

The effect of ACTH4-10Pro⁸-Gly⁹-Pro¹⁰ on neurotrophin-3 expression in Sprague Dawley rat on acute spinal cord injury



Made Gemma Daniswara Maliawan¹, Eko Agus Subagio^{1*}, Budi Utomo²,
Muhammad Arifin Parenrengi¹, Asra Al Fauzi¹, I Ketut Sudiana³

ABSTRACT

Background: Neurotrophic factors such as NT-3 play an important role in spinal cord regeneration, contributing to spinal cord regeneration assisting functional improvement in SCI. ACTH4-10Pro⁸-Gly⁹-Pro¹⁰, an analog of the N-terminal fragment (4-10) adrenocorticotrophic hormone, is one of the neuroprotective compounds that can increase NT-3 levels in brain ischemia. Therefore, we conducted a study with experimental animals to determine the effect of NT-3 expression on ACTH4-10Pro⁸-Gly⁹-Pro¹⁰ administration in acute compression SCI.

Method: We used Sprague Dawley rats with acute compression SCI model using 20 gr and 35 gr aneurysm clips. ACTH4-10Pro⁸-Gly⁹-Pro¹⁰ was administered intranasally to the treatment group, and 0.9% NaCl was administered intranasally to the positive control group. Both of the groups then terminated at 3 and 6 hours.

Results: In rats with mild SCI that were given ACTH4-10Pro⁸-Gly⁹-Pro¹⁰, the NT-3 expression after 3 hours and 6 hours was 14 (12-17) and 10 (7-13). In rats with severe SCI given ACTH4-10Pro⁸-Gly⁹-Pro¹⁰, the NT-3 expression after 3 hours and 6 hours was 9 (6-11) and 8 (7-10). Intranasal administration of ACTH4-10Pro⁸-Gly⁹-Pro¹⁰ showed higher NT-3 expression than the group with 0.9% NaCl in mild and severe SCI.

Conclusion: Intranasal administration of ACTH4-10Pro⁸-Gly⁹-Pro¹⁰ can increase NT-3 expression in mild and severe acute SCI at 3 and 6 hours. Expression of NT-3 in the group with ACTH4-10Pro⁸-Gly⁹-Pro¹⁰ mild acute SCI was higher than in the group with severe acute SCI. Further research needs to be done to determine the neuroregeneration effect of ACTH4-10Pro⁸-Gly⁹-Pro¹⁰ on SCI.

Keywords: spinal cord injury, central nervous system, neurotrophin-3.

Cite This Article: Maliawan, M.G.D., Subagio, E.A., Utomo, B., Parenrengi, M.A., Fauzi, A.A., Sudiana, I.K. 2022. The effect of ACTH4-10Pro⁸-Gly⁹-Pro¹⁰ on neurotrophin-3 expression in Sprague Dawley rat on acute spinal cord injury. *Bali Medical Journal* 11(1): 18-22. DOI: 10.15562/bmj.v11i1.3143

¹Department of Neurosurgery, Faculty of Medicine, Universitas Airlangga, Dr. Soetomo General Hospital, Surabaya, Indonesia;

²Department of Public Health and Preventive Medicine, Faculty of Medicine, Universitas Airlangga, Dr. Soetomo General Hospital, Surabaya, Indonesia;

³Department of Anatomical Pathology, Faculty of Medicine, Universitas Airlangga, Dr. Soetomo General Hospital, Surabaya, Indonesia;

*Corresponding author:

Eko Agus Subagio;
Department of Neurosurgery, Faculty of Medicine, Universitas Airlangga, Dr. Soetomo General Hospital, Surabaya, Indonesia;

eko.agus@fk.unair.ac.id

Received: 2022-11-25

Accepted: 2022-01-26

Published: 2022-02-04

INTRODUCTION

Spinal cord injury (SCI) is an injury responsible for most disabilities, ranging from damage to sensory and motor functions to multiorgan dysfunction. The incidence of SCI in developing countries ranges from 13.1 to 163.4 per million population with prevalence rates between 490 to 560 per million population.¹ In Indonesia, there were 104 SCI cases registered at Fatmawati General Hospital in 2014, with the most common etiology being traffic accidents and falling from a height.²

The highest incidence of traumatic SCI occurs in men of productive age with an average age at the time of injury between 32 – 55.4 years, 37 – 47.9 years, and 26.8– 56.6 years, for North America, Europe,

and Asia, respectively.³ The high incidence of SCI at a young age certainly impacts the emergence of serious economic loss for families, communities, and countries. Functional defects caused by SCI significantly impact decreasing quality of life and patient life expectancy.⁴

Nerve cells in the central nervous system (CNS) in humans have a very limited regeneration response that does not allow for complete healing after injury.⁵ However, it is possible to develop neuroprotective therapies to maximize the functional integrity of the spinal cord remaining after injury.⁶ The most important thing in the treatment of spinal cord injury is how to achieve axonal regeneration in the damaged area.⁷ NT-3 is important for developing and maintaining

neuronal populations and promotes differentiation. Also, NT-3 is important in forming the substantia nigra and pyramidal pathways responsible for motor activity and have the best response in corticospinal axon regeneration compared to other neurotrophins, even considered superior.⁸

A neuroprotective compound, ACTH4-10Pro⁸-Gly⁹-Pro¹⁰, also known as heptapeptide Semax an analog of the N-terminal fragment (4-10) of adrenocorticotrophic hormone, is known to enhance the transcription of NT-3 mRNA 24 hours after the ischemia of the rat brain cortex.⁹ ACTH4-10Pro⁸-Gly⁹-Pro¹⁰ has also been shown to be effective in treating vascular disease, allergic and toxic inflammation, and partial atrophy

of the human optic nerve.¹⁰ Intranasal administration of ACTH4-10Pro⁸-Gly⁹-Pro¹⁰ in rats with SCI showed an anti-inflammatory response expected to prevent secondary injury to nerve.^{11,12} Our study was aimed to determine the effect of ACTH4-10Pro⁸-Gly⁹-Pro¹⁰ administration on the expression of NT-3 in the spinal cord after mild and severe compression SCI.

METHOD

This study used a spinal cord injury model of male Sprague Dawley rats, weighing 250-300 g. Forty-five samples were divided into nine groups of five samples each. One group became the control group with the spinal cord was left uninjured as a baseline. The rest became the treatment group in which laminectomy at the level of 2nd thoracic vertebra was performed, followed by spinal cord compression using an aneurysm clip with a clamping force of 20 g for mild SCI (4 groups) and 35 g for severe SCI (4 groups) in 1 minute. The laminectomy site was then closed with sutures.

Each treatment group was divided into two subgroups. Positive control groups were given 0.9% NaCl intranasally, while the treatment groups were given ACTH4-10Pro⁸-Gly⁹-Pro¹⁰ intranasally at a 300 mg/kg dose. Then each group was divided again into two groups to be terminated, and then myelum transection was performed at 3 hours and 6 hours after compression. The preparations were fixed in formalin and examined for IHC. The NT-3 was calculated per 100 cells using associated anti-monoclonal antibodies and viewed with a light microscope using a 1000x magnification. Cells that showed positive expression gave the results of a brown color image in the cell cytoplasm (Figure 1).

Data collection is carried out in a controlled environment, assuming that all conditions are managed equally and controlled. Data of NT-3 level was presented in a relative expression graph. Data normality analysis was performed using Shapiro-Wilk, and then the collected data were analyzed using the nonparametric Kruskal-Wallis test with Mann-Whitney difference test.

RESULTS

The result of the normality test with the Shapiro-Wilk method showed abnormal data distribution with the Kruskal-Wallis test found significant differences ($p < 0,001$) between groups (table 1). In rats with mild SCI given ACTH4-10Pro⁸-Gly⁹-Pro¹⁰, the NT-3 expression after 3 hours and 6 hours was 14 (12-17) and 10 (7-13). In rats with severe SCI given ACTH4-10Pro⁸-Gly⁹-Pro¹⁰, the median NT-3 expression after 3 hours and 6 hours was 9 (6-11) and 8 (7-10).

Mann-Whitney test was carried out to pinpoint the differences between groups. Overall NT-3 expressions are shown in figure 2. Except between the control group and group administered with NaCl 0,9% 3 hours, the median expression of NT-3 was significantly different between all comparison groups. Overall, it appears that administration of ACTH4-10Pro⁸-Gly⁹-Pro¹⁰ induced higher NT-3 expression compared to NaCl 0,9%. Also, the expression of NT-3 appeared to increase over time in groups treated with NaCl 0,9% but significantly decreased overtime in groups treated with ACTH4-10Pro⁸-Gly⁹-Pro¹⁰.

Higher expression of NT-3 was also achieved significantly in ACTH4-10Pro⁸-Gly⁹-Pro¹⁰ groups with severe SCI

compared to NaCl 0,9% group at 3 hours and 6 hours after compression ($p < 0,05$) as shown in table 3. However, the time factor appears to be less significant in severe SCI because no difference was found when comparing the group with NaCl 0,9% at 3

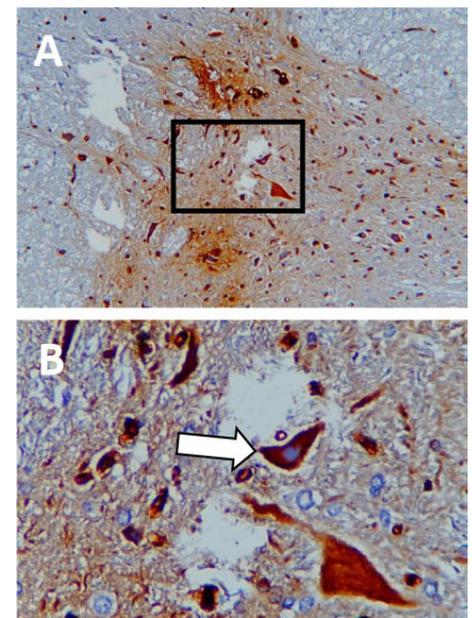


Figure 1. Neurons expressing NT-3 react with anti-NT-3, marked by brown cytoplasm (arrow) by IHC. A) 100x magnification. B) 400x magnification in the box marked area in figure A.

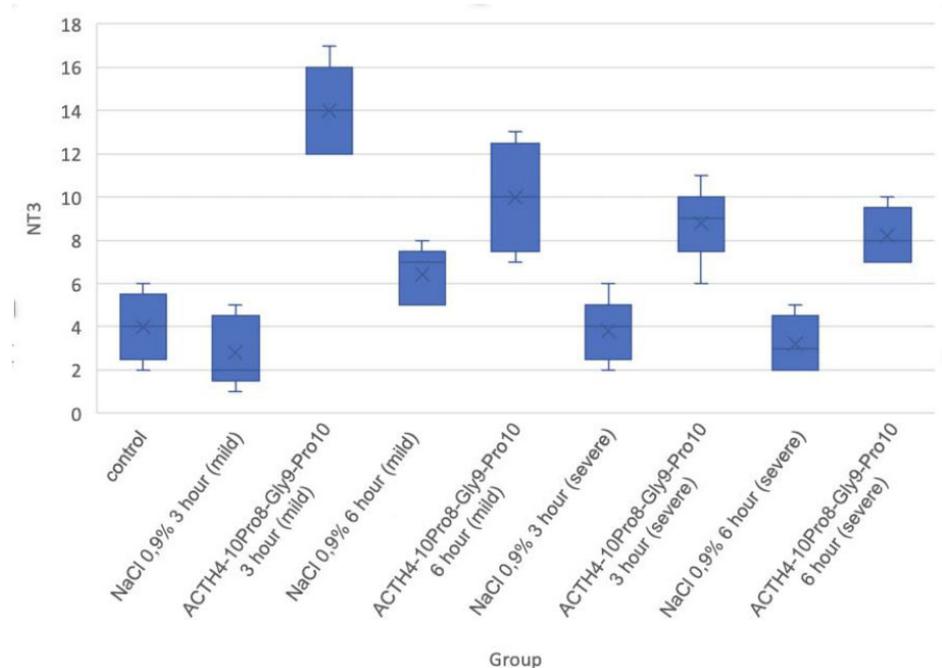


Figure 2. Median value of NT-3 expression.

hours and 6 hours and ACTH4-10Pro⁸-Gly⁹-Pro¹⁰ at the same time frame.

Our study found significantly higher NT-3 expression in the ACTH4-10Pro⁸-Gly⁹-Pro¹⁰ group with mild SCI at 3 hours compared to groups with severe SCI at both 3 hours and 6 hours shown in table

4. However, at 6 hours, the differences were not significant anymore. Therefore, it seems that the NT-3 inducing effect of ACTH4-10Pro⁸-Gly⁹-Pro¹⁰ is optimum at 3 hours after administration in the mild SCI, and the effect is sustained or slightly reduced over time.

DISCUSSION

SCI causes an imbalance in the microenvironment that can exacerbate and accelerate nerve damage, impairing regeneration and functional recovery.¹³ The balance between proneurotrophins and neurotrophins is disrupted after SCI,

Table 1. NT-3 expression in all study groups.

Groups	NT3 Expression (cells/visual fields)			P
	Median	Min	Max	
Control	4	2	6	
ACTH4-10Pro ⁸ -Gly ⁹ -Pro ¹⁰ 3 hours mild	14	12	17	
ACTH4-10Pro ⁸ -Gly ⁹ -Pro ¹⁰ 6 hours mild	10	7	13	
NaCl 0,9% 3 hours mild	2	1	5	
NaCl 0,9% 6 hours mild	7	5	8	<0,001*
ACTH4-10Pro ⁸ -Gly ⁹ -Pro ¹⁰ 3 hours severe	9	6	11	
ACTH4-10Pro ⁸ -Gly ⁹ -Pro ¹⁰ 6 hours severe	8	7	10	
NaCl 0,9% 3 hours severe	4	2	6	
NaCl 0,9% 6 hours severe	3	2	5	

Table 2. Comparison of NT-3 expression in rats with mild SCI.

Groups	Median	Groups	Median	p
Control	4	NaCl 0,9% 3 hours	2	0,242
		ACTH4-10 3 hours	14	0,009*
		NaCl 0,9% 6 hours	7	0,044*
		ACTH4-10 6 hours	10	0,009*
NaCl 0,9% 3 hours	2	ACTH4-10 3 hours	14	0,009*
		NaCl 0,9% 6 hours	7	0,014*
		ACTH4-10 6 hours	10	0,009*
ACTH4-10 3 hours	14	NaCl 0,9% 6 hours	7	0,008*
		ACTH4-10 6 hours	10	0,045*
NaCl 0,9% 6 hours	7	ACTH4-10 6 hours	10	0,033*

Table 3. Comparison of NT-3 expression in severe SCI.

Groups	Median	Groups	Median	p
Control	4	NaCl 0,9% 3 hours	4	0,831
		ACTH4-10 3 hours	9	0,011*
		NaCl 0,9% 6 hours	3	0,393
		ACTH4-10 6 hours	8	0,009*
NaCl 0,9% 3 hours	4	ACTH4-10 3 hours	9	0,011*
		NaCl 0,9% 6 hours	3	0,519
		ACTH4-10 6 hours	8	0,009*
ACTH4-10 3 hours	9	NaCl 0,9% 6 hours	3	0,008*
		ACTH4-10 6 hours	8	0,517
NaCl 0,9% 6 hours	3	ACTH4-10 6 hours	8	0,009*

Table 4. Comparison of NT-3 expression in mild and severe SCI.

Kelompok	Median	Kelompok	Median	p
ACTH4-10 3 hours mild	14	ACTH4-10 6 hours mild	10	0,045*
		ACTH4-10 3 hours severe	9	0,008*
		ACTH4-10 6 hours severe	8	0,009*
ACTH4-10 6 hours mild	10	ACTH4-10 3 hours severe	9	0,459
		ACTH4-10 6 hours severe	8	0,242
ACTH4-10 3 hours severe	9	ACTH4-10 6 hours severe	8	0,517

resulting in increased proneurotrophins and decreased neurotrophins, leading to cellular apoptosis, reducing synaptic plasticity, promoting the inflammatory response, and degeneration.¹⁴

NT-3, BDNF, and NGF expression decrease significantly as early as 6 hours after spinal cord contusion in rat model.¹⁵ Our study showed that NT-3 expression in rats with mild SCI was significantly higher in ACTH4-10Pro⁸-Gly⁹-Pro¹⁰ groups compared to NaCl 0,9% group at 3 hours and 6 hours after compression ($p < 0,05$), as shown in table 2. Higher expression of NT-3 was also achieved significantly in ACTH4-10Pro⁸-Gly⁹-Pro¹⁰ groups with severe SCI compared to NaCl 0,9% group at 3 hours and 6 hours after compression ($p < 0,05$), as shown in table 3. ACTH4-10Pro⁸-Gly⁹-Pro¹⁰ is known to act by binding to the melanocortin receptor, such as MC4R found in the spinal cord.¹⁶ In vitro studies on astrocytes showed that activation of the MC4R receptor can increase the expression of BDNF through the cAMP-PKA-CREB.¹⁷ A previous study in rats showed that administration of ACTH4-10Pro⁸-Gly⁹-Pro¹⁰ intranasally can increase BDNF expression in 3 hours after SCI.¹⁸ The mechanism for increasing NT-3 expression in this study has not been elucidated, but it is possible to have the same mechanism as the increase in BDNF.

Our study found significantly higher NT-3 expression in the ACTH4-10Pro⁸-Gly⁹-Pro¹⁰ group with mild SCI than in the ACTH4-10Pro⁸-Gly⁹-Pro¹⁰ group with severe SCI at 3 hours after injury ($p < 0,05$) as shown in table 4. Nerve damage that occurs after SCI can result from primary or secondary injury. More severe injury would result in more severe primary damage, causing a more severe imbalance at the tissue, cellular, and molecular levels,

which could affect the expression of NT-3. Intranasal administration of ACTH4-10Pro⁸-Gly⁹-Pro¹⁰ in rats with SCI model also showed anti-inflammatory effects by reducing the expression of IL-1, TNF- α , NF-KB, neutrophil, and induced reduction in the apoptotic mechanism through increased ratio of Bcl-2/HSP70.^{11,12,19}

Our study's peak of NT-3 expression was shown in the ACTH4-10Pro⁸-Gly⁹-Pro¹⁰ group 3 hours after mild and severe SCI. Intranasal administration of ACTH4-10Pro⁸-Gly⁹-Pro¹⁰ is known to reach the rat brain within 2 minutes post-administration and has a concentration 10-15 times higher than intravenous administration²⁰. Another study in rats said that the nootropic and analgesic effects after intranasal administration of ACTH4-10Pro⁸-Gly⁹-Pro¹⁰ could be observed 15-30 minutes after administration.²¹ Human studies have shown that ACTH4-10 concentrations in spinal CSF increase within 10 minutes and peak within 30 minutes after intranasal administration, suggesting the possibility of using an extraneuronal pathway for the peptide to reach its target, pass through the intercellular gap in the olfactory epithelium and then diffuse into the subarachnoid space.²²

In this study, no locomotor assessment was carried out in rats, so the difference in the functional recovery effect of mild and severe SCI is not known after ACTH4-10Pro⁸-Gly⁹-Pro¹⁰ treatment. However, one study showed improved BBB scores in rats with the SCI model after intranasal administration of ACTH4-10Pro⁸-Gly⁹-Pro¹⁰.² The receptor NT-3 also changes after the occurrence of SCI, so research on the TrkA, TrkB, TrkC, p75NTR, proneurotrophin, PC1, PC2, and furin receptors is also necessary to understand

more clearly the action of ACTH4-10Pro⁸-Gly⁹-Pro¹⁰.¹⁵

CONCLUSIONS

Intranasal administration of ACTH4-10Pro⁸-Gly⁹-Pro¹⁰ increased NT-3 expression in mild and severe acute SCI at 3 and 6 hours. Expression of NT-3 was higher in the mild acute SCI group after administration of ACTH4-10Pro⁸-Gly⁹-Pro¹⁰ than in the severe acute SCI group. Further studies are needed to determine the neuroregeneration effect of ACTH4-10Pro⁸-Gly⁹-Pro¹⁰ on SCI.

CONFLICT OF INTEREST

No competing interests were declared.

ETHICS CONSIDERATION

Ethics approval has been obtained from Animal Care and Use Committee (ACUC), Faculty of Veterinary Medicine, Airlangga University, Surabaya, with EC number 325-KE.

FUNDING

This research received no specific grant from any funding agency.

AUTHOR CONTRIBUTIONS

All authors contribute to the study from the conceptual framework, data acquisition, and data analysis until reporting the study results through publication.

REFERENCES

- Kang Y, Ding H, Zhou H, et al. Epidemiology of worldwide spinal cord injury: a literature review. *J Neurorestoratol*. 2017; 6:1-9. doi:10.2147/JN.S143236
- Sutrisno A, Patellongi I, Yusuf I, et al. The Effect of ACTH 4-10 ProGlyPro as Anti-

- inflammatory on Astrocyte Cell Repair in Spinal-Cord- Injured Mouse by Assessing Locomotor Function. *IJSBAR (International J Sci Basic Appl Res)*. 2017;4531:31-41.
3. Ning G-Z, Wu Q, Li Y-L, Feng S-Q. Epidemiology of traumatic spinal cord injury in Asia: a systematic review. *J Spinal Cord Med*. 2012;35(4):229-239. doi:10.1179/2045772312Y.00000000021
 4. Carlson GD, Gorden C. Current developments in spinal cord injury research. *Spine J*. 2002;2(2):116-128. <http://www.ncbi.nlm.nih.gov/pubmed/14588270>. Accessed November 22, 2018.
 5. Samantaray S, N.P. T, D.D. M, A. V, S.K. R, N.L. B. Neuroprotective drugs in traumatic CNS injury. *Open Drug Discov J*. 2010;2(SPEC. ISS.3):174-180. <http://www.embase.com/search/results?subaction=viewrecord&from=export&id=L365268755>.
 6. Kwon BK, Tetzlaff W, Grauer JN, Beiner J, Vaccaro AR. Pathophysiology and pharmacologic treatment of acute spinal cord injury. *Spine J*. 2004;4(4):451-464. doi:10.1016/j.spinee.2003.07.007
 7. Cao HQ, Dong ED. An update on spinal cord injury research. *Neurosci Bull*. 2013;29(1):94-102. doi:10.1007/s12264-012-1277-8
 8. Narazaki DK, Barros Filho TEP de, Oliveira CRGCM de, et al. Spinal cord regeneration: the action of neurotrophin-3 in spinal cord injury in rats. *Clinics (Sao Paulo)*. 2006;61(5):453-460. doi:10.1590/S1807-59322006000500013
 9. Dmitrieva VG, Povarova O V., Skvortsova VI, Limborska SA, Myasoedov NF, Dergunova L V. Semax and Pro-Gly-Pro activate the transcription of neurotrophins and their receptor genes after cerebral ischemia. *Cell Mol Neurobiol*. 2010;30(1):71-79. doi:10.1007/s10571-009-9432-0
 10. Agapova TY, Agniullin Y V., Shadrina MI, et al. Neurotrophin gene expression in rat brain under the action of Semax, an analogue of ACTH4-10. *Neurosci Lett*. 2007;417(2):201-205. doi:10.1016/j.neulet.2007.02.042
 11. Ardananurdin A, Subagio EA, Utomo B. Spinal Cord' s IL-1 , TNF- α and NF- κ B Expression in Sprague-Dawley Rat on Acute Spinal Cord Injury. 2020;3(3):109-114. doi:10.15562/ijn.v3i3.130
 12. Faris M, Utomo B, Fauzi A Al, et al. ACTH4-10PRO8-GLY9-PRO10 improves Neutrophil profile in spinal cord injury of rat models. *J Adv Pharm Educ Res*. 2021;11(2):61-65. doi:10.51847/LWGHW1YN1Q
 13. Fan B, Wei Z, Yao X, et al. Microenvironment Imbalance of Spinal Cord Injury. *Cell Transplant*. 2018;27(6):853-866. doi:10.1177/0963689718755778
 14. Wong I, Liao H, Bai X, et al. ProBDNF inhibits infiltration of ED1+ macrophages after spinal cord injury. *Brain Behav Immun*. 2010;24(4):585-597. doi:10.1016/j.bbi.2010.01.001
 15. Hajebrahimi Z, Mowla SJ, Movahedin M, Tavallaei M. Gene expression alterations of neurotrophins, their receptors and prohormone convertases in a rat model of spinal cord contusion. *Neurosci Lett*. 2008;441(3):261-266. doi:10.1016/j.neulet.2008.06.046
 16. Van Der Kraan M, Tatro JB, Entwistle ML, et al. Expression of melanocortin receptors and pro-opiomelanocortin in the rat spinal cord in relation to neurotrophic effects of melanocortins. *Mol Brain Res*. 1999;63(2):276-286. doi:10.1016/S0169-328X(98)00291-5
 17. Caruso C, Carniglia L, Durand D, Gonzalez P V., Scimonelli TN, Lasaga M. Melanocortin 4 receptor activation induces brain-derived neurotrophic factor expression in rat astrocytes through cyclic AMP - Protein kinase A pathway. *Mol Cell Endocrinol*. 2012;348(1):47-54. doi:10.1016/j.mce.2011.07.036
 18. Baskoro W. Pengaruh Pemberian ACTH4-10 Pro8Gly9 Pro10 Terhadap Ekspresi Brain Derived Neurotrophic Factor (BDNF) Medula Spinalis Pada Acute Spinal Cord Injury Penelitian Eksperimental Laboratorik. 2017. <https://repository.unair.ac.id/72828/>.
 19. Subagio EA. Mekanisme Penurunan Jumlah Sel Apoptosis Melalui Peningkatan Rasio Bcl-2/ HSP 70 Oleh ACTH4-10 Pro8-Gly9-Pro10 Pada Cedera Kompresi Korda Spinalis Akut. 2011. <http://repository.unair.ac.id/id/eprint/71183>.
 20. Shevchenko K V., Nagaev IY, Alfeeva LY, et al. Kinetics of semax penetration into the brain and blood of rats after its intranasal administration. *Russ J Bioorganic Chem*. 2006;32(1):57-62. doi:10.1134/S1068162006010055
 21. Manchenko DM, Glazova NY, Levitskaya NG, Andreeva LA, Kamenskii AA, Myasoedov NF. The nootropic and analgesic effects of semax given via different routes. *Neurosci Behav Physiol*. 2012;42(3):264-270. doi:10.1007/s11055-012-9562-6
 22. Born J, Lange T, Kern W, McGregor GP, Bickel U, Fehm HL. Sniffing neuropeptides: a transnasal approach to the human brain. *Nat Neurosci*. 2002;5(6):514-516. doi:10.1038/nn849



This work is licensed under a Creative Commons Attribution