ORIGINAL ARTICLE

Prostate Disease

Prostate-derived IL-1β upregulates expression of NMDA receptor in the paraventricular nucleus and shortens ejaculation latency in rats with experimental autoimmune prostatitis

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Experimental autoimmune prostatitis (EAP)-induced persistent inflammatory immune response can significantly upregulate the expression of N-methyl-D-aspartic acid (NMDA) receptors in the paraventricular nucleus (PVN). However, the mechanism has not yet been elucidated. Herein, we screened out the target prostate-derived inflammation cytokines (PDICs) by comparing the inflammatory cytokine levels in peripheral blood and cerebrospinal fluid (CSF) between EAP rats and their controls. After identifying the target PDIC, qualified males in initial copulatory behavior testing (CBT) were subjected to implanting tubes onto bilateral PVN. Next, they were randomly divided into four subgroups (EAP-1, EAP-2, Control-1, and Control-2). After 1-week recovery, EAP-1 rats were microinjected with the target PDIC inhibitor, Control-1 rats were microinjected with the target PDIC, while the EAP-2 and Control-2 subgroups were only treated with the same amount of artificial CSF (aCSF). Results showed that only interleukin-1β (*IL-1β*) had significantly increased mRNA-expression in the prostate of EAP rats compared to the controls ($P < 0.001$) and significantly higher protein concentrations in both the serum ($P = 0.001$) and CSF ($P < 0.001$) of the EAP groups compared to the Control groups. Therefore, IL-1β was identified as the target PDIC which crosses the blood-brain barrier, thereby influencing the central nervous system. Moreover, the EAP-1 subgroup displayed a gradually prolonged ejaculation latency (EL) in the last three CBTs (all $P < 0.01$) and a significantly lower expression of NMDA NR1 subunit in the PVN ($P = 0.043$) compared to the respective control groups after a 10-day central administration of IL-1β inhibitors. However, the Control-1 subgroup showed a gradually shortened EL ($P < 0.01$) and a significantly higher NR1 expression ($P = 0.004$) after homochronous IL-1β administration. Therefore, we identified IL-1β as the primary PDIC which shortens EL in EAP rats. However, further studies should be conducted to elucidate the specific molecular mechanisms through which IL-1β upregulates NMDA expression.

INTRODUCTION

It is estimated that 30%–40% of patients with chronic prostatitis/chronic pelvic pain syndrome (CP/CPPS; category III prostatitis) suffer from premature ejaculation (PE),[1–3] which is difficult to cure and greatly reduces the quality of life. Currently, the exact etiology of CP/CPPS has not yet been elucidated, and the mechanism of autoimmunity is considered to be the most likely pathogenesis.[4–7]

In our previous study, we showed that the ejaculation latency (EL) of experimental autoimmune prostatitis (EAP) rat models was significantly shortened compared to the controls after conducting copulatory behavior testing (CBT).[8] This indicates that the EAP rats suffer the same PE symptom as CP/CPPS patients and thus they form

an ideal model for elucidating the mechanism of PE in CP/CPPS patients. Moreover, our previous study confirmed that the persistent EAP-induced inflammatory immune response (IIR) can significantly upregulate the expression of N-methyl-D-aspartic acid (NMDA) receptors in the paraventricular nucleus (PVN) by the NR1 subunit, which shortens the EL by enhancing sympathetic nervous system (SNS) sensitivity.[8] However, the mechanism of chronic inflammation in the prostate which causes the upregulated expression of NMDA receptors is still unclear.

Accumulating evidences have revealed many clues, with prostate-derived inflammation cytokine (PDIC), which crosses the blood–brain barrier (BBB) to influence the central nervous system

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(CNS), being considered as the key factor.[9–12] Therefore, this study aimed at exploring the following factors in the EAP rat model: (1) whether PDICs can display a significantly elevated concentration in peripheral blood and simultaneously cross the BBB; (2) whether the selected PDIC can significantly shorten the EL in the control rats by PVN microinjection; and (3) whether the inhibitor of selected PDIC can significantly prolong the EL of EAP rats in CBTs.

MATERIALS AND METHODS

Animals and housing
Fifty male and 16 female Wistar rats (age: 12–14 weeks old; weight: 250–320 g) were obtained from the Experimental Animal Center of Nanjing Medical University (Nanjing, China). All rats were housed in a specific pathogen-free room, and the conditions of accommodation are described in detail in our previous study.[8] All procedures in this study were approved by the Experimental Animal Care and Use Committee of Nanjing Medical University (No. IACUC-1911011) and were in accordance with the Guide for the Care and Use of Laboratory Animal published by the US National Institutes of Health (8th edition, 2011).

Experimental designs
First, we sacrificed eight male rats to obtain the male accessory glands for the preparation of prostate protein extract (PPE). The other 42 males were randomly divided into the EAP group and the control group ($n = 21$ for each group). After a 30-day EAP modeling, five EAP rats and five controls were randomly sacrificed to obtain peripheral blood and cerebrospinal fluid (CSF) samples for screening the target PDICs on the 32nd day of the experiment timeline. Next, we simultaneously conducted three CBTs with a 3-day interval to exclude males unqualified in copulatory behaviors. When screening the target PDICs, all qualified males in the initial 3-time CBTs were subjected to implanting tubes onto the bilateral PVN. They were then randomly divided into the EAP-1, EAP-2, Control-1, and Control-2 subgroups ($n = 8$ for each group). After 1-week recovery, the target PDIC inhibitor and the target PDIC were administered every day through PVN microinjection to the rats in the EAP-1 and Control-1 subgroups from the 49th day to the 58th day, respectively. In addition, the EAP-2 and Control-2 subgroups were only treated with the same amount of artificial CSF (aCSF; MlBio, Shanghai, China). In other words, EAP-1 rats were microinjected with the target PDIC inhibitor, Control-1 rats were microinjected with the target PDIC, while the EAP-2 and Control-2 subgroups were only treated with the same amount of aCSF. Five minutes after each administration, CBT was performed to observe and record the copulatory behaviors of male rats and then repeated four times with a 3-day interval. On the 62nd day of the experimental timeline, the remaining male rats were sacrificed to collect blood and brain samples for further experiments. All experimental steps, except EAP modeling, were completed in a double-blind manner. It is worth noting that all animals were numbered, and it was impossible to visually recognize their grouping information. **Figure 1** shows the schematic illustration of the experimental timeline of this study.

EAP model establishment
Preparation of PPE and EAP modeling was in accordance with a protocol described in our previous study.[8] The autoimmune injections (AIIs) were conducted on day 0 and repeated on the 15th and 30th days of the experimental timeline (**Figure 1**).

Tactile allodynia assessment (TAA)
We measured the sense threshold of tactile allodynia to determine the development of EAP modeling on the 2 days before AIIs performance
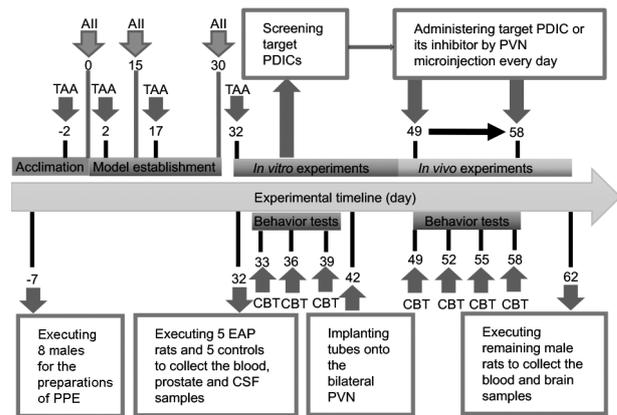

Figure 1: The experimental timeline. AII: autoimmune injections; CBT: copulatory behavior testing; CSF: cerebrospinal fluid; EAP: experimental autoimmune prostatitis; PDIC: prostate-derived inflammation cytokine; PPE: prostate protein extraction; PVN: paraventricular nucleus; TAA: tactile allodynia assessment.

(baseline), 2 days, 17 days, and 32 days (2 days after each AII, respectively), as shown in **Figure 1**. The TAA protocol was described in our previous study.[8]

Collection of blood, CSF, prostate, and brain samples
Rats were anesthetized by intraperitoneal injection of 80 mg kg^{-1} pentobarbitone (Xingzhi factory, Shanghai, China) and kept in a lateral position to allow full exposure of the back of neck. Next, 2 ml of peripheral blood was exsanguinated from the carotid artery, followed by collection of 0.5 ml of CSF through a puncture made at the foramen magnum. Rats were then sacrificed to isolate the brain and prostate tissues according to our previously reported procedure.[8] Finally, the PVN area was punched out by microdissection with reference to the Paxinos and Watson atlas.[13]

Screening target PDICs
After successful modeling (on the 32nd day), we first used quantitative real-time polymerase chain reaction (qRT-PCR) to screen out the pro-inflammation cytokine candidates which were highly expressed in the prostate tissues of EAP rats compared to the controls. Next, we performed enzyme-linked immunosorbent assay (ELISA) to further verify whether the levels of candidate cytokines were also significantly increased in the serum and CSF of the EAP model, with the overarching goal of screening out the target PDICs for the subsequent experiments.

We extracted total RNA from the TRIzol homogenates (Ambion, Austin, TX, USA) of prostate tissues and purified it using trichloromethane and isopropanol. Next, we synthesized cDNA using iScript™ cDNA Synthesis kits (Bio-Rad Laboratories, Shanghai, China) according to the manufacturer's protocol. The mRNA levels of pro-inflammation cytokines were detected using Platinum™ RTS SYBR™ Green qPCR SuperMix-UDG kits (Invitrogen, Carlsbad, CA, USA) with primer sequences of interleukin-1α (*IL-1α*), *IL-1β*, *IL-4*, *IL-6*, *IL-8*, *IL-10*, *IL-13*, interferon-γ (*IFN-γ*), monocyte chemotactic protein-1 (*MCP-1*), macrophage inflammatory protein-1α (*MIP-1α*), and tumor necrosis factor-α (*TNF-α*) reported in previous studies (**Supplementary Table 1**).[9,14–16] The basic protocol for qRT-PCR was an initial incubation at 95°C for 5 min, followed by 40 cycles of denaturation at 95°C for 15 s, annealing at 60°C for 30 s, and extension at 72°C for 60 s. All samples were run in triplicate, and relative mRNA expression was normalized to the expression of glyceraldehyde phosphate dehydrogenase (*GAPDH*) mRNA (an endogenous control).

Rat ELISA Kits (Abcam, Cambridge, UK) were used to further verify the levels of the cytokine candidates in the serum and CSF. The tests were performed by double-antibody sandwich ELISA according to the manufacturer's instructions. Notably, assay sensitivity for all detected cytokines was <1 pg ml^{-1}.

Implanting tubes onto bilateral PVN and microinjection

The protocols used for implanting tubes and PVN microinjection in this study were in accordance with our previous study,[17,18] and referred to the Paxinos and Watson atlas. After successful tube implantation, we administered 50 nl each side of target PDIC or its inhibitor or aCSF every day to the four subgroups, respectively, through bilateral PVN microinjection. At the end of the behavior evaluation, 50 nl each side methylene blue was injected into the PVN microinjection sites, followed by histological identification. Notably, only the males with correct injection into the PVN were included in our final analyses.

Copulatory behavior testing

We conducted CBTs according to the protocol reported in our previous studies.[8,17,19] Males would be excluded from behavior evaluation due to no copulation with females at least twice in the initial three CBT sessions. After administration by PVN microinjection, the following behavioral parameters were recorded and analyzed based on the videos of the last four CBT sessions: mount latency (ML), mount frequency (MF), intromission latency, intromission frequency (IF), EL, ejaculation frequency (EF), postejaculatory interval (PEI), and intromission ratio (IR) = IF/(MF + IF).

NMDA NR1 subunit detection in the PVN

When the CBTs were completed, all remaining males were sacrificed to isolate their brains. Next, the PVN area was punched out by microdissection. We then used immunohistochemistry and western blot analysis to detect NMDA NR1 subunit in the PVN. The detailed procedures and the antibodies used were described in our previous study.[8]

Statistical analyses

All data were analyzed using SPSS software version 19 (IBM, New York, NY, USA). Continuous variables were presented as mean ± standard deviation (s.d.). Levene test and Kolmogorov–Smirnov test were used to determine the normality and homogeneity of variance, respectively. Parametric variables were compared between two groups using Student's *t*-test, while nonparametric variables were compared using Mann–Whitney U test and Wilcoxon tests. $P < 0.05$ was considered to be statistically significant.

RESULTS

EAP modeling

In total, 40 rats in the two groups successfully underwent three-time AIIs for modeling. Two rats (No. 11 and 16) died from suspected allergic reaction after the second and the third injection, respectively. After unblinding, the two rats were confirmed to belong to the EAP group.

Assessment of pelvic pain development

No significant difference was found in the pain threshold of lower abdominal skin between the two groups on the basal condition prior to EAP modeling (2 days before AIIs performance [baseline], $P = 0.812$) or on the 2nd day after first AII ($P = 0.484$). However, the lower abdominal pain thresholds in the EAP group significantly decreased on the 17th and 32nd days compared to the thresholds in the controls (both $P < 0.001$; **Figure 2a**).
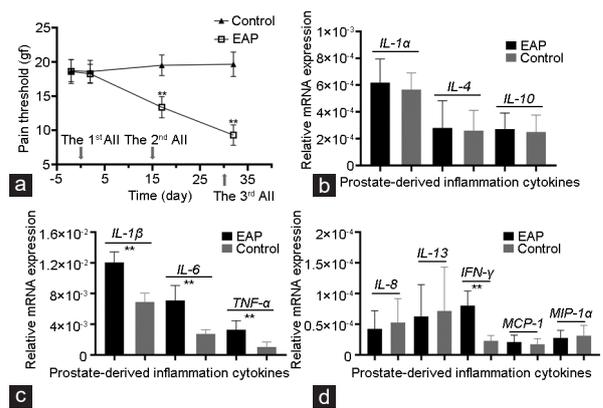

Figure 2: (**a**) Comparison of lower abdominal pain thresholds between the EAP and control groups on the 2 days before AIIs performance, 2nd day, 17th day and 32nd day of the experimental timeline ($n = 19$ rats in the EAP group *vs* $n = 21$ in the control group). Comparisons of (**b**) *IL-1α*, *IL-4*, and *IL-10*, (**c**) *IL-1β*, *IL-6*, and *TNF-α*, and (**d**) *IL-8*, *IL-13*, *IFN-3*, *MCP-1*, and *MIP-1α* to *GAPDH* mRNA expression in prostate tissues between the two groups after successful modeling ($n = 5$ rats for each group). Data are presented as mean ± s.d.; **$P < 0.01$. AII: autoimmune injections; EAP: experimental autoimmune prostatitis; gf: gram-force; *IL-1α*: interleukin-1α; *IL-4*: interleukin-4; *IL-10*: interleukin-10; *IL-1β*: interleukin-1β; *IL-6*: interleukin-6; *TNF-α*: tumor necrosis factor-α; *IL-8*: interleukin-8; *IL-13*: interleukin-13; *IFN-γ*: interferon-γ; *MCP-1*: monocyte chemotactic protein-1; *MIP-1α*: macrophage inflammatory protein-1α; *GAPDH*: glyceraldehyde phosphate dehydrogenase

Cytokine mRNA levels in prostate tissue

After successful modeling, we detected 11 pro-inflammation cytokines expressions in prostate tissues and compared the relative cytokine/*GAPDH* mRNA levels between the EAP and control groups. Results showed that four cytokines (*IL-1β*, *IL-6*, *IFN-γ*, and *TNF-α*) were significantly increased in the prostate tissues of EAP rats ($P < 0.001$, $P = 0.001$, $P = 0.001$, and $P = 0.006$, respectively), as shown in **Figure 2b**–**2d**.

Serum/CSF cytokine levels

Based on the qRT-PCR results, we used ELISA method to further detect and compare the protein levels of IL-1β, IL-6, IFN-γ, and TNF-α in the serum and CSF of the two groups. The expressions of IL-1β and IL-6 in the peripheral circulation of EAP rats were significantly elevated compared to those in the controls ($P = 0.001$ and $P = 0.015$, respectively; **Figure 3a**). In the CSF, we found significantly higher levels of IL-1β and TNF-α in EAP rats than in controls (both $P < 0.001$; **Figure 3b**).

Copulatory behavior testing

Three (No. 10, 19, and 30) of the 30 males were excluded from the subsequent four-time behavior evaluation because of no copulation with females at least twice in the initial three CBTs. The remaining 12 EAP rats and 15 controls were further grouped into the EAP-1 ($n = 6$) subgroup, EAP-2 ($n = 6$) subgroup, Control-1 ($n = 8$) subgroup, and Control-2 ($n = 7$) subgroup.

Based on the qRT-PCR and ELISA results, we identified IL-1β as the target PDIC because it crossed the BBB to influence the CNS. Given 1-week recovery after implanting tubes onto bilateral PVN, rats in the EAP-1 subgroup received IL-1β inhibitor (Gevokizumab, XOMA, Berkeley, CA, USA) every day through PVN microinjection. Similarly, the Control-1 subgroup received recombinant rat IL-1β (Sangon Biotech, Shanghai, China). On the other hand, the EAP-2 and Control-2 subgroups only received aCSF.

Results showed that the EL of EAP-1 rats was significantly prolonged in the last three CBTs via a stepwise process ($P = 0.005$, $P < 0.001$, and $P =$

0.001, respectively; **Figure 4a**) compared to rats in the EAP-2 subgroup. Furthermore, the IF and IR values of rats in the EAP-1 subgroup were significantly increased in the last CBT (both $P < 0.001$). Rats in the Control-1 subgroup showed a significantly gradually shortened EL value compared to rats in the Control-2 subgroup in the last three CBTs ($P < 0.001$, $P < 0.001$, and $P = 0.005$, respectively; **Figure 4b**). Moreover, the IF and IR values of rats in the Control-1 subgroup were significantly decreased in the last CBT ($P < 0.001$ and $P = 0.001$, respectively). **Table 1** shows the details of these behavioral parameters.

Expression of NMDA NR1 subunit in the PVN
Immunohistochemical detection revealed that the average number of NR1(+) neurons in the PVN in four consecutive sections was significantly reduced in the EAP-1 rats compared to the EAP-2 rats (mean ± s.d.: 77.2 ± 7.2 *vs* 104.3 ± 6.3, $P < 0.001$). However, the number was higher in the Control-1 rats compared to the Control-2 rats (mean ± s.d.: 88.5 ± 9.4 *vs* 66.9 ± 5.9, $P < 0.001$; **Figure 5a** and **5b**). In addition, quantitative analyses of western blot bands showed that the expression ratio of NR1 to GAPDH was significantly lower in the EAP-1 rats than that in the EAP-2 rats (mean ± s.d.: 0.88 ± 0.09 *vs* 1.03 ± 0.13, $P = 0.043$), but the expression was significantly higher in the Control-1 rats than that in the Control-2 rats (mean ± s.d.: 0.90 ± 0.11 *vs* 0.72 ± 0.08, $P = 0.004$), as shown in **Figure 5c**.

DISCUSSION
EAP rats are mature animal models mimicking human CP/CPPS[9–12,20] and suffering the same PE symptom as CP/CPPS patients.[8] In our previous study, we proved that the persistent EAP-induced IIR can significantly upregulate the expression of NMDA receptors in the PVN.[8] Therefore, the main aim of this study was to identify the specific PDIC that can influence the CNS in EAP rats. The target PDIC should simultaneously meet the following three conditions: (1) upregulated expression in the prostate tissue of EAP rats (derived from the prostate); (2) significantly increased serum concentration (secreted into the peripheral blood); and (3) significantly increased CSF concentration (crossing the BBB).

First, we used qRT-PCR to screen out the target PDIC. Results showed that the mRNA levels of four pro-inflammation cytokines (*IL-1β*, *IL-6*, *IFN-γ*, and *TNF-α*) were significantly increased in the prostate tissues of EAP rats compared to the controls (**Figure 2b–2d**). We then performed ELISA to further detect and compare the protein levels of the four cytokines in the serum and CSF of the EAP and control groups. The final results showed that only the protein concentration of IL-1β was significantly increased in both the serum and CSF of the EAP group (**Figure 3**), indicating that IL-1β is not only released in large quantities into the peripheral circulation, but it can also cross the BBB into the CNS when chronic inflammation in the prostate persists. Therefore, we initially identified IL-1β as the target PDIC. Moreover, results showed that the protein level of IL-6 increased significantly in the serum of EAP rats but not in their CSF, indicating that it may be released into the peripheral circulation in large quantities instead of the CNS and is not the target PDIC. We also excluded IFN-γ due to the insignificant difference of its protein levels in both the serum and CSF between the two groups. Interestingly, the protein level of TNF-α was significantly increased in the CSF of EAP rats but not in their serum, suggesting that TNF-α can be excluded. However, this phenomenon seems to be difficult to explain.

According to previous studies,[21,22] TNF-α is initially synthesized as a membrane-bound protein (transmembrane TNF-α [tmTNF-α]) and is then cut off from the extracellular domain by a sheddase (TNF-α-converting enzyme [TACE]) to release the fully functional form (soluble TNF-α [sTNF-α]). sTNF-α is responsible for most of the adverse actions of TNF-α.[23] Wei *et al*.[24] reported that upregulated expression of IL-1β in the hypothalamus could be drivers of
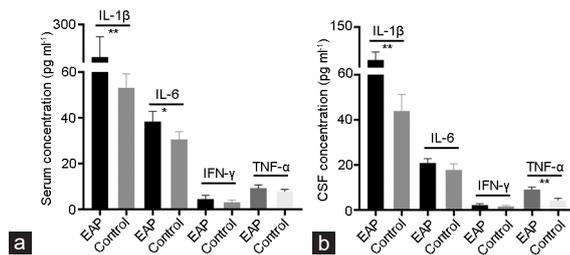
Figure 3: Comparison of protein levels of IL-1β, IL-6, IFN-γ and TNF-α in the (**a**) serum and (**b**) CSF between the two groups after successful modeling ($n = 5$ for each group). Data are presented as mean ± standard deviation; $^{*}P < 0.05$, $^{**}P < 0.01$. CSF: cerebrospinal fluid; EAP: experimental autoimmune prostatitis; IL-1β: interleukin-1β; IL-6: interleukin-6; IFN-γ: interferon-γ; TNF-α: tumor necrosis factor-α.
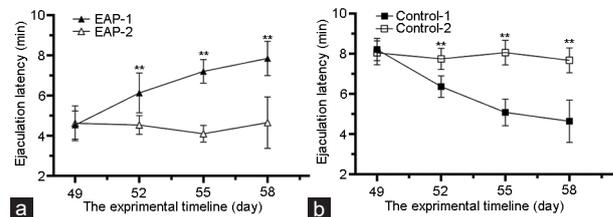
Figure 4: Subgroup comparisons of ejaculation latency in the last four copulatory behavior testing. (**a**) Comparisons between EAP-1 ($n = 6$) and EAP-2 ($n = 6$) subgroups. (**b**) Comparisons between Control-1 ($n = 8$) and Control-2 ($n = 7$) subgroups. Data are presented as mean ± standard deviation; $^{**}P < 0.01$. EAP: experimental autoimmune prostatitis.

Table 1: Comparisons of parameters of the last copulatory behavior testing session on the 58th day of the experimental timeline among the subgroups

Parameters	EAP group (mean±s.d.)		P	Control group (mean±s.d.)		P
	EAP-1 (n=6)	EAP-2 (n=6)		Control-1 (n=8)	Control-2 (n=7)	
ML (s)	13.4±1.5	15.5±2.8	0.148	14.6±2.3	13.9±1.3	0.487
MF	16.1±1.6	15.6±0.8	0.561	15.4±1.5	15.8±1.9	0.648
Intromission latency (s)	31.8±2.8	34.0±1.1	0.108	31.8±3.1	28.5±3.4	0.070
EL (min)	7.4±0.8	4.7±1.3	0.001**	5.2±1.9	7.9±0.9	0.005**
EF	2.6±0.3	3.0±0.3	0.059	2.8±0.4	2.4±0.2	0.072
PEI (min)	4.8±0.6	5.5±0.6	0.107	5.3±0.7	4.9±1.0	0.392
IF	15.8±1.6	9.9±1.3	<0.001**	10.4±1.8	16.7±2.3	<0.001**
IR	0.50±0.02	0.39±0.04	<0.001**	0.40±0.06	0.51±0.01	0.001**

IR=IF/(MF+IF). $^{**}P<0.01$. EAP: experimental autoimmune prostatitis; EF: ejaculation frequency; EL: ejaculation latency; IF: intromission frequency; IR: intromission ratio; MF: mount frequency; ML: mount latency; PEI: postejaculatory interval; s.d.: standard deviation
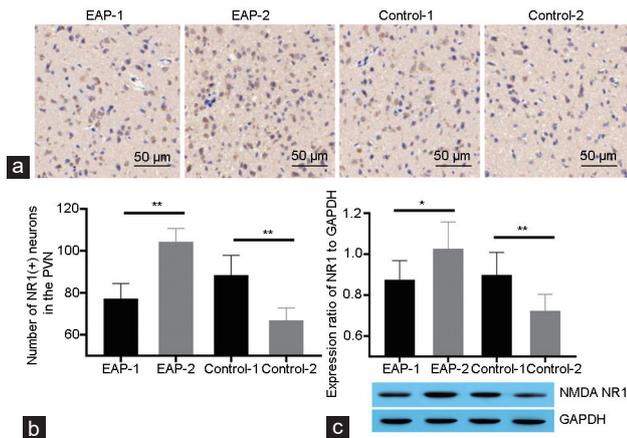
Figure 5: Subgroup comparisons of NMDA NR1 expression in punched PVN samples. (**a**) Immunohistochemistry detecting NR1 distribution (brown color) in the four subgroups; (**b**) a bar graph showing the average numbers of NR1(+) neurons in the PVN; (**c**) western blot analysis of NR1 normalized against GAPDH in the PVN ($n = 6$ rats in the EAP-1 and EAP-2 groups each, $n = 8$ in the Control-1 and $n = 7$ in the Control-2). $^*P < 0.05$, $^{**}P < 0.01$. EAP: experimental autoimmune prostatitis; GAPHD: glyceraldehyde-3-phosphate dehydrogenase; NMDA NR1: N-methyl-D-aspartic acid NR1; PVN: paraventricular nucleus of the hypothalamus.

TACE activity in the brain. In addition, Yu *et al.*[25] found that intracerebroventricular injection of IL-1β increased TACE expression by neurons or glial cells in the PVN and subfornical organ of normal rats. Based on the above findings, we speculate that the significantly increased TNF-α level in the CSF of EAP rats is not directly derived from the prostate but is due to the indirect cause of massively released IL-1β crossing the BBB into the CNS when chronic prostatitis persists. Therefore, the phenomenon, TNF-α protein concentration increasing significantly in the CSF of EAP rats but not in the serum, should be further evidence that IL-1β is the target PDIC.

Increased IL-1β synthesis has also been found in CP/CPPS clinical studies[26,27] and in animal models.[9,28] Consistent with our results, Hu *et al.*[9] found elevated IL-1β in the CSF of CP/CPPS rats, which can interact with its receptors in specific brain areas, thereby leading to CP/CPPS-related anxiety, depression, and spatial and associative memory impairment. Moreover, Leonard and Song[29] suggested that IL-1β could be transported from peripheral blood into the brain by an active transport mechanism. Collectively, all these results support the fact that prostate-derived IL-1β is the target PDIC.

Although we have confirmed that IL-1β can cross the BBB into the CNS in large quantities, it is not known whether it contributes to the pathogenesis of PE. To explore this issue, we contrived to adopt direct central administration of recombinant IL-1β by implanting tubes and microinjection onto the bilateral PVN of some control rats (the Control-1 subgroup). Results indicated that the Control-1 subgroup had a significantly gradually shortened EL (**Figure 4b**) from the fourth day of daily administration, and on the tenth day, it was found to be similar to the value of EAP-2 rats that were only administered with aCSF (**Table 1**). Meanwhile, we also adopted central administration of gevokizumab (IL-1β inhibitor) to treat some EAP rats (EAP-1 subgroup), with results showing a significantly gradually prolonged EL value (**Figure 4a**). On the tenth day of administration, the EL of EAP-1 subgroup seemed to have recovered to the level of Control-2 rats that only received aCSF (**Table 1**). Therefore, these two mutually corroborating results confirmed the role of IL-1β in influencing the CNS to induce EL shortening.

Zhu *et al.*[30] reported that IL-1β could stimulate chronic release of excitatory neurotransmitters, activate NMDA receptors, and evoke neuronal hyperexcitability in the brain. In addition, IL-1β may negatively regulate GABA receptors and inhibit K^+ efflux.[31,32] These findings suggest that the high concentration of IL-1β in the cortex and thalamus contributes to sympathetic excitation. However, our results reveal some new findings that differ from the above reported results. We discovered that the expression of PVN NMDA receptors could be significantly upregulated on the tenth day after central administration of IL-1β, which could be inhibited by gevokizumab, an IL-1β inhibitor (**Figure 5b** and **5c**). Combined with the findings of our previous animal studies which showed that injecting traces of NMDA into the PVN can significantly increase lumbar splanchnic nerve activity,[18,19] the present results seem to be a good explanation why IL-1β can enhance SNS sensitivity. Furthermore, SNS hyperactivity has been confirmed to be closely associated with primary PE after measuring penile-skin sympathetic response in our previous clinical studies.[33,34] However, the mechanism through which IL-1β upregulates NMDA receptors in the PVN has not yet been elucidated.

There are two main limitations in this study. First, only 11 of the most common pro-inflammatory cytokines were explored as candidates in the first step of screening target PDICs using qRT-PCR. Therefore, it was difficult to detect and compare more inflammatory-related cytokines with high flux, which probably leads to the omission of target PDICs. Further studies should adopt inflammatory-cytokine expression profiling microarrays to expand the screening range. Second, the specific mechanism through which IL-1β upregulates NMDA receptors in the PVN has not been clarified. Subsequent studies may employ IL-1β as the chronic stimulation of primary cultured PVN neurons *in vitro* to elucidate the mechanism.

CONCLUSION

This study has shown that chronic inflammation of the prostate can release a large amount of IL-1β into the peripheral circulation, which further crosses the BBB into the CNS. In the PVN, IL-1β significantly upregulates the expression of NMDA receptors to enhance SNS sensitivity and then shortens the EL, and this phenomenon can be reversed by central administration of IL-1β inhibitors. Therefore, we identified IL-1β as the primary PDIC which shortened EL in EAP rats. However, further studies should be conducted to elucidate the specific molecular mechanisms through which IL-1β upregulates the expression of NMDA receptors.

AUTHOR CONTRIBUTIONS

JY and JDX designed this experiment. JY, JCL, QJZ, YMW, GQZ, and NHS performed the research. Statistical analyses were conducted by JY, JCL, ZJW, and JXX. JY wrote the first draft of the manuscript. JDX and JHC revised the manuscript for intellectual content. All authors reviewed, edited, read, and approved the final manuscript.

COMPETING INTERESTS

All authors declare no competing interests.

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Supplementary Information is linked to the online version of the paper on the *Asian Journal of Andrology* website.

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Supplementary Table 1: Primer sequences of the detected inflammation cytokines and endogenous control

Genes	Primers from the 5′- to 3′-ends
IL-1α	Forward: TGAGTCGGCAAAGAAATCAA
	Reverse: GACAGATGGTCAATGGCAGA
IL-1β	Forward: GCCAACAAGTGGTATTCTCCA
	Reverse: CCGTCTTTCATCACACAGGA
IL-4	Forward: ACCTTGCTGTCACCCTGTTC
	Reverse: GTGTTCCTTGTTGCCGTAAG
IL-6	Forward: TCCTACCCCAACTTCCAATGCTC
	Reverse: CCAACTGACTTTGAGCCAACGAG
IL-8	Forward: AGACAGTGGCAGGGATTCAC
	Reverse: GAGTGTGGCTATGACTTCGGT
IL-10	Forward: AGAAGCTGAAGACCCTCTGGATAC
	Reverse: GCTCCACTGCCTTGCTTTTATT
IL-13	Forward: CTCAGGGAGCTTATCGAGGA
	Reverse: GCAACTGGAGATGTTGGTCA
IFN-γ	Forward: CATCGCCAAGTTCGAGGTGA
	Reverse: TCTGGTGACAGCTGGTGAATC
MCP-1	Forward: ATGCAGTTAATGCCCCACTC
	Reverse: TTCCTTATTGGGGTCAGCAC
MIP-1α	Forward: CATTCCTGCCACCTGCAAAT
	Reverse: CAAGTGAAGAGTCCCTGGATGTG
TNF-α	Forward: TGCCTCAGCCTCTTCTCATT
	Reverse: GCTTGGTGGTTTGCTACGAC
GAPDH	Forward: GCCTTCCGTGTTCCTA
	Reverse: AGACAACCTGGTCCTCA

IL: interleukin; IFN: interferon; MCP: monocyte chemotactic protein; MIP: macrophage inflammatory protein; TNF: tumor necrosis factor; GAPDH: glyceraldehyde phosphate dehydrogenase