Research Article

Injection of colchicine intra-hippocampal cortical area 1 enhances novelty seeking behavior

Manizheh Karami[1,2], Nosaibeh Riahi[2], Mohammad Reza Jalali Nadoushan[3]

[1]Neurophysiology Research Center, Shahed University,
[2]Department of Biology, Faculty of Basic Sciences, Shahed University
[3]Department of Pathology, School of Medicine, Shahed University, Tehran, Iran

Received: 01-12-2012
Revised: 08-01-2013
Accepted: 27-02-2013

Correspondence to:
Dr. Manizheh Karami,
E-mail: karami@shahed.ac.ir

ABSTRACT

Objective: Colchicine, a potent neurotoxin derived of plant has been recently identified as a degenerative toxin of small pyramidal cells in the hippocampal cortical area 1 (CA1). In this study, the effect of the alkaloid intra hippocampal CA1 on the novelty seeking behavior in the conditioning task was measured.

Materials and Methods: Injections of colchicine (1-75 μg/rat, intra-CA1) were performed in cannulated male Wistar rats while being settled in the stereotaxic apparatus. Control group was solely injected saline (1 μl/rat, intra-CA1). One week later, after recovery, all the animals passed the novelty seeking paradigm using an unbiased conditioning task. They were habituated with the conditioned place preference (CPP) apparatus on day 1. Then they were confined in one part of the CPP box for 3 more days. Finally, the animals were tested in the last day. To evaluate, the possible cell injury effect of the toxin on the pyramidal cells of the CA1 both the motivational staying signal in the parts of the box and the non-motivational locomotive signs of the rats were measured.

Results: Based on the present study, the alkaloid caused significant novelty seeking behavior at higher doses. It also affected the compartment entering behavior in the colchicine received group. However, the alkaloid did not show the significant effect on sniffing, rearing or grooming in the rats.

Conclusion: Injection of colchicine intra-CA1 may impair the neuronal transmission of motivational information by the pyramidal cells in the dorsal hippocampus.

KEY WORDS: Colchicine, hippocampus, novelty behavior, pyramidal cell

Introduction

Lesion by toxic analogs of neurotransmitters is useful because of lack of lesion selectivity in the specific cell populations. This fact raises much serious problem whenever one attempts to ascribe the effects of specific cell population removal on the neurophysiology and the neuronal transmission. Colchicine is a plant alkaloid,[1] which is known as a potent inhibitor of physiological processes. The alkaloid specifically binds to the receptor site on the tubulin[2] and blocks mitosis.[3] Goldschmidt and Steward[4] have previously demonstrated the colchicine as a substance, which preferentially destructs the granule cells in the dentate gyrus. However, the toxic effect of colchicine on other cellular processes rather tubulin subunits has been specified later.[5] After Correia and Lobert,[5] damage of pyramidal cells in the cortical area 1 (CA1) of the hippocampus was shown by this laboratory.[6] This neurointoxication is surprisingly important because neuronal functions depend on an intact cytoskeleton. The cytoskeletal alterations are associated with neurological disorders,[7] which is notably common in cases of neurodegenerative pathology.[8] This lesion also may refer to the learning and spatial memory impairment,[9] because the hippocampal sub-regions are referenced as those that function in spatial and working memory processes.[10] We aim to show a selective neurotoxic effect of colchicine in the small pyramidal cell populations in CA1 area of rat hippocampus through evaluation of novelty seeking behavior

Access this article online	
Website: www.ijp-online.com	Quick Response Code:
DOI: **********	

Materials and Methods

Animals

The animals used were male Wistar rats (Pasteur Institute of Iran, Tehran, Iran) weighing between 250 g and 350 g. They were housed two per cage in a controlled colony room (temperature

21°C ± 3°C). They were maintained under a 12:12 h light-dark cycle with water and food provided *ad libitum*. 6 animals were used in each experiment and all experiments were approved by the local ethics committee.

Drugs

The drug colchicine (Merck Co., Germany) used in the present study was prepared in sterile 0.9% NaCl solution. A mixture level of 5:2 of ketamine (100 mg/kg) and xylazine (20 mg/kg) purchased from Veterinary organization of Iran were intraperitoneally (i.p.) injected to anesthetize the experimental animals. Vehicle injections were of the appropriate volume of 0.9% physiological saline.

Surgery and colchicine injection

The animals were anesthetized and placed in a stereotaxic apparatus, with the incisor bar set at approximately, 3.3 mm below horizontal zero to achieve a flat skull position. An incision was made to expose the rat skull. Two holes were drilled in the skull at stereotaxic coordinates: AP-3.8 mm posterior to bregma, and L ± 1.8 to ± 2.2 mm according to the atlas of Paxinos and Watson.[11] Two guide cannulae (21-gauge) were inserted into the holes. For animals receiving bilateral injections of colchicine into the CA1 of the hippocampus, the guide cannula were lowered 2 mm below bregma through the holes drilled at the above-mentioned coordinates.

The colchicine (1, 5, 25, and 75 μg/rat) was administered intra-CA1 of the hippocampus while the cannulated rats were still settled down by the stereotaxic apparatus. The guide cannula was anchored with a jeweler's screw, and the incision was closed with the dental cement. After that, the injection cannula that extended 1 mm beyond the guide cannula was inserted into the guide cannula through, which the alkaloid (1-75 μg/rat) was gently injected into the site. The injection cannula then was left in the place for another 60 s to facilitate the diffusion of the drug. One week after the recovery the rats passed the novelty seeking task by the place conditioning procedure.

Histological verification

After completion of experiments, the animals were decapitated on the usage of overdose of chloroform. The brains were removed and fixed in a 10% formalin solution for 48 h before sectioning. Sections were taken through the brain areas of cannula placements, and the cannula placements were verified using the atlas of Paxinos and Watson.[11] Data from rats with injection sites located outside the hippocampal CA1 area was excluded of the analysis.

Cresyl violet stain

Thin (4-5 μm) brain sections provided by paraffin blocks were cleared of paraffin by xylol and xylol-alcohol and placed in 95%, 75%, and 50% ethanol and distilled water for 30 s each and administered with cresyl violet solution (Merck Co., Germany) for 2 min. Subsequently, the sections were placed in 0.1% acetic acid (about 10 s) and in 50%, 75%, and 95% ethanol for 30 s each, cleared in xylene and mounted with entallen glue (Merck Co., Germany).

Statistical analysis

The data were analyzed with One-way analysis of variance (ANOVA) followed by the Tukey-Kramer multiple comparison tests. P values < 0.05 were considered as significant.

Place conditioning apparatus

A two compartment conditioned place preference apparatus (30 cm × 60 cm × 30 cm) was used in the experiment. Place conditioning was conducted using an unbiased procedure, with the design and the apparatus previously been described.[12]

Novelty seeking task by the place conditioning paradigm

The experiment consisted of three following phases:

Familiarizing

On day 1 (before confining) animals received one habituation session. They were placed in the middle-line of the apparatus to freely move into the entire apparatus for 10 min. In this phase, the removable wall was raised 12 cm above the floor. The time spent by rats in each compartment was recorded by an Ethovision system (Auto iris Video Camera LVC-DV323ec, LG Electronics, South Korea) located 120 cm above the apparatus. The recorded behavior by the system was then analyzed by an observer had no knowledge of the treatments. None of the groups displayed a significant preference for one of the compartments, confirming that this procedure is unbiased.

Confining

This phase was started 1 day after the familiarization. It consisted of 6-part pairings; the animals were simply confined for 40 min in one part of the box twice a day with a 6-h interval. This phase was carried out during the light phase of a 12-h light/dark cycle (e.g., at 09.00 a.m. and at 15.00 p.m.). This protocol was as performed in the control (saline treated) group as carried out for the experimental groups (colchicine 1-75 μG/rat treated intra-CA1).

Testing

Test session was carried out on day 5, 1 day after the last confining session. Each animal was tested only once. For testing, the removable wall was raised 12 cm above the floor and each animal was allowed free access to both compartments of the apparatus for 10 min. The time spent (s) in the compartments on day of testing were subtracted between those obtained for the familiarization and the testing phases and the result was expressed as mean ± S.E.M. Count of behavioral signs as the change in number of the sign/10 min performed in the apparatus was also counted.

Results

Cell damage induced by colchicine in the area of CA1 of Wistar rats

The colchicine (1-75 μg/rat) was effective in inducing of lesion in the layer CA1 [Figure 1].

Effect of injection of colchicine intra-CA1 on score of staying

Bilateral injection of colchicine (1-75 μg/rat, intra-CA1), a plant derived alkaloid, before (1 week) starting the process of novelty seeking, resulted in a significant difference in the score of staying in the novel part in the rats compared to the control group ($F_{4,24} = 6.584$, $P < 0.01$) [Figure 2].

Effect of injection of colchicine intra-CA1 on behavioral signs

Figure 3 shows the effect of colchicine injection (1-75 μg/rat, intra-CA1) on behavioral signs in Wistar rats. Administration of neurotoxin showed significant effect on compartment entering ($F_{4,24} = 3.911$; $P < 0.05$). However, no significant effect on sniffing, rearing or grooming was recorded ($P > 0.05$).

Figure 1: The histological verifications of colchicine lesion effect (1-75 μg/rat, intra-CA1) in Wistar rats. The injection of colchicine was carried out only once. After receiving the drug each animal was allowed to recover. Then the animal passed the behavioral experiments. The alkaloid damaging effect in the experimental animals is shown in the figure [Figure 1b] in contrast to the control [Figure 1a]

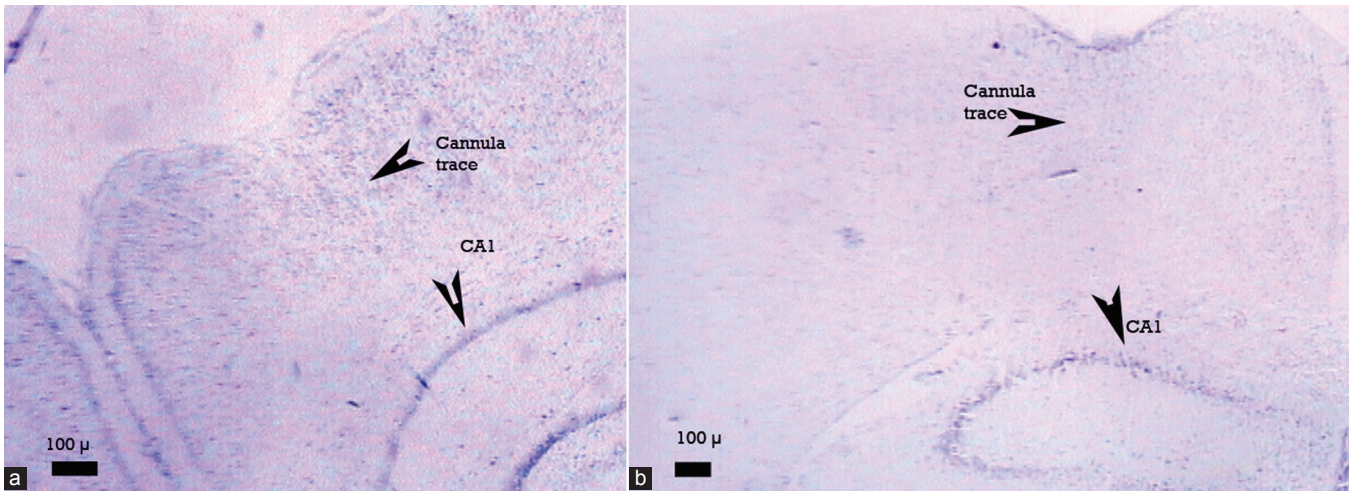


Figure 2: Response to colchicine or saline (control), intra-CA1, in the novelty behavior using the place conditioning task. Each animal, after being recovered, passed the task detailed in the Materials and Methods. The animals were tested in last day to evaluate a simple type of learning. Data are expressed as the score of change in seeking for the novel place and expressed as mean ± S.E.M. A difference between drug-administered groups versus the vehicle was observed. *Post hoc* analysis by Tukey showed the differences (*$P < 0.05$) to the control

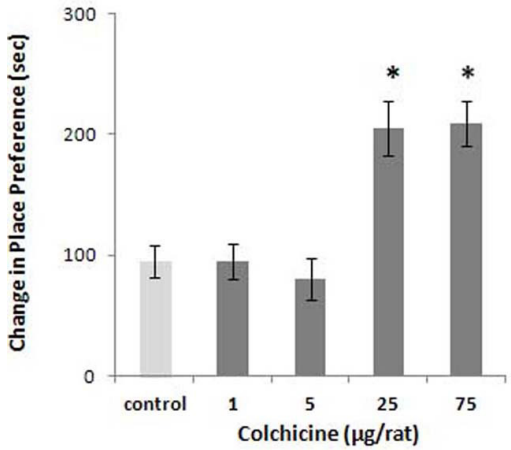
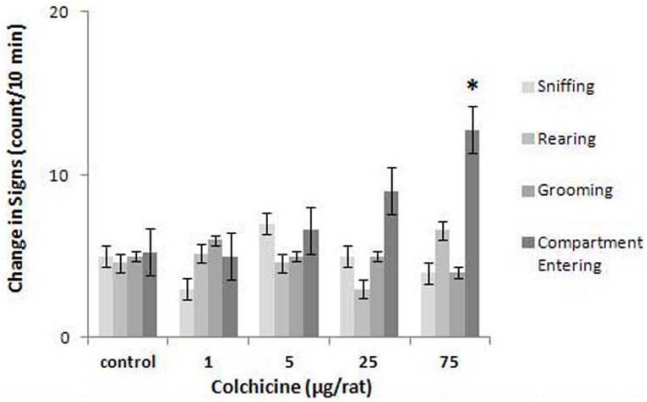


Figure 3: The behavioral signs in male Wistar rats that received colchicine or saline (control) intra-CA1. The colchicine (1-75 μg/rat) or saline (1 μl/rat) was given 1 week before of starting of the novelty paradigm. The animals then passed the task as detailed in M and M. Data are expressed as change in count of behavioral signs per 10 min ± SEM. *Post hoc* analysis by Tukey showed the difference (*P <0.05) to the control



Discussion

This research was designed to survey the effect of a potent plant neurotoxin, colchicine, on small pyramidal cells in the hippocampal CA1 and the seeking behavior in the conditioning task as well. We discuss that the increased locomotion (horizontal activity) mainly characterizes the rat's behavioral response to novelty.

According to this research, the toxin showed degenerative effects on the pyramidal cells and produced a significant novelty behavior as well as change in the compartment entering.

Colchicine is an alkaloid, which is extracted from *Colchicum autumnale* L. This toxin binds to tubulin dimers, which results in the formation of a tubulin – colchicine complex that acts primarily to prevent microtubule assembly.[13] In animal cells, colchicine is usually considered as the lethal agent even at the lowest concentrations.[14] This alkaloid impairs appearance of several learning paradigms by morris water maze, radial arm maze, aversive, and operant conditioning tasks.[15,16]

This work showed a damage effect of the colchicine on the small pyramidal cell population in the rat CA1 area. Supporting finding provides that the toxin colchicine irreversibly damages dendrites by disrupting their micro-tubular supporting network,[16] a mechanism, which is expressed for the selective toxic effect of colchicine of dentate granule cells.[17] Some other studies, furthermore, using similar doses of colchicine to ours in the present study have reported only the reversible

effects.[18] This alkaloid inhibits, the rapid axonal transport[19] and produces long-lasting morphological changes in neurons and glia; it has been suggested that the axonal membrane as well as dendrites are the site for the action of colchicine.[20,21] Because these sites are rich in microtubules and contain specific receptors

As lesion studies specify, a critical role of CA1 in learning and memory processes[22] we planned the learning tasks so effectively as needed to discuss on toxic effect of colchicine in the CA1. All rats were evaluated for sniffing, rearing, grooming, and compartment entering and the results provided some significant effects.

After place conditioning testing, the experimental animals, those suffering from the lesion made by colchicine (1-7.5 μg/rat) in the CA1 area, exhibited a different type of locomotor activity (compartment entering) in comparison to the control saline treated group (intra-CA1).

The axonal transport can be blocked by the microtubule-depolymerizing agent colchicine, so, this plant alkaloid can impair the functional properties of nerve cells. Individual difference in sensation-seeking responses has been reported as for instance, when rats are exposed to a novel environment, some rats (high responders) exhibit high locomotor response, while others (low responders) show low rates of locomotor activity.[23] The odor sampling by sniffing behavior during the paradigm has encountered the idea that sniffing plays a critical role in odor information processing by shaping spatial and temporal patterns of afferent input to the olfactory bulb and through the patterns of higher level neural activity as well.[24] Furthermore, in a survey in the novelty task rats with the CA1 lesions, impairment in the place conditioning test has been recorded.[10]

Further, hippocampal lesions are associated with impairments in spatial navigation acquisition of conditional discriminations.[25] The rats suffering from CA1 lesion cannot separate training events across time because they unable to lessen interfering of actions and undergo deficits in spatial learning.

Thus, the effects reported in the present study may be the consequence of an impaired neuronal transmission in hippocampal pyramidal cells, a process, which consequently may be related to the observed data. Based on the finding of this work, the small pyramidal cells can be chosen as another target for the action of colchicine, while the investigators have suggested the granular cells as the main candidate for destruction by the alkaloid.

References

1. Alali FQ, El-Elimat T, Li C, Qandil A, Alkofahi A, Tawaha K, *et al.* New colchicinoids from a native Jordanian meadow saffron, *colchicum brachyphyllum*: Isolation of the first naturally occurring dextrorotatory colchicinoid. J Nat Prod 2005;68:173-8.
2. Lockwood AH. Molecules in mammalian brain that interact with the colchicine site on tubulin. Proc Natl Acad Sci U S A 1979;76:1184-8.
3. Mundy WR, Tilson HA. Neurotoxic effects of colchicine. Neurotoxicology 1990;11:539-47.
4. Goldschmidt RB, Steward O. Preferential neurotoxicity of colchicine for granule cells of the dentate gyrus of the adult rat. Proc Natl Acad Sci U S A 1980;77:3047-51.
5. Correia JJ, Lobert S. Physiochemical aspects of tubulin-interacting antimitotic drugs. Curr Pharm Des 2001;7:1213-28.
6. Porkhodadad S, Karami M, Jalali Nadoushan MR. Positive effect of nitric oxide on morphine-induced place conditioning in wistar rats treated by colchicine intra-hippocampal CA1. J Clinic Toxicol 2011;1:1.
7. Selkoe DJ. Translating cell biology into therapeutic advances in Alzheimer's disease. Nature 1999;399:A23-31.
8. Uchida K, Samejima M, Okabe A, Fukuda A. Neuroprotective effects of melatonin against anoxia/aglycemia stress, as assessed by synaptic potentials and superoxide production in rat hippocampal slices. J Pineal Res 2004;37:215-22.
9. McNaughton BL, Barnes CA, Meltzer J, Sutherland RJ. Hippocampal granule cells are necessary for normal spatial learning but not for spatially-selective pyramidal cell discharge. Exp Brain Res 1989;76:485-96.
10. Okada K, Okaichi H. Functional differentiation and cooperation among the hippocampal subregions in rats to effect spatial memory processes. Behav Brain Res 2009;200:181-91.
11. Paxinos G, Watson C. The Rat Brain in Stereotaxic Coordinates. 5th ed. Ontario: Academic Press, Harcourt Brace Jovanovich Publisher; 2005. Digital version, Figure 65.
12. Karami M, Zarrindast MR, Sepehri H, Sahraei H. Role of nitric oxide in the rat hippocampal CA1 area on morphine-induced conditioned place preference. Eur J Pharmacol 2002;449:113-9.
13. Panda D, Goode BL, Feinstein SC, Wilson L. Kinetic stabilization of microtubule dynamics at steady state by tau and microtubule-binding domains of tau. Biochemistry 1995;34:11117-27.
14. Eigsti OJ, Dustin P Jr. Colchicine in Agriculture, Medicine, Biology and Chemistry. Ames, Iowa: The Iowa State College Press; 1955. p. 496.
15. Nakayama T, Sawada T. Involvement of microtubule integrity in memory impairment caused by colchicine. Pharmacol Biochem Behav 2002;71:119-38.
16. Priel A, Tuszynski JA, Woolf NJ. Neural cytoskeleton capabilities for learning and memory. J Biol Phys 2010;36:3-21.
17. Walsh TJ, Schulz DW, Tilson HA, Schmechel DE. Colchicine-induced granule cell loss in rat hippocampus: selective behavioral and histological alterations. Brain Res 1986;98:23-36.
18. Giuditta A, Chun JT, Eyman M, Cefaliello C, Bruno AP, Crispino M. Local gene expression in axons and nerve endings: The glia-neuron unit. Physiol Rev 2008;88:515-55.
19. Gajate C, Barasoain I, Andreu JM, Mollinedo F. Induction of apoptosis in leukemic cells by the reversible microtubule-disrupting agent 2-methoxy-5-(2',3',4'-trimethoxyphenyl)-2,4,6-cycloheptatrien-1-one: Protection by Bcl-2 and Bcl-X(L) and cell cycle arrest. Cancer Res 2000;60:2651-9.
20. Partida-Sánchez S, Garibay-Escobar A, Frixione E, Parkhouse RM, Santos-Argumedo L. CD45R, CD44 and MHC class II are signaling molecules for the cytoskeleton-dependent induction of dendrites and motility in activated B cells. Eur J Immunol 2000;30:2722-8.
21. Kumar A, Dogra S, Prakash A. Neuroprotective Effects of Centella asiatica against Intracerebroventricular Colchicine-Induced Cognitive Impairment and Oxidative Stress. Int J Alzheimers Dis 2009;2009.pii:972178.
22. Gilbert PE, Kesner RP, Lee I. Dissociating hippocampal subregions: Double dissociation between dentate gyrus and CA1. Hippocampus 2001;11:626-36.
23. Piazza PV, Deminière JM, Le Moal M, Simon H. Factors that predict individual vulnerability to amphetamine self-administration. Science 1989;245:1511-3.
24. Wesson DW, Donahou TN, Johnson MO, Wachowiak M. Sniffing behavior of mice during performance in odor-guided tasks. Chem Senses 2008;33:581-96.
25. Cain DP, Boon F, Corcoran ME. Thalamic and hippocampal mechanisms in spatial navigation: A dissociation between brain mechanisms for learning how versus learning where to navigate. Behav Brain Res 2006;170:241-56.

Cite this article as: Citation will be included before issue gets online***

Source of Support: Nil. **Conflict of Interest:** None declared.