FAILING OF INFORMATION TRANSMISSION BY DORSAL HIPPOCAMPUS DUE TO MICROINJECTION OF COLCHICINE IN RAT'S CORTICAL AREA 1

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ABSTRACT

Objective: Colchicine has been introduced recently as a neurotoxin with damage effect on neurons of hippocampal cortical area 1 (CA1). Effect of colchicine, a plant derived neurotoxin on memory retrieval was explored experimentally by means of novelty seeking task in intact Wistar rats.

Methods: The subjects were cannulated by stereotaxic apparatus at coordinates adjusted for the CA1 area. After recovery, all animals experienced the novelty seeking paradigm using an unbiased conditioning device. First, they were habituated with the conditioned place preference (CPP) apparatus. They were then confined in one part of the CPP box for 3 consecutive days. Finally, the animals were microinjected colchicine (1-25 µg/rat) intra-hippocampal CA1 prior to testing. Control group was cannulated too, but, solely injected saline (1-µl/rat, intra-CA1). The time spent in the novel part of the device and the motivational signs of the rats were measured. Furthermore, the possible cell injury effect of the toxin on the CA1 layer was verified.

Results: The alkaloid caused significant novelty seeking behavior in the experimental animals though did not show a significant effect on the compartment entering. The destruction effect of the neurotoxin on the treated rats' dendrites spines was evidenced.

Conclusion: Based on this finding the information transmission by dorsal hippocampal pyramidal cells may impair with an administration of neurotoxin colchicine, intra-CA1.

Keywords: Colchicine, Memory retrieval, Novelty seeking behavior, Cortical area 1, Pyramidal cell layer.

INTRODUCTION

We have introduced recently a neurotoxin, colchicine, as an alkaloid with damage effect on neurons of hippocampal cortical area 1 (CA1). In this research, we assessed the destructive influence of the alkaloid colchicine on the CA1 cell appendages.

Colchicine, a plant derived neurotoxin, inhibits microtubule assembly by binding to tubulin [1], a manner that may impair the learning processes. The colchicine microinjection into the rat hippocampus results in preferential damage to hippocampal granule cells in the dentate gyrus [2]. Other researchers have shown that the alkaloid specifically binds to the receptor site on the tubulin [3]. Considering that the neuronal functions are dependent on cytoskeleton entity, the cytoskeletal alterations have been related with neurodegenerative pathology [4].

In view of the fact that the hippocampal sub-regions are involved in spatial and working memories [5], the intoxication of the areas can impair the learning and memory processes. It should be notified that the memory formations by hippocampal sub-regions are time-dependently contributed to the areas [6], because the CA3 and CA1 mediate the short-term and the intermediate-term memories, respectively.

After Correia and Lobert (2001) [3], this laboratory has proposed the pyramidal cell damage in the CA1 of the hippocampus [7]. We have characterized the response to the novelty in the rats treated by colchicine before of the commencement of the behavioral measurements.

Regarding that use of colchicine is useful for cell removal to specifically ascribe the neurophysiology and the neuronal transmission, we aimed to measure experimentally the hazard effect of colchicine on rat's memory retrieval by microinjection of colchicine into the rat

hippocampal CA1 to address for the pyramidal cell destruction in the CA1. We provided the straight evidence of the agent toxicity.

METHODS

Animals

The subjects were male Wistar rats bought from Pasteur Institute of 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 hrs light-dark cycle with water and food provided *ad libitum*. Each animal (n=6 per group) was used only once and the experimental protocol was performed based on Shahed University Review Committee for the use of human or animal subjects (Document No. 358, October, 2013).

Drugs

Colchicine (Merck Co., Germany) was prepared in sterile 0.9% NaCl solution and microinjected into the CA1. Ketamine (100 mg/kg) and xylazine (20 mg/kg) (Veterinary Organization, Iran) were intraperitoneally injected to anesthetize the experimental animals. Vehicle injection was 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: Anterior-posterior - 3.8 mm posterior to bregma, and L: ±1.8 to ±2.2 mm according to the atlas of Paxinos and Watson (2005) [8]. 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 was lowered 2 mm below bregma through the holes drilled at the above-mentioned coordinates. The guide cannula was

anchored with a jeweler's screw, and the incision was closed with the dental cement.

The colchicine (1-25 µg/rat) was administered intra-CA1 of the hippocampus prior to testing of animals' novelty seeking behavior. The injection cannulae that extended 1-mm beyond the guide cannula were inserted into the guide cannula through polyethylene tubing linked with a 5 µ Hamilton syringe. The alkaloid (1-25 µg/rat) was gently (lasting 60 seconds) injected into the site. The injection cannulae then were left in the place for another 60 seconds to facilitate the diffusion of the drug.

Histological verification

After experiments, the experimental animals were decapitated after usage of overdose of anesthetics. The animals' brains were collected in a 10% formalin solution for further histological examination. The cannulae placements were checked in the brain slices with the rat brain atlas [8]. Data from rats with injection sites located outside the hippocampal CA1 area was excluded of the analysis (5 rats).

Staining of slices

Thin (3 µm) brain sections were processed with tissue processor through paraffin embedding. The serially sections (3 µm) were prepared with a rotary microtome. The slides were then stained using cresyl violet. The dendrites' spine in the brain slices were additionally verified by Golgi staining. The sections were finally mounted with entellan (Merck Co., Germany). They were evaluated with light video photomicroscope (Olympus) at the desired magnification.

Statistical analysis

All data were analyzed with analysis of variance followed by the Tukey-Kramer's comparison test. $p < 0.05$ were considered as significant.

Place conditioning apparatus

An apparatus with two compartments (30 cm × 60 cm × 30 cm) was used in the experiment. Place conditioning was conducted using an unbiased conditioned place preference [9].

Novelty seeking task by the place conditioning paradigm

It was performed with the following three - phase program:

Habituation

All animals received one habituation session on day 1; they were placed in the middle-line of the device to move freely in the entire apparatus for 10 minutes. 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 records were then analyzed by an observer who was unfamiliar to the treatments. All groups displayed no significant preference for the parts of apparatus, confirming the unbiased procedure.

Confining

This phase was started 24 hrs after the adaptation stage. It consisted of 6-confined part pairings; the animals were simply confined in one part of the box for 40 minutes twice a day with 6 hrs interval [7].

Testing

Test session was done on day 5. Each animal was tested only once; similar to that for the familiarization, 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 minutes. The time spent in each compartments was subtracted between those obtained from the familiarization and expressed as mean ± standard error of mean. Change in number of the behavioral sign performed in the phases/10 minutes was also counted.

RESULTS

Pathological evidence induced by colchicine in the Wistar rats CA1

As the Fig. 1 shows the colchicine (1-25 µg/rat) induced the dendrites' spine lesion in the layer CA1 (Fig. 1a-b). However, it did not produce meaningful change in cell density of the layer compared to the control, the fact indicating a time-taking lesion effect of the toxin (Fig. 1c-e).

Effect of injection of colchicine intra-CA1 on expression of non-motivational staying time

Injection of a plant-derived alkaloid, colchicine (1-25 µg/rat, intra-CA1), prior to testing of novelty seeking behavior, resulted in a significant difference in the score of staying in the novel part between the colchicine received rats to the control group ($F_{4,25} = 7.187$, $p = 0.0015$) (Fig. 2).

Effect of injection of colchicine intra-CA1 on expression of behavioral signs

The effect of colchicine injection (1-25 µg/rat, intra-CA1) prior to the novelty-seeking behavior testing, on the expression of the behavioral signs are shown in Fig. 3a-e. According to the result, the neurotoxin caused significant effect on sniffing ($F_{4,25} = 4.098$, $p = 0.019$), and rearing ($F_{4,25} = 3.719$, $p < 0.034$), and grooming ($F_{4,25} = 3.022$, $p = 0.04$), and wet dog shaking ($F_{4,25} = 3.684$, $p = 0.025$), but, it produced no significant result on compartment entering ($p > 0.05$).

DISCUSSION

We designed to measure behaviorally the prompt effect of a potent plant neurotoxin, colchicine, on the seeking behavior in the conditioning task to differentially contribute the cells to specific behavioral components of simple learning tasks. In addition, we evidenced the destruction effect of the toxin on the small pyramidal cells of the hippocampal CA1.

Based on the present data, the alkaloid produced a significant effect on expression of novelty behavior in the colchicine received animals. Moreover, it showed a prominent change in expression of sniffing, and rearing, and grooming, and wet dog shaking in the rats. Furthermore,
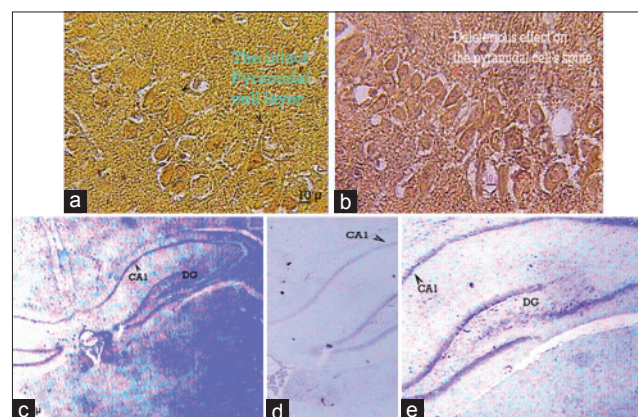

Fig. 1: Verifications for colchicine damage effect (1-25 µg/rat, intra-cortical area 1 [CA1]) in Wistar rats. The rats were trained in the novelty seeking task lasted 5 days. Colchicine was injected only once prior to testing of the novelty behavior. After completion of the experiments, the animals were killed with an overdose of anesthetics. The rats' brain samples were collected in formalin for histological processing. The slices then were stained by cresyl violet to specifically indicate the cells in the CA1 layer. The samples were furthermore stained by Golgi satin to show the exact dendrites' appendages damage. The alkaloid demolition effect in the experimental animals' CA1 cells is shown in the Fig. 1a (control) and b (colchicine treated). The amount of the cells in the layer was evidenced as no changed as can be seen in Fig. 1e (colchicine treated) in contrast to 1c and d (controls)
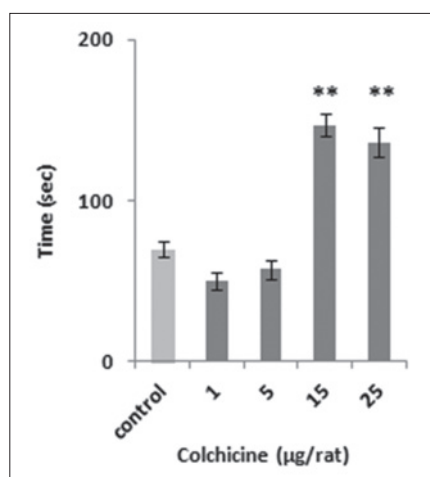
Fig. 2: The response to colchicine or saline (control), intra-cortical area 1. At first, the animals were cannulated at the area of studying by stereotaxis apparatus. 1-week later, after recovery, they passed the task detailed in the materials and methods. The experimental animals were injected drug or vehicle pre-testing of novelty behavior using the place conditioning task (day 5). Data are expressed as the score of change in seeking for the novel place and expressed as mean ± standard error of mean. A difference between the drug-administered groups versus the vehicle was observed. *Post-hoc* **analysis by Tukey's comparison test showed the differences **p<0.01 to the control.**
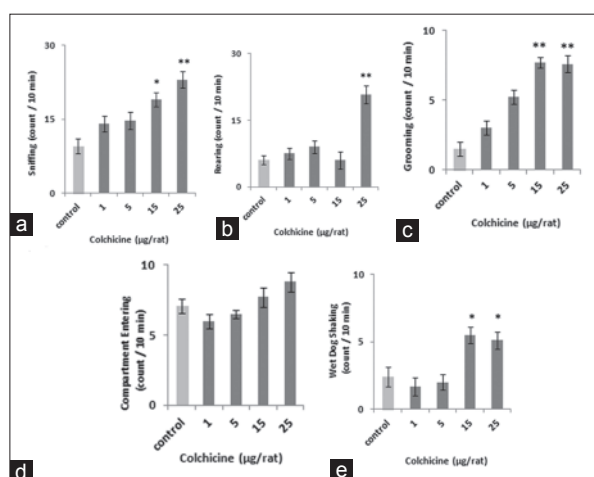

Fig. 3a-e: The behavioral signs in male Wistar rats that received colchicine or saline (control) intra-cortical area 1. Colchicine (1-25 µg/rat) or saline (1-µl/rat) was given pre-testing of novelty paradigm. The animals then passed the task as detailed in materials and methods. Data are expressed as change in count of behavioral signs per 10 minutes ± standard error of mean. *Post-hoc* **analysis by Tukey's** *post-hoc* **showed the difference (*p<0.05, **p<0.01) to the control**

it showed a destructive effect on the pyramidal cells' projections. However, the neurotoxin did not reduce the cell density of the CA1 as compared with the control.

In support, impairment in the novelty task has previously been recorded [7] in rats with the CA1 lesions. In the experience, we have shown that the alkaloid impairs the appearance of the learning paradigm. The effect may induce by the microtubule assembly prevention has been proposed previously [10].

The present findings may demonstrate the disturbance of simple spatial memory in the conditioning device. To discuss the colchicine

prompt effect when microinjected into the CA1 pre-testing of the novelty behavior, the authors may notify that colchicine binds to tubulin dimmers, the phenomenon leading to formation of a tubulin-colchicine complex [10]. The toxin occupies the axonal membrane as well as dendrite, which are rich in microtubules and contain specific receptors [11]. This alkaloid, therefore, inhibits the rapid axonal transport [12] and produces long-lasting morphological changes in neurons and glia.

So far studies have specified a critical role of neural cytoskeleton in molecular-level processing of information required for learning and memory storage [13]. The colchicine, as an alkaloid extracted from *Colchicum autumnale* L., binds primarily to the tubulin dimmers and disrupts the axoplasmic transport [14], and induces the neurofibrillary degeneration [15,16]. Thus, the experimental animals, those received colchicine (1-25 µg/rat in the CA1 area), may exhibit a prominent staying time in the present novel environment, illustrating the impairment in the learning and memory processes. Importantly, evidence by others has shown another effect of the toxin in the brain areas; a fall in level of choline acetyltransferase, the enzyme catalyzing production of acethylcholine [17]. Other previously published work has provided a local decrease in the enzyme activity beside a significant loss of 55-kDa protein in the soluble fraction and of 50-kDa protein in myelin and synaptosomal fractions in the colchicine-received rats' hippocampi [16]. The research has additionally marked an increase level in (3H) glutamate binding in the interested area alike those seen in Alzheimer's disease [16,18].

Other chief findings, the meaningful change in expression of sniffing, and rearing, and grooming, can be discussed by rat's increased seeking behavior when encounter to the new environ. Similarly, the individual difference in the sensation-seeking responses has been reported by others. It has been shown that the sniffing behavior plays a significant role in odor information processing by shaping spatial and temporal patterns of afferent input to the olfactory bulb [19]. We may further explain that the toxin, colchicine, is introduced as a lethal agent even at the lowest concentrations [20,21]. This alkaloid has been evidenced that impairs the learning [13,22]. To clear the mechanism, we may discuss that the microinjection of the colchicine into the CA1 most likely forms a harmful compound, the tubulin-colchicine complex [15,23], which binds primarily to tubulin dimmers to prevent microtubule assembly and disturbs the axonal transmission.

There was no significant difference in the compartment entering response. Although, the change in number of squares crossed with forepaws (compartment entering) was insignificant, but, shaking the body similar to shake the water from the wet dog fur (wet-dog shaking) was significant. They were assessed to show the toxin effect on the locomotor activity or fear-like response of the treated rats. Based on the given result, the alkaloid did not induce a paralyzing response in an acute usage intra-CA1.

This work did not show a prominent drop effect of the colchicine on the small pyramidal cell population in the rat CA1 area unless for the cell' projections. We have previously indicated that the toxin, colchicine, brings damages to the neuronal entity [7]. Now, we may confirm that the neurotoxin's mass destructive effect is a time-taking outcome. Furthermore, we may demonstrate that the alkaloid can rapidly induce damages to the dendrites' projections and spines probably by disrupting their micro-tubular supporting network [12,13].

In view that this plant alkaloid impaired the functional properties of CA1 nerve cells we conclude that the microtubule-depolymerizing agent, colchicine, may swiftly block the axonal transport in the layer CA1. In contrast, it has been introduced as the selective toxin for dentate granule cells [24].

In brief, our finding may candidate the small pyramidal cells as proper for studying of memory dysfunction by colchicine.

ACKNOWLEDGMENT

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