RESEARCH PAPER

Acta Neurobiol Exp 2020, 80: 358–363
DOI: 10.21307/ane-2020-032

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The modulatory role of β-amyloid in the regulation of nociception in mice

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β-amyloid is an important factor in the pathophysiology of Alzheimer's disease.This study investigates β-amyloid's role in the regulation of nociception in mice. Pretreated once, 2 weeks prior to testing with β-amyloid, male ICR mice were examined on various nociceptive tests. Pretreatment with β-amyloid reversed the nociceptive effects induced by intraperitoneally administered acetic acid (writhing response) and intraplantar injection of 5% formalin into the hind paw. β-amyloid pretreatment also elevated the threshold for nociception in the mechanical von Frey test. Additionally, p-CREB and p-ERK levels in the spinal cord and the adrenal gland increased after formalin injection. Pretreatment with β-amyloid attenuated formalin-induced overexpression of p-CREB and p-ERK in the spinal cord and the adrenal gland. Our results suggest that chemical and mechanical nociception appear to be altered in β-amyloid-treated animals. Furthermore, the reduction of nociception by β-amyloid in the formalin pain model appears to be mediated, at least in part, by the suppression of p-CREB and p-ERK level in the spinal cord and the adrenal gland.

Key words: nociception, pain, β-amyloid, spinal cord, signal molecules

INTRODUCTION

Alzheimer's disease (AD) is a neurodegenerative disorder characterized by dementia, cognitive impairment, and memory loss. β-amyloid is one of the pathological mediators leading to AD (Sisodia and Price, 1992). A major neuropathological component of AD progression is deposition of β-amyloid, which is derived from larger amyloid precursor proteins. Postmortem brains of AD patients reveal neuropathological features including the presence of β-amyloid plaques and neurofibrillary tangles, which also contain β-amyloid peptide (Sojkova and Resnick, 2011).

Several lines of clinical evidence have demonstrated nociceptive changes in patients with AD. For example, Benedetti et al. (1999) reported that pain tolerance was increased in Alzheimer's patients. However, the relationship between AD pathology and pain perception is still rather unclear (Monroe et al., 2012). An earlier study demonstrated that β-amyloid acts as a blocking mediator in amyloid precursor protein (APP) over-expressing CRND8 transgenic mice (Shukla et al., 2013), suggesting that β-amyloid may be responsible for blunted pain perception observed in Alzheimer's patients.

Recent studies have shown that the extracellular signal-regulated kinase P44/42 (ERK1/2) and cyclic AMP response element-binding protein (CREB) are involved in various types of pain transmission. Both CREB and ERK protein expression are elevated in dorsal root ganglia (DRG) and spinal cord in rat neuropathic pain models (Crown et al., 2006; Ji et al., 2009). For ex-

Received 17 January 2020, accepted 23 June 2020

ample, the expression of spinal ERK and CREB proteins are correlated with the development allodynia after spinal cord injury (Crown et al., 2006). ERK protein also contributes to pain sensitization after tissue and nerve injury (Ji et al., 2009), as well as in the production of neuropathic pain (Han et al., 2011). Additionally, both CREB and ERK protein expression are also elevated in a diabetic neuropathy model and in a capsaicin-treated pain model (Miyabe and Miletic, 2005; Song et al., 2005; Wu et al., 2005; Dang et al., 2014). However, the role of spinal CREB and ERK proteins in the modulation of nociception in β-amyloid-pretreated animal models has not yet been explored.

Although previous studies have suggested that pain perception is altered in Alzheimer's patients, the exact role and mechanism of β-amyloid in the brain in the regulation of nociception has not been thoroughly characterized. Thus, the present study was designed to examine the effect of supraspinal β-amyloid pretreatment on nociceptive behavior in several mouse pain models. Furthermore, the potential roles of ERK and CREB in β-amyloid's regulation of pain were investigated.

METHODS

Experimental animals

Male ICR mice (6 weeks of age) weighing 25-30 g at the beginning of experiments (Myung-Jin, Inc., Seoul, Korea) were used in the experiments. The mice were housed 5 per cage in a room maintained at 22±1°C with an alternating 12-hour light-dark cycle. Food and water were available ad libitum. The animals were allowed to adapt to the laboratory for at least 2 h before testing and were only used once. To reduce variation, all experiments were performed during the light phase of the cycle (10:00~17:00).

Drug

β-amyloid from Sigma-Aldrich (St. Louis, MO, USA) was dissolved in saline (0.9% NaCl). The β-amyloid (410 pmol/mice) was administered i.c.v. as described previously (Song et al., 1998; Yan et al., 2001; Cho et al., 2005). β-amyloid was prepared just prior to using.

Experimental design

To examine the change of pain perception after β-amyloid injection, mice were performed in three different nociceptive models: visceral pain model, formalin pain model, and von Frey test. In the behavior test, the mice were divided randomly into two groups: the normal mice group (control group) and the β-amyloid injected group.

The i.c.v. administration of β-amyloid (410 pmol/5 μl) was performed following the procedure established by Laursen and Belknap (1986). Briefly, each mouse was injected at bregma with a 50 μl Hamilton micro syringe fitted with a 26-gauge needle that was inserted to a depth of 2.4 mm. The i.c.v. injection volumes were 5 μl, and the injection sites were verified by injecting a similar volume of 1% methylene blue solution and determining the distribution of the injected dye in the ventricular space. The experiments were performed only when the success rate of i.c.v. injection was over 95%.

We next measured whether the expression level of signal molecular proteins is altered in the spinal cord and the adrenal gland after β-amyloid injection. Mice were randomly divided into four groups. Control group: the normal mice. β-amyloid group: these mice were tested after 2 weeks of i.c.v. pretreatment with β-amyloid. Saline + 5% formalin group: mice were i.c.v. pretreatment with 5 μl saline, after 2 weeks mice were injected 5% formalin in the left hind paw. β-amyloid + 5% formalin group: mice were i.c.v. pretreatment with β-amyloid (410 pmol), after 2 weeks mice were injected 5% formalin in the left hind paw.

Nociceptive models and nociceptive behavior measurements

These nociceptive behavior tests were performed after 2 weeks of the pretreatment of β-amyloid. For the visceral pain model (Feng et al., 2019), 1% acetic acid was injected intraperitoneally (10 mL/kg of body weight). The number of writhing responses (as a measure of visceral pain) was counted for 30 min after acetic acid injection. For the formalin pain model (Hunskaar et al., 1985), 10 μl of 5% formalin was injected subcutaneously under the plantar surface of the left hind paw. Nociceptive behaviors such as shaking and licking the hind paws were counted during the first phase (0-5 min) and the second phase (20-40 min) using a stop watch. Mechanical allodynia was assessed by von Frey testing (Bonin et al., 2014). For the von Frey test, mice were individually placed in a clear glass cell with a metal mesh floor, allowed to adapt to the testing environment for 30 min, and then von Frey filaments (North Coast Medical, Inc., Gilroy, CA, USA) were applied to the plantar surface using an up and down paradigm.

Then the mice hind paw withdrawal threshold was compared. In these behavior experiments, 8 animals were used per group.

Western blot

The mouse adrenal gland and spinal cord were dissected at 20 min after 5% formalin injection. Tissue was washed twice with cold Tris-buffered saline (20 mmol/L Trizma base and 137 mmol/L NaCl, pH 7.5). Immediately after washing, tissues were lysed with sodium dodecyl sulfate lysis buffer (62.5 mmol/L Trizma base, 2% w/v sodium dodecyl sulfate, 10% glycerol) containing 0.1 mmol/L Na_3VO_4, 3 mg/mL aprotinin, and 20 mmol/L NaF. After brief sonication, the concentration of protein was determined with a detergent-compatible protein assay reagent (Bio-Rad Laboratories, Hercules, CA, USA) using bovine serum albumin as the standard. After adding bromophenol blue (0.1% w/v), the proteins were boiled, separated by electrophoresis in 10-12% polyacrylamide gels, and transferred onto a polyvinylidene difluoride membrane (Millipore, Bedford, MA, USA). The membranes were immunoblotted with antibodies p-ERK1/2 (Abcam, USA; 1:1000), p-CREB (Abcam, USA; 1:1000) and β-actin (Cell Signaling Technology, USA; 1:1000) in a blocking buffer for 1 h at room temperature. Membranes were then washed 4 times with Tris-buffered saline containing 20% Tween-20 (TBST; 10 mM Trizma base (pH 8.0), 150 mM NaCl, and 20% Tween 20) for 20 min and then incubated with the anti-rabbit IgG-horseradish peroxidase conjugate (1:5000) in blocking buffer at room temperature for 1 h. After washing the membranes with TBST for 20 min (4 times), ECL-plus solution (Millipore, Billerica, MA, USA) was added. Then the membranes were exposed to Hyperfilm-MP (Amersham Phamacia Biotech) for detection of light emission. The specific signals were quantified with the Multi-Gauge Version 3.1 (Fuji Film, Japan) and expressed as the percentage of the control (Sim et al., 2014; Kang et al., 2015).

Statistics

All values were expressed as the mean ± standard deviation (SEM). The statistical significance of differences among multiple variables were assessed with two-way ANOVA with Tukey's *post hoc* test using GraphPad Prism (Version 8.0.2, GraphPad Software, USA). Differences between the groups were assessed using unpaired t-tests. Statistical significance was indicated by p-values less than 0.05.

RESULTS

Changes in nociceptive behaviors in the β-amyloid-pretreated group in various pain models

ICR mice were pretreated once, 2 weeks prior to testing with 410 pmol of β-amyloid. In the writhing test, 1% acetic acid was administered i.p. and the number of writhing events was counted for 30 min. As shown in Fig. 1A, the i.c.v. pretreatment with β-amyloid attenuated the number of writhing events induced by acetic acid, compared to the control group (t_8=9.439, P<0.0001). In the formalin test, 5% formalin was injected into the hind-paw. As shown in Fig. 1B, compared to the control group, the i.c.v. pretreatment with β-amyloid attenuated the nociceptive behaviors during both 1st and 2nd phases (t_8=8.498, P<0.0001; t_8=12.31, P<0.0001, respectively). As shown in Fig. 1C, β-amyloid pretreatment for 2 weeks caused an elevation in the mechanical stimulation threshold as measured by the von Frey test (t_8=2.637, P=0.0298), compared to the control group.

Changes in phosphorylated CREB and ERK proteins in the spinal cord and adrenal gland after β-amyloid pretreatment in a formalin-induced pain model

The lumbar spinal cord and adrenal gland were dissected 20 min after 5% formalin was injected into hind paws, and protein was extracted for Western blot analysis. As shown in Fig. 2A, B, formalin injection caused an elevation of p-CREB ($F_{1,5}$=298.3, P<0.001) and p-ERK ($F_{1,5}$=305.0, P<0.001) protein levels in the spinal cord, compared to the control group. However, two-way ANOVA (formalin × β-amyloid) revealed that i.c.v. pretreatment with β-amyloid caused reductions in formalin-induced elevations of p-CREB ($F_{1,5}$=206.5, P<0.001) and p-ERK ($F_{1,5}$=237.8, P<0.001) protein levels in the spinal cord, compared to the saline + 5% formalin group (Fig. 2A, B). Additionally, in Fig. 2C, D, compared to the control group, p-CREB ($F_{1,5}$=89.48, P<0.001) and p-ERK ($F_{1,5}$=13.26, P<0.05) protein levels in the adrenal gland were also altered by formalin. A two-way ANOVA (formalin × β-amyloid) showed that β-amyloid produced a significant effect of the expression level of p-CREB ($F_{1,5}$=148.1, P<0.001) and p-ERK ($F_{1,5}$=258.3, P<0.001) proteins, this indicated that i.c.v. pretreatment with β-amyloid suppressed the elevation of p-CREB and p-ERK protein levels induced by formalin in the adrenal gland (Fig. 2C, D).

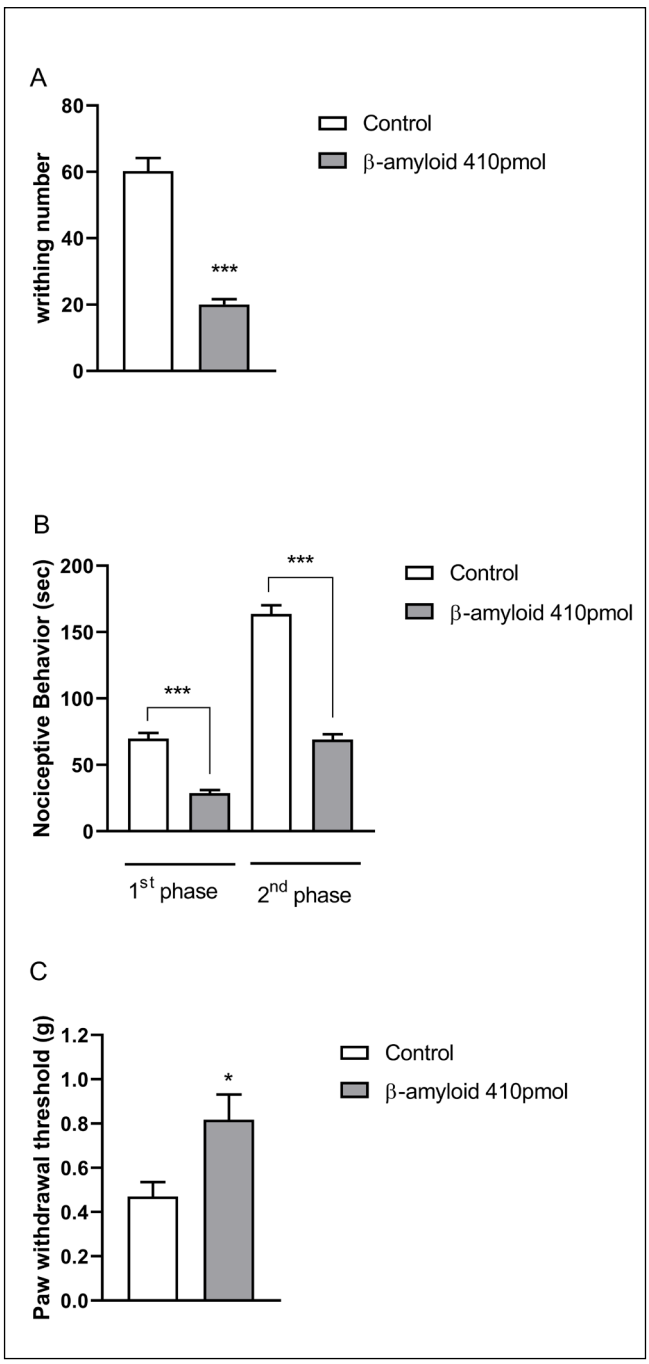

Fig. 1. β-amyloid-induced nociceptive behavioral changes in various pain models. ICR mice were β-amyloid-pretreated (i.c.v.) once 2 weeks before testing. Then, nociceptive behaviors induced by (A) 1% acetic acid (i.p.), (B) 5% formalin (intraplantar injection into the hind-paws), or (C) mechanical pain stimulation by von Frey filaments were assessed. (A) The number of writhing responses was counted for 30 min after acetic acid injection. (B) In the formalin pain test, nociceptive behaviors such as vigorous licking and paw shaking were counted during the first (0-5 min) and the second (20-40 min) phase using a stop watch. (C) The mechanical pain threshold was measured by von Frey test. Vertical bars indicate the mean ± SEM. Differences between the two means were assessed by unpaired t-test. The number of mice per group was 8. *** $P<0.001$, * $P<0.05$.

DISCUSSION

We found that supraspinal pretreatment with β-amyloid caused a reduction in nociceptive behaviors, manifested in writhing- and formalin-pain models. In addition, the threshold for mechanical pain was elevated in the β-amyloid-treated group, suggesting that β-amyloid may play an important modulatory role in the regulation of nociception in β-amyloid-induced AD. In support of our present finding, Shukla et al. (2013) recently reported that β-amyloid acts as a blocking mediator for pain perception in APP over-expressing CRND8 transgenic mice. Likewise, Pamplona et al. (2010) reported that i.c.v.-injected, β-amyloid-treated mice exhibited an increased jump threshold in the foot shock-sensitivity test. Although the results from several clinical studies (Husebo et al. 2016; Cao et al. 2019) have revealed that nociception in Alzheimer's patients is complex, our study suggests that the exact role of β-amyloid in the regulation of nociception, especially in β-amyloid-induced Alzheimer's patients, should be clarified through yet further clinical investigation. While we investigated the β-amyloid-induced AD model, the results from this study are partially supported by findings in other AD models. For example, colchicine, okadaic acid, and streptozotocin (STZ) are also used to induce AD (Shree et al., 2017; Malekzadeh et al., 2017).

Several lines of evidence have previously demonstrated that CREB and ERK proteins are closely associated with pain transmission. For example, both p-CREB and p-ERK expression in the spinal cord or dorsal root ganglia are up-regulated in various types of chronic pain models, such as neuropathic pain and neuropathy (Miyabe and Miletic, 2005; Song et al., 2005). Furthermore, p-CREB and p-ERK expression in the spinal cord or brain are up-regulated in acute inflammatory pain models, such as the formalin pain model (Hermanson and Blomqvist, 1997; Seo et al., 2008; Hagiwara et al., 2009; Mao et al., 2013; Kang et al., 2015). Up-regulation of both p-CREB and p-ERK expression in a formalin-induced pain model was observed in this study. Furthermore, we found that supraspinal pretreatment with β-amyloid almost completely abolished the p-CREB and p-ERK expression induced by formalin injection, suggesting that the reduction of nociception by β-amyloid treatment in a formalin pain model appears to be mediated, at least in part, by the reduction of p-CREB and p-ERK level in the spinal cord. Although the experiment was not carried out, we speculate that β-amyloid located at the supraspinal level may further enhance the descending pain inhibitory system, which could lead to decreased p-CREB and p-ERK expression in the spinal cord.

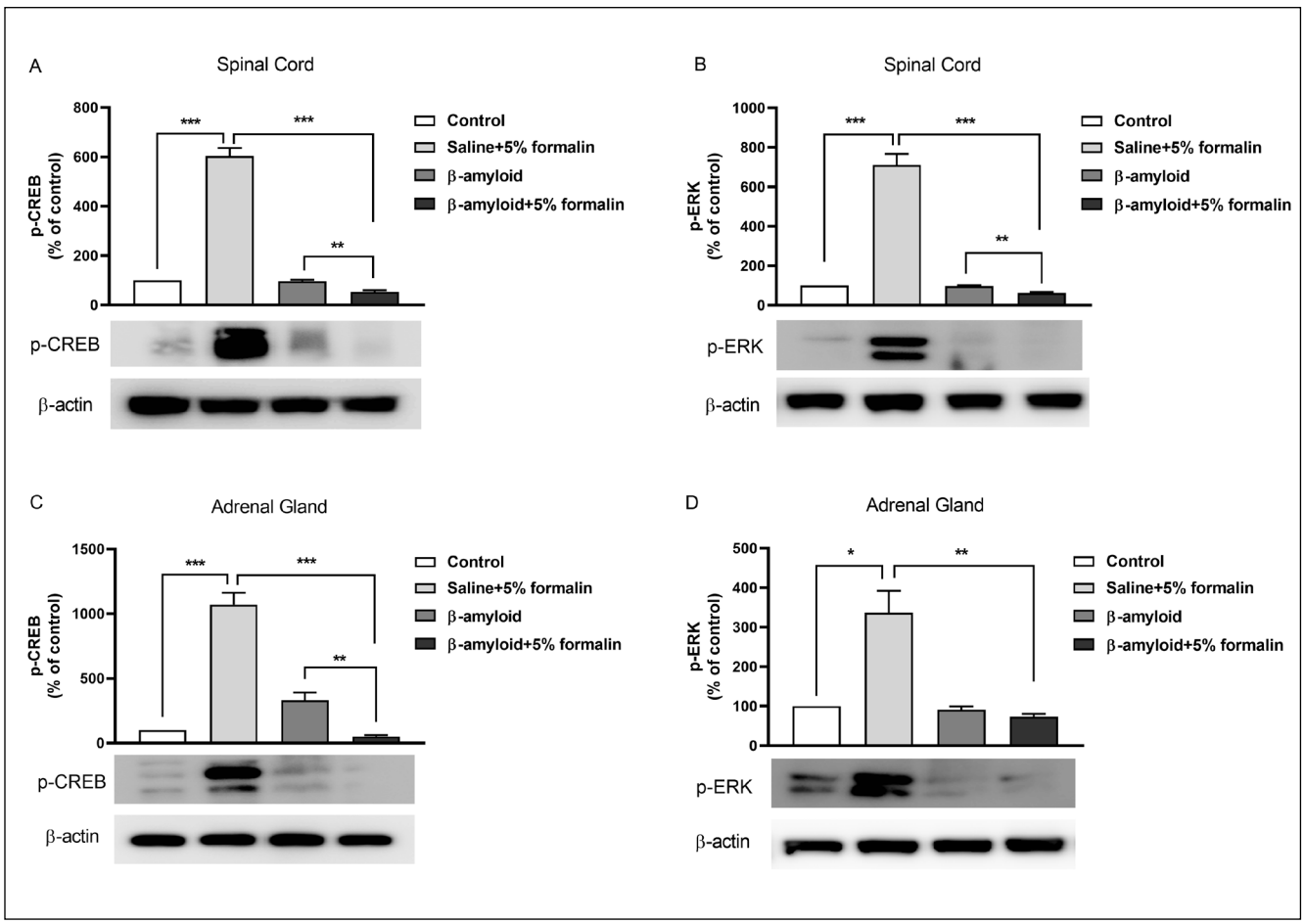

Fig. 2. Effects of β-amyloid pretreatment on p-CREB and p-ERK expression in the spinal cord and the adrenal gland. ICR mice were β-amyloid-pretreated once 2 weeks before testing. Then, the lumbar region of the spinal cord and adrenal gland were dissected. p-CREB and p-ERK expression in the spinal cord (A and B, respectively) and the adrenal gland (C and D, respectively) were analyzed by Western blot. β-actin (1:1000 dilution) was used as an internal loading control. Signals were quantified with the use of laser scanning densitometry and expressed as a percentage of the control. Values are mean ± SEM. Each quantified result was analyzed by two-way ANOVA with a Bonferroni's *post hoc* test. Differences between the two means were assessed by unpaired t-test. The number of animals in each group was 6. *** $P<0.005$, ** $P<0.01$, * $P<0.05$.

Our group and others have previously reported that the adrenal gland is closely involved with regulation of nociception and antinociception (Sim et al., 2013; Kang et al., 2014). An earlier study demonstrated that both CREB and ERK proteins are involved the regulation of adrenal steroidogenesis (Chen et al., 2005). In addition to the spinal cord, we found that p-CREB and p-ERK expression in the adrenal gland are up-regulated in the formalin pain model. Furthermore, increased blood glucocorticoid levels are an important aspect of blood glucose up-regulation in several pain models (Sim et al., 2013). Thus, it is possible that the up-regulation of CREB and ERK phosphorylation, induced by formalin injection, may be related to elevations in blood glucocorticoid levels, which may exert a negative feedback influence to alleviate the nociceptive behavior.

CONCLUSION

Taken together, our results describe a decrease in chemically- or mechanically-induced nociceptive behaviors in β-amyloid-treated mice, suggesting that the pain transmission system can be modulated in a β-amyloid-induced AD animal model. Additionally, we observed down-regulation of p-CREB and p-ERK, which may be involved in the reduction of nociception by β-amyloid in the formalin pain model.

ACKNOWLEDGEMENT

This research was supported by the Hallym University Fund (HRF-201807-011).

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