

반복 모성 분리가 이유 전기 백서 뇌의
Glucocorticoid Receptor와 Cyclooxygenase-2에
미치는 영향*

THE EFFECT OF REPEATED MATERNAL SEPARATION ON
GLUCOCORTICOID RECEPTOR AND CYCLOOXYGENASE-2
IN PREWEANLING RAT BRAIN

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목 적 :

가

반복 모성 분리를 경험한 Sprague-Dawley 태아는 모성 분리를 경험하지 않은 Sprague-Dawley 태아에 비해 뇌에서 Glucocorticoid Receptor (GR)와 Cyclooxygenase-2 (COX-2)의 발현이 증가하는 것으로 나타났다.

방 법 : (태아) 8 Sprague-Dawley 4
반복 모성 분리를 경험한 태아 4, 모성 분리를 경험하지 않은 태아 4, 총 8마리의 Sprague-Dawley 태아를 사용하였다. 태아를 사육한 후, 뇌를 채취하여 COX-2와 GR의 발현을 측정하였다.

결 과 : GR 발현이 DG(785.75 ± 65.11, 1183.63 ± 111.00)에서 증가하였다. COX-2 발현이 CA1(32.25 ± 2.85, 81.75 ± 12.05), CA3(27.25 ± 3.48, 41.62 ± 3.36)에서 증가하였다. DG(104.38 ± 11.98, 167.25 ± 22.72)에서 COX-2와 GR의 발현이 증가하였다.

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GR COX-2

가

결론:

가 COX-2 GR ,
가 DG

GR COX-2가 가 , DG가

중심 단어:

· Glucocorticoid receptor · Cyclooxygenase - 2.

3).

서론

가 2)8),

(種)

가 1).

(maternal depriva-

tion)

(maternal separation)

가 1-3).

decarboxylase

1)10), DNA

9), ornithine

serotonin(5-HT), norepinephrine, dopamine

11), c-myc, max

10), im-mediate early genes(IEGs) c-Fos NGFI-B 12)

4-6).

가

hypothalamic - pituitary - adrenal axis(HPA axis)

가 ,

HPA axis

, Maeney

HPA

3).

가

HPA

2)3)7).

13). Glucocorticoid HPA axis
가 , GR glucocorticoid
HPA axis

가 가 .

24

14). GR

HPA axis

가

¹⁴⁾ cyclooxygenase - 2(COX - 2)
(inducible form) cyclooxygenase
(endotoxin), inflammatory cyto-
kine,
¹⁵⁾ COX - 2

가 Alzheimer 's disease,
COX - 2 가가

¹⁶⁾¹⁷⁾ COX - 2

가 COX - 2

가 HPA axis

¹⁸⁾ GR COX - 2
¹⁵⁾¹⁹⁾

가

4 10 4

GR COX - 2
가

COX - 2
가

방 법

1. 실험동물
Rat pups 8 Sprague -

Dawley (Harlan Sprague Dawley, Inc. Indiana-
polis, Indiana) 20~
24 , 12 /
3
4
4
(N=4)
(N=4) 10 3
7 4

2. 뇌조직 처리

14 pentobarbital sodium(20
mg/kg)
0.05M (phosphate buf-
fered saline, PBS) 1
(0.1M) 4% paraformaldehyde - 0.1% glutar-
aldehyde (4) 10
50~60ml/min
4 6mm
가 4 12~16
30% sucrose 2~5
Cryostat(American Optical) 40 μm
GR COX -
2 8
. GR COX - 2
(free - floating)

3. GR 및 COX-2의 면역조직화학 염색

peroxid-
ase PBS 1% H2O2
15 10 3 PBS
, rabbit anti - rat GR(Santa Cruz Biotechnology
Inc., USA) 1 : 500 1% bovine
serum albumin, 10% goat serum, 0.3% Triton
X - 100 1 24 4
. 1
PBS 10 3 , 2 (Ve-
ctastatin - Elite kit biotinylated anti - rabbit IgG
1 : 200 , 0.3% Triton X - 100) 1

GR COX - 2

2 PBS 10 3
 avidin - biotin - peroxidase complex (Vectastain - Elite kit A 1 : 100, B 1 : 100, 0.3% Triton X - 100) 1
 3,3'-diaminobenzidine tetrahydrochloride(DAB) 0.05M Tris 0.02%, H₂O₂ 0.003% 5
 PBS 10 3
 gelatin - coated slide
 2 ethanol
 가 xylene po-
 lymount
 COX - 2 , 1
 goat anti - rat COX - 2(Santa Cruz Biotechnology Inc., USA) 1 : 2000 1%
 bovine serum albumin, 10% rabbit serum, 0.3% Triton X - 100 , 2
 Vectastatin - Elite kit biotinylated anti - rabbit IgG

1 : 200 0.3% Triton X - 100
 . DAB 3

4. 조직관찰 및 영상 분석

GR COX - 2
 DAB
 COX - 2 GR
 18) CA1, CA2, dentate gyrus(DG)

GR COX - 2

Franklin

Paxinos

20)

5. 통계학적 분석

± (mean ± standard error)
 SPSS for windows ver. 10.0
 GR
 COX - 2 Mann - Whitney test

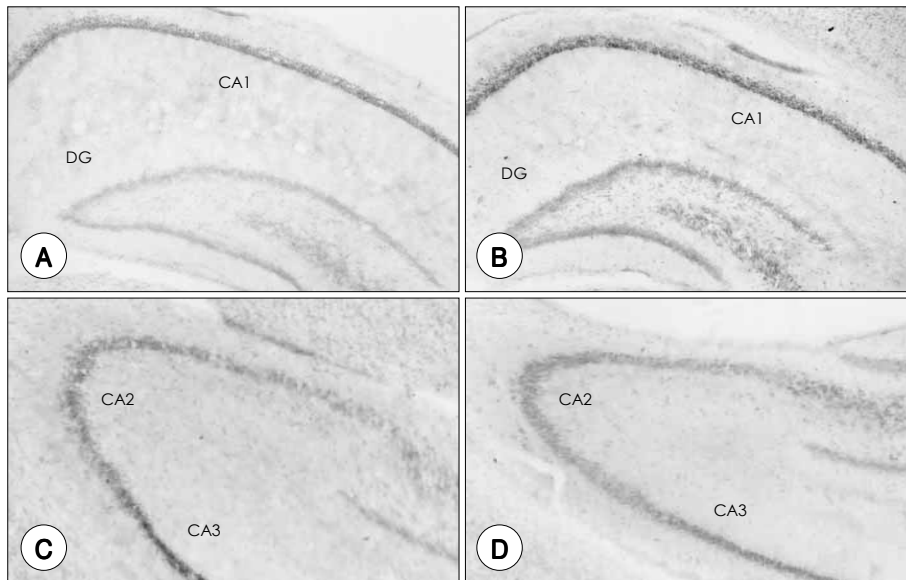


Fig. 1. Microscopic photographs depicting GR concentration in the hippocampus of the Control(A,C) and Repeated Maternal Separation(B, D) groups (×50). GR-immunopositive cells, colored dark brown by DAB, were observed in the CA1, CA2, CA3 and DG regions of the hippocampus. The number of GR-immunopositive cells was significantly higher only in the DG(dentate gyrus) of the Repeated Maternal Separation group(1183.63 ± 111.00) than in Control group(785.75 ± 65.11). Overall GR expression was still increased also in CA1 and CA3 of the hippocampus in the Repeated Maternal Separation group.

p<.05

결 과

1. GR의 발현

GR DAB

Table 1. Number of GR-immunopositive cells in each area of hippocampus (Mean \pm Standard Error) in Control group (CG) and Repeated Maternal Separation group (RMS)

Region of Hippocampus	GR		
	CG	RMS	p-value
CA1	1203.88 \pm 105.98	1290.88 \pm 104.06	0.60
CA2	129.63 \pm 11.33	120.38 \pm 6.26	0.53
CA3	209.75 \pm 29.45	220.25 \pm 14.61	0.75
DG	785.75 \pm 65.11	1183.63 \pm 111.00	0.01*

p-values were obtained by Mann-Whitney test. * : p<.05. GR : Glucocorticoid Receptor, CG : Control group, RMS : Repeated maternal separation group, DG : Dentate gyrus

GR

Fig. 1

GR CA1
1203.88 \pm 105.98 (Mean \pm Standard Error) ,
1290.88 \pm 104.06 , CA2 129.64
 \pm 11.33 , 120.38 \pm 6.26 , CA3
209.75 \pm 29.45 , 220.25 \pm 14.61 ,
DG 785.75 \pm 65.11 , 1183.63 \pm
111.00 GR
, DG
(p<.05)(Table 1).

2. COX-2의 발현

COX - 2 DAB

COX - 2

Fig. 2

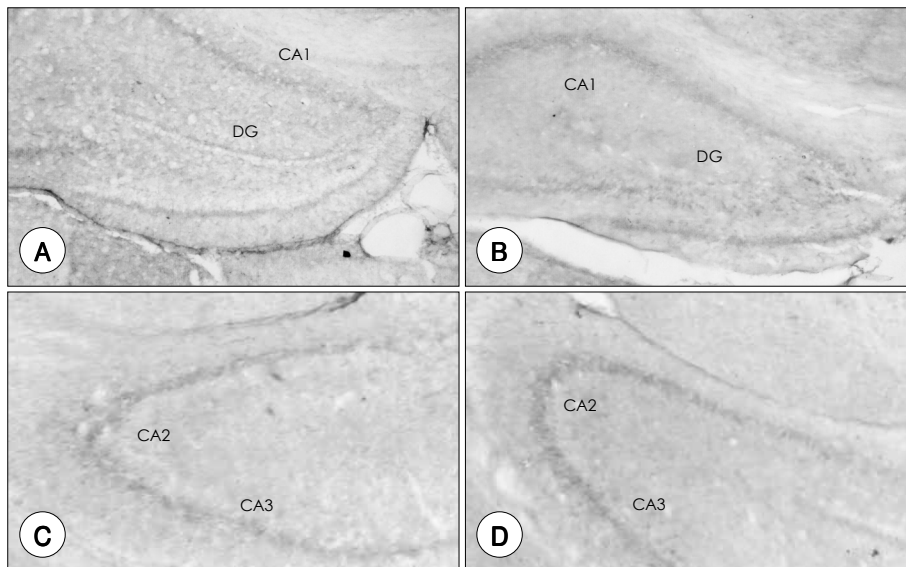


Fig. 2. Microscopic photographs depicting COX-2 concentration in the hippocampus of the Control (A, C) and Repeated Maternal Separation (B, D) groups (\times 50). COX-2-immunopositive cells, colored dark brown by DAB, were observed in the CA1, CA2, CA3 and DG regions of the hippocampus. The number of COX-2-immunopositive cells in the CA1 and CA3 regions of the Repeated Maternal Separation group (81.75 \pm 12.05 and 41.63 \pm 3.36 respectively) were significantly increased when compared to the Control (32.25 \pm 2.85 and 27.25 \pm 3.48 respectively).

GR COX-2

Table 2. Number of COX-2-immunopositive cells in each area of hippocampus (Mean (Standard Error) in Control group (CG) and Repeated Maternal Separation group (RMS)

Region of Hippocampus	COX-2		
	CG	RMS	p-value
CA1	32.25 ± 2.85	81.75 ± 12.05	0.00*
CA2	14.63 ± 2.15	25.63 ± 4.81	0.09
CA3	27.25 ± 3.48	41.63 ± 3.36	0.02*
DG	104.38 ± 11.98	167.25 ± 22.72	0.06

p-values were obtained by Mann-Whitney test. * : p<0.05. COX-2 : Cyclooxygenase-2, CG : Control group, RMS : Repeated maternal separation group, DG : Dentate gyrus

COX-2, CA1
 32.25 ± 2.85 (Mean ± Standard error),
 82.75 ± 12.05, CA2 14.63 ± 2.15
 , 25.63 ± 4.81, CA3 27.25 ±
 3.48, 41.63 ± 3.36, DG
 104.38 ± 11.98, 167.25 ± 22.72
 GR
 , CA1, CA3
 , DG
 (p<.05) (Table 2).

고 찰

, GR 가
 , DG GR
 가 HPA axis
 HPA axis
 18) 가 가
 , HPA axis
 , HPA axis
 , HPA axis cate-
 cholamine
 18)
 가 HPA
 ,
 21)

2~3
 (“handling”
 “handling”
 GR
 가 HPA
 axis
 GR
 14
 (stress nonrespon-
 sive period)
 HPA axis가
 24)
 14
 HPA axis가
 24) Gilles 5) 7
 HPA axis가
 , 7 2
 corticosterone 가 가 . Suchecki
 25) 6, 9, 12 24
 ACTH
 가 ,
 가 가
 14 HPA axis
 가 가
 HPA axis
 , CRH, ACTH, corticosterone
 , GR
 , GR
 14)
 , HPA axis
 corticosterone GR
 (reactive) , HPA
 26), GR 가 HPA
 Herman

27) , GR glucocorticoid 5-HT , GR (neuronal integrity) , GR glucocorticoid neurogenesis 18) , 6)28) , 15)29) Alzheimer's disease, pyramidal cell layer, COX-2 가 16) , 17) COX-2 가 , Cyclooxygenase prostaglandin thromboxane , COX-1 , COX-2 가 isoform 15) COX-1 constitutive form IEG , COX-2 indu-cible form 15) COX-2 DG GR COX-2 가 (neural plasticity) DG 15) COX-2 periven-tricular nucleus, 15)19) HPA axis 2 26)30) steroid COX-2 aride cytokine , bacterial lipopolysacch- aride forced cold , 가 15) , GR water swim , , COX-2가 30) , GR COX-2 upregulation , COX-2 northern blotting in situ hybridization

GR COX - 2
 axis 가 , GR COX - 2가 HPA 가
 , DG rat pups
 가 , glu- statisti-
 cocorticoid가 GR COX - 2 cal power
 가 .

References

4-6)
 가
 4)28)
 28)
 7)
 HPA axis
 7)
 GR
 COX - 2 가
 COX - 2가 가
 COX - 2가
 가 ,
 COX - 2가
 가
 GR COX - 2
 가
 rat pups

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IN PREWEANLING RAT BRAIN**

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Objectives : The effects of repeated maternal separation on the expression of glucocorticoid receptor (GR) and cyclooxygenase-2 (COX-2) in the hippocampus of rat pups at preweanling stage were evaluated.

Methods : The experimental, Repeated Maternal Separation group (N=4) was separated from the mother for four hours a day over a period of ten days beginning with postnatal day 4. The Control group (N=4), on the other hand, did not separated from the mother at all. GR and COX-2 expression in the hippocampus was examined by immunohistochemistry on postnatal day 14.

Results : It was determined that the number of GR-immunopositive cells in the dentate gyrus of the hippocampus was significantly increased in the Repeated Maternal Separation group. The numbers of COX-2-immunopositive cells in the CA1 and CA3 were also significantly higher in the Repeated Maternal Separation group.

Conclusion : These results suggest that maternal separation may be a significant developmental stress that induces GR and COX-2 expression in the hippocampus of developing pups.

KEY WORDS : Maternal separation · Stress · Glucocorticoid receptor · Cyclooxygenase-2.