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Protein–energy malnutrition increases activation of the transcription factor, nuclear factor κB, in the gerbil hippocampus following global ischemia☆

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Abstract

Protein–energy malnutrition (PEM) exacerbates functional impairment caused by brain ischemia. This is correlated with reactive gliosis, which suggests an increased inflammatory response. The objective of the current study was to investigate if PEM increases hippocampal activation of nuclear factor κB (NFκB), a transcription factor that amplifies the inflammatory response involved in ischemic brain injury. Mongolian gerbils (11–12 weeks old) were randomly assigned to control diet (12.5% protein) or protein-deficient diet (2%) for 4 weeks. The 2% protein group had a 15% decrease in voluntary food intake ($P<.001$; unpaired t test), resulting in PEM. Body weight after 4 weeks was 20% lower in the PEM group ($P<.001$). Gerbils were then exposed to sham surgery or global ischemia induced by 5-min bilateral common carotid artery occlusion. PEM independently increased hippocampal NFκB activation detected by electrophoretic mobility shift assay at 6 h after surgery ($P=.014$; 2-factor ANOVA). Ischemia did not significantly affect NFκB activation nor was there interaction between diet and ischemia. Serum glucose and cortisol concentrations at 6 h postischemia were unaltered by diet or ischemia. A second experiment using gerbils of the same age and feeding paradigm demonstrated that PEM also increases hippocampal NFκB activation in the absence of surgery. These findings suggest that PEM, which exists in 16% of elderly patients at admission for stroke, may worsen outcome by increasing activation of NFκB. Since PEM increased NFκB activation independent of ischemia or surgery, the data also have implications for the inflammatory response of the many individuals affected globally by PEM.
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Keywords: Protein–energy malnutrition; Global ischemia; Nuclear factor κB; Hippocampus; Stroke

1. Introduction

The complex cascade of events causing neural cell death following brain ischemia or stroke involves ATP depletion, glutamate excitotoxicity, membrane depolarization, increased intracellular calcium, oxidative stress and inflammation [1,2]. Nuclear factor κB (NFκB) is a transcription factor that is key in amplifying the inflammatory response involved in stroke pathophysiology [3,4]. Increased NFκB activation has been demonstrated in human stroke [5,6] and experimental models of brain ischemia [5,7–14].

Attempts to decrease stroke-induced brain injury and associated death and disability with drugs targeting single early cellular events in the ischemic cascade have failed [15]. Combination therapy is inevitable, given the complexity of the cell death cascade [15]. Nutritional status at the time of the stroke and in the subsequent few days could alter mechanisms important in the cascade. Approximately 16% of elderly patients present with protein–energy malnutrition (PEM) at the time of hospital admission for stroke (reviewed in Ref. [16]). Although most clinical studies addressing the impact of PEM on stroke outcome are limited by small sample size and study design, correlations have been drawn between PEM at stroke admission and increased risk of morbidity and mortality [17,18]. A definitive answer was expected from three large Feed or Ordinary Diet Collaboration Trials [19–21]. Analysis of data from the initial cohort recruited suggested that compromised baseline nutritional

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status of patients was associated with decreased chance of survival and increased functional dependency at 6 mo after the stroke [19]. However, the completed trials failed to answer this question because of experimental flaws (reviewed in Ref. [22]).

Our previous study in a model of global ischemia, which has many of the same pathophysiological mechanisms as focal ischemia [1], demonstrated a causal link between nutritional status and outcome. PEM impaired functional outcome measured as lack of habituation in the open field following bilateral carotid artery occlusion in the gerbil [23], a model in which the hippocampal CA1 region is particularly vulnerable to damage [24]. Although PEM did not significantly exacerbate ischemia-induced hippocampal CA1 neuron loss, a subset of protein–energy malnourished ischemic gerbils showed dramatic reactive gliosis that correlated with functional outcome. Since CA1 neuronal cell death can continue for several months after global ischemia [25], these findings suggest that our 10-day histological end point underestimated the ultimate extent of neuronal death.

Given that inflammatory mechanisms modulate proliferation of reactive astrocytes [26] and microglia coordinate the inflammatory response [27], the presence of reactive gliosis suggests that PEM might lower the set-point for the inflammatory response following brain ischemia. Increased inflammatory mediators have been reported in PEM [28,29], yet many other studies show an attenuated inflammatory response to infectious stimuli, depending on type, age of onset and severity of PEM [30,31]. The current study was therefore designed to address a potential mechanistic link between PEM and ischemia-induced inflammation. Since protein malnutrition can increase lipopolysaccharide-induced NFκB activation and proinflammatory cytokines in liver [32], we investigated whether PEM increases hippocampal NFκB activation in response to global ischemia. A second objective of the study was to address the possibility that PEM alters serum glucose or glucocorticoid levels since these physiological variables modify ischemic brain injury [33,34].

2. Materials and methods

2.1. Experiment 1

2.1.1. Animals and diets

Adult male Mongolian gerbils (Charles River Canada, QC, Canada), aged 11–12 weeks, were acclimated for 4–7 d and randomly assigned for 4 weeks to adequate protein (CON, 12.5% protein) or low protein diet (PEM, 2% protein). The diet was modified from the AIN-93M rodent diet [35] and does not contain the antioxidant tertiary-butylhydroquinone (Table 1). Diets were purchased from Dyets (Bethlehem, PA). Gerbils fed a 2% protein diet voluntarily decrease their food intake, which results in a mixed PEM [23]. Gerbils were housed in groups of three at 22°C with a 12-h light/dark cycle in shoebox cages with bedding and free access to food and water. Weekly body weights were monitored as was daily food intake. All animal care and procedures followed the Canadian Council on Animal Care guidelines and were approved by the University of Saskatchewan Committee on Animal Care and Supply.

Table 1
Experimental diets [a]

Component	Adequate protein (CON), g/kg	Low protein (PEM), g/kg
Vitamin-free casein	140	22.4
L-Cystine	1.8	0.29
Sucrose	100	100
Cornstarch	465.7	543.049
Dextrinized cornstarch	155	181.001
Soybean oil	40	40
Cellulose	50	50
Mineral mix	35 [b]	35 [c]
Calcium phosphate, dibasic	0	12.4
Calcium carbonate	0	3.36
Vitamin mix [d]	10	10
Choline bitartrate	2.5	2.5

[a] Diets were purchased from Dyets.
[b] AIN-93M mineral mix [35].
[c] AIN-93M modified mineral mix: calcium and phosphorus deleted, potassium citrate · H_2O increased from 28 to 226.55 g/kg and sucrose increased from 209.806 to 618.256 g/kg mineral mix.
[d] AIN-93M vitamin mix [35].

2.1.2. Surgical procedures

After 4 weeks on their diets, the animals were exposed to either global ischemia or sham surgery, which resulted in four experimental groups that were assessed at two post-ischemic time points (6 and 24 h): control diet with sham surgery (CON-Sham, n=3), control diet with global ischemia (CON-Isch, n=6), PEM with sham surgery (PEM-Sham, n=3) and PEM with global ischemia (PEM-Isch, n=6). Global ischemia was achieved with 5-min bilateral carotid artery occlusion under 1.5–2% isoflurane anesthetic with 1 L/min O_2. The common carotid arteries were isolated through a ventral midline incision. A surgical silk thread was passed under both arteries to allow gentle lifting of the artery. Core body temperature was monitored with a rectal probe, and the animal was warmed with a homeothermic blanket (Harvard Apparatus Canada, Saint-Laurent, QC, Canada). Tympanic temperature was monitored intra-ischemically as an indicator of brain temperature by inserting a fine temperature probe (Barnant Type T Digi-Sense Thermometer, Barrington, IL, USA) through the tympanic membrane. Tympanic temperature was maintained during the ischemic period at 36.5±0.6°C by a blanket (Global Medical Products, Burlington, ON, Canada) circulating with warm water wrapped around the gerbil's head. When brain temperature was stable, two 8×1.5 mm microaneurysm clips (World Precision Instruments, Sarasota, FL, USA) were applied to both common carotid arteries for 5 min to occlude blood flow. The blockage was confirmed visually. After

removal of the clips, carotid artery reflow was verified visually and the incision was closed. Sham surgery procedures were identical except that arteries were not occluded after isolation. Gerbils were placed under a heat lamp for 2 h after surgery, and they could choose whether or not to access the lamp after they became mobile. Animals were then returned to shoebox cages to be monitored for activity.

2.1.3. Activity monitoring for complete forebrain ischemia

A recent study shows that a significant proportion of commercially available gerbils no longer lack posterior communicating arteries [36], resulting in incomplete forebrain ischemia in this model of global ischemia. To assess consistency of the ischemic insult induced in this experiment, we measured activity for 20 h postischemia since persistent motor hyperactivity occurring after occlusion is a reliable indicator of ischemic severity [37]. We have demonstrated that PEM does not confound the screening tool, as it neither independently affects the hyperactivity (P=.897) nor alters the ischemic effect (P=.782) (unpublished observations). Activity was measured with an Opto-M3 Activity Meter (Columbus Instruments, Columbus, OH, USA) utilizing infrared beam interruption in two dimensions. We used the following two criteria to identify animals with complete forebrain ischemia, which correctly classifies 84.6% of animals (unpublished data): (1) activity had to be greater than 3 Standard deviations above the mean activity of sham animals in our laboratory (n=30); (2) the hyperactivity had to be sustained throughout the 20-h period of monitoring. Although gerbils from the 6-h post-ischemic time point followed the same activity monitoring protocol, the data could not be assessed accurately because 6 h is not sufficiently long to determine if the hyperactivity is sustained.

2.1.4. Serum cortisol and serum glucose

Blood samples were collected at 6 h following ischemia to measure these two physiological variables. Serum glucose and cortisol, the major glucocorticoid in gerbil [38], were analyzed by Prairie Diagnostic Services, College of Veterinary Medicine, University of Saskatchewan. Serum glucose was analyzed by the hexokinase method spectrophotometrically at 505 nm (Roche/Hitachi 704/911/912, Roche Diagnostics, IN, USA). Total serum cortisol was measured by radioimmunoassay (Coat-A-Count Cortisol Kit, Diagnostic Products, Los Angeles, CA, USA).

2.1.5. Hippocampal nuclear extraction and protein determination

Gerbils were perfused with heparinized saline by intracardiac injection at 6 or 24 h post-ischemia. The nuclear extraction method was modified from Xu et al. [39]. Hippocampus was dissected on ice and homogenized in 0.5 ml phosphate-buffered saline (0.1 mol/L) using a Brinkmann Polytron Model PT10-35 (Rexdale, ON, Canada). Each sample was then centrifuged (Eppendorf Centrifuge 5403, Westbury, NY, USA) at 600×g for 10 min and the pellet was resuspended in 0.5 ml of buffer containing 10 mmol/L HEPES (pH 7.9), 10 mmol/L KCl, 1.5 mmol/L $MgCl_2$, 0.5 mmol/L dithiothreitol and 0.5 mmol/L phenylmethylsulphonylfluoride. The mixture was incubated on ice for 10 min and centrifuged again at 600×g for 10 min. The pellet was again resuspended in 0.3 ml of 0.05% Nonidet P-40 in buffer and homogenized. The samples were centrifuged at 600×g for 10 min and the pellet resuspended in 0.5 ml of buffer containing 5 mmol/L HEPES (pH 7.9), 25% glycerol, 1.5 mmol/L $MgCl_2$, 0.2 mmol/L EDTA, 0.5 mmol/L dithiothreitol, 0.5 mmol/L phenylmethylsulphonylfluoride and 0.3 mol/L NaCl. After mixing by inverting the tubes, the samples were incubated on ice for 30 min before being centrifuged at 14,000×g for 30 min. The supernatant fluid (nuclear extract) was stored at −80°C.

Total protein concentration of brain nuclear extracts was determined by the bicinchoninic acid assay [40].

2.1.6. NFκB activation by electrophoretic mobility shift assay

Protein–DNA complexes were resolved by electrophoretic mobility shift assay (EMSA) as described [41] using the gel-shift NFκB family kit (Active Motif, Carlsbad, CA, USA). All the reagents and the oligonucleotide probe were supplied in the kit. The double-stranded oligonucleotide (5′-AGCTTGGGGTATTTCCAGCCG-3′) was end-labeled using γ[-^{32}P]-ATP and T4 polynucleotide kinase (Invitrogen, CA) and purified. Binding reactions were carried out with end-labeled, double-stranded DNA probe (>1×10^5 cpm/μl) and nuclear extracts according to the protocols indicated in the gel-shift NFκB family kit. The reaction mixture was subjected to EMSA using 5% polyacrylamide gel (1.5 mm thickness) at 4°C in 24.8 mmol/L Tris (pH 8.5), 190 mmol/L glycine and 1 mmol/L EDTA for 1 h at 110 V. Gels were dried and exposed to X-ray film. Optical density was measured using a densitometry system (Alpha Imager System, Alpha Innotech, San Leandro, CA, USA).

Each gel contained a sample from Experiment 2 (see below) from an animal on control diet without exposure to any type of surgery, which was designated the reference group. The results were expressed as a ratio, which was calculated as the optical density (ADU) of the sample from an experimental group expressed per microgram of protein divided by the optical density (ADU) of a sample from the reference group per microgram of protein. This ratio was used to control for variability caused by differences between gels. All samples were analyzed using the same labeled probe, and all gels were run on the same day. Four gels were run, and all gels were developed on the same film twice. With these variables controlled, it was possible to analyze the following number of samples for each of the two post-ischemia time points (6 and 24 h): PEM-Isch (n=6); CON-Isch (n=6 for 6 h and n=5 for 24 h); PEM-Sham (n=2); CON-Sham (n=2).

2.1.7. Statistical analysis

All statistical analyses were conducted using SPSS 12.0 for Windows (SPSS, Chicago, IL, USA). Pre-ischemic body weight and food intake were analyzed by unpaired Student's *t* test. Post-ischemic body weight and biochemical data were analyzed by two-factor ANOVA. Differences were considered statistically significant at $P<.05$. All data are presented as mean±S.E.M.

2.2. Experiment 2

Since increased hippocampal NFκB activation was observed in PEM-Sham animals in Experiment 1, a second experiment was designed to investigate whether PEM increases NFκB activation in the hippocampus independent of sham surgery. Male Mongolian gerbils of the identical strain and age as used in Experiment 1 were randomly assigned to either CON diet or PEM ($n=4$) for 4 weeks, but the gerbils did not receive any surgery. Nuclear extracts were prepared from fresh hippocampus, stored at −80°C, and EMSA measurements were performed for NFκB activation as described for Experiment 1. However, unlike the EMSA results from Experiment 1 that were presented as a ratio, the data for hippocampal NFκB activation in Experiment 2 have not been corrected for gel-to-gel variability by expressing them relative to data from a reference animal. This is because samples from both Experiments 1 and 2 were run on the same gels, and the Experiment 2 samples obtained from animals exposed to CON diet actually constituted the reference samples for Experiment 1; there were no additional reference samples run for Experiment 2 samples. Thus, to correct for variability among gels, a CON sample from a particular gel was compared against the PEM sample run on the same gel by pairing samples by gel, and the results were analyzed by Student's paired *t* test.

3. Results

3.1. Experiment 1

3.1.1. Body weight and food intake

Body weight and food intake data from Experiment 1 are presented in Table 2. The initial body weight was not significantly different between CON and PEM groups. After the 4-week period of feeding, the control group gained on average 4.9 g and the PEM group lost on average 7.4 g. The differences in final body weight and body weight change between the groups were significant ($P<.001$). Total food intake per cage was significantly different between the CON and PEM groups ($P<.001$).

Following ischemia, there was a significant effect of diet ($P<.001$) on body weight but there was no significant effect of ischemia ($P=.260$ and 0.379 at 6 and 24 h, respectively) or interaction between ischemia and diet at any time point ($P=.373$ and .806 at 6 and 24 h, respectively) (Table 3). All experimental groups lost weight during the 24-h period following surgery. Food intake was decreased at 24 h post-ischemia in all experimental groups except the CON-Sham group, but the sample size (on a cage basis) was insufficient to analyze these data statistically (data not shown).

Table 2
Body weight and food intake prior to ischemia

	Dietary treatment	
	CON	PEM
Initial body weight (g) [1]	63.4±0.5[a]	62.2±0.6[a]
Final body weight (g) [1]	68.3±0.6[a]	54.7±0.8[b]
4-week body weight change (g) [1]	+ 4.9±0.5[a]	− 7.4±0.7[b]
Total 4-week food intake (g) [2]	109.6±1.4[a]	93.7±1.8[b]

Results in the table are shown as mean±S.E.M. Means with different letters in the same row differ significantly by unpaired *t* test.

[1] $n=24$.

[2] $n=8$ cages (three animals per cage).

3.1.2. Serum glucose

Serum glucose concentration at 6 h post-ischemia is shown in Fig. 1. There was no significant effect of diet ($P=.242$) or ischemia ($P=.359$) on serum glucose and no interaction between diet and ischemia ($P=.844$).

3.1.3. Serum cortisol

Serum cortisol concentration at 6 h post-ischemia is shown in Fig. 2. There was no significant effect of diet ($P=.878$) or ischemia ($P=.702$) on serum cortisol, and no interaction was found between ischemia and diet ($P=.832$).

3.1.4. Activity monitoring for complete forebrain ischemia

The percentage of gerbils with bilateral common carotid artery occlusion that met criteria for complete forebrain ischemia was 45.8% (11/24).

3.1.5. NFκB activation by EMSA

Fig. 3 shows representative autoradiograms of NFκB activation in hippocampal nuclear extracts measured by EMSA. In general, activation of NFκB was more pronounced as a result of PEM than from global ischemia.

Figs. 4 and 5 show the results from semiquantification of the EMSA results. At 6 h post-surgery, PEM significantly increased NFκB activation ($P=.014$) (Fig. 4). Ischemia did not significantly increase NFκB activation ($P=.169$), and there was no interaction between diet and ischemia ($P=.270$). At 24 h postsurgery, there was a trend for PEM to independently increase NFκB activation ($P=.097$) (Fig. 5). There was no significant effect of ischemia ($P=.644$) nor was there a significant interaction between diet and ischemia ($P=.360$).

3.2. Experiment 2

Body weight and food intake showed the same pattern over the 4 weeks of feeding as in Experiment 1 (data not shown).

Unlike the previous EMSA results that were presented as a ratio (relative to data from a reference animal), the data for hippocampal NFκB activation in Experiment 2 have not

Table 3
Pattern of body weight post-ischemia[a]

Time point (h)	Body weight (g)			
	PEM-Isch	PEM-Sham	CON-Isch	CON-Sham
6	54.1±0.8	56.3±1.2	66.1±1.0	66.4±0.8
24	53.5±1.2	55.5±1.8	64.8±1.8	65.9±0.7

[a] Results in the table are presented as mean±S.E.M.; n=6 for each ischemic group and n=3 for each sham group. There was a significant effect of diet only at both time points by two-factor ANOVA (P<.001).

been adjusted for intergel variation (Fig. 6). However, when samples were paired by gel, PEM significantly increased NFκB activation over that demonstrated in CON samples (P=.026, n=4, one-tailed paired t test).

4. Discussion

Feeding a 2% protein diet to the gerbil for a 4-week period causes a voluntary reduction in food intake, thus inducing PEM [23]. We have previously demonstrated that this dietary paradigm reduces food intake by 15%, body weight by 17%, liver reduced-glutathione by 49% [23] and serum albumin by 18% [42], with a 66% increase in liver lipid [23]. A similar magnitude of depression in food intake and weight loss was observed in the current study. However, both sham and ischemic groups fed control diet also lost weight following surgery, and food intake was decreased up to 24 h after surgery in all groups except sham animals fed control diet. Although depressed food intake and weight loss are potential confounding variables during the post-ischemic period in rodent surgical models of stroke, these data are often not reported.

PEM caused a significant increase in hippocampal NFκB activation at 6 h following global ischemia or sham surgery with a similar trend at 24 h. This provides a mechanism by which the inflammatory response may be exacerbated if protein–energy deficiency is present at the time of stroke. Our results provide good evidence for the translocation of activated NFκB proteins to the nucleus but no information on the transcription regulatory effect of NFκB. This should be investigated to confirm that there is also enhanced expression of the pro-inflammatory target genes that are regulated by NFκB and important in stroke pathophysiology. The latter include molecules such as intercellular cell adhesion molecule 1, inducible nitric oxide synthase, cyclooxygenase-2, interleukin-1β and tumor necrosis factor α [3,4]. A vicious cycle can ensue, as cytokines induced by NFκB activation can then act as stimuli for more NFκB activation [43].

The absence of a significant ischemia-induced increase in NFκB activation is in contrast to findings from the majority of experimental global and focal brain ischemia studies [5,7–14]. Our result is most likely accounted for by the inconsistent ischemic severity demonstrated in our model. This is supported by Fig. 5, which shows NFκB activation increasing by 24 h, but in a variable manner, in the ischemic group fed control diet. Although the screening

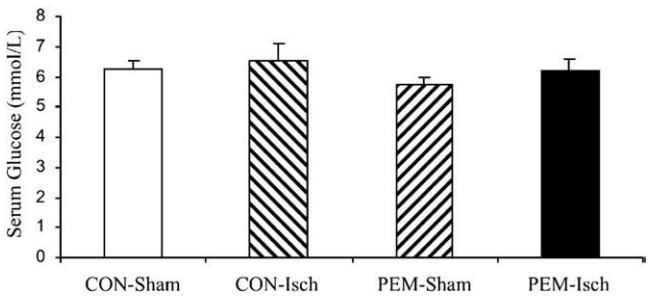

Fig. 1. The effect of PEM and ischemia on serum glucose concentrations at 6 h post-ischemia. Results are presented as mean±S.E.M.; n=6. No significant effects of treatments were found by a two-factor ANOVA (P>.05).

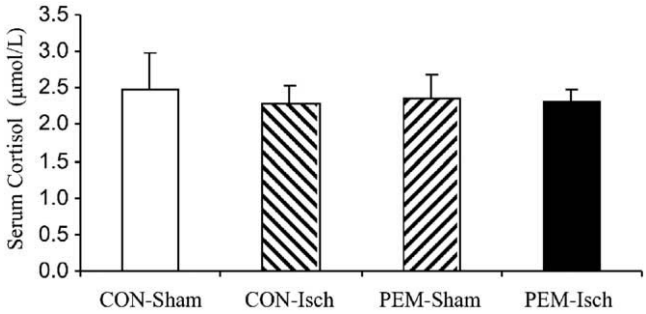

Fig. 2. The effect of PEM and ischemia on serum cortisol concentration at 6 h post-ischemia. Results are mean±S.E.M.; n=6. No significant effects of treatments were found by a two-factor ANOVA (P>.05).

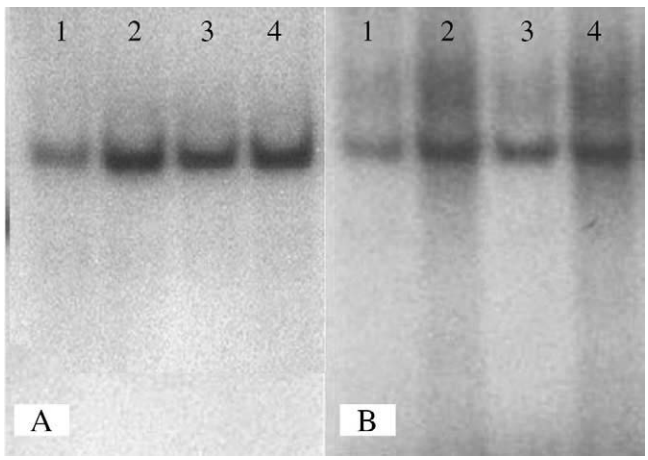

Fig. 3. Representative autoradiograms of NFκB activation in hippocampal nuclear extracts measured by electrophoretic mobility shift assay. (A) Effect of PEM on NFκB activation during the post-ischemic period. Times post-ischemia are 6 h (Lanes 1 and 2) and 24 h (Lanes 3 and 4); Lanes 1 and 3 are CON-Isch; Lanes 2 and 4 are PEM-Isch. (B) Effect of PEM and sham surgery on NFκB activation. Times postsurgery are 6 h (Lanes 1 and 2) and 24 h (Lanes 3–4); Lanes 1 and 3 are CON-Sham; Lanes 2 and 4 are PEM-Sham.

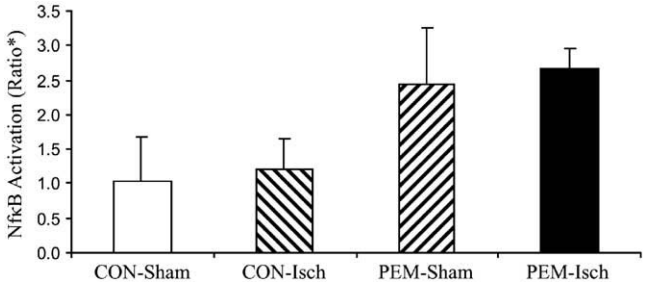

Fig. 4. The effect of PEM and ischemia on NFκB activation at 6 h post-ischemia. Results are presented as mean± S.E.M.; n=6 for each ischemic group and n=2 for each sham group. There was a significant effect of diet by a two-factor ANOVA (P=.014). *Ratio=optical density (ADU) of the experimental sample per microgram of protein divided by the optical density (ADU) of the reference sample per microgram of protein.

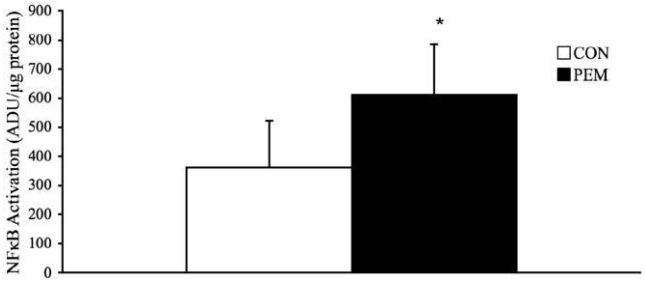

Fig. 6. The influence of PEM on NFκB activation in the absence of surgery. The results are presented as mean±S.E.M. *P=.026; significant elevation of NFκB activation (one-tailed paired t test; n=4).

procedure detected complete (or severe) ischemia in only 46% of the study animals exposed to bilateral carotid artery occlusion, all gerbils were included in the statistical analysis. This was because of the inability to judge if hyperactivity was sustained in gerbils from the 6-h survival time and the obvious increase in NFκB activation observed in all protein–energy malnourished animals, regardless of surgical treatment. With the unexpected observation of an independent effect of PEM on NFκB activation, it is acknowledged that the sample size for sham controls was underestimated. This study limitation likely also contributed to the absence of a statistically significant effect of ischemia on NFκB activation.

Despite these limitations, the data suggest that PEM is an independent predictor of NFκB activation at 6 h following ischemia, whereas at 24 h, PEM does not appear to further enhance the small ischemia-induced NFκB activation observed. However, without the expected substantial increase in NFκB activation following ischemia, our hypothesis that PEM would enhance brain ischemia-induced activation of this transcription factor was incompletely tested. Further studies will be needed under conditions of more consistent forebrain ischemia to ascertain whether PEM and ischemia interact or act independently to increase NFκB activation.

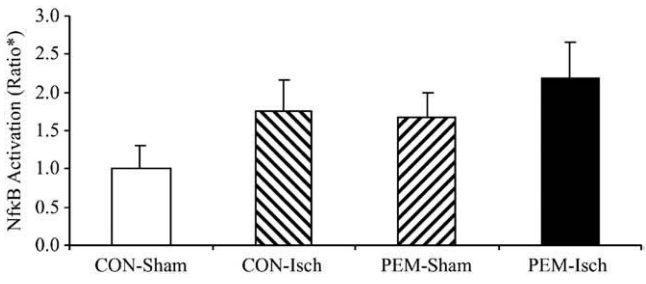

Fig. 5. The effect of PEM and ischemia on NFκB activation at 24 h post-ischemia. The results are presented as mean±S.E.M.; n=6 for PEM-Isch group, n=5 for CON-Isch group, n=2 for each sham group. There was a trend for PEM to increase NFκB activation as analyzed by a two-factor ANOVA (P=.097). *Ratio=optical density (ADU) of the experimental sample per microgram of protein divided by the optical density (ADU) of the reference sample per microgram of protein.

While PEM may exert multiple effects on both the acute cascade of neuronal death and the later recovery phase following stroke, our study has demonstrated that increased NFκB activation is one potentially important contributor. The binding of NFκB to specific DNA sequences occurs via dimers of NFκB subunits [43]; p65 and p50 subunits appear to be translocated following brain ischemia [8,10]. Super-shift assays will be used in future to determine how ischemia and PEM affect the relative involvement of these specific proteins in the DNA–protein complex. Whether PEM allows increased NFκB translocation into the nucleus by increasing degradation of the inhibitory protein, IκB, will also need to be addressed. Since NFκB activation is regulated by redox processes [44] and PEM increases oxidative stress in many tissues [45,46], including ischemic brain [23], we speculate that PEM may exacerbate NFκB activation by influencing cell redox status.

There is considerable evidence that NFκB activation is a key mediator of ischemic cell death. Reduced neuronal damage following focal ischemia has generally been described in mice lacking the p50 subunit of NFκB [5,10]. In transgenic mice expressing IκBα-SR (a specific dominant inhibitor of NFκB) in neurons, infarct volume is also decreased after focal ischemia [47]. Studies using proteosomal inhibitors of NFκB, although not completely specific, also support this idea [48]. Important limitations of all of these studies, however, are that they rely exclusively on histological assessment to evaluate outcome, and survival times are too short to be certain that decreased NFκB decreases and not merely delays brain damage. In vitro data have also suggested some neuroprotective roles for NFκB [49], as has one recent study of focal ischemia in a p50 knockout mouse [50]. Our results also show constitutive NFκB activation in the hippocampus, which is in agreement with other studies in the central nervous system [14,43,49] and emerging functions for NFκB [43,49]. Prolonged activation of NFκB may be necessary to cause neuronal death in global ischemia [14], and thus studies with longer survival times will be needed.

Our results have been hampered by the variability in ischemic severity. Until recently, there were major advantages to using the well-established model of global ischemia employed in this study [24]. The absence of posterior communicating arteries in Mongolian gerbils allowed for a reliable degree of forebrain ischemia with 5-min bilateral carotid artery occlusion [24]. The minimal surgical intervention was also a benefit for nutritional studies since the stress state and tissue wasting that accompanies surgery and neurotrauma [51] is not a significant feature of human stroke [52]. However, a recent report has demonstrated that posterior communicating arteries have now developed in 61% of gerbils [36]. Our indirect measure of ischemic severity by postischemic hyperactivity similarly detected incomplete ischemia in 54% of gerbils exposed to bilateral carotid artery occlusion. While screening on the basis of hyperactivity or measurement of cerebral blood flow could be used to reduce interanimal variability, it is likely that the model will be abandoned because of the high proportion of animals affected.

Given the limitation of small sample size for sham groups in Experiment 1, the results of Experiment 2 greatly strengthen the initial conclusion by corroborating the finding that PEM increases NFκB activation in hippocampus. Experiment 2 has further demonstrated that this is likely a general feature of PEM that can arise independently of a surgical stress. Whether PEM causes a sustained increase in NFκB activation in hippocampus or alters a short-term response to the stress of anesthesia is unknown. In either case, the findings have potentially far-reaching implications for the inflammatory response of the hundreds of millions of protein–energy malnourished individuals worldwide [53].

PEM, depending on the degree of malnutrition and acuteness of onset, can impair insulin response and glucose homeostasis and increase serum glucocorticoids [54,55]; any such effects are likely to be further influenced by an ischemic insult. Our results suggest that PEM does not mediate its effects on ischemic brain injury by altering either of these variables. However, additional time points will be necessary to rule out this possibility. Given the somewhat variable and unphysiologically high values obtained for serum cortisol, we will also need to address the possibility that stress-induced inflation of basal cortisol levels masked an effect of PEM as has been previously shown with acute preanesthesia stress [56,57].

In summary, the results of this study demonstrate that PEM increases hippocampal NFκB activation following global ischemia, suggesting that patients who are protein–energy compromised at the time of presentation for stroke will have an increased inflammatory response. No evidence was obtained to suggest that alterations in serum glucose or cortisol are responsible for the increase in ischemic brain injury in PEM. Our findings further suggest that increased hippocampal NFκB activation is a general feature of PEM that is not dependent on ischemic brain injury or surgical stress.

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