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Hippocampal infusions of pyruvate reverse the memory-impairing effects of septal muscimol infusions

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Abstract

Hippocampal infusions of glucose reverse memory deficits in spontaneous alternation and in a continuous multiple trial inhibitory avoidance task. The current experiments tested whether glucose metabolism may participate in these effects of glucose. Specifically, these experiments determined whether the glycolytic metabolite pyruvate would mimic these effects of glucose. Male Sprague–Dawley rats were given septal infusions of vehicle or the gamma-aminobutyric acid (GABA) receptor agonist muscimol (0.15 nmol for spontaneous alternation or 5 nmol for continuous multiple trial inhibitory avoidance) combined with hippocampal infusions of vehicle or pyruvate (200 nmol) 15 min prior to assessing spontaneous alternation or training in a continuous multiple trial inhibitory avoidance task. The infusions of muscimol decreased percent alternation scores and continuous multiple trial inhibitory avoidance retention latencies tested 48 h after training. More importantly, hippocampal infusions of pyruvate reversed the deficits produced by septal infusions of muscimol on both tasks. These findings show for the first time that hippocampal infusions of pyruvate influence memory and suggest that glucose may affect memory via glycolytic metabolism.

Keywords

Muscimol; Pyruvate; Glucose; Metabolism; Spontaneous alternation; Shock avoidance; Hippocampus

1. Introduction

Extensive evidence suggests that glucose is involved in memory processes (Benton and Nabb, 2003; Korol and Gold, 1998; Messier and Gagnon, 2000). In both humans and rodents, glucose impairs or enhances memory in a dose-dependent manner (Kopf and Baratti, 1994; Li et al., 1998; Parsons and Gold, 1992; Sunran-Lea et al., 2002). Specifically, moderate increases in glucose enhance memory, whereas extremely elevated glucose levels are associated with memory deficits (Biessels et al., 1996; Craft et al., 1994; Flood et al., 1990; Gold, 1995; Li et al., 1998; Ryan and Geckle, 2000). The effects of changes in blood glucose levels on memory are mediated, at least in part, by an influence on the brain. Glucose readily crosses the blood brain barrier through brain type glucose transporters (Rahner-Welsch et al., 1995; Takata et al., 1997). More importantly, direct injections of glucose into specific brain regions, including the septum or hippocampus, enhance memory (Ragozzino et al., 1998; Stefani and Gold, 1998; Stefani et al., 1999), and reverse drug-induced deficits (Krebs and Parent, 2005; Parent et al., 1997; Ragozzino and Gold, 1995; Stefani and Gold, 1998, 2001). Septal co-infusions of glucose with gamma-aminobutyric acid (GABA) receptor agonists produce memory deficits,

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suggesting that the memory-impairing effects of glucose are also mediated, at least in part, by the brain (Parent and Gold, 1997; Parent et al., 1997; Shah and Parent, 2003, 2004).

Extensive evidence suggests that glucose influences memory through an interaction with specific neurotransmitter systems. Specifically, glucose may enhance memory through a process that involves an increase in acetylcholine synthesis or release in the brain, including the hippocampus (Durkin et al., 1992; Kopf and Baratti, 1994; Pavone et al., 1998; Ragozzino et al., 1994). For instance, systemic glucose administration and localized increases in hippocampal glucose augment hippocampal extracellular acetylcholine levels and enhance spontaneous alternation performance, a measure of spatial working memory (Degroot et al., 2003; Ragozzino et al., 1996, 1998; Stefani and Gold, 2001). Also, hippocampal infusions of glucose mimic the effects of acetylcholinesterase inhibitors and reverse memory deficits produced by septal GABA receptor activation (Degroot and Parent, 2000, 2001; Krebs and Parent, 2005; Parent et al., 1997).

In contrast, we have obtained evidence suggesting that glucose may impair memory by augmenting GABA function. Specifically, co-infusions of glucose with muscimol (a GABA_A receptor agonist and partial GABA_C receptor agonist; Johnston, 2000) impair spontaneous alternation and inhibitory avoidance, a measure of long-term aversive memory (Parent and Gold, 1997; Parent et al., 1997; Shah and Parent, 2003, 2004). These studies show that glucose exacerbates memory deficits produced by muscimol or interact with sub-effective doses of muscimol to produce a deficit. Consistent with the idea that glucose may impair memory by increasing GABA activity, evidence indicates that acute administration of large amounts of glucose and experimentally induced hyperglycemia increase GABA levels in the brain (Amoroso et al., 1990; During et al., 1995; Fink and Gothert, 1993; Fink et al., 1994; Ohtani et al., 1997).

Elevated glucose levels may impair memory through a process that involves glycolytic metabolism. Glucose metabolism yields two molecules of pyruvate and two molecules of adenosine-tri-phosphate (ATP; Hertz and Dienel, 2002). Pyruvate metabolism, in turn, yields byproducts necessary for GABA synthesis (Hertz and Dienel, 2002). We have found that brain infusions of pyruvate mimic the memory-impairing effects of glucose. That is, as in the case of septal co-infusions of glucose with muscimol, co-infusions of pyruvate with muscimol impair spontaneous alternation performance and inhibitory avoidance in rats (Shah and Parent, 2003, 2004). These deficits are not the result of hyperosmolar conditions, because equiosmolar concentrations of fructose or sorbitol do not mimic these deficits produced by glucose and pyruvate (Shah and Parent, 2003, 2004).

Glucose may also enhance memory and increase acetylcholine through a process that involves glycolytic metabolism. In addition to participating in GABA synthesis, pyruvate also contributes to the synthesis of acetylcholine. Specifically, decarboxylation of pyruvate produces acetyl coenzyme A (CoA), one of the biosynthetic precursors for acetylcholine (Dolezal and Tucek, 1981), and preventing pyruvate oxidation decreases acetylcholine synthesis (Gibson et al., 1975; Lefresne et al., 1973). Combining glucose with choline, another acetylcholine precursor, improves memory performance and increases hippocampal acetylcholine release (Kopf et al., 2001). As well, co-infusions of pyruvate or glucose with morphine into the medial septum reverse the memory-impairing effects of morphine on spontaneous alternation (Ragozzino et al., 1995; Ragozzino and Gold, 1995).

The evidence reviewed above raises the possibility that glycolytic metabolism is involved in the enhancing effects of glucose on hippocampal-dependent memory formation. If this is the case, then pyruvate, the glycolytic metabolite of glucose, should mimic the enhancing effects of glucose on memory. As a result, the present experiments determined whether hippocampal

infusions of pyruvate would mimic the ability of glucose to reverse memory deficits produced by septal GABA receptor activation. Specifically, the following experiments examined the ability of hippocampal infusions of pyruvate to reverse spontaneous alternation and shock avoidance deficits produced by septal infusions of the GABA receptor agonist muscimol. Spontaneous alternation is a measure of spatial working memory that is dependent on the hippocampus (Johnston et al., 1977; Lalonde, 2002). Shock avoidance is a commonly used measure of emotional, long-term memory that is less dependent on spatial processes (McGaugh, 2004), but still involves the hippocampus (Lovely et al., 1971; Martinez et al., 2002).

2. Materials and methods

2.1. Subjects

Male Sprague–Dawley-derived rats weighing 200–250 g upon arrival (Charles River, Wilmington, MA) were used. Thirty ($n=5-9$ per group: spontaneous alternation), and 51 ($n=10-15$ per group: continuous multiple trial inhibitory avoidance) rats were used. The rats were housed individually on a 12 h light–dark cycle (lights on at 7:00 a.m.) with food and water ad libitum. The Georgia State University Institutional Animal Care and Use Committee (IACUC) approved all procedures involving rats.

2.2. Surgery

At least 1 week after arrival, the rats were given atropine sulfate (0.4 mg/kg, i.p.), anesthetized with sodium pentobarbital (50 mg/kg, i.p.), and then given an injection of penicillin (1500 units, i.m., Crystiben). Stereotaxic (David Kopf Instruments, Tujunga, CA) surgical procedures were used to implant one 22-gauge stainless-steel guide cannulae (Plastics One, Inc., Roanoke, VA) aimed at the medial septum (0.5 mm anterior [AP] to bregma, 4.9 mm ventral to dura [DV]) and one guide cannula aimed at the dorsal hippocampus (4.5 mm AP, 1.6 mm DV, and 4.0 mm from the interaural line; Paxinos and Watson, 1986). The hemisphere in which the unilateral hippocampal cannulae were implanted was counterbalanced. The cannulae were secured to the skull with three jeweler's screws and cranioplastic cement and a dummy cannula was inserted to keep the cannulae free of debris. Immediately after surgery, the rats were given an injection of 0.9% sterile saline (3.0 cc, s.c.) and then wrapped with a paper towel and kept under a warm lamp until recovery from anesthesia. Two days following surgery, the patency of each cannula was checked and betadine was applied to the surgical wound. If signs of infection were evident, the rats were anesthetized with isoflurane gas (5%) delivered in 1000 ml/min of oxygen and given an additional injection of penicillin (1500 units i.m., Crystiben).

2.3. Drug preparation and drug infusions

Two days prior to behavioral testing, the experimenter handled each rat for 2 min. Behavioral testing occurred at least 1 week after surgery and was conducted between 8:00 a.m. and 6:00 p.m. Drug treatments were counterbalanced over the course of the day. Before and after all handling and behavioral testing, the rats were allowed 30 min to habituate to the laboratory environment. Fifteen minutes prior to behavioral testing, different groups of rats were given unilateral hippocampal infusions of vehicle (1 μ l, 0.5 μ l/min; phosphate-buffered saline [PBS], pH=7.4) or pyruvate (200 nmol). The dose of pyruvate was selected based on pilot experiments showing that 200 nmol pyruvate reversed memory deficits with the least amount of variability. One minute after the hippocampal infusion was initiated, the rats were given a septal injection of vehicle (PBS; 0.5 μ l, 0.5 μ l/min) or muscimol (0.15 nmol for spontaneous alternation; 5 nmol for continuous multiple trial inhibitory avoidance). The hippocampal and septal injections overlapped for the last minute and ended simultaneously. The dose of muscimol was selected based on pilot experiments showing that this dose impairs spontaneous alternation performance without decreasing activity measures. The 5 nmol dose of muscimol was selected on the basis

of previous findings indicating that this dose impaired avoidance retention performance without affecting acquisition (Degroot and Parent, 2001; Krebs and Parent, 2005).

All drugs were prepared on the day of testing. The drugs were infused through a 28-gauge injection needle that extended 1.2 mm (hippocampus) or 1.0 mm (septum) beyond the guide cannulae. The needle was connected to a 25 μ l Hamilton syringe by polyethylene tubing (PE-50), and the infusions were delivered using an infusion pump (Harvard Apparatus 11). Following the completion of both injections, the needle was left in place for 1 min to facilitate drug diffusion. The experimenter was blind to the identity of the solutions that were administered.

2.4. Spontaneous alternation

Spontaneous alternation is a hippocampal-dependent task (Johnston et al., 1977) that is assumed to be a measure of spatial working memory, because in order to alternate between locations one must remember where one has been. This assumption is supported by the finding that spontaneous alternation is impaired by removing directional cues or by increasing the interval between arm choices (Dember, 1989; Lalonde, 2002). Fifteen minutes after the drug injections, spontaneous alternation performance was assessed by placing each rat in a Y-maze composed of three equally spaced arms (60°; 60 cm long $\times$ 17.5 cm high). The floor of each arm was composed of stainless steel (3.5 cm wide) and the top (14 cm wide) was covered with a colorless, transparent Plexiglas lid. The rats were placed in the same starting arm of the Y-maze and allowed to explore the maze for 8 min. The experimenter, who was blind to drug treatment, recorded the sequence and number of arms the rats entered. The maze was cleaned with 70% ethanol after each rat was tested. The number of arms each rat entered was used as a measure of activity. A percent alternation score was computed for all rats that entered at least 10 arms. An alternation was defined as entering three different arms consecutively. The percent alternation score was computed by dividing the number of alternations each rat made by the number of arms entered minus two (i.e., the number of alternations possible) and then multiplying that resulting quotient by 100.

2.5. Continuous multiple trial inhibitory avoidance

The avoidance apparatus consisted of a trough-shaped alley (91 cm long, 15 cm high, 20 cm wide at the top, and 6.4 cm wide at the bottom) that was divided into a lighted (31 cm long) and a dark (60 cm long) compartment by a retractable door. The dark compartment had a metal floor through which shock could be delivered. A 15-W lamp was placed over the lighted compartment and was the only source of illumination in the room. The table underneath the avoidance apparatus was lined with bench paper and the apparatus was cleaned with 70% ethanol after each rat was trained.

For the training the rat was placed in the lighted compartment with its head facing away from the door. Once the rat turned around to face the door or after 12 s passed, the retractable door was opened and the rat was allowed to cross over to the dark (shock) compartment. After the rat crossed with all four paws, the rat was given a footshock (1.2 mA) until it returned to the lighted compartment (maximum 4 s). This sequence constituted one training trial. Subsequent trials started immediately after the rat moved back into the lighted compartment; the rat was never removed from the apparatus between trials. All rats escaped the dark compartment within 4 s on each trial. Training continued until the rat remained in the lighted compartment for 100 consecutive seconds or for a maximum of 5 trials. The number of trials needed to reach the criterion was recorded and used as a measure of acquisition.

Retention of the training was tested 48 h ($\pm$ 1 h) later. For the retention test, each rat was placed in the lighted compartment of the avoidance chamber with its head facing away from the closed

door. After the rat turned to face the door or 12 s passed, the door was opened and the latency (s) to cross over to the dark (shock) compartment was recorded and used as a measure of retention. Each rat was given a maximum of 600 s to enter the dark compartment during the retention test. Footshock was not delivered on the retention test.

2.6. Histology

After behavioral testing, the rats were euthanized with an overdose of sodium pentobarbital (400 mg/kg, i.p.) and perfused intracardially with 0.9% saline followed by 10% formalin. Their brains were stored in a 10% formalin solution for at least 2 days before sectioning. All brains were sectioned on a cryostat (Leica CM 30510 S) and 45–60 μ m sections were taken through the septal and hippocampal cannulae tracts. The brain sections were stained with thionin and an unbiased observer determined the cannulae placement using a light microscope (Olympus BX41). Acceptable medial septal cannulae placement was defined as injection tips located within the medial septum, but not within the lateral septum or the ventral diagonal band of Broca. Moreover, the cannula must not have penetrated the fimbria. Acceptable placement for hippocampal cannulae was defined as injection sites located within hippocampal fields CA1, CA2, CA3, or dentate gyrus. Only rats with acceptable cannulae placements in both brain regions were included in the statistical analyses.

2.7. Statistical analysis

The spontaneous alternation data were expressed as means and standard errors of the mean (S.E.M.) and analyzed using 2 (septal drug treatment) $\times$ 5 (hippocampal drug treatment) univariate analysis of variance (ANOVA) and Tukey post hoc tests where appropriate. The number of trials to criterion during acquisition and the retention latency data were not normally distributed. This was because several rats required the maximum number of trials to reach the acquisition criterion or reached the maximum retention latency cut-off of 600 s. Consequently, these data were expressed as medians and inter-quartile ranges (I.Q.) and the non-parametric Kruskal–Wallis and Mann–Whitney *U* tests were used to detect differences between treatment groups. An alpha level of 0.05 was used as the criterion for statistical significance.

3. Results

3.1. Pyruvate reverses spontaneous alternation deficits

The approximate locations of the septal and hippocampal infusions are shown in Fig. 1. Drug infusions into the septum [$F(1,29)=7.62$; $p<.01$] significantly affected spontaneous alternation performance, and the effects of the infusions into the septum and hippocampus interacted significantly [$F(1,29)=8.46$; $p<.01$; see Fig. 2A]. As in previous research, infusions of muscimol into the septum impaired spontaneous alternation performance. Specifically, the percent alternation scores of rats given muscimol in the septum and vehicle in the hippocampus (M–V) were significantly lower than those of rats given infusions of vehicle in both brain areas (V–V; $p<.01$). Hippocampal infusions of pyruvate alone did not significantly affect spontaneous alternation performance [$F(1,29)=1.72$; $p>.05$]. The percent alternation scores of rats given vehicle in the septum and pyruvate in the hippocampus (V–P) did not differ significantly from those of V–V rats ($p>.05$). Importantly, the findings indicated that hippocampal infusions of pyruvate reversed alternation deficits produced by the septal muscimol infusions. Specifically, the percent alternation scores of rats given septal muscimol infusions combined with hippocampus infusions of pyruvate (M–P) were not significantly different than those of V–V rats ($p>.05$), and were significantly higher than those of M–V rats ($p<.05$). The findings of this experiment indicated that drug infusions into the septum [$F(1,29)=.05$; $p>.05$] and hippocampus [$F(1,29)=.10$; $p>.05$] did not significantly affect the number of arms the rat entered in the maze (see Fig. 2B).

3.2. Pyruvate reverses continuous multiple trial inhibitory avoidance retention deficits

Fig. 3A demonstrates that the drug infusions significantly affected trials to criterion during continuous multiple trial inhibitory avoidance training [$\chi^2(3,50)=10.27, p<.05$]. Surprisingly, septal infusions of muscimol enhanced acquisition performance. Specifically, M-V rats required significantly fewer trials to reach criterion during continuous multiple trial inhibitory avoidance training than did V-V rats [$U(1,23)=21.5; p<.01$]. Hippocampal infusions of pyruvate alone did not affect acquisition. There were no significant differences in the number of trials to criterion between V-V and V-P rats [$U(1,25)=48.0; p>.05$]. Hippocampal infusions of pyruvate attenuated the effects of muscimol on acquisition. Specifically, for trials to criterion, M-P rats did not differ from either M-V rats ($U(1,24)=50.0; p>.5$) or V-V rats, although there was a tendency for M-P rats to take fewer trials to reach criterion than V-V rats [$U(1,28)=61.0; p=.06$].

Fig. 3B illustrates that the pretraining drug infusions into the septum and hippocampus significantly affected subsequent continuous multiple trial inhibitory avoidance retention [$\chi^2(3,50)=11.74, p<.01$]. Septal infusions of muscimol impaired continuous multiple trial inhibitory avoidance retention. Specifically, M-V rats had significantly shorter retention latencies than did V-V rats [$U(1,23)=16; p<.01$]. Hippocampal infusions of pyruvate alone did not affect continuous multiple trial inhibitory avoidance retention. The retention latencies of V-P rats were not significantly different from those of V-V rats [$U(1,25)=66.0; p>.05$]. More importantly, hippocampal infusions of pyruvate reversed continuous multiple trial inhibitory avoidance retention deficits produced by muscimol. M-P rats had significantly longer retention latencies than did M-V rats [$U(1,24)=30; p<.01$], and their latencies did not significantly differ from those of V-V rats [$U(1,28)=81.5; p>.05$].

4. Discussion

4.1. Pyruvate reverses spontaneous alternation deficits

These results demonstrate that septal infusions of the GABA receptor agonist muscimol impair spontaneous alternation performance. More importantly, the findings show that these deficits are reversed by hippocampal infusions of pyruvate. These data are consistent with previous research indicating that infusions of pyruvate into the septum reverse morphine-induced spontaneous alternation performance deficits (Ragozzino et al., 1995). The present findings extend these previous results by showing that hippocampal infusions of pyruvate also affect memory. These findings are consistent with the possibility that glycolytic metabolism is involved in the ability of hippocampal glucose to influence mnemonic processes.

4.2. Pyruvate reverses continuous multiple trial inhibitory avoidance retention deficits

The results also demonstrate that hippocampal infusions of pyruvate reverse continuous multiple trial inhibitory avoidance retention deficits produced by pretraining septal infusions of muscimol. Combined with the results found in spontaneous alternation, these data show that hippocampal infusions of pyruvate affect spatial working memory and emotional, long-term memory. Combined, these findings show that hippocampal infusions of pyruvate reverse deficits in memory situations that vary in terms of cognitive and motivational demands. They also provide additional evidence that is consistent with the possibility that glucose enhances memory through a process that involves glycolytic metabolism.

The results also showed that septal infusions of muscimol decrease the number of trials rats needed to learn the continuous multiple trial inhibitory avoidance criterion. This is in contrast to our previous results showing that septal infusions of muscimol do not affect acquisition of shock avoidance (Degroot and Parent, 2001; Krebs and Parent, 2005). It is not clear why septal infusions of muscimol affected acquisition performance in the present experiment. Although

measures of shock duration were not recorded, it is possible that there were differences in the duration of the footshock that rats received on individual trials, which might have contributed to the effects of muscimol on acquisition. That is, one might expect that if muscimol increased the length of time rats spent in the shock compartment, then those rats would have learned better and needed less trials to reach the criterion. Although our previous findings show that septal infusions of muscimol do not affect flinch-jump thresholds, (Parent and Gold, 1997), it is also possible that in the present experiment, septal infusions of muscimol may have enhanced acquisition performance by increasing shock sensitivity. The enhancing effects of septal infusions of muscimol are congruent, though, with previous findings showing that a compromised hippocampus may facilitate acquisition in some memory tasks (Eichenbaum et al., 1988; McDonald and White, 1995), including acquisition of a trace two-way active avoidance task (Guillazo-Blanch et al., 2002). The results showed that the hippocampal pyruvate infusions partially attenuated the effects of muscimol on acquisition, but completely reversed the muscimol-induced retention deficits. Combined with the present evidence showing that hippocampal infusions of pyruvate influence spontaneous alternation, the results of the continuous multiple trial inhibitory avoidance task provide converging evidence that elevations in hippocampal pyruvate have important mnemonic consequences.

4.3. General discussion

The present results show that hippocampal infusions of pyruvate, a product of glucose metabolism, mimic the effects of glucose in both a spontaneous alternation and a shock avoidance task. These findings are the first to show that hippocampal infusions of pyruvate influence behavioral measures of memory, and are consistent with the hypothesis that glycolytic metabolism participates in the effects of glucose on memory. The motivational, temporal, and cognitive differences between the spontaneous alternation and continuous multiple trial inhibitory avoidance task support the hypothesis that pyruvate influences memory rather than some other process that influences performance in a memory task. The finding that increases in hippocampal pyruvate enhance memory in both spontaneous alternation and continuous multiple trial inhibitory avoidance indicate that pyruvate is involved in several mnemonic processes. Specifically, the findings from the spontaneous alternation task suggest that hippocampal pyruvate is involved in on-line spatial associations, and the findings from the continuous multiple trial inhibitory avoidance task reveal that hippocampal pyruvate also affects emotional and long-term memory. The fact that the pretraining infusions of pyruvate did not affect acquisition in the continuous multiple trial inhibitory avoidance task suggests further that elevating pyruvate in the hippocampus influences consolidation of newly formed emotional memories.

The present findings also show that higher doses of septal infusions of muscimol are needed to impair avoidance retention than spontaneous alternation. This is consistent with previous evidence investigating the effects of septal infusions of muscimol on different behavioral measures of memory (Krebs and Parent, 2005; Nagahara and McGaugh, 1992; Nagahara et al., 1992; Parent and Gold, 1997). Collectively this research shows that higher doses of muscimol are needed in the septum to impair shock avoidance than are needed to produce deficits in a rewarded alternation, spontaneous alternation, or spatial water maze task. It is not clear why higher doses are needed to impair shock avoidance. One possibility is that the higher doses of muscimol may influence avoidance via non-GABAergic mechanisms. The finding that lower doses of muscimol are effective in impairing avoidance retention when they are co-infused with glucose (Parent and Gold, 1997; Shah and Parent, 2003) suggests that this possibility is unlikely. Thus, these findings suggest that more GABA receptors or more prolonged receptor activation is required to impair avoidance memory than other types of memory. The task-dependent effects of muscimol do not appear to be related to the stress level of the task, because both water maze and shock avoidance produce stress responses (De Boer

et al., 1990; Mabry et al., 1995; Van der Borgh et al., 2005). The differences also do not appear to be related to the presence of shock, because low doses of septal infusions of muscimol impair shock avoidance in a shock-probe burying test of anxiety (Degroot and Treit, 2003). Additional research is needed to determine the factors that contribute to these task-dependent, dose-response differences of septal GABA receptor activation.

Combined with previous findings (Shah and Parent, 2003, 2004), the present findings show that pyruvate mimics both the memory-impairing and -enhancing effects of glucose. As indicated in the Introduction, evidence suggests that glucose may enhance memory through a process that involves acetylcholine, but may impair through a process that involves an increase in GABA function. Combined with the results obtained with pyruvate, these findings suggest that different products of glycolytic metabolism could mediate the different effects of glucose on memory. These different products could vary as a function of glucose concentration or brain region. For instance, we have observed that septal infusions of glucose can lead to memory impairments (Parent and Gold, 1997; Parent et al., 1997; Shah and Parent, 2003, 2004), whereas elevating glucose levels in the hippocampus typically enhances memory or prevents deficits (Krebs and Parent, 2005; Parent et al., 1997; Ragazzino et al., 1998; Stefani and Gold, 2001).

In order to determine whether glucose metabolism is necessary for the effects of glucose on memory, it would be necessary to directly manipulate glycolytic metabolism. For example, it would be ideal to co-infuse glucose with a drug that inhibits metabolism in order to determine whether inhibiting metabolism prevents the memory-enhancing effects of glucose. There are, however, currently no satisfactory methods for doing so. For instance, iodoacetate is a drug that prevents the enzymatic breakdown of glyceraldehyde 3-phosphate and thus the production of pyruvate (Malcolm et al., 2000; Guo et al., 2001; Massieu et al., 2000). We recently attempted to inhibit metabolism using iodoacetate but found that it produced hippocampal tissue damage at a variety of concentrations (Krebs, D. L. and Parent, M. B., unpublished observations). Two-deoxyglucose (2-DG) is a drug that also prevents the metabolism of glucose; however, intra-ventricular and intra-hypothalamic infusions of 2-DG induce hyperglycemia (Engeset and Ritter, 1980; Takahashi et al., 1997), a condition known to cause memory deficits (Biessels et al., 1996; Flood et al., 1990; Leung and Bryant, 2000; Ryan et al., 1995).

Experiments that have induced in vitro increases in brain pyruvate levels have shown that pyruvate also reverses the neurotoxic effects of hydrogen peroxide and zinc (Mazzio and Soliman, 2003; Kawahara et al., 2002). Alzheimer's disease is a neurodegenerative dementia associated with β -amyloid plaques, which are exacerbated by inhibition of glycolysis in the rat hippocampus (Arias et al., 2002). Combined with the findings of the present study, these results raise the possibility that pyruvate may serve as a treatment for the neurotoxic effects and memory impairments observed in Alzheimer's disease.

In summary, intra-hippocampal infusions of pyruvate reverse memory deficits produced by septal GABA receptor activation. The effects of pyruvate were observed in a hippocampal-dependent task that involves short-term, spatial working memory and one that is more dependent on long-term, emotional memory. These findings show that pyruvate in the hippocampus influences memory in its own right and support the possibility that the effects of glucose on memory may be mediated through a process involving glycolytic metabolism.

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Fig. 1. Schematic illustration of coronal sections of the rat brain showing the approximate location of (A) medial septal and (B) hippocampal infusion sites in experiment 1. Atlas plates were adapted from Paxinos and Watson (1986).

Fig. 2.

(A) Effects of septal and hippocampal drug treatment on mean percent alternation scores ($\pm$ S.E.M.). Septal infusions of muscimol decreased percent alternation scores ($*p < .01$ vs. V–V). Hippocampal infusions of pyruvate, at a dose that did not affect percent alternation scores when infused alone, reversed the deficits produced by muscimol ($#p < .05$ vs. M–V). (B) Effects of septal and hippocampal drug treatment on mean number of arm entries ($\pm$ S.E.M.). There were no significant effects of any of the manipulations on number of arm entries.

Fig. 3.

(A) Effects of septal and hippocampal drug treatments on trials to criterion ($\pm$ I.Q.) in the continuous multiple trial inhibitory avoidance task. Septal infusions of muscimol decreased the number of trials to reach criterion ($*p < .01$ vs. V-V). There was a tendency for combined infusions of pyruvate into the hippocampus with infusions of muscimol in the septum to also decrease the number of trials to criterion ($p = .06$ vs. M-V). (B) Effects of septal and hippocampal drug manipulations on continuous multiple trial inhibitory avoidance retention latencies ($\pm$ I.Q.). Septal infusions of muscimol decreased retention latencies ($*p < .01$ vs. V-V). Hippocampal infusions of pyruvate, at a dose that did not have an effect when infused alone, reversed the retention latency deficits produced by septal infusions of muscimol ($\#p < .05$ vs. M-V).