Journal of Medical Sciences

ISSN 1682-4474

science alert

ANSInet
an open access publisher
http://ansinet.com

Research Paper

JMS (ISSN 1682-4474) is an International, peer-reviewed scientific journal that publishes original article in experimental & clinical medicine and related disciplines such as molecular biology, biochemistry, genetics, biophysics, bio-and medical technology. JMS is issued eight times per year on paper and in electronic format.

For further information about this article or if you need reprints, please contact:

Moazedi Ahmad Ali
Department of Biology,
School of Science,
Shahid Chamran University,
Ahvaz, Iran

Tel: 0098-9163080198
Fax: 0098-611-3331045

J. Med. Sci., 8 (1): 22-27
1st January, 2008

Co-Administration of Epinephrine and Glucose Do Not Have Synergic Effects on the Improvement of Spatial Learning Task in Young Male Rats

[1]A.A. Moazedi, [1]M. Belaran, [2]A. Hemmaty and [3]A. Rasekh

This study was designed to evaluate the influence of co-administered epinephrine and glucose on spatial learning in the Y-maze task. Young male wistar rats were given daily injections of epinephrine (0.1 mg kg^{-1} 30 min before training, sc), glucose (500 mg kg^{-1} 10 min before training, i.p.) and co-administration of epinephrine (0.1 mg kg^{-1}) and glucose (500 mg kg^{-1}). Sham groups received saline at the same volumes and conditions. The results showed that epinephrine significantly increase spatial learning at first ($p<0.05$), second ($p<0.05$), third ($p<0.01$) and fourth ($p<0.05$) days. Also, spatial learning improve at first ($p<0.05$) day in glucose group. Comparison between co-administration epinephrine and glucose and epinephrine groups were significant in first day ($p<0.001$). No synergic effect observed on the enhancement of spatial learning task in co-administration of epinephrine and glucose.

Key words: Glucose, epinephrine, spatial learning, Y-maze, rat

[1]Department of Biology, School of Science,
Shahid Chamran University, Ahvaz, Iran
[2]Department of Pharmacology, School of Pharmacology,
Jundishapour University, Ahvaz, Iran
[3]Department of Statistic, School of Mathematic,
Shahid Chamran University, Ahvaz, Iran

ANSInet
Asian Network for Scientific Information

INTRODUCTION

The memory-improving action of glucose has now been studied for almost 20 years and the study of this phenomenon has led to a number of important developments in the understanding of memory and brain physiology (Messier, 2004). Over the past several years, considerable evidence has accumulated from rodents and humans suggesting that modest increases in circulating glucose regulate many brain and behavior functions, including learning and memory (Gold, 2004, 2005; Nabb and Benton, 2006). Systemically administered drugs like D-glucose near the time of training, enhance learning and memory for a broad spectrum of tasks (Gold, 2004; McNay *et al*., 2006; Salinas and Gold, 2005). Also, glucose effectively enhances cognition in persons with Alzheimer disease or Down syndrome (Watson and Craft, 2004). Injections of glucose prior to behavioral testing enhance memory and block the testing-associated drop in ECF glucose in the hippocampus (Messier, 2004; Gold, 2005). Although glucose improvement of memory is well established, there are controversial reports in this ground. For example, as shown by Ragozzino *et al*. (1992), glucose (100 mg kg^{-1}) did not enhance Y-maze spontaneous alternation performance, when injected intra-septally. In addition, administration of glucose (250 mg kg^{-1}) did not influence norepinephrine release in the hippocampus in plus maze test (Men *et al*., 1999).

On the other hand, endogenous factors such as hormonal responses to training, known as potential modulators of the formation of memory (Gold, 2004). Epinephrine as an adrenergic agonist is one of the most reliable enhancers of memory formation, with evidences showing that increases in peripheral epinephrine levels induced by external stimuli or exogenous administration of this hormone, improve memory in both rodents and humans (Korol and Gold, 1998; Miyashita and Williams, 2004, 2006).

As suggested by Korol and Gold (1998), epinephrine release at or around the time of training, is implicated in regulating memory formation. A contribution of the peripheral hormone epinephrine in modulating memory for emotionally-arousing experiences demonstrates across species and in a wide range of learning conditions (Clayton and Williams, 2000; Lieberman *et al*., 2005). In addition, findings suggest that memories for events at times of high motivation, stress, or arousal levels are retained for longer (Watson and Craft, 2004). Norepinephrine also has long been suggested as a modulator of learning and memory (Harley, 2004). There are controversial reports about the effect of epinephrine on learning. For example, as shown by Talley *et al*. (2000), epinephrine fails to enhance performance on a delayed spontaneous alternation task, in rats were deprived of food for 24 h. Also, As shown by Gold and van Buskirk (1978) exogenous injection of epinephrine to rats, impairs memory for an aversive task when the electric shock use to motivate animal cause release of much endogenous epinephrine.

Concerning the memory enhancing effects of glucose and epinephrine, the present study evaluates the effect of co-administration of glucose and epinephrine on spatial learning.

MATERIALS AND METHODS

Animals: Male wistar rats, weighting 90±30 g, were prepared from animal house of Jundishapour University. The rats were individually housed in a clean room, with constantly controlled temperature (23±1°C) and light (12 h light-dark cycles, with lights on at 8:00) and free access to tap water and pelleted food. They were held in the colony room for 2 days before training. Also were handled during these days. All experimental manipulations were carried out during the light period from 07:00 am to 06:00 pm.

Drug administration: Glucose and epinephrine were obtained from Merck chemical Co. Glucose prepared in sterile 0.9% NaCl vehicle but we add 4 drops of NaOH (1.0 N) to sterile 0.9% NaCl vehicle for solving epinephrine (Swetman, 2002). About 500 mg kg^{-1} glucose (Ebrahimy Vostakolaee *et al*., 2002) by i.p injection and 0.1 mg kg^{-1} epinephrine (Clayton and Williams, 2000) by s.c injection were administered daily for 5 days prior to onset of training and testing and was continued on the same schedule throughout the duration of experiment. During behavioral manipulations on training days, glucose 10 min or epinephrine 30 min were injected, before training. Rats randomly were divided into 7 groups.

Control group as no injection group was trained only, glucose injection group, epinephrine injection group, co-administration glucose and epinephrine and two sham groups were received saline and one sham group was received saline with NaOH at the same volume and conditions.

Blood glucose level: Blood glucose concentration in control, sham and glucose groups were determined 10 min after injection of glucose (500 mg kg^{-1}) or saline. The mean of blood glucose concentration were 110 mg dL^{-1} in control, 98 mg dL^{-1} in sham and 140 mg dL^{-1} in glucose groups.

Apparatus: A Y-maze apparatus was used for active avoidance conditioning (Ebrahimy Vostakolaee *et al*., 2002). The Y-maze was composed of three equally spaced, through shaped arms joined to a triangular central platform. Each arm was 60 cm in length and 17.5 cm in height, with a floor width of 3.5 cm and a ceiling width of 14 cm. the ceiling was covered with translucent black Plexiglas (Stefani and Gold, 2001).

Behavioral testing procedures: To begin each experimental session, the animal was transported in its living cage from the colony room to the testing room. After a few minutes, the animal was transferred to the unit and procedures were initiated as follows. Y-maze apparatus was put in a completely dark and silent place. To knowing the apparatus, at the first day of the training, rats were allowed to have a freely moving for 15 min. Then, training began from the arm that rat was there. Each rat was trained 30 trials every day and training was continued for 5 days. At the end of each session, Correct Response Percentages (CRP) were calculated. Minimum CRP was considered as 86.6% (Moazedi and Motamedi, 1995).

Statistical analyses: Data were analyzed with SPSS software (ver :10.0) using One Way ANOVA procedure, then for determining the significant difference between groups, Least Significant Difference (LSD) procedure were used. The minimally acceptable level was set at $p<0.05$.

RESULTS AND DISCUSSION

The results showed that there is a significant difference between glucose (n = 7) and sham (n = 7) groups at the first day ($p<0.05$) of training. Indeed, glucose injection caused improvement of spatial learning (Fig. 1). Also, comparison between epinephrine (n = 8) and sham groups (n = 7) show a significant difference at the first ($p<0.05$), second ($p<0.05$), third ($p<0.01$) and fourth ($p<0.05$) days (Fig. 2). On the other hand, comparison between co-administration of glucose and epinephrine group (n = 8) and epinephrine group, show a significant difference at first ($p<0.001$) day of training (Fig. 3), the mean of correct responses in co-administered is lower than epinephrine group. There was no significant difference between glucose and co-administered groups (Fig. 3).

In this study, the effect of co-administration of glucose and epinephrine on the spatial learning was investigated. The results showed that exogenous

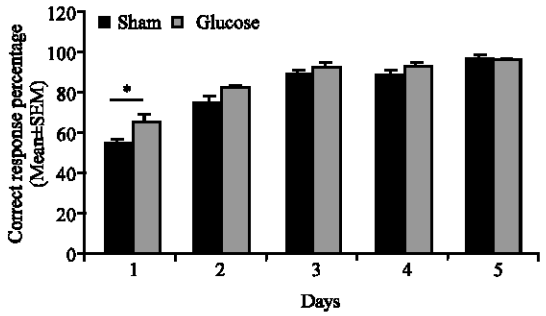

Fig. 1: The effect of glucose administration on correct response percentage (Mean±SEM) in the 5 days of training*: $p<0.05$

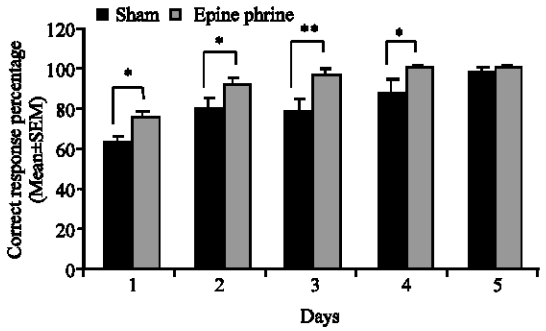

Fig. 2: The effect of epinephrine administration on correct response percentage (Mean±SEM) in the 5 days of training *: $p<0.05$ ***: $p<0.01$

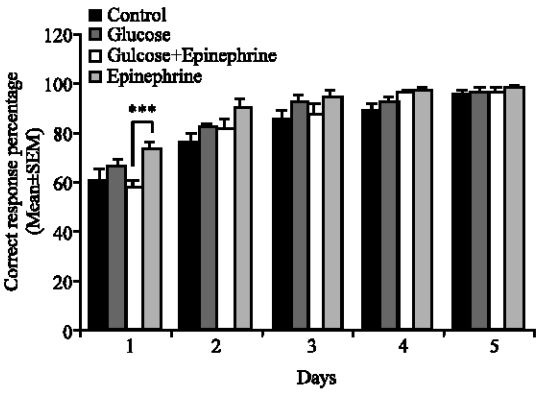

Fig. 3: Comparison between the mean of correct response percentage (Mean±SEM) in the 5 days of training of glucose, epinephrine and co-administration of glucose and epinephrine ****: $p<0.001$

injection of glucose improves spatial learning in young male rats and this result, confirms several reports of other researchers.

According to Gold (2005), administration of glucose enhances cognitive functions in humans and rodents,

including reversing age-related impairments in learning and memory. On the other hand, there are controversial reports about the positive effect of glucose on spatial learning. As shown by Gold *et al.* (1986), post training injection of glucose (100 mg kg^{-1}, s.c) to Spragu-Dawley rats, impairs memory for an aversive task when the electric shock use to motivate animal is a high-intensity shock (0.7 mA, 3.4 sec) while facilitates memory for an aversive task when the electric shock uses to motivate animal is a low intensity shock (0.5 mA, 0.7 sec).

The mechanisms of glucose's actions on memory have been under intense investigation for the past several years. It seems unlikely that glucose affects cognition via only one mechanism and findings indicate that it is possible that both central and peripheral mechanisms operate to produce the optimal physiological states that will lead to memory facilitation (Messier, 2004). Glucose freely crosses the blood-brain barrier (Pych *et al.*, 2006) via its transporters. So; it is plausible that glucose affects central processes directly to modulate memory performance (Messier, 2004; Stefani *et al.*, 1999). Microinjections of glucose directly into the lateral ventricles of the brain, hippocampus (Stefani and Gold, 2001), medial septum (Stefani and Gold, 1998) or amygdala (Schroeder and Packard, 2003) can also enhance memory and reverse drug-induced impairments in learning and memory (Korol and Gold, 1998; McNay *et al.*, 2006; Berridge and Waterhouse, 2003) The other possible mechanism by which glucose exerts it's effect, is the enhancement of acetylcholine (Ach) release in the brain in learning and memory situations and conclude that cholinergic system is involved in glucose regulation of learning and memory and other cognitive functions (Korol and Gold, 1998; Gulpinar and Yegen, 2004). Also, Glucose can affects memory processes via K-ATP channels. This class of inwardly rectifying potassium channel is present in the mammalian brain and expresses at high levels in the hippocampus (McNay and Gold, 2002; Stefani *et al.*, 1999). In addition, there are in the cortex, striatum and septal region (Stefani *et al.*, 1999; Rashidy-Pour, 2001).

Also, our results showed that exogenous injection of epinephrine significantly enhances spatial learning in young male rats and this result confirms several studies in this field. There are reports have been suggested that in rodents, epinephrine enhances memory for inhibitory avoidance, active avoidance, spontaneous alternation, visual discrimination and one-trial appetitive task (Talley *et al.*, 2000). On the other hand, there are controversial reports about the effect of epinephrine on spatial learning.

For example, as shown by Sonner *et al.* (2005), administration of exogenous epinephrine (0.01 mg kg^{-1}, i.p.) did not decrease amnesia produced by inhaled isoflurane or desflurane.

Understanding of how epinephrine affects memory processing remains obscure because epinephrine does not freely enter into the central circulation to produce any direct effects on the brain (Miyashita and Williams, 2006; Clayton and Williams, 2000). Thus, a peripheral action of the hormone most likely mediates its effects on cognition (Gold, 2004). In addition, epinephrine has been shown to produces a number of central actions that facilitate the encoding of new events into long-term memory (Miyashita and Williams, 2006).

Recently, extensive evidences suggest that a possible pathway by which epinephrine exerts at least a part of its peripheral actions may involve the actions of this hormone on hepatic stores of glucose (McGaugh and Roozendaal, 2002) and the subsequent penetration of glucose into the brain to affect acetylcholine release in the hippocampus (Miyashita and Williams, 2006).

Other findings have revealed a peripheral-central neuronal pathway mediating the effects of epinephrine on memory consolidation (McGaugh and Roozendaal, 2002). Several lines of evidence suggest that epinephrine may act on vagal afferents to the brain to regulate memory formation (Ghacibeh *et al.*, 2006; Miyashita and Williams, 2004). The vagus is densely embedded with β-adrenergic receptors and neural activity along peripheral afferent vagal fibers is significantly elevated in response to systemic injection of epinephrine (Miyashita and Williams, 2006). The nucleus of the solitary tract (NTS) is a terminal region of ascending vagal fibers and appears to contribute to the memory facilitating affects of peripheral hormones (Clayton and Williams, 2000). NTS neurons that receive vagal input travel through the brainstem and their axonal terminals innervate and release norepinephrine within a widespread region of the amygdala (Miyashita and Williams, 2006; Clayton and Williams, 2000). Norepinephrine binds to both β-adrenoceptors and α_1-adrenoceptors at postsynaptic sites and activates cyclic adenosine monophosphate (cAMP) and protein kinase formation (McGaugh and Roozendaal, 2002). The terminal projections of both NTS and Locus Ceruleus (LC) neurons, release norepinephrine in structures that are involved in either increasing peripheral sympathetic output or in structures that play important roles in modulating the storage of new experiences into long-term memory (Miyashita and Williams, 2004, 2006). Through these anatomical connections, input received from peripheral vagal fibers, can initiate all of the central

changes that are observed following systemic administration of epinephrine (Miyashita and Williams, 2004, 2006).

Present results of comparison between epinephrine and co-administration of glucose and epinephrine groups, showed an impairment of spatial learning in co-administered rather than epinephrine group. These findings indicate that although co-administration of glucose and epinephrine can improves spatial learning, the memory enhancing effect is less than glucose or epinephrine injection alone. In the other words, possibly, glucose and epinephrine don't have a synergism effect on the spatial learning.

This result, confirms previous experiments in our laboratory indicated that the rats that received glucose (500 mg kg^{-1}) immediately posttraining, shows decreased learning in comparison with sham groups (Ebrahimy Vostakolaee *et al*., 2002).

High doses of epinephrine or glucose result in blood glucose levels significantly greater than those seen under conditions which predict enhancement of memory storage and can result in retrograde amnesia. Also, the dose-response curve for the enhancing effect of epinephrine and glucose is inverted-U in shape with amnesia at high doses (Hall and Gold, 1992). In addition, as shown by Ragozzino *et al*. (1996), 24 min pretraining administration of glucose (1000 mg kg^{-1}) did not influence spatial learning in a four-arm cross maze.

Regarding these reports, it is possible that systemic administration of epinephrine enhances blood glucose levels and this amount of glucose, add to the exogenous glucose and impair spatial learning in comparison with glucose or epinephrine injection alone.

ACKNOWLEDGMENT

All experiments were performed in the Laboratory of Learning and Memory in Biology Department of Shahid Chamran University in Iran.

REFERENCES

Berridge, C.W. and B.D. Waterhouse, 2003. The locus coeruleus-noradrenergic system: Modulation of behavioral state and state-dependent cognitive processes, a review. Brain Res. Rev., 42: 33-84.

Clayton, E.C. and C.L. Williams, 2000. Noradrenergic receptor blockade of the NTS attenuates the mnemonic effects of epinephrine in an appetitive light-dark discrimination learning task. Neurobiol. Learn Mem., 74: 135-145.

Ebrahimy Vostakolaee, S., A. Moazedi and R. Chinipardaz, 2002. Comparison of the effects of pretraining and posttraining administration of glucose on the spatial learning in male rats. Shahid Chamran Univ. J. Sci., 9: 15-25.

Ghacibeh, G.A., J.I. Shenker, Shenal, M. Basim and M. Kenneth, 2006. The influence of vagus nerve stimulation on memory. Cogn. Behav. Neuro., 19 (3): 119-122.

Gold P.E. and R.B. van Buskirk, 1978. Effects of alpha and β-adrenergic receptor antagonists on post-trial epinephrine modulation of memory: Relationship to posttraining brain norepinephrine concentrations. Behav. Biol., 24: 168-184.

Gold, P.E., J. Vogt and J.L. Hall, 1986. Glucose effects on memory: Behavioral and pharmacological characteristics. Behav. Neural. Biol., 46 (2): 145-155.

Gold, P.E., 2004. Coordination of multiple memory systems, mini review. Neurobiol. Learn. Mem., 82: 230-242.

Gold, P.E., 2005. Glucose and age-related changes in memory. Neurobiol. Aging., 26: 60-64.

Gulpinar, M.A. and B.C. Yegen, 2004. The physiology of learning and memory: Role of peptides and stress. Curr. Peptid Protein Sci., 5: 457-473.

Hall, J.L. and P.E. Gold, 1992. Plasma glucose levels predict the disrupting effects of adrenoceptor antagonists on enhancement of memory storage. Eur. J. Phycol., 221: 365-337.

Harley, C.W., 2004. Norepinephrine and dopamine as learning signals. Neural. Plast., 11 (3-4): 191-204.

Korol, D.L. and P.E. Gold, 1998. Glucose, memory and aging. Am. J. Clin. Nutr., 67: 764-771.

Lieberman, H.R., J.H. Georgelis, T.J. Maher and S.K. Yeghiayan, 2005. Tyrosine prevents effects of hyperthermia on behavior and increases norepinephrine. Physiol. Behav., 84 (1): 33-38.

McGaugh, J.L. and B. Roozendaal, 2002. Role of adrenal stress hormones in forming lasting memories in the brain. Curr. Opin Neurobiol., 12: 205-210.

McNay, E.C. and P.E. Gold, 2002. Food for thought: Fluctuations in brain extracellular glucose provide insight into the mechanisms of memory modulation. Behav. Cogn. Neurosci. Rev., 1 (4): 264-280.

McNay, E.C., C.E. Canal, R.S. Sherwin and P.E. Gold, 2006. Modulation of memory with septal injections of morphine and glucose: Effects on extracellular glucose levels in the hippocampus. Phisiol. Behav., 87 (2): 298-303.

Men, D., R. Mccarty and P.E. Gold, 1999. Enhanced release of norepinephrine in rat hippocampus during spontaneous-alternation tests. Neurbiol. Learn Mem., 71 (3): 289-300.

Messier, C., 2004. Glucose improvement of memory: A review. Eur. J. Phycol., 490: 33-57.

Miyashita, T. and C.L. Williams, 2004. Peripheral arousal-related hormones modulate norepinephrine release in the hippocampus via influences on brainstem nuclei. Behav. Brain Res., 153: 87-95.

Miyashita, T. and C.L. Williams, 2006. Epinephrine administration increases neural impulses propagated along the vagus nerve: Role of peripheral β-adrenergic receptors. Neurobiol. Learn Mem., 85: 116-124.

Moazedi, A. and F. Motamedi, 1995. Effect of GM1 on discrimination learning in rat. Iranian J. Biol., 1 (1): 10-24.

Nabb, S. and D. Benton, 2006. The influence on cognition of the interaction between the macro-nutrient content of breakfast and glucose tolerance. Physiol. Behav., 87: 16-23.

Pych, J.C., M. Kim and P.E. Gold, 2006. Effects of injections of glucose into the dorsal striatum on learning of place and response mazes. Behav. Brain Res., 167: 373-378.

Ragozzino, M.E., M.E. Parker and P.E. Gold, 1992. Spontaneous alternation and inhibitory avoidance impairments with morphine injections into the medial septum. Attenuation by glucose administration. Brain Res., 597: 241-249.

Ragozzino, M.E., K.E. Unick and P.E. Gold, 1996. Hippocampal acetylcholine release during memory testing in rats: Augmentation by glucose. Proc. Nat. Acad. Sci., USA., 93: 4693-4698.

Rashidy-Pour, A., 2001. ATP-sensitive potassium channels mediates the effects of a peripheral injection of glucose on memory storage in an inhibitory avoidance task. Behav. Brain Res., 126: 43-48.

Salinas, J.A. and P.E. Gold, 2005. Glucose regulation of memory for reward reduction in young and aged rats. Neurobiol. Aging., 26 (1): 45-52.

Schroeder, J.P. and M.G. Packard, 2003. Systemic or intra-amygdala injections of glucose facilitate memory consolidation for extinction of drug-induced conditioned reward. Eur. J. Neurosci., 17: 1482-1488.

Sonner, J.M., Y. Xing and M.D. Yi Zhang, 2005. Administration of epinephrine does not increase learning of fear to tone in rats anesthetized with isoflurane or desflurane. Anesth Analg., 100: 1333-1337.

Stefani, M.R. and P.E. Gold, 1998. Intra-septal injections of glucose and glibenclamide attenuate galanin-induced spontaneous alternation performance deficits in the rat. Brain Res., 813: 50-56.

Stefani, M.R., G.M. Nicholson and P.E. Gold, 1999. ATP-sensitive potassium channel blockade enhances spontaneous alternation performance in the rat: A potential mechanism for glucose-mediated memory enhancement. Neuroscience, 93: 557-563.

Stefani, M.R. and P.E. Gold, 2001. Intrahippocampal infusions of K-ATP channel modulators influence spontaneous alternation performance: Relationships to acetylcholine release in the hippocampus. J. Neurosci., 21: 609-614.

Swetman, S.C., 2002. Martindale: The Complete Drug Reference. Pharmaceutical Press.

Talley, C.E.P., S. Kahn, L. Alexander and P.E. Gold, 2000. Epinephrine fails to enhance performance of food-deprived rats on a delayed spontaneous alternation task. Neurobiol. Learn Mem., 73: 79-86.

Watson, G.S. and S. Craft, 2004. Modulation of memory by insulin and glucose: Neuropsychological observations in Alzheimer's disease. Eur. J. Phycol., 490: 97-113.