


Original Article

# The effect of intracerebroventricular administration of insulin on memory impairment-induced by scopolamine in male rats

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## Abstract

**Introduction:** Cholinergic neuronal deficiency is one of the main causes of Alzheimer's pathology, which leads to learning and memory impairment. Scopolamine is a muscarinic cholinergic antagonist commonly used to induce Alzheimer's disease (AD). Insulin also regulates learning and memory function. Thus, the aim of this study was to determine the effect of central administration of insulin on passive avoidance learning.

**Methods:** In this experiment, fifty-nine rats were divided into 6 groups: (1) intact, (2) sham, (3) scopolamine-saline, (4) scopolamine-insulin4, (5) scopolamine-insulin8 and (6) scopolamine-insulin16. In addition, scopolamine (70nmol/2μl) was injected into the right lateral ventricle, before the retrieval test of the inhibitory avoidance task. Then the effects of three doses of insulin (4, 8 or 16 mU/2μl) were investigated on the passive avoidance learning in an amnesic model induced by scopolamine.

**Results:** Our results indicate that the retrieval of passive avoidance memory was significantly improved by intracerebroventricular administration of insulin in 4 and 8 mU/2μl doses but not in 16 mU/2μl.

**Conclusion:** These results confirmed that insulin could improve the retrieval phase of passive avoidance memory that was impaired by scopolamine.

## Keywords:

Insulin;  
Scopolamine;  
Learning;  
Memory;  
Passive avoidance learning

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## Introduction

Alzheimer's disease (AD), the most common form of dementia among the elderly, usually begins with a decrease in memory and leads to a weakening of cognitive functions and adaptation of the individual. Several neuropathological characteristics are evident in AD and may include changes in behavior (Terry et al., 2011). Most of the studies about Alzheimer's pathophysiology focused on the neurofibrillary

tangles, senile plaques, loss of brain volume and changes in cholinergic system. Among these, cholinergic impairments are involved in several AD symptoms (Mufson and Kordower, 2000). Given these cholinergic changes, it is noteworthy that cholinergic activity is correlated with the effects of glucose memory enhancement (Watson and Craft, 2004). However, glucose metabolism and insulin activity impairments also contribute to cognitive deterioration in patients with Alzheimer's. It has been demonstrated that cerebral insulin receptor inhibition

acts as an *in vivo* model of Alzheimer's (Hoyer and Lannert, 1999). According to this concept, clinical studies in the past indicated that induced hyperinsulinemia with euglycemia preservation in the periphery can ameliorate memory in Alzheimer's patients and normal adults (Craft et al., 2003).

Regarding the growing proofs of several studies, these outcomes confirm the association between peripheral (Gasparini et al., 2002) and central (Park et al., 2000) insulin effects on cognitive disorder. Insulin had an effective role in improving verbal memory in groups of patients with AD and mild cognitive impairments. However, insulin therapy, among those patients who possess the APOE (epsilon4+) genotype (a strong predictor of AD), had different effects than patients who do not carry this allele (Reger et al., 2006). Moreover, it has been shown that intranasal insulin administration had different effects on memory impairments, given the APOE genotype (Reger et al., 2008). Intranasal insulin has also demonstrated the efficacy and preventing effects on some symptoms and pathology of Alzheimer's. A large body of experiments have shown that intranasal insulin can improve cognition (Craft et al., 2012; Rosenbloom et al., 2014; Salameh et al., 2015). Animal studies indicated that insulin in the nose is able to cross the cribriform plate, that easily spreads across the brain and is able to improve learning and memory impairments (Salameh et al., 2015). Most importantly, a clinical study has shown that memory impairment progression was delayed in AD patients with poor cognitive impairment, following intranasal insulin administration (Craft et al., 2012). Further research showed that due to glucose uptake enhancement following the increase in the amount of insulin in the frontal and parietotemporal cortex, the attentional state and general performance of these patients were boosted (Craft et al., 2012).

Despite the effectiveness of insulin to improve memory impairment in AD, some ambiguities still exist. In addition, the underlying mechanisms of both insulin and cholinergic activity interaction, in passive avoidance (PA) learning are not fully understood. This study investigated how these mechanisms are created to prevent AD development. Thus, there may be alternative mechanisms that can be useful in lightening the way. Amnesic effects were induced in the animals using central infusion of a muscarinic

cholinergic antagonist, scopolamine. Subsequently, the effects of three doses of insulin on retrieval were evaluated in a PA learning task.

## Materials and methods

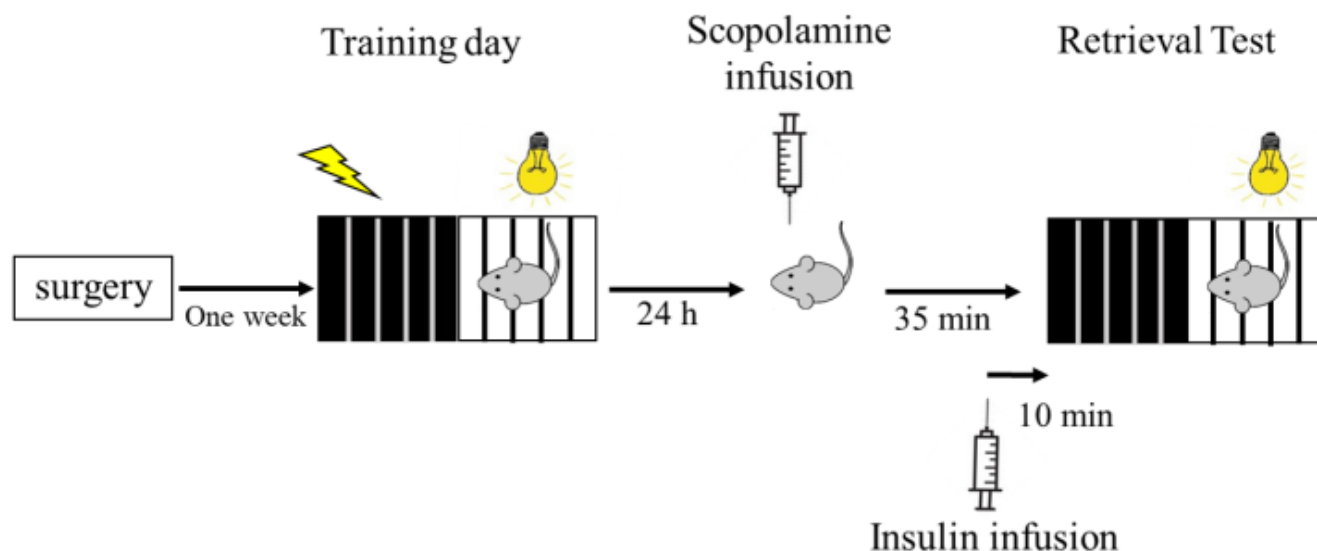
### Animals

Male Wistar rats (200–250g) that were provided from our own breeding colony, were used in this study. All experimental animals had free access to food and tap water. The environmental conditions consisted of constant temperature ( $25\pm 2^{\circ}\text{C}$ ) and controlled humidity with a 12h light/dark cycle. The procedures in the passive avoidance tests were performed in accordance with the international principles for the experimental use of animals (Olfert et al., 1993). In this study, the experiments were done in a manner conforming to internationally accepted principles for the use of experimental animals (National Research Council Committee for the Update of the Guide for the Care and Use of Laboratory Animals, 2011) and were in accordance with the guidelines of the ethical committee at Mazandaran University of Medical Sciences.

### Surgical method and drug microinjection

One week prior to the behavioral testing, the animals were anesthetized by a mixture of ketamine and xylazine (100 and 2.5mg/kg intraperitoneal, respectively). They were then placed in stereotaxic device (Stoelting, USA) and a guide cannula was fixed exactly above the right lateral ventricle of each rat, based on Paxinos and Watson atlas (AP:  $-0.8$  mm from bregma; ML: 1.5 mm from midline; DV:  $-2.6$  mm from the surface of skull). Dental acrylic cement was used for cannula fixation. Behavioral tests were performed a week after the recovery.

To deliver the drugs, an injection needle (27 gauge), joined at one end to the polyethylene tube and at the other end to a 5 $\mu\text{l}$  Hamilton micro syringe was used. Treatment infusion was done through the 21-gauge guide cannula. When the injection needle was inserted correctly, a final volume of 2 $\mu\text{l}$  of the saline, scopolamine, 4, 8, or 16mU of insulin was delivered. The needle remained in place for at least 1 minute, to deliver the medication and avoid leaking towards the outside of the cannula. The injection time lasted 3 to 4 minutes. The doses of insulin were chosen based on previous studies that examined the enhanced



**Fig.1.** A graphical abstract experimental design for the study.

insulin-induced cognitive activity (Park et al., 2000; Grillo et al., 2009).

The 70nmol scopolamine hydrobromide was dissolved in 2 $\mu$ l of sterile 0.9% saline based on earlier literature which investigated the effect of scopolamine-induced learning deficit (Albiston et al., 2004). Sterile 0.9% saline with the same volume was used as a solvent.

### Experimental design

To investigate the effect of insulin, 6 experimental groups were used in this study. These six groups were divided into: (1) intact (n=5); (2) sham (sal-sal, n=10); (3) scopolamine-saline (sco-sal, n=11); (4) scopolamine- insulin4 (sco-Ins4, n=11); (5) scopolamine- insulin8 (sco-Ins8, n=11) and (6) scopolamine- insulin16 (sco-Ins16, n=11). Memory impairment was induced by scopolamine administration (70nmol, ICV) 35min before the retrieval test. Drugs infusions of insulin (4, 8 and 16mU, ICV), or saline were delivered 10min before the retrieval test. A graphical design for this study is shown in Figure 1.

### Passive avoidance apparatus

The PA device was made up of two light and dark chambers with the same size (20 $\times$ 20 $\times$ 40 cm) and a rectangular guillotine door in the middle (8 $\times$ 8cm) that connected the two parts. The stainless steel rods were embedded on the floor of both parts spaced 1cm apart. From the floor of the dark portion, an electric current could pass through an electrical

stimulator. The behavioral test was performed in standard conditions in a sound-isolated room. A 100W lamp was placed at 40cm above the light chamber (Ardeshiri et al., 2017).

### Behavioral testing

#### PA training

All animals were habituated to the device in two trials. For these sessions, the animals were put on the light chamber and after 10s the guillotine door was opened. The rats entered the dark part of the device due to their inherent tendency to darkness. After placing the rat in a dark chamber, the door was closed and after 30s, the rat was moved from the dark chamber to its cage. The habituation trial was repeated after 30min. After a similar period of time, the acquisition trial was conducted. The delay in entering the dark chamber in the acquisition trial (step-through latency, STLa) was recorded when the animal had entered the dark chamber completely. In the training session, immediately after the animals entered the dark chamber, the door was released and an electric shock (50-Hz, 1mA for 1.5s) was used. After 20s, the rats were returned into their home cage. The procedure was repeated after 2min. An electrical shock was delivered every time that the rat re-entered the dark chamber. Whenever the rat remained in the light chamber for 120 consecutive seconds, the training session was finished.

#### Retention test

Twenty-four hours after the training trial, the retention

test was performed. The rat was left in the lighted part of the apparatus for 10s, then the door was opened and the step-through latency (STLr) and the time spent in the dark compartment (TDC) were recorded over 300s. We have recorded the time of STLr with a chronometer.

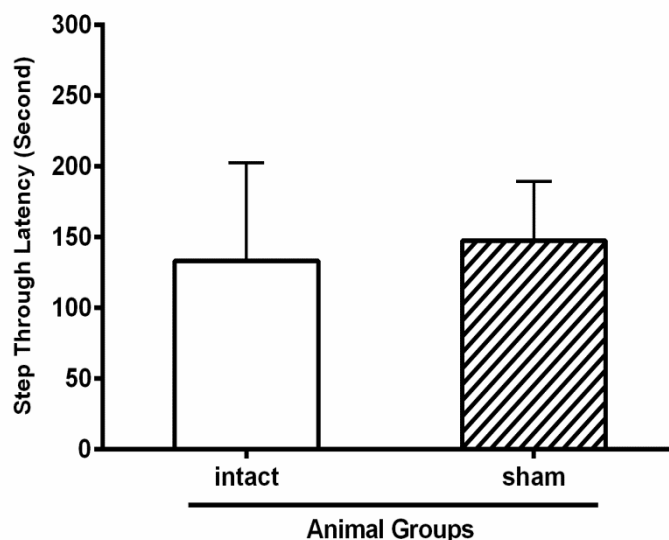
### Statistical analysis

First, the data was analyzed by Kolmogorov and Smirnov in order to test the normality of distribution. Then, non-parametrical tests, the Kurskal-Wallis, Dunn's multiple comparisons test and the Mann-Whitney U test were used. The STLr and TDC were expressed as the mean±SEM.  $P<0.05$  was considered to be significant.

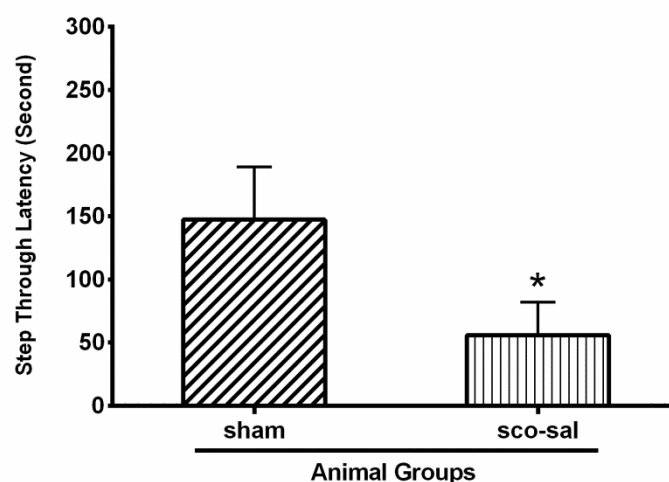
## Results

STLa (step through latency in acquisition trial) has shown no significant difference among the groups before the animals received an electrical shock. These results confirmed the innate tendency of all animal groups to the dark compartment ( $P=0.719$ , chi-square=2.878; data are not shown). There is no significant difference between sham (saline treated) and intact groups in the step-through latency analyzed by the Mann-Whitney U test. ( $P=0.5631$ , Fig. 2).

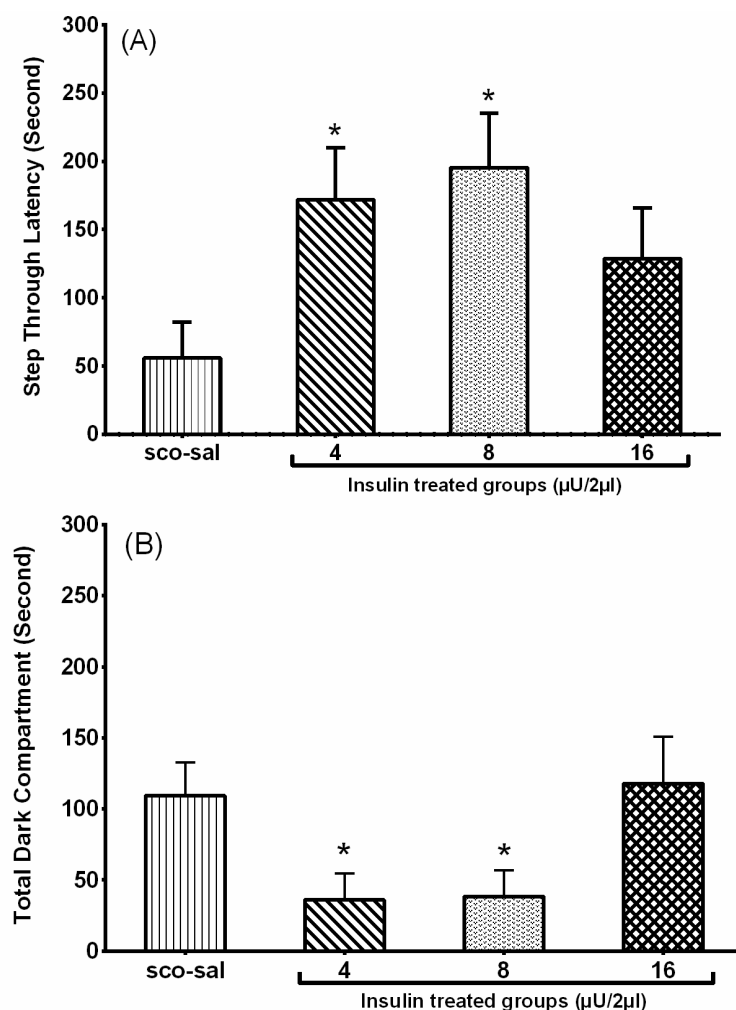
### The effect of scopolamine on retrieval of passive avoidance learning



**Fig.2.** The step-through latency time in saline treated and intact groups. The normal saline did not affect step-through latency. Data are expressed as mean±SEM.



**Fig.3.** The step-through latency time in scopolamine treated and saline treated groups. Data are expressed as mean±SEM. \* $P<0.05$  is considered as a significant value with respect to the sham group. Abbreviations: sham: saline treated group, sco-sal: scopolamine treated group.



**Fig.4.** The effects of insulin administration on memory impairment induced by scopolamine. (A) Step-through latency (STLr) and (B) time in the dark compartment (TDC) are shown here. Data are expressed as mean $\pm$ SEM. \* $P$ <0.05 is considered as a significant value with respect to the scopolamine-saline treated group. Abbreviations: sco-sal: scopolamine treated group, sco-Ins4: insulin 4 treated group, sco-Ins8: insulin 8 treated group and sco-Ins16: insulin 16 treated group.

The Mann–Whitney U test showed that the step through latencies to enter the dark chamber were significantly lower in the scopolamine treated group in comparison with the sham group ( $P=0.0225$ , Fig. 3).

#### The effect of pre-retrieval insulin ICV administration on memory impairment induced by scopolamine in PAL task

The effects of insulin (4, 8, 16mU/2 $\mu$ l) or saline (2 $\mu$ l) injection into the right ventricle on retention in the PAL task were investigated. Data analysis by Kurskal-Wallis showed a significant difference between groups ( $P=0.0294$ , chi-square=8.995). A Dunn's multiple comparisons test demonstrated that STLr significantly increased in the insulin4 ( $P=0.02$ ) and 8 ( $P=0.02$ ) groups but not in the insulin16 group ( $P=0.3$ , Fig. 4A). In addition, a Kurskal-Wallis analysis demonstrated significant difference between groups

in the TDC results ( $P=0.01$ , chi-square=10.35). A Dunn's multiple comparisons test also showed that pre-retrieval infusion of insulin into the right ventricle significantly lowered the TDC time in the 4 ( $P=0.03$ ) and 8 mU/2 $\mu$ l ( $P=0.04$ ) groups, but the TDC time in the 16 mU/2 $\mu$ l did not significantly decrease compared to the sham group ( $P>0.05$ , Fig. 4B). These results therefore indicate that blockage of cholinergic receptors in the brain has been reversed by insulin in the PAL task.

## Discussion

The main finding in the present study was pre-retrieval administration of insulin with 4 and 8 mU/2 $\mu$ l doses but not 16 mU/2 $\mu$ l, could alleviate amnesic effects induced by scopolamine in passive avoidance learning. In this study, the same behavior of the



animals in the acquisition trial (data are not shown) without any delivered shock indicate that the animals were homogeneous in learning (Ambrogi Lorenzini et al., 1997).

The positive effects of 4 and 8 mU/2 $\mu$ l, pre-retrieval administration of insulin in these experiments on passive avoidance learning were consistent with the results of previous studies in which the effects of several doses of insulin on memory and learning were investigated (Park et al., 2000; Grillo et al., 2009). Based on previous investigations the granule cells of dentate gyrus, the pyramidal cells of Ammon's horn and cerebral cortex possess high levels of insulin receptor mRNA (Marks et al., 1990). Also, intracerebroventricular administration of insulin translocate GLUT4 to the plasma membrane of hippocampal neuronal cells, lead to a rapid increase in glucose utilization during neuronal activity (Grillo et al., 2009). In this study, insulin could affect memory via two general approaches: an interaction with cholinergic cells or directly on insulin receptors in the areas involved in passive avoidance memory. In fact, the widespread expression of insulin receptors in the brain and their signal transduction cascades are important in Alzheimer's pathology.

Also, in this report, the higher level of insulin (16 mU/ $\mu$ l) has no significant effect on passive avoidance retrieval. The relationship between higher dose and response was not observed, considering that there was no significant effect on the dose of 16 mU/ $\mu$ l insulin. Regarding the pattern of the memory enhancing effect of insulin, the best results were observed in low doses of insulin. A memory facilitator pattern of insulin was somewhat in line with previous investigation (Craft et al., 2003). According to our results, high dose of insulin might reduce the levels of blood glucose to a degree that does not make glucose available for cells involved in memory (i.e. cholinergic neuronal cells), but a slight increase in insulin may increase the amount of glucose for the cells involved in the memory processes. Thus, it is highly likely that insulin activates several mechanisms in the central nervous system. It might be that a low dose of insulin activates cognitive mechanisms, while doses above the baseline level in the normal physiological state may otherwise activate metabolic mechanisms. However, the molecular mechanisms through which the effects of insulin occur during retrieval of passive avoidance learning need more

investigation.

## Conclusion

In conclusion, our results indicate that insulin could improve memory impairments induced by scopolamine. The mechanisms through which insulin could ameliorate the memory of passive avoidance task were presumably not by a direct action on glucose metabolism. Insulin-enhancing effects on cognition probably occur through both insulin effects on glucose metabolism and its unique cognitive effects. We also witnessed that a low dose of insulin improved the amnesic effects of cholinergic blockade better than a higher insulin dose. Probably, the cerebral insulin level within the physiologic level was related to memory facilitation.

## Acknowledgments

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## Conflict of interest

The authors declare that there are no conflicts of interest.

## References

- Albiston AL, Pederson ES, Burns P, Purcell B, Wright JW, Harding JW, et al. Attenuation of scopolamine-induced learning deficits by LVV-hemorphin-7 in rats in the passive avoidance and water maze paradigms. *Behav Brain Res* 2004; 154: 239-43.
- Ambrogi Lorenzini CG, Baldi E, Bucherelli C, Sacchetti B, Tassoni G. Role of ventral hippocampus in acquisition, consolidation and retrieval of rat's passive avoidance response memory trace. *Brain Res* 1997; 768: 242-8.
- Ardeshiri MR, Hosseinmardi N, Akbari E. The effect of orexin 1 and orexin 2 receptors antagonisms in the basolateral amygdala on memory processing in a passive avoidance task. *Physiol Behav* 2017; 174: 42-48.
- Craft S, Asthana S, Cook DG, Baker LD, Cherrier M, Purganan K, et al. Insulin dose-response effects on memory and plasma amyloid precursor protein in Alzheimer's disease: interactions with apolipoprotein E genotype. *Psychoneuroendocrinology* 2003; 28: 809-22.
- Craft S, Baker LD, Montine TJ, Minoshima S, Watson GS, Claxton A, et al. Intranasal insulin therapy for Alzheimer disease and amnesic mild cognitive impairment: a pilot clinical trial. *Arch Neurol* 2012; 69: 29-38.

- Gasparini L, Netzer WJ, Greengard P, Xu H. Does insulin dysfunction play a role in Alzheimer's disease?. *Trends Pharmacol Sci* 2002; 23: 288-93.
- Grillo CA, Piroli GC, Hendry RM, Reagan LP. Insulin-stimulated translocation of GLUT4 to the plasma membrane in rat hippocampus is PI3-kinase dependent. *Brain Res* 2009; 1296: 35-45.
- Hoyer S, Lannert H. Inhibition of the neuronal insulin receptor causes Alzheimer-like disturbances in oxidative/energy brain metabolism and in behavior in adult rats. *Ann N Y Acad Sci* 1999; 893: 301-3.
- Marks JL, Porte D Jr, Stahl WL, Baskin DG. Localization of insulin receptor mRNA in rat brain by in situ hybridization. *Endocrinology* 1990; 127: 3234-6.
- Mufson E, Kordower JH. Cholinergic basal forebrain systems in the primate central nervous system: anatomy, connectivity, neurochemistry, aging, dementia, and experimental therapeutics. *Functional Neurobiology of Aging*. Academic Press, San Diego 2000, p. 243-76.
- National Research Council (US) Committee for the Update of the Guide for the Care and Use of Laboratory Animals. *Guide for the care and use of laboratory animals*. Washington (DC): National Academies Press (US) National Academy of Sciences., 2011; P.154-5.
- Olfert ED, Cross BM, McWilliam AA. *Guide to the care and use of experimental animals*. Ottawa: Canadian Council on Animal Care; 1993; P.161-2.
- Park CR, Seeley RJ, Craft S, Woods SC. Intracerebroventricular insulin enhances memory in a passive-avoidance task. *Physiol Behav* 2000; 68: 509-14.
- Reger MA, Watson GS, Frey WH, Baker LD, Cholerton B, Keeling ML, et al. Effects of intranasal insulin on cognition in memory-impaired older adults: modulation by APOE genotype. *Neurobiol Aging* 2006; 27: 451-8.
- Reger MA, Watson GS, Green PS, Baker LD, Cholerton B, Fishel MA, et al. Intranasal insulin administration dose-dependently modulates verbal memory and plasma amyloid-beta in memory-impaired older adults. *J Alzheimers Dis* 2008; 13: 323-31.
- Rosenbloom MH, Barclay TR, Pyle M, Owens BL, Cagan AB, Anderson CP, et al. A single-dose pilot trial of intranasal rapid-acting insulin in apolipoprotein E4 carriers with mild-moderate Alzheimer's disease. *CNS Drugs* 2014; 28: 1185-9.
- Salameh TS, Bullock KM, Hujoel IA, Niehoff ML, Wolden-Hanson T, Kim J, et al. Central nervous system delivery of intranasal insulin: mechanisms of uptake and effects on cognition. *J Alzheimers Dis* 2015; 47: 715-28.
- Terry AV Jr, Callahan PM, Hall B, Webster SJ. Alzheimer's disease and age-related memory decline (preclinical). *Pharmacol Biochem Behav* 2011; 99: 190-210.
- Watson GS, Craft S. Modulation of memory by insulin and glucose: neuropsychological observations in Alzheimer's disease. *Eur J Pharmacol* 2004; 490: 97-113.