia (20 mm Hg CO_2 for 6 h) induced caspase-3 activation and apoptosis; the PS1Δ9 FAD mutation significantly potentiated these effects. Moreover, the combination of hypocapnia (20 mm Hg CO_2) and hypoxia (5% O_2) induced caspase-3 activation and apoptosis in a synergistic manner. Hypocapnia (5 and 20 mm Hg CO_2 for 6 h)

also led to an increased Aβ production. *Conclusion:* The findings suggest that hypocapnia (e.g. during general anesthesia) could exacerbate AD neuropathogenesis.

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Introduction

Alzheimer's disease (AD) is the most common form of age-related dementia and a rapidly growing health problem. Mutations in three genes, presenilin-1 (PS1) on chromosome 14, presenilin-2 (PS2) on chromosome 1, and the amyloid β-protein precursor (APP) on chromosome 21 have been shown to be responsible for roughly half of all cases of early-onset (<60 years old) familial AD (FAD) [1]. Amyloid β-protein (Aβ) production is a major pathological hallmark of AD and is potentiated by caspase activation [2–4]. Increasing evidence suggests a role for caspase activation and apoptotic cell death in AD as well as in a large number of neurodegenerative disorders such as Huntington's disease, amyotrophic lateral sclerosis, and spinocerebellar ataxia [5–7]. Interestingly, all three early-onset FAD gene products, PS1, PS2, and APP, undergo caspase cleavage during apoptosis [8–10].

Hypocapnia (extracellular PCO_2 level <40 mm Hg), a physiological stressor, has been shown to cause cognitive deficits [11], neurodevelopment deficits [12], and postop-

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erative psychomotor dysfunction [13, 14]. Hypocapnia can also worsen cerebral hypoxia through the induction of cerebral vasoconstriction and exacerbate hypoxic-ischemic brain damage [15]. Importantly, a recent study by Ohyu et al. [16] showed that hypocapnia (20 mm Hg CO_2) together with hypotension (50 mm Hg mean arterial pressure) for 0.5 h induced apoptotic neuronal cell death in the hippocampus of newborn rabbits. Thus, we set out to examine whether hypocapnia can contribute to AD neuropathogenesis via induction of caspase activation, apoptosis, and increased Aβ production. Mutations in PS1 increase cell susceptibility to apoptosis induced by staurosporine [17] and neuronal death by hypoxia [18]. Thus, we also examined whether the PS1Δ9 FAD mutation can render increased hypersensitivity to caspase-3 activation and apoptosis induced by hypocapnia.

For this purpose, we employed human H4 neuroglioma cell lines stably transfected with wild-type (wt) and FAD mutant (Δ9) PS1 constructs to test for effects of hypocapnia on cellular apoptosis and caspase-3 activation as well as the influence of the PS1Δ9 FAD mutation on these effects [19]. As in Kovacs et al. [17], we used stably transfected H4 human neuroglioma cells expressing a relatively low level of PS1 to minimize overexpression artifacts (e.g. spontaneous apoptosis). H4 cells were also transfected with *APP* containing the Sweden mutation [20] to test the effects of hypocapnia on Aβ production.

Material and Methods

Cell Lines

We used H4 human neuroglioma cells stably transfected to express low levels of either an FAD mutant PS1Δ9 (PS1 mutant cells) or wild-type (PS1 wt cells) in the caspase-3 activation and apoptosis experiments. These cells were previously shown to express sufficiently low levels of PS1 so as to avoid spontaneous apoptosis that can result from robust overexpression of PS1 [17]. H4 cells stably transfected with APP (H4 Swedish mutation cells) [20] were used in the experiments in which Aβ levels were assayed. All cell lines were cultured in DMEM (high glucose) containing 9% heat-inactivated fetal calf serum, 100 units/ml penicillin, 100 µg/ml streptomycin, 2 m*M* L-glutamine and 200 µg/ml G418.

Conditions of Hypocapnia and Hypocapnia plus Hypoxia

Varying concentrations of O_2 and CO_2 were delivered to a sealed plastic box in a 37°C incubator containing 6-well plates seeded with cell cultures. Datex infrared gas analyzer (Puritan-Bennett, Tewksbury, Mass., USA) was used to continuously monitor the delivered CO_2 and O_2 concentrations. Control conditions included 40 mm Hg CO_2 plus 21% O_2, which mimics the gas phase of 'normal' air in the standard cell incubator. A pH blood gas machine was used to measure pH and PCO_2 of the cell culture media following exposure to conditions of hypocapnia.

Cell Lysis and Protein Amount Quantification

Detergent lysates were prepared by sonication of cells in ice-cold 5 m*M* Tris (pH 8) containing protease inhibitors (1 µg/ml aprotinin, 1 µg/ml leupeptin, 1 µg/ml pepstatin A). The lysates were collected, centrifuged at 1,000 *g* for 10 min, and quantified for total proteins by the BCA protein assay kit (Pierce, Iselin, N.J., USA).

Cell Apoptosis Assay

Cell apoptosis was assessed by a cell death detection ELISA kit (Roche, Palo Alto, Calif., USA), which assays cytoplasmic histone-associated DNA fragmentation associated with cellular apoptosis.

Western Blot Analysis of Caspase-3 Cleavage

Western blot analysis was performed as described by Kovacs et al. [17]. Briefly, 40 µg of total protein of each sample was subjected to SDS-polyacrylamide gel electrophoresis using 4–20% gradient Tris/glycine gels under reducing conditions (Invitrogen, Carlsbad, Calif., USA). Next, proteins were transferred to a polyvinylidene difluoride membrane (Bio-Rad, Hercules, Calif., USA) using a semi-dry electrotransfer system (Amersham Biosciences, San Francisco, Calif., USA). Nonspecific proteins were blocked using 5% nonfat dry milk in TBST for 1.5 h. Blots were then incubated with a primary antibody, followed by a secondary antibody (horseradish peroxidase-conjugated antirabbit antibody 1:10,000; Pierce, New York, N.Y., USA). Blots were washed with 1 × TBST for 30 min between steps. A caspase-3 antibody (1:1,000 dilution; Cell Signaling, Beverly, Mass., USA) was used to recognize the large fragment of cleaved caspase-3 (17–20 kD) resulting from cleavage at aspartate position 175.

Colorimetric Caspase-3 Activity Assay

Caspase-3 activity was quantified by a caspase-3 activity kit (R&D, Minneapolis, Minn., USA), which can determine chromophore p-NA released in association with cleavage of peptide by caspase-3.

pH and PCO_2 Measurements

Following exposure to hypocapnia, cell culture medium was drawn into a blood gas syringe, and the syringe was immediately closed with a plug. Extracellular pH and CO_2 was then measured in a pH blood gas machine.

Quantitation of Aβ Using Sandwich ELISA Assay

Following exposure to hypocapnia, conditioned media were collected, and secreted Aβ was measured by a Sandwich ELISA assay. Briefly, 96-well plates were coated with mouse monoclonal antibodies specific to $Aβ_{40}$ (Ab266) or $Aβ_{42}$ (21F12). Following blocking with BSA, wells were incubated overnight at 4°C with test samples of conditioned cell culture media, and then an anti-Aβ (α-Aβ-HR1) conjugated to horseradish peroxidase was added. Plates were developed with TMB reagent and well absorbance measured at 450 nm. Aβ levels in test samples were determined by comparison with signal from unconditioned media spiked with known quantities of $Aβ_{40}$ or $Aβ_{42}$.

Statistics

Given the presence of background caspase-3 activity and apoptosis in the transfected cells, we did not use absolute values to describe changes in caspase-3 activity and apoptosis. Instead, cell apoptosis and caspase-3 activity were presented as a percentage of those of the control group. 100% caspase-3 activity or apoptosis refers to control levels for purposes of comparison to experimental conditions. Aβ

production is presented as ng/ml. ANOVA with repeated measurements was used to compare the difference from the control group. p values less than 0.05 were considered statistically significant.

Results

Induction of Caspase-3 Activation and Apoptosis by Hypocapnia and Potentiation by the PS1Δ9 FAD Mutation

We first investigated the effects of hypocapnia (20 mm Hg CO_2 for 6 h) on caspase-3 activation and apoptosis and then examined the influence of a FAD PS1 mutation on these effects. Normal CO_2 (40 mm Hg CO_2) was used as a control condition. Based on levels of cleaved (activated) caspase-3 (17–20 kD), hypocapnia induced caspase-3 cleavage in both PS1 wt and PS1 mutant cells, but this was greatly exacerbated in the PS1 mutant cells (fig. 1a).

As shown in figure 1b, hypocapnia also led to a 1.3-fold increase in caspase-3 activity in PS1 wt ($p < 0.05$), but with a significantly greater (1.7-fold) increase in the mutant PS1 cells ($p < 0.05$). Finally, hypocapnia led to a 1.4-fold increase in apoptosis in PS1 wt ($p < 0.05$), but to a significantly greater (2.2-fold) extent ($p < 0.05$) in the PS1 mutant cells (fig. 1c).

Synergistic Induction of Caspase-3 Activation and Apoptosis by Hypocapnia and Hypoxia and Potentiation by the PS1Δ9 FAD Mutation

Hypoxia and hypocapnia often occur simultaneously (e.g. during hyperventilation). Thus, we investigated the effects of hypoxia plus hypocapnia on caspase-3 activation and apoptosis, and the influence of a PS1 FAD mutation on these effects. For hypoxia studies, cells were exposed to 5% O_2 for 3 h. Caspase-3 cleavage and caspase-3 activity were determined by caspase-3 antibody and by colorimetric caspase-3 activity assay kit, respectively. Hypoxia alone neither induced caspase-3 cleavage (fig. 2a) nor increased caspase-3 activity (fig. 2b).

To assess the effects of hypocapnia, cells were exposed to 20 mm Hg CO_2 for 3 h and cleaved caspase-3 and caspase-3 activity were determined using the same methods. Hypocapnia neither induced caspase-3 cleavage (fig. 3a), nor increased caspase-3 activity (fig. 3b). In the positive control, 1 μM STS caused caspase-3 cleavage, while normal CO_2 (40 mm Hg PCO_2), the negative control, did not (fig. 3a).

Finally, to assess the combined effects of hypoxia and hypocapnia, cells were exposed to hypoxia (5% O_2) plus
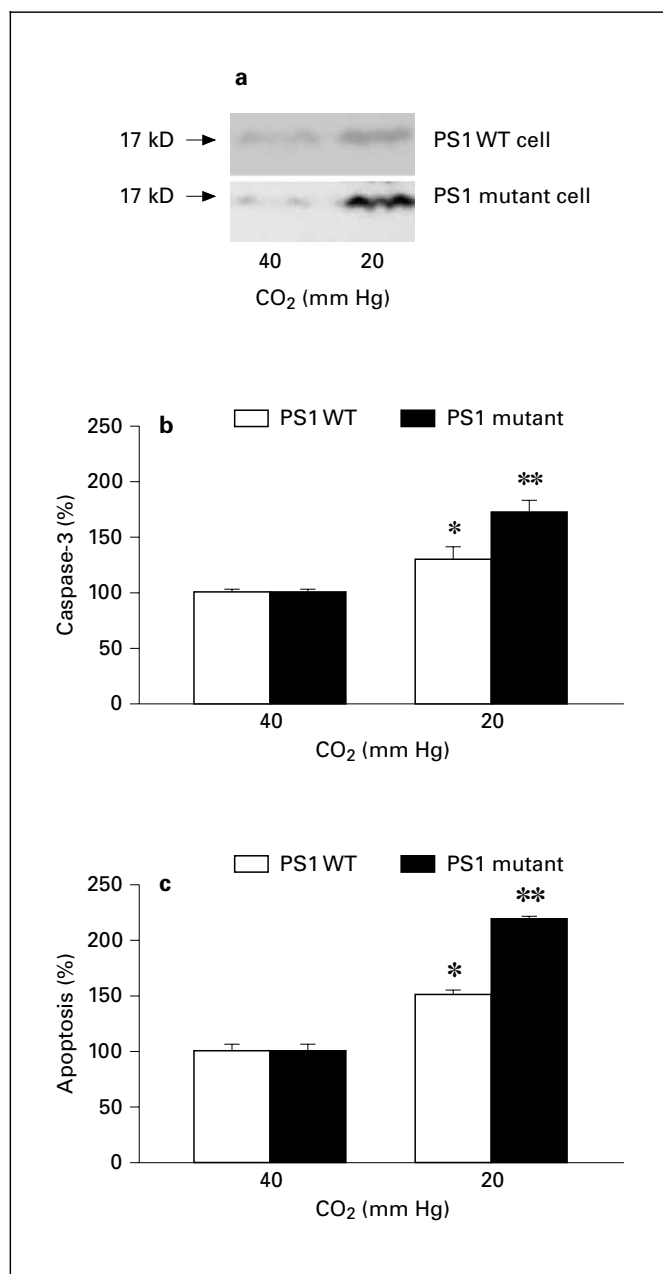

Fig. 1. Caspase-3 activation and apoptosis following hypocapnia in H4 wt PS1-expressing and PS1Δ9 mutant cells. In the PS1Δ9 FAD mutant cells, caspase-3 activation and apoptosis were more robustly induced by hypocapnia as compared to the wt PS1-expressing cells. **a** Caspase-3 staining revealed minimal levels of cleaved caspase-3 in untreated cells, whereas exposure to hypocapnia for 6 h resulted in an increased level of cleaved caspase-3 in both PS1Δ9 mutant and wt PS1-expressing cells, but with a greater increase in the mutant cells. **b** Colorimetric caspase-3 activity assay showed an increased caspase-3 activity in both PS1Δ9 mutant and wt PS1-expressing cells (* $p < 0.05$), but with a greater increase in the mutant cells (** $p < 0.05$). **c** ELISA cell death assay showed an increase in apoptosis in both PS1Δ9 mutant and wt PS1-expressing cells (* $p < 0.05$), but with a greater increase in the mutant cells (** $p < 0.05$).
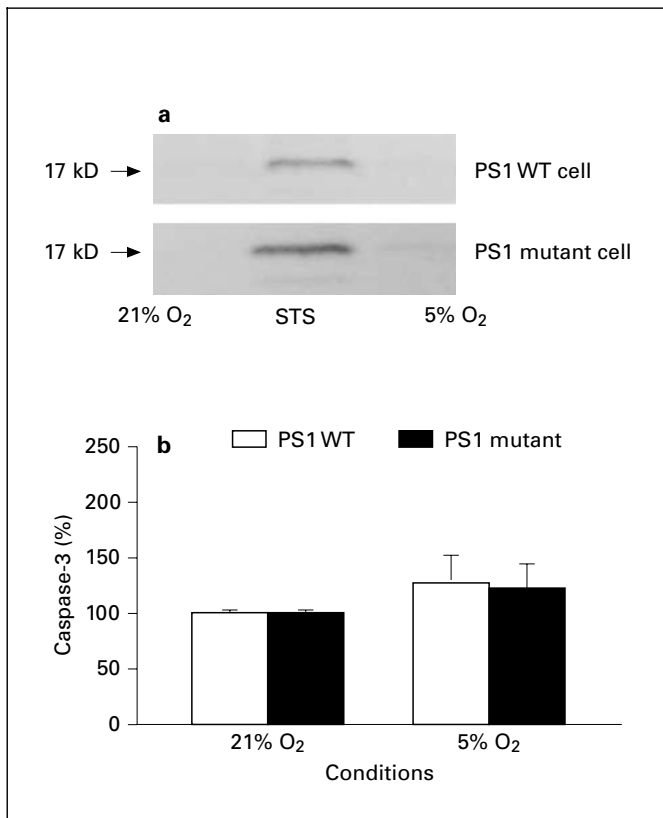
Fig. 2. Caspase-3 activation following hypoxia in PS1Δ9 mutant and wt PS1-expressing cells. Hypoxia (5% O_2 for 3 h) did not increase caspase-3 activation. **a** Cleaved caspase-3 as detected by immunoblotting. Caspase-3 staining showed cleaved caspase-3 induced by STS in both PS1Δ9 mutant and wt PS1-expressing cells, but was greatly exacerbated in the mutant cells. No cleaved caspase-3 was detected following exposure to hypoxia (5% O_2 for 3 h). **b** Caspase-3 activity as assessed by colorimetric caspase-3 activity assay. No significant increase of caspase-3 activity was detected following exposure to hypoxia.

Fig. 3. Caspase-3 activation following hypocapnia in PS1Δ9 mutant and wt PS1-expressing cells. Hypocapnia (20 mm Hg PCO_2 for 3 h) did not increase caspase-3 activation. **a** Cleaved caspase-3 as detected by immunoblotting. Caspase-3 staining showed cleaved caspase-3 induced by STS in both PS1Δ9 mutant and wt PS1-expressing cells, but was greatly exacerbated in the mutant cells. No cleaved caspase-3 was detected following exposure to hypocapnia (20 mm Hg PCO_2 for 3 h). **b** Caspase-3 activity as assessed by colorimetric caspase-3 activity assay. No significant increase of caspase-3 activity was detected following exposure to hypocapnia (20 mm Hg PCO_2 for 3 h).

hypocapnia (20 mm Hg CO_2) for 3 h. Caspase-3 cleavage, caspase-3 activity, and extent of cellular apoptosis were then measured. While neither hypoxia nor hypocapnia alone induced caspase-3 cleavage, the combination of hypoxia and hypocapnia induced caspase-3 cleavage in both PS1 wt and PS1 mutant cells, and this was exacerbated in the PS1 mutant cells (fig. 4a). We next measured caspase-3 activity following 3 h exposure to the combination of hypoxia (5% O_2) plus hypocapnia (20 mm Hg CO_2). Although hypoxia or hypocapnia alone did not induce caspase activity after 3 h (fig. 2b, 3b), the combination of hypoxia plus hypocapnia induced a 1.3- and 1.7-fold increase of caspase-3 activity in wt PS1 and mutant
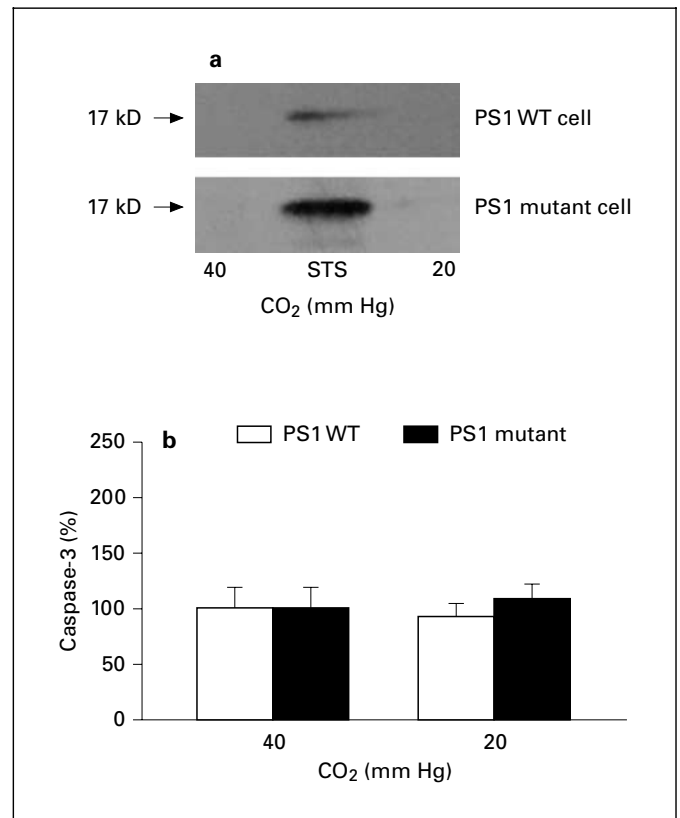
PS1 cells, respectively ($p < 0.05$) (fig. 4b). Again, caspase-3 activity was significantly greater in the mutant PS1 cells as compared to the wt cells ($p < 0.05$). The combination of hypoxia (5% O_2) plus hypocapnia (20 mm Hg CO_2) did not result in a significant increase in apoptosis in PS1 wt cells, but the combination caused significantly (1.4-fold) greater apoptosis in the PS1 mutant cells as compared to the control condition ($p < 0.05$) (fig. 4c).

To confirm that apoptosis and caspase-3 activation were indeed induced by either hypocapnia conditions alone for 6 h, or by the combination of hypoxia plus hypocapnia for 3 h, cells were exposed to 'normal' air conditions (40 mm Hg CO_2 plus 21% O_2) for 6 h, and caspase-3
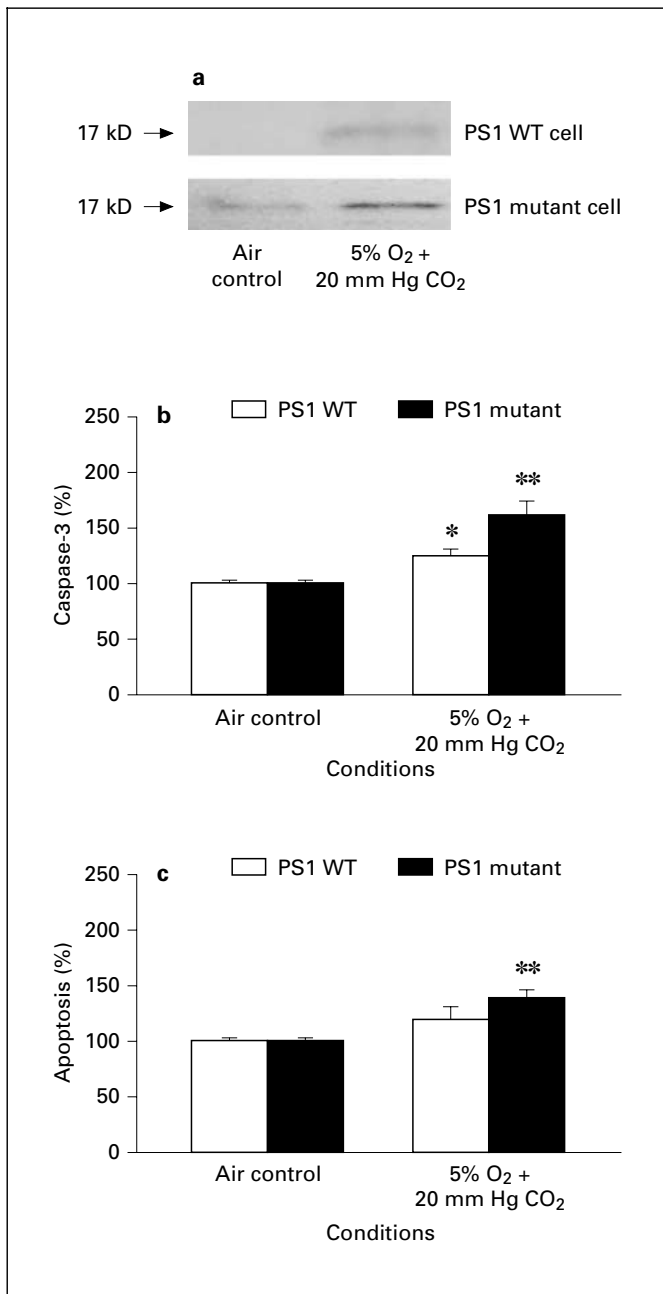
Fig. 4. Caspase-3 activation and apoptosis following exposure of wt PS1 and PS1Δ9 mutant H4 cells to conditions of hypocapnia plus hypoxia. In the PS1Δ9 FAD mutant cells, caspase-3 activation and apoptosis were more robustly induced by hypocapnia plus hypoxia as compared to the wt PS1-expressing cells. **a** Cleaved caspase-3 as detected by immunoblotting. Caspase-3 staining showed minimal levels of cleaved caspase-3 in untreated cells, whereas exposure to hypocapnia plus hypoxia for only 3 h resulted in an increased level of cleaved caspase-3 in both PS1Δ9 mutant and wt PS1-expressing cells, but with a greater increase in the mutant cells. **b** Caspase-3 activity as assessed by colorimetric caspase-3 activity assay. Colorimetric caspase-3 activity assay showed an increased caspase-3 activity in both PS1Δ9 mutant and wt PS1-expressing cells (* $p < 0.05$), but with a greater increase in the mutant cells (** $p < 0.05$). **c** Apoptosis as detected by an ELISA cell death assay. ELISA cell death assay showed an increase in apoptosis in both PS1Δ9 mutant and wt PS1-expressing cells, but with a significantly greater increase in the mutant cells (** $p < 0.05$).

cleavage, caspase-3 activation, and cellular apoptosis were assessed. Cells undergoing no treatment served as negative controls, while cells treated with 1 μM STS to induce caspase activation served as positive controls. In 'normal' air, neither caspase-3 cleavage nor caspase-3 activity was increased, and apoptosis was not observed (fig. 5). Collectively, these data indicate that hypocapnia alone for 6 h, or the combination of hypocapnia and hypoxia for 3 h, specifically induce caspase-3 cleavage, caspase-3 activation, and apoptosis. Moreover, all of these events were significantly potentiated by the presence of the PS1Δ9 FAD mutation.

Increased Aβ Production in Response to Hypocapnia

H4 neuroglioma cells transfected with *APP* containing the Swedish FAD mutation [20] (H4 APP$_{Swe}$ cells) were exposed to either normal CO_2 (40 mm Hg CO_2) or hypocapnia (20 and 5 mm Hg CO_2) conditions for 6 h. Exposure to hypocapnia conditions led to increased Aβ production in a dose-dependent manner (fig. 6) ($p < 0.05$). After 6 h of treatment with 40, 20 and 5 mm Hg CO_2, Aβ$_{40}$ concentrations in the conditioned media were 10.2, 18.4 and 22.5 ng/ml, respectively, while Aβ$_{42}$ concentrations were 1.5, 2.1 and 3.8 ng/ml, respectively. No significant changes in the ratio of Aβ$_{42}$:Aβ$_{40}$ were observed.

Elevation of pH following Exposure to Hypocapnia

We next measured extracellular pH and PCO_2 values following exposure to normal PCO_2 (40 mm Hg PCO_2) and hypocapnia (20 mm Hg PCO_2) conditions for 6 h. Hypocapnia led to a significant decrease of extracellular PCO_2 levels and a significant elevation of extracellular pH. Notably, in response to hypocapnia, we observed no difference in either the resulting elevation of pH or reduction of PCO_2 for PS1 wt versus the PS1Δ9 FAD mutant cells (table 1).
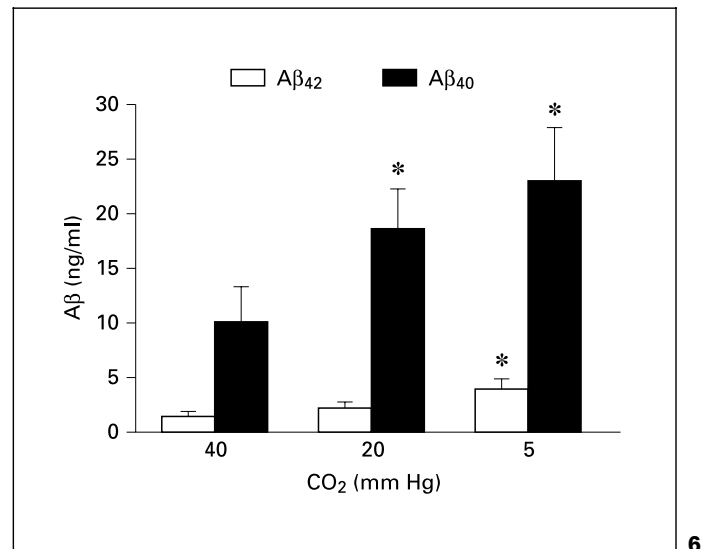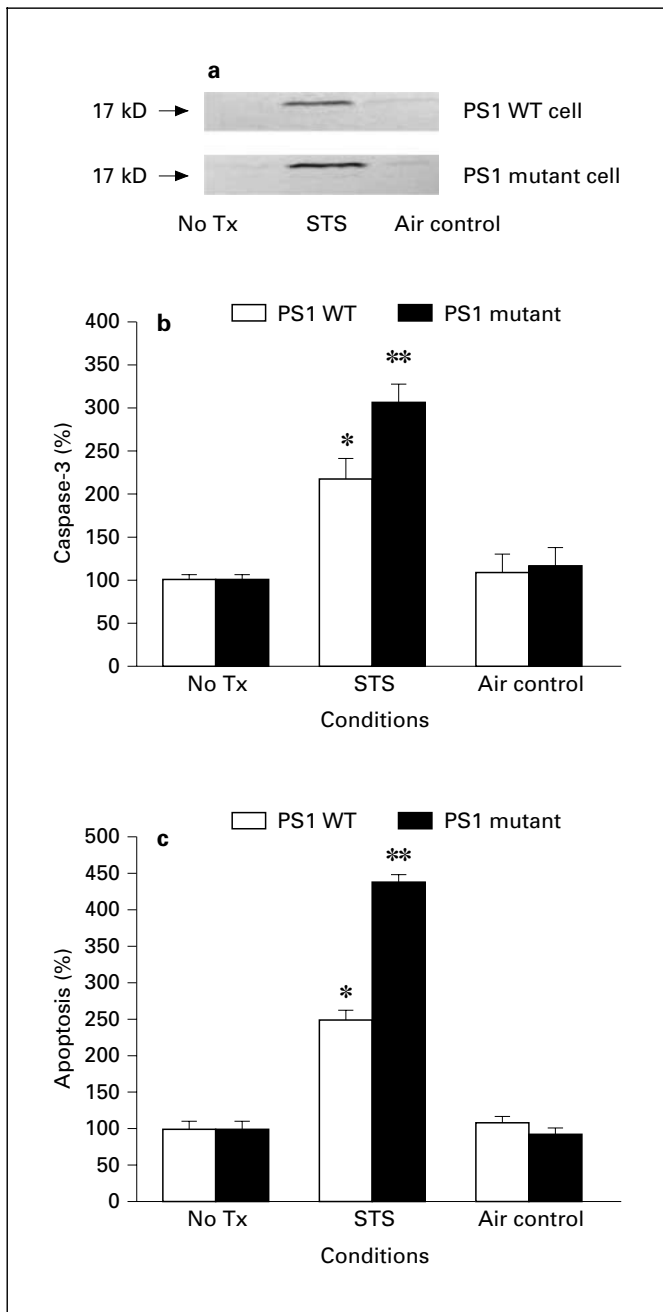
Fig. 5. Caspase-3 activation and apoptosis following exposure of PS1Δ9 mutant and wt PS1-expressing cells to control air conditions (40 mm Hg PCO_2 plus 21% O_2). These conditions neither increased caspase-3 activation nor increased apoptosis. **a** Cleaved caspase-3 as detected by immunoblotting. Caspase-3 staining showed cleaved caspase-3 induced by STS in both PS1Δ9 mutant and wt PS1-expressing cells, but was greatly exacerbated in the mutant cells. No cleaved caspase-3 was detected following exposure to control air. **b** Caspase-3 activity as assessed by colorimetric caspase-3 activity assay. STS treatment induced caspase-3 activity in both PS1Δ9 mutant and wt PS1-expressing cells, but more so in the mutant cells. No increase in caspase-3 activity was detected following exposure to control air. *, ** $p < 0.05$. **c** Apoptosis as assessed by ELISA cell death assay. STS treatment induced apoptosis in both PS1Δ9 mutant and wt PS1-expressing cells, but was greatly increased in the mutant cells. No increase in apoptosis was detected following exposure to control air. *, ** $p < 0.05$.

Fig. 6. Aβ production in H4 APP$_{Swe}$ cells in response to hypocapnia. Six hours of exposure to conditions of hypocapnia (5 and 20 mm Hg CO_2) increased production of Aβ$_{42}$ and Aβ$_{40}$ in a dose-dependent manner as compared to normal CO_2 pressure (40 mm Hg CO_2; * $p < 0.05$). No significant changes in the ratio of Aβ$_{42}$:Aβ$_{40}$ were observed.

Discussion

Hypocapnia, a physiological stressor, remains a prevalent concern in clinical practice [14] as it can result from a large number of disorders including hypoxemia, pneumonia, congestive heart failure, anxiety, central nervous system infection, and from drugs such as salicylates. Hypocapnia has been shown to cause cognitive deficits [11],

neurodevelopment deficits [12], and postoperative psychomotor dysfunction [13, 14].

The data presented here constitute the first demonstration that hypocapnia can increase caspase-3 activation and apoptosis, as well as Aβ production in H4 human neuroglioma cells. Additionally, we have demonstrated that hypocapnia in combination with hypoxia synergistically activates caspase-3 and induces apoptosis. With

Table 1. PCO_2 and pH values in response to hypocapnia

	40 mm Hg CO_2		20 mm Hg CO_2	
	PCO_2	pH	PCO_2	pH
PS1 wt cells	33.2 ± 3.6	7.44 ± 0.05	$19.2 \pm 2.1^*$	$7.80 \pm 0.04^+$
PS1 mutant cells	32.6 ± 1.2	7.41 ± 0.05	$19.2 \pm 0.9^*$	$7.80 \pm 0.02^+$

H4PS1Δ9 mutant and wt PS1-expressing cells were exposed to either a normal CO_2 pressure (40 mm Hg) or a condition of hypocapnia (20 mm Hg CO_2) for 6 h. Extracellular pH and PCO_2 values were determined immediately following exposure. Both PCO_2 and pH values were within normal physiological ranges following exposure to a normal CO_2 pressure (40 mm Hg). The PCO_2 level was significantly decreased (* $p < 0.05$) and the pH value was significantly elevated ($^+$ $p < 0.05$) following exposure to a condition of hypocapnia (20 mm Hg CO_2) in both PS1 wt and PS1Δ9 mutant cells. There was no significant difference in either elevation of pH value or reduction of PCO_2 level between H4 PS1Δ9 mutant and wt PS1-expressing cells.

regard to AD, we have found that in comparison to wt PS1, the PS1Δ9 FAD mutation significantly potentiates caspase-3 activation and apoptosis induced by conditions of hypocapnia or the combination of hypocapnia plus hypoxia. Finally, we have shown that hypocapnia increases pH elevation and reduces PCO_2 in the media suggesting that the observed increases in caspase-3 activation, apoptosis, and Aβ production in response to hypocapnia could be due, at least in part, to extracellular alkalosis.

Neuropathological hallmarks of AD include the increased accumulation of Aβ (e.g. in senile plaques), abnormally hyperphosphorylated *tau* protein in neurofibrillary tangles, synaptic degradation, and neuronal cell loss [1, 21]. Increasing evidence suggests a role for caspase activation and apoptotic cell death in a large number of neurodegenerative disorders including AD [5–10], Parkinson's disease [22, 23], Huntington's disease [24, 25], amyotrophic lateral sclerosis [26] and stroke [27, 28]. Our current findings showing that hypocapnia can induce caspase-3 activation and apoptosis, and also increase Aβ production suggest that hypocapnia (e.g. as a result of general anesthesia) could conceivably contribute to neuropathogenesis in AD (and perhaps, other neurodegenerative disorders in which apoptosis has been implicated). However, the physiological relevance of these in vitro findings regarding induction of apoptosis by hypocapnia and alkalosis would obviously depend heavily on the buffering capacity of the individual organism.

Our data showing that the PS1Δ9 FAD mutation potentiates caspase activation and apoptosis induced by hypocapnia, or by hypocapnia plus hypoxia are consistent with the previously reported activity of this and other PS1 FAD mutations in promoting enhanced susceptibility to apoptosis and neuronal death [17, 18]. While the molecular mechanisms by which PS1 mutations enhance cell apoptosis have not been determined, several have been reported to interfere with cellular calcium homeostasis and calcium release from the ER [18, 29, 30] thereby promoting cell death. PS1 mutations have also been shown to abrogate the interaction of PS1 with β-catenin rendering increased susceptibility to apoptosis [17, 31]. Finally, accumulations of PS1 have been suggested to cause caspase activation and apoptosis [17, 32–34]. The expression level of PS1 in the transfected mutant and wt PS1 cells used in these experiments is obviously higher than that in FAD patients. Thus, it is not clear whether physiological accumulations of PS1 full-length or the mutant PS1 protein could likewise potentiate apoptosis induced by stressors such as STS, hypocapnia or the combination of hypocapnia plus hypoxia. Future studies will be required to determine potential associations between changes in PS1 full-length and alterations in caspase cleavage and apoptosis.

The increases in Aβ observed in this study could be due to either increased Aβ production or release following hypocapnia and alkalosis. Future studies comparing the CTF-γ/AICD level to the Aβ level will be required to determine whether this increase in Aβ is due solely to altered production or also to changes in secretion.

Finally, the finding that elevated exposure to hypocapnia elevates pH is not unexpected, but suggests that extracellular alkalosis, at least in part, contributes to increased caspase-3 activation, apoptosis, and Aβ production fol-

lowing hypocapnia. Interestingly, increased extracellular pH has previously been shown to enhance neuronal cell excitability through the activation of excitatory amino acid receptors [35], while decreased extracellular pH inhibits these same receptors as well as voltage-dependent calcium channels presumably to prevent neuronal injury [36, 37]. Other studies suggest that alkalosis enhances production of reactive oxygen species and the release of cytochrome c as a result of mitochondrial transmembrane voltage disruption [38], which is essential for the induction of apoptosis [39, 40]. Our current findings suggest that alkalosis may potentiate neuronal cell apoptosis in response to hypocapnia and the combination of hypocapnia and hypoxia (e.g., experienced during general anesthesia).

Hypocapnia can cause brain damage by worsening cerebral hypoxia through induction of cerebral vasoconstriction to exacerbate hypoxic-ischemic brain damage [15]. However, our current findings suggest that hypocapnia, itself, is sufficient to induce apoptosis, at least in part by promoting extracellular alkalosis. Further, our cell-based studies reveal a synergistic effect of hypoxia and hypocapnia on caspase-3 activation and cell apoptosis.

Collectively, these data raise the possibility of an alternative mechanism to in vivo hypocapnia-induced vasoconstriction as an explanation for how hypocapnia may exacerbate the adverse effects of hypoxia on neurons: hypocapnia induces alkalosis, which then potentiates neuronal injuries induced by hypoxia.

In conclusion, our findings that hypocapnia and hypoxia can induce caspase-3 activation, apoptosis, and increased Aβ production, and that an FAD mutation, PS1Δ9, potentiates these events, suggest that these two physiological stressors that occur during general anesthesia and various clinical conditions could contribute to AD neuropathogenesis.

Acknowledgements

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