SHORT REPORTS

Posttraining glucose in inhibitory avoidance facilitates memory consolidation in rats

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The present research examines the effects of glucose on memory consolidation in rats over time. Several groups of rats were trained on inhibitory avoidance tasks and were then injected with 500 mg/kg of glucose or saline intraperitoneally under different footshock-drug injection intervals. Memory-facilitating effects were observed only at intervals of less than 30 min, suggesting that glucose affects the memory consolidation process. Our results with rats agree with the results of Kopf, Opezzo, and Baratti (1993), who worked with mice. The present research also showed that glucose does not have nonspecific effects on memory but does facilitate the memory of a shock experience.

Extensive evidence shows that glucose facilitates memory and learning and ameliorates memory/learning deficits caused by aging, drugs, and Alzheimer's disease in rodents and humans (for rodents, see Gold, 1986; Messier & White, 1987; Stone, Rudd, & Gold, 1992; for humans, see Benton, Owens, & Parker, 1994; Craft, Murphy, & Wemstrom, 1994; Manning, Ragozzino, & Gold, 1993). Various tasks have been used to examine the effects of glucose on memory. Among them, the most often used have been inhibitory avoidance tasks (Gold, 1986; Gold, Vogt, & Hall, 1986; Kopf, Opezzo, & Baratti, 1993; Lee, Graham, & Gold, 1988; Ragozzino & Gold, 1991; Rodriguez, Horne, Mondragon, & Phelps, 1994; Stone, Croul, & Gold, 1988; Stone, Rudd, & Gold, 1990) and spontaneous alternation tasks (Lennartz & Gold, 1995; Parsons & Gold, 1992; Ragozzino, Unick, & Gold, 1996; Stone et al., 1992; Stone, Walser, Gold, & Gold, 1991). Other tasks have also been used, such as conditional discrimination (Winocur, 1995), the M-shaped water maze (Means & Fernandez, 1992), the eight-arm radial maze (Packard & White, 1990), and the appetitive leverpress (Messier & Destrade, 1988).

The timing of glucose administration can also vary. It can be administered before training (Means & Fernandez, 1992; Stone et al., 1990), after training (Gold, 1986; Rodriguez, Van Audsle, Dhanens, & Mondragon, 1993), or before testing (Stone et al., 1990). Administration can be systemic (Gold, 1986; Packard & White, 1990; Rodriguez et al., 1994) or intraventricular (Lee et al., 1988). Effective doses also vary. Reported effective doses are between 10 mg/kg (Gold, 1986) and 2 g/kg (Messier &

White, 1987) for rats and between 30 mg/kg (Kopf et al., 1993) and 4 g/kg (Messier & Destrade, 1988) for mice. These wide-ranging differences in tasks and glucose treatments suggest glucose involvement in several different mechanisms that are related to various functions of memory and learning.

In the present research, we tried to examine the effects of glucose on memory consolidation using inhibitory avoidance tasks. Previous glucose studies on inhibitory avoidance tasks have shown that glucose administered immediately after the shock seems to facilitate memory consolidation of the shock experience. This facilitated memory consolidation effect of glucose decreases over time. The effect disappears when the shock-glucose administration interval is longer than 1 h (Gold, 1986; Kopf et al., 1993; Lee et al., 1988; Stone et al., 1988).

However, to our knowledge, no inhibitory avoidance experiments using rats have been reported that assess the memory consolidation effects of glucose with shock-glucose intervals of less than 1 h. Using mice, Kopf et al. (1993) conducted a step-through inhibitory avoidance experiment with 0-, 10-, 30-, and 180-min shock-glucose intervals. In their experiment, glucose administered 0 and 10 min after shock facilitated retention of the shock experience, but this effect disappeared when glucose was given 30 and 180 min later. In an extension of Kopf et al.'s work with mice, in the present research, we attempted to determine the time-dependent effects of glucose on memory consolidation in rats using several shock-glucose intervals.

As noted, effective doses for rats differ greatly—from 10 mg/kg (Gold, 1986) to 2 g/kg (Messier & White, 1987). In our preliminary experiments (unpublished), rats were administered 100 or 500 mg/kg of glucose or saline immediately after the shock experience in inhibitory avoidance tasks. The mean latency to the goal in the retention

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Figure 1. Effects of glucose injections on the retention test conducted 24 h after the inhibitory avoidance training. Drugs were administered immediately after the final training trial. Three bars with the same legend in a group indicate the group median latencies with quartiles on the first, second, and third trials.

test for the glucose 500-mg/kg group was longer than the latency for the glucose 100-mg/kg group or the saline group, and there was no difference between the glucose 100-mg/kg group and the saline group (no statistical differences). Therefore, we used only 500 mg/kg of glucose in the experiments reported here.

GENERAL METHOD

Subjects

Experimentally naive male Wistar rats obtained from Shimizu Lab Animals (Kyoto) were used. They were housed in individual wire cages with free access to water. The animal room was maintained at $23^{\circ} \pm 1^{\circ}\text{C}$, with a 12:12-h light:dark cycle starting from 7:00 a.m. Training and testing were conducted between 11:00 a.m. and 3:00 p.m.

Apparatus

The training apparatus was an acrylic box ($47 \times 23 \times 22$ cm) with a stainless steel grid floor. The steel grids were connected to the shock generator (Biomedica Inc., Osaka, BSG-107), which could deliver a scramble footshock. The apparatus was divided into two compartments by a wooden wall at the middle. Into this wall, a 10×10 -cm opening was cut from the floor, and a guillotine door separated the compartments. One compartment was white and was illuminated by a 10W light bulb; it served as the startbox. The other compartment was dark, and a foodcup was attached to its far wall, 1.5 cm above the floor. A pair of photocells placed on the side walls created a photo beam crossing 1 cm above the foodcup to permit measurement of time from the placement of a rat in the startbox to the moment it reached the foodcup.

Drugs

Glucose solution was prepared daily. Fifty grams of D-(+)-glucose, anhydrous (Nacalai Tesque, Inc., Kyoto), was dissolved in 100 ml of saline. For the glucose animals, a volume of 1.32 ml/kg of glucose solution was injected intraperitoneally so that a dose level of

500 mg/kg of glucose was achieved. For saline animals, the same volume of saline was injected intraperitoneally.

Procedure

Prior to the training, rats were reduced to approximately 85% of ad lib feeding weights and handled for a total of 15 min. As habituation, each rat was placed individually in the apparatus with the guillotine door open for 5 min or until it consumed eight, 45-mg food pellets (P. J. Noyes Co., Inc., Lancaster, New Hampshire) from the foodcup, whichever came first.

Beginning the following day, rats received three trials of training per day for 5 days. In the training and subsequent retention test trials, five pellets were supplied for each trial. For each trial, animals were placed in the white compartment with the guillotine door open, and the door was closed when they entered the dark compartment. When animals ate food pellets, they were taken out of the apparatus. After the 4th day's training, rats were divided into subgroups in such a way that the 4th day's mean latency for each subgroup was counterbalanced. On the third trial of the 5th day, a footshock (0.5 mA, 1.2 sec) was delivered, except to rats in the nonshocked group, through the floor grids at the moment they broke the photo beam above the foodcup. The rats were then removed from the apparatus and received drug injections intraperitoneally according to the experimental design. Twenty-four hours later, they were put through three retention test trials. The retention test trials were the same as the training trials: Rats were placed in the apparatus and allowed to consume food pellets. On each training trial and test trial, the latency to the foodcup was measured. The cut-off time was 300 sec. Rats that did not enter the dark compartment within 300 sec were removed from the apparatus and received a score of 300 sec.

Data Analysis

Group medians were used considering skewed distributions of statistics. Mann-Whitney *U* and Kruskal-Wallis tests for the between-groups comparisons and the Wilcoxon signed-ranks tests for the within-subject comparisons were employed for each of three trials on the final training day and the retention test day.

EXPERIMENT 1

Glucose administration immediately after a shock experience in rodents lengthens the latency to the goal in retention testing (Gold, 1986; Rodriguez et al., 1994). In Experiment 1, we tried to determine whether glucose enhances the memory of a shock experience, resulting in stronger resistance to entering the place where the shock was received, or whether the longer latency was due to the nonspecific effects of glucose.

Method

Forty rats, 2.5 months old with a mean weight of 307.1 g ($SD = 13.6$ g) at the beginning of the experiment, served as subjects. After 4 days of training, they were divided into four groups (10 rats in each group): nonshocked saline, nonshocked glucose, shocked saline, and shocked glucose. On the 5th day, animals in shocked groups received a footshock at the moment they reached the foodcup on their third trial. They were then taken out of the apparatus and immediately administered saline or glucose. For animals in nonshocked groups, the experimental procedure was the same except that no shock was given on their third trial. On the following day, all subjects received three retention test trials.

Results

Median latencies to the foodcup for the four groups on the retention test trials were compared with those on the final training day (Figure 1). Nonshocked animals, both

Figure 2. (a) Median latencies for each group on the final training day. Drugs were administered immediately (0 min), 10 min, 30 min, or 60 min after the final training trial. (b) Effects of delayed injections of glucose on the retention tests 24 h later. Three bars with the same legend in a group indicate the group median latencies with quartiles on the first, second, and third trials.

those administered saline and those administered glucose, exhibited almost the same running performance on the final training day as on the test day. Their median latencies were very similar, indicating that there is no effect of glucose on the test trials when shock is not given on the training day. In contrast, both groups of shocked animals showed longer latencies on the test day than on the final training day [$T_s(n = 10) = 0$, $p_s < .001$ for both groups on each of three trials]. When the shocked saline and the shocked glucose animals were compared on the test day, the glucose-administered rats showed longer latencies [$U_s(10 \text{ and } 10) = 16, 15$, and 24 ; $p_s < .01, .01$, and $.10$ for the first, second, and third trials, respectively].

These statistical analyses confirmed shock effects on the retention tests conducted 24 h later, with shock effects being greater for the glucose group than for the saline group.

EXPERIMENT 2

Experiment 2 examined how the effects of glucose changed as a function of shock–glucose intervals.

Method

Sixty-four rats, 2.5 months old and weighing an average of 303.9 g ($SD = 17.8$ g) at the beginning of the experiment, were used as subjects. On the third trial of the 5th training day, all subjects received a footshock when they reached the foodcup. Then, either saline or glucose was injected immediately (0 min), 10, 30, or 60 min after the footshock, according to the grouping design decided on the previous day. Saline-administered groups contained 6 rats each, and glucose-administered groups contained 10 rats each. All subjects received three retention test trials on the following day.

Results

Figure 2 shows the median latencies on the final training day and the retention test day. On the final training day (Figure 2a), the eight groups performed in a very similar and stable manner. As compared with the final training day, the latencies on the test day (Figure 2b) were significantly longer for all groups on every trial except the saline-10-min and the saline-30-min groups on the second trial. However, on the retention tests, the latencies for the saline groups and for the glucose groups revealed different tendencies. The latencies for all saline groups were approximately the same regardless of the length of the intervals [$H_s(3) = 4.22, 3.56$, and 0.62 ; all n.s. on every trial]. In contrast, the latencies for the glucose groups changed depending on intervals. As the interval became longer, the latency became shorter [$H_s(3) = 15.26, 10.44$, and 11.62 ; $p_s < .01, .05$, and $.01$ for the first, second, and third trials, respectively].

When the latencies for the retention tests were compared between the saline group and the glucose group of matching intervals, significant differences were found only at 0- and 10-min intervals [$U_s(6 \text{ and } 10) = 2, 9$, and 18 ; $p_s < .01, .05$, and $.20$ (n.s.) for the first, second, and third trials at the 0-min interval; $U_s(6 \text{ and } 10) = 7, 10$, and 7 ; $p_s < .05$ for the first, second, and third trials at the 10-min interval, respectively]. When the intervals were longer than 10 min, the effect of glucose disappeared on every trial.

DISCUSSION

In the present research, we assessed the memory consolidation effects of glucose in inhibitory avoidance tasks using rats that had received 500-mg/kg glucose in-

jections after a trial in which a footshock was delivered. Many researchers have reported that glucose administered immediately after a shock experience in inhibitory avoidance tasks lengthens the latency to the goal in a retention test conducted 24 or 48 h later (Gold, 1986; Kopf & Baratti, 1994; Lee et al., 1988; Stone et al., 1988). Experiment 1 confirmed the results of these experiments. At the same time, Experiment 1 indicated that the longer latency in the retention test was not due to the nonspecific effects of glucose, but rather that glucose enhanced the memory of the shock experience.

In Experiment 2, we tried to determine whether there exists a shorter time limit than 1 h for memory enhancement by glucose. The results showed that the effect of glucose decreases as a function of the shock-glucose interval and that the effect disappears when the interval is longer than 30 min. These results indicate that the time required to consolidate the memory of the experience—receiving a 0.5-mA footshock for 1.2 sec—is less than 30 min and that glucose administered during the consolidation period facilitates memory consolidation. The results also suggest that glucose does not affect memory after the completion of consolidation. The present results on the time course of memory consolidation agree with Kopf et al.'s (1993) results with mice, suggesting that rats and mice undergo similar memory consolidation processes.

Many researchers recognize that this glucose effect is dose dependent. In studies of rats, reported effective doses differ widely, from 10 mg/kg (Gold, 1986) to 2g/kg (Messier & White, 1987), although doses of 100 to 250 mg/kg have most often been found effective (Gold et al., 1986; Rodriguez et al., 1993; Stone et al., 1990). To the best of our knowledge, this is the only study in which 500 mg/kg has been found to be effective. As Gold et al. showed, the footshock intensity is an important variable for inhibitory avoidance learning. The footshock we gave was 0.5 mA for 1.2 sec. This intensity is the same as that used by other researchers (Gold, 1986; Gold et al., 1986), and the duration is also within the range used (from 0.7 sec in Gold, 1986, and Gold et al., 1986, to until the animal escapes in Rodriguez et al., 1994). Therefore, it is not likely that the duration of our footshock can be the reason why 500 mg/kg was effective. This effectiveness may be due to the difference in the strain of rats used. Gold and his colleagues used Sprague-Dawley rats, and White and his colleagues used hooded rats. Our rats were of the Wistar strain. Another variable that might have affected the result is the reward we used during training. We used food pellets, which contain some amount of starch. Gold and his colleagues often used water as reward in their inhibitory avoidance training, and Kopf et al. (1993) employed a step-through method, which does not use reward at all. These differences in the experimental design need to be examined more fully before the difference in the effective dose in our experiments can be explained.

In summary, on the basis of the results of the present experiments and other studies on time-dependent character-

istics of memory processing, we conclude that one effect of posttraining glucose, among others, is the facilitation of the consolidation of the memory of an experience. We also conclude that glucose exerts such a facilitatory effect when it is administered within 30 min after the experience.

REFERENCES

- BENTON, D., OWENS, D. S., & PARKER, P. Y. (1994). Blood glucose influences memory and attention in young adults. *Neuropsychologia*, **32**, 595-607.
- CRAFT, S., MURPHY, C., & WEMSTROM, J. (1994). Glucose effects on complex memory and nonmemory tasks: The influence of age, sex, and glucoregulatory response. *Psychobiology*, **22**, 95-105.
- GOLD, P. E. (1986). Glucose modulation of memory storage processing. *Behavioral & Neural Biology*, **45**, 342-349.
- GOLD, P. E., VOGT, J., & HALL, J. L. (1986). Glucose effects on memory: Behavioral and pharmacological characteristics. *Behavioral & Neural Biology*, **46**, 145-155.
- KOPF, S. R., & BARATTI, C. M. (1994). Memory-improving actions of glucose: Involvement of a central cholinergic muscarinic mechanism. *Behavioral & Neural Biology*, **62**, 237-243.
- KOPF, S. R., OPEZZO, J. W., & BARATTI, C. M. (1993). Glucose enhancement of memory is not state-dependent. *Behavioral & Neural Biology*, **60**, 192-195.
- LEE, M. K., GRAHAM, S. N., & GOLD, P. E. (1988). Memory enhancement with posttraining intraventricular glucose injections in rats. *Behavioral Neuroscience*, **102**, 591-595.
- LENNARTZ, R. C., & GOLD, P. E. (1995). Glucose does not reverse impairments on spontaneous alternation induced by the noncompetitive NMDA antagonist MK-801. *Neurobiology of Learning & Memory*, **63**, 107-110.
- MANNING, C. A., RAGOZZINO, M. E., & GOLD, P. E. (1993). Glucose enhancement of memory in patients with probable senile dementia of the Alzheimer's type. *Neurobiology of Aging*, **14**, 523-528.
- MEANS, L. W., & FERNANDEZ, T. J. (1992). Daily glucose injections facilitate performance of a win-stay water-escape working memory task in mice. *Behavioral Neuroscience*, **106**, 345-350.
- MESSIER, C., & DESTRADE, C. (1988). Improvement of memory for an operant response by post training glucose in mice. *Behavioural Brain Research*, **31**, 185-191.
- MESSIER, C., & WHITE, N. M. (1987). Memory improvement by glucose, fructose, and two glucose analogs: A possible effect on peripheral glucose transport. *Behavioral & Neural Biology*, **48**, 104-127.
- PACKARD, M. G., & WHITE, N. M. (1990). Effects of posttraining injections of glucose on acquisition of two appetitive learning tasks. *Psychobiology*, **18**, 282-286.
- PARSONS, M. W., & GOLD, P. E. (1992). Scopolamine-induced deficits in spontaneous alternation performance: Attenuation with lateral ventricle injections of glucose. *Behavioral & Neural Biology*, **57**, 90-92.
- RAGOZZINO, M. E., & GOLD, P. E. (1991). Glucose effects on mecamylamine-induced memory deficits and decrease in locomotor activity in mice. *Behavioral & Neural Biology*, **56**, 271-282.
- RAGOZZINO, M. E., UNICK, K. E., & GOLD, P. E. (1996). Hippocampal acetylcholine release during memory testing in rats: Augmentation by glucose. *Proceedings of the National Academy of Sciences*, **93**, 4693-4698.
- RODRIGUEZ, W. A., HORNE, C. A., MONDRAGON, A. N., & PHELPS, D. D. (1994). Comparable dose-response functions for the effects of glucose and fructose on memory. *Behavioral & Neural Biology*, **61**, 162-169.
- RODRIGUEZ, W. A., VAN AUDEL, L. R., DHANENS, K., & MONDRAGON, A. N. (1993). Glucose modulates recently reactivated memories. *Psychobiology*, **21**, 93-100.
- STONE, W. S., CROUL, C. E., & GOLD, P. E. (1988). Attenuation of scopolamine-induced amnesia in mice. *Psychopharmacology*, **96**, 417-420.
- STONE, W. S., RUDD, R. J., & GOLD, P. E. (1990). Amphetamine, epi-

- nephine, and glucose enhancement of memory retrieval. *Psychobiology*, **18**, 227-230.
- STONE, W. S., RUDD, R. J., & GOLD, P. E. (1992). Glucose attenuation of scopolamine- and age-induced deficits in spontaneous alternation behavior and regional brain [^3H]2-deoxyglucose uptake in mice. *Psychobiology*, **20**, 270-279.
- STONE, W. S., WALSER, B., GOLD, S. D., & GOLD, P. E. (1991). Scopolamine- and morphine-induced impairments of spontaneous alternation performance in mice: Reversal with glucose and with cholinergic and adrenergic agonists. *Behavioral Neuroscience*, **105**, 264-271.
- WINOCUR, G. (1995). Glucose-enhanced performance by aged rats on a test of conditional discrimination learning. *Psychobiology*, **23**, 270-276.

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