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Effect of Sub-Acute Insulin Treatment on Short-Term Non-Spatial Working Memory in Mice

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ABSTRACT

Insulin is well known as a trophic hormone that regulates glucose, protein and lipid metabolism in target tissues. It was, until recently, considered as a peripheral hormone that had no effect on the central nervous system. It is now well established that insulin occurs in the brain where it exerts many physiological effects. The aim of the study was to determine the effect of sub-acute insulin administration on non-spatial working memory in mice. Twelve mice of both sexes, weighing between 18 - 22 g, were divided into two groups and treated with either insulin or de-ionized water (control) for seven days. Short-term working memory was assessed using novel object recognition task. Time spent exploring the objects did not differ between the groups. Novel object recognition and discrimination ratio was also similar for the control and insulin-treated animals. It is concluded that sub-acute administration of insulin has no effect on short-term non-spatial working memory in treated mice.

Key Words: *Insulin, Short-Term Memory, Non-Spatial Memory, Working Memory, Sub-Acute Treatment*

INTRODUCTION

Insulin is well known as a trophic hormone that regulates glucose, protein and lipid metabolism in target tissues (Venkatesh 2012). It is also known to be used as replacement therapy in type 1 diabetes mellitus. Until recently, insulin was considered only as a peripheral hormone, unable to cross the blood brain barrier and to affect the central nervous system (Laron 2009). It is now well established that insulin occurs in the brain where it exerts regulatory and trophic effects (Blázquez et al. 2014). In addition to its metabolic effects, insulin is reported to have other physiological effects, including effect on cognition (Ghasemi et al. 2013). Despite active research into the role of insulin in cognition, there is still much to be discovered on the effects of insulin on the various forms of memory and learning. In particular, there is dearth of information on the effect of sub-acute insulin administration on non-spatial working memory.

The aim of the study was, therefore, to determine the effect of sub-acute insulin administration on non-spatial working memory in mice.

MATERIALS AND METHODS

Care, Treatment and Grouping of Animals

Young mice of both sexes, weighing between 18-21 g

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(4-6 weeks old), were used for the study. Experimental protocols were approved by Ahmadu Bello University Research Committee. The mice were allowed free access to feed and drinking water during acclimatization and throughout the experimental period. They were maintained under the prevailing natural 12-h light/12-h dark cycle (photo phase: 6:30 – 18:30). Insulin (Actrapid, Novo Nordisk A/S, Denmark), reconstituted 1:3 with de-ionized water (dH2O) for ease of dosing, was used. Twelve mice, divided into two groups were used. They were treated for 7 days with daily subcutaneous injection of de-ionized water (control group) or insulin at the dose of 10 I.U./kg/day (insulin-treated group) (Sharma et al. 2007).

Behavioural tests were done 30 minutes after the last insulin injection, after one week of insulin administration (Francis et al. 2008). The manifestation of learning and memory obtained from insulin-treated mice were compared with that of control.

Assessment of short-term non-spatial working memory using novel object recognition task

The novel object recognition (novel object preference) task (NORT) capitalized on the findings of Berlyne (1950) that rats prefer to explore objects that they have not previously encountered over objects that are familiar. Preferences to explore the various objects are recorded, and a tendency to explore the novel object over the familiar sample is interpreted as evidence of memory for the training exposure (Ennaceur and Delacour 1988). Object recognition experiments were performed in a locally-constructed white wooden box of 60 x 40 x 30 cm dimension. Objects to be discriminated were of about the same size, made of plastic, deferring in shape and colour. Objects had no genuine significance and were not previously associated to rewarding or aversive stimuli. Two days before the test, mice were allowed to explore the box twice for 5 minutes, in order to acclimatize. On the testing day each mouse was placed in the box for two 4-minute sessions and left to explore objects freely. During the first session (S1), two copies of the same object were present, whereas in the second session (S2), mice were exposed to a copy of the objects, presented previously in S1 plus a novel object. The S1 and S2 were separated by a 15-min interval. Exploration was defined as the mice sniffing, gnawing or touching the object with the nose, or head orientation within <1.0 cm, whereas sitting and/or turning around the object was not considered as exploratory behaviours. To avoid the presence of olfactory cues, objects and floor were thoroughly cleaned with 70% ethanol after each session. Moreover, the combination of objects (novel vs. old) and their respective position (right versus left) were counter balanced to prevent biased preferences for particular objects or positions. The performance of the rats was video-taped and the following parameters were evaluated:

a) Time spent by the rats in exploring the objects during either S1 or S2;

b) Novel object recognition. The latter was calculated as the percentage of time spent in exploring the new object with respect to the total amount of time spent in exploring the two objects during S2; and

c) The discrimination-ratio was the duration of exploration of the novel object, divided by the total exploration duration of both objects during the test phase. A discrimination ratio equal to 0.5 indicated chance behaviour; with scores above 0.5; indicating preference for the novel object and, therefore, memory of the familiar object.

Statistical Analyses

Values were expressed as mean ± S.E.M. The t-test of independent samples was used to compare means of novel object recognition, while General Linear Model-repeated measures ANOVA was used to compare time spent exploring objects (NORT). Bonferroni test was employed for post-hoc multiple comparisons. Values of $P < 0.05$ were considered significant. All data were analysed using Statistical Package for the Social Sciences (SPSS) version 20.0 statistical package (SPSS Inc., IL, USA).

RESULTS

Time Spent Exploring the Objects

The time spent exploring objects by the control and insulin-treated animals during the two sessions of the NORT is presented in Figure 1. Insulin-treated mice spent 1.83 ± 1.0, 1.50 ± 0.8, 5.00 ± 1.2and 13.00 ± 2.7seconds on object 1, object 2, object 3 and novel object, respectively. Mice in the control group spent 4.50 ± 2.5, 3.67 ± 1.9, 6.83 ± 2.0, and 18.17 ± 2.9seconds on object 1, object 2, object 3 and novel objects respectively. Although the animals spent more time exploring the novel object, there was no significant difference in time spent on familiar and novel objects between the two groups ($P > 0.05$). This indicates that the performance of the two groups were similar, suggesting that insulin did not have a significant effect on the memory of the treated mice.

Novel Object Recognition and Discrimination Ratio

Novel object recognition was 76.34 ± 5.8 and 69.82 ± 4.7 for the control and insulin groups, respectively (Figure 2). Discrimination ratio was 1.62 and 1.64 for the control and insulin groups, respectively. There was no significant difference in the novel object recognition ($P> 0.05$) between the two groups. The animals in both groups have shown preference for the novel object, which indicates intact memory for the animals. This implies that insulin treatment did not affect memory.

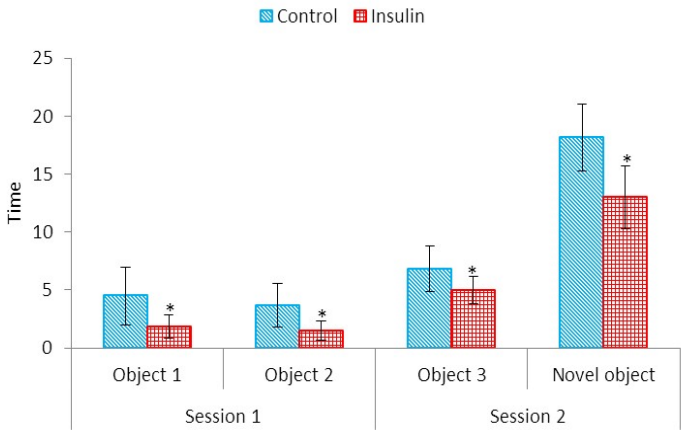

Fig. 1: Time spent by control and insulin-treated mice exploring familiar and novel objects during novel object recognition task (Mean ± S.E.M, n = 6). * = statistically the same (*P*> 0.05) compared to control group.

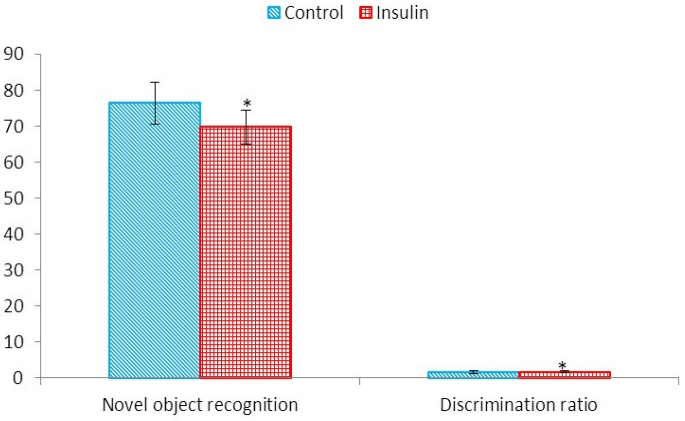

Fig. 2: Novel object recognition and discrimination ratio of control and insulin-treated mice during novel object recognition task (Mean ± S.E.M, n = 6). * = statistically the same (P> 0.05) compared to control group.

DISCUSSION

Measurement of novel object recognition is widely used for evaluating non-spatial working memory in rodents (Ennaceur and Delacour 1988); not involving the use of primary reinforcement (for example, food, shock), and comparable to similar procedures employed in human and non-human primate subjects (Ennaceur 2010). In this study, the animals in the two groups showed preference for the novel object which indicated memory of the earlier exposure with the other object. This was corroborated further by the values of discrimination ratio (above 0.5) which established the reliability of such behaviour. This performance was as expected of mice with intact memory. The results indicate that the insulin treatment did not impair or improve short-term non-spatial working memory in the exposed animals, and agree with the findings of Backeström et al. (2015), who showed that plasma insulin levels were not associated with lower episodic memory in a non-diabetic and non-demented human population.

Evidence exists that demonstrate the fact that insulin signalling is required for normal memory process in humans and lower animals (Lin et al. 2010; Liu et al. 2013; Bloemer et al. 2014). However, reports on the effect of insulin on different types of learning and memory remain scanty and inconsistent, with some reporting improvement (McNay et al. 2010), others – impairment (Kamal et al. 2013), and yet others – no effect (Backeström et al. 2015). As it is inherent in most biological processes, the effects vary based on many influencing circumstances such as dose, duration of exposure (acute, sub-acute or chronic) and route of insulin administration (including intra-peritoneal, intra-cerebro-ventricular, intra-hippocampal). Further studies are required to elucidate the effect of insulin on learning and memory animals under different circumstances.

CONCLUSION

Based on the findings of this study, it was concluded that sub-acute administration of insulin exerted no significant effect on short-term, non-spatial working memory in the treated mice.

Conflict of Interest

None declared.

REFERENCES

Backeström, A., Eriksson, S., Nilsson, L., Olsson, T. and Olov-Rolandsson, O. (2015) Glucose but not insulin or insulin resistance is associated with memory performance in middle-aged non-diabetic women: a cross sectional study. Diabetology and Metabolic Syndrome. 7:20-28.

Berlyne, D. E. (1950) Novelty and curiosity as determinants of exploratory behaviour. British Journal of Psychology. 41:68-80.

Blázquez, E., Velázquez, E., Hurtado-Carneiro, V. and Ruiz-Albusac, J. M. (2014) Insulin in the brain: its pathophysiological implications for states related with central insulin resistance, type 2 diabetes and Alzheimer's disease. Frontiers in Endocrinology. 5:161-172.

Bloemer, J., Bhattacharya, S., Amin, R. and Suppiramaniam, V. (2014) Impaired insulin signaling and mechanisms of memory loss. Progress in Molecular Biology and Translational Science. 121:413-449.

Ennaceur, A. (2010) One-trial object recognition in rats and mice: methodological and theoretical issues. Behavioural Brain Research. 215(2):244-254.

Ennaceur, A. and Delacour, J. (1988) A new one-trial test for neurobiological studies of memory in rats. 1: behavioral data. Behavioral Brain Research. 31:47-59.

Francis, G. J., Martinez, J. A., Liu, W. Q., Xu, K., Ayer, A., Fine, J., Tuor, U. I., Glazner, G., Hanson, L. R., Frey II, W. H. and Toth, C. C. (2008) Intranasal insulin prevents cognitive decline, cerebral atrophy and white matter changes in murine type I diabetic encephalopathy. Brain. 131:3311-3334.

Ghasemi, R., Haeri, A., Dargahi, L., Mohamed, Z. and Ahmadiani, A. (2013) Insulin in the brain: sources, localization and functions. Molecular Neurobiology. 47(1):145-171.

Kamal, A., Ramakers, G. M. J., Gispen, W. H., Biessels, G. J. and Al Ansari, A. (2013) Hyperinsulinemia in rats causes impairment of spatial memory and learning with defects in hippocampal synaptic plasticity by involvement of postsynaptic mechanisms. Experimental Brain Research. 226: 45 - 51.

Laron, Z. (2009) Insulin and the brain. Archives of Physiology and Biochemistry. 115(2):112-116.

Lin, C. H. A., Tomioka, M., Pereira, S., Sellings, L., Iino, Y. and van der Kooy, D. (2010) Insulin signaling plays a dual role in caenorhabditis elegans memory acquisition and memory retrieval. The Journal of Neuroscience. 30(23):8001– 8011.

Liu, P., Zou, L., Jiao, Q., Chi, T., Ji, X., Qi, Y., Xu, Q. and Wang, L. (2013) Xanthoceraside attenuates learning and memory deficits via improving insulin signaling in STZ-induced AD rats. Neuroscience Letters. 543:115-120.

McNay, E. C., Ong, C. T., McCrimmon, R. J., Cresswell, J., Jonathan, S. Bogan, J. S. and Sherwin, R. S. (2010) Hippocampal memory processes are modulated by insulin and high-fat-induced insulin resistance. Neurobiology of Learning and Memory. 93(4):546–553.

Sharma, S., Sharlene, R. and Holly, B. (2010) Assessment of spatial memory in mice. Life Sciences. 87:521-536.

Venkatesh, D. (2012) Endocrine system. In Tandon, O. P and Tripathi, Y. (eds.). Best and Taylor's Physiological Basis of Medical Practice, 13th Ed. Gurgaon: Wolters Lower/Lippincott Williams and Wilkins.

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