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Hippocampal acetylcholine release during memory testing in rats: Augmentation by glucose

(cholinergic/learning/hippocampus/microdialysis)

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ABSTRACT Several lines of evidence indicate that a modest increase in circulating glucose levels enhances memory. One mechanism underlying glucose effects on memory may be an increase in acetylcholine (ACh) release. The present experiment determined whether enhancement of spontaneous alternation performance by systemic glucose treatment is related to an increase in hippocampal ACh output. Samples of extracellular ACh were assessed at 12-min intervals using *in vivo* microdialysis with HPLC-EC. Twenty-four minutes after an intraperitoneal injection of saline or glucose (100, 250, or 1000 mg/kg), rats were tested in a four-arm cross maze for spontaneous alternation behavior combined with microdialysis collection. Glucose at 250 mg/kg, but not 100 or 1000 mg/kg, produced an increase in spontaneous alternation scores (69.5%) and ACh output (121.5% versus baseline) compared to alternation scores (44.7%) and ACh output (58.9% versus baseline) of saline controls. The glucose-induced increase in alternation scores and ACh output was not secondary to changes in locomotor activity. Saline and glucose (100–1000 mg/kg) treatment had no effect on hippocampal ACh output when rats remained in the holding chamber. These findings suggest that glucose may enhance memory by directly or indirectly increasing the release of ACh. The results also indicate that hippocampal ACh release is increased in rats performing a spatial task. Moreover, because glucose enhanced ACh output only during behavioral testing, circulating glucose may modulate ACh release only under conditions in which cholinergic cells are activated.

Extensive evidence now indicates that modest increases in circulating glucose levels enhance the formation of new memories in rodents and humans (1, 2). In rodents, glucose enhances memory on several different tasks, including those motivated by appetitive and aversive conditions (3–6). In humans, glucose enhances memory in healthy young and elderly, as well as in subjects with Alzheimer disease or Down syndrome (7–10).

The breadth of circulating glucose effects on cognitive functions—i.e., across species and tasks—suggests that glucose might act on brain systems important for memory formation. Circulating glucose is readily transported from the blood to the brain and serves as the major source of energy for the central nervous system (11, 12). Consistent with the view that glucose has direct actions in the brain, microinjections of glucose into specific brain areas of rats—i.e., septohippocampal system—enhance mnemonic functioning (13, 14). However, a clear understanding of the neurobiological mechanisms underlying glucose facilitation of memory remains to be determined. Besides its essential metabolic function, glucose provides substrate for the synthesis of acetylcholine (ACh) and other neurotransmitters, actions that may contribute to the effects of glucose on memory. The metabolism of glucose is critical for the production of acetyl-CoA, a precursor of ACh (15), and may be closely linked to ACh synthesis (16). Also, decreases in blood glucose levels result in decreases in brain ACh synthesis (17). Thus, one possibility is that glucose enhances memory processes by increasing ACh synthesis and release, perhaps by augmenting the availability of acetyl-CoA.

Consistent with the view that glucose acts through a cholinergic mechanism, previous findings indicate that glucose injections modify the effects of cholinergic drugs on various behavioral and neural measures. For example, glucose attenuates spontaneous alternation deficits produced by cholinergic antagonists on a Y-maze, although glucose administration does not itself enhance performance on this task (18, 19). Glucose also reduces hyperactivity and paradoxical sleep deficits produced by muscarinic cholinergic antagonists (20, 21). Conversely, glucose increases the onset and severity of physostigmine-induced tremors, although glucose itself does not induce tremors (22). Thus, glucose interacts with cholinergic agents across a variety of measures.

Results from biochemical experiments suggest that glucose may modulate cholinergic neurons only under conditions in which the activity of cholinergic neurons is substantially increased or decreased. At doses that enhance memory, glucose injections reverse opioid-induced reductions in hippocampal ACh release, but glucose does not affect hippocampal ACh release in rats tested while in their home cages (23, 24). Conversely, glucose potentiates an increase in hippocampal ACh output produced by scopolamine, a muscarinic cholinergic antagonist (25). Furthermore, extracellular brain glucose levels vary with neuronal activity, suggesting that circulating glucose may be critical in modulating neural processes important for memory functioning (26).

These behavioral and biochemical findings led to the idea that glucose may modulate ACh output only under conditions in which cholinergic neurons are activated. However, determining the neurochemistry changes underlying glucose effects on memory requires biochemical measurements in the context of behavioral testing and in a task in which performance is enhanced by glucose injections. Because alternation performance of rats tested on a standard Y-maze is not enhanced by glucose, we assessed spontaneous alternation performance in a more difficult four-arm cross maze, in which glucose effectively enhanced performance. If glucose effects on memory are due to the activity of cholinergic cells, then glucose should increase ACh release on memory tasks in which the activation of cholinergic neurons occurs. Glucose should have no effect on ACh release under conditions in which the activity of cholinergic cells remains stable. Previous studies found an increase in hippocampal high-affinity choline uptake immediately following testing in spatial working memory tasks (27,

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Abbreviation: ACh, acetylcholine.

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28), suggesting that hippocampal ACh release is increased during performance in such tasks.

The present experiment addresses the following questions: (*i*) Is hippocampal ACh release increased during spontaneous alternation testing in a cross maze? (*ii*) Do systemic glucose injections potentiate an increase in hippocampal ACh output during behavioral testing? (*iii*) Do glucose injections improve memory on a four-arm version of spontaneous alternation testing? (*iv*) Does glucose enhance hippocampal ACh output while rats remain in their holding chamber?

MATERIALS AND METHODS

Subjects. Male Sprague–Dawley rats (Charles River Breeding Laboratories) weighing 300–400 g at the time of surgery served as subjects. Rats were housed individually with food and water available ad libitum. A 12-hr light/dark cycle (lights on 0700) was maintained.

Surgery. Rats received atropine sulfate (0.2 ml of a 540 μg/ml solution, i.p.) 20 min before being anesthetized with sodium pentobarbital (50–60 mg/kg, i.p.). Subsequent to being anesthetized, each rat was placed in a stereotaxic frame with the nose-bar set at 5.0 mm above the interaural line according to the atlas of Pellegrino *et al.* (29). Using standard stereotaxic procedures, a plastic guide cannula (CMA/12 type; Carnegie Medicin, Stockholm) was lowered into the hippocampal formation. The stereotaxic coordinates were 3.8 caudal to bregma, 5.0 mm medial-lateral, and 3.8 ventral from dura. Five jeweler's screws were placed in the skull surrounding the cannula and cemented in place with dental acrylic (Plastics One, Roanoke, VA).

Microdialysis Procedure. Beginning the day after surgery, rats were handled each day for ≈5 min. At least 1 week following surgery, rats were randomly chosen to be tested in either a spontaneous alternation or "resting" condition. A minimum of 3 days after the first testing, the same rats were then tested in the alternate condition. All rats were tested during their light phase (0800–1600). A 3-mm dialysis probe (CMA/12; Carnegie Medicin) was inserted through the guide cannula. The dialysis probe was connected to plastic tubing and driven by a microinfusion system (CMA/100; Carnegie Medicin). The dialysis probe was perfused continuously at a rate of 2.1 μl/min with cerebrospinal fluid (128 mM NaCl/2.5 mM KCl/1.3 mM $CaCl_2$/2.1 mM $MgCl_2$/21 mM NaH_2PO_4/1.3 mM Na_2HPO_4/3.3 mM glucose and brought to pH 7.0 by NaOH) which contained the acetylcholinesterase inhibitor neostigmine bromide (6 μM).

Spontaneous Alternation Condition. Rats were placed in a black Plexiglas chamber (23 × 30 × 30 cm) and microdialysis was begun. Perfusate collected for the first 45–60 min was not analyzed, allowing equilibration between the brain tissue and perfusion solution before sampling. Subsequently, samples were collected at 12-min intervals. ACh content of samples collected during 1 hr (5 hr total) served as baseline rates for ACh output. At the beginning of the second hour, rats were randomly assigned to one of the following treatment groups: saline (n = 10); glucose (100 mg/kg, n = 8); glucose (250 mg/kg, n = 9), or glucose (1000 mg/kg, n = 8). The glucose doses were mixed in saline and all treatments were injected i.p.

At the beginning of the third postinjection sample (24 min after injection) rats were removed from the holding chamber and placed in a four-arm cross maze. The maze (85-cm height) was constructed of wood painted grey and contained a central platform (25-cm diameter) from which radiated four symmetrical arms (55 cm long × 10 cm wide) with 12 cm walls. The treatment test interval was based on past behavioral experiments in which rats were tested 20–30 min following systemic injections (18, 19). After being placed in the central platform, rats were allowed to traverse the maze freely for 12 min. The number and sequence of entries were recorded; an alternation was defined as entry into four different arms on overlapping quintuple sets. Five consecutive arm choices within the total set of arm choices made up a quintuple set. A quintuple set consisting of arm choices A,B,D,A,C was considered an alternation. A quintuple set consisting of arm choices A,B,D,A,D was not considered an alternation. Using this procedure, possible alternation sequences are equal to the number of arm entries minus 4. The percent alternation score is equal to the ratio of (actual alternations/possible alternations) × 100; chance performance on this task is 22%. Only data from rats that made at least 12 arm choices (8 possible alternations) were included in the behavioral and accompanying biochemical analyses.

After behavioral testing, rats were returned to the holding chamber. Two additional microdialysis samples were collected. Thus, 5 baseline and 5 postinjection samples were collected for a total of 10 samples.

To test whether implantation of the probe and/or the microdialysis procedure altered spontaneous alternation scores, a group of unoperated controls (n = 15) was injected with saline and tested for spontaneous alternation performance using the same procedure as above.

Resting Condition. Habituation, baseline collection, and treatment groups (n = 5) were the same as in the spontaneous alternation condition. Following the i.p. injection, microdialysis samples were collected for an additional 60 min (five samples) while the rat remained in the holding chamber.

Acetylcholine Assay. Samples (20 μl) were assayed for ACh by using HPLC with electrochemical detection. ACh was separated from choline by a reverse-phase analytical column (Chromspher 5 C18, 100 × 3 mm, Chrompack, Middelburg, The Netherlands). Subsequently, an enzymatic postcolumn reactor containing acetylcholinesterase (EC 3.1.1.7; Sigma type VI-S) and choline oxidase (EC 1.1.3.17; Sigma) converted the ACh to choline and acetate and the choline to betaine. The final conversion to hydrogen peroxide was electrochemically detected by a platinum electrode held at a potential of +525 mV. The mobile phase containing 0.2 mM dibasic potassium phosphate, 1.0 mM tetramethylammonium hydroxide, 0.3 mM EDTA, and 0.005% Kathon CG (to prevent bacterial growth) was delivered at a rate of 0.6 ml/min by a solvent delivery system (PM-80; Bioanalytical Systems, West Lafayette, IN). ACh and choline peaks were quantified by comparison to peak heights of ACh and choline standard solutions. The detection limit was 50 fmol and the assay was completed within 7 min.

Delay Spontaneous Alternation Procedure. If spontaneous alternation in the cross maze has a memory component, then implementing a delay between arm choices should reduce alternation scores. To determine this, some of the rats were randomly chosen after microdialysis testing to be tested on the cross maze for a second time (without injection or dialysis). Rats were assigned to either a no-delay or 60-sec delay condition. In the no-delay condition, rats were allowed to traverse the maze for 12 min while the number and sequence of arm entries was recorded. In the 60-sec delay condition, rats were first allowed to make three arm choices. After entering the third arm, and each subsequent arm, a wood block was placed at the end of the arm preventing the rat from exiting the arm for 60 sec. Testing occurred for 25 min in the delay condition. A rat needed to make a minimum of 12 arm choices for its data to be included in the analyses.

Histology. After testing, rats received a lethal dose of sodium pentobarbital. Subsequently, a dialysis probe, dipped in ink, was inserted through the hippocampal guide cannula. This was followed by an intracardial perfusion with 0.9% saline and a 10% formalin solution. Brains were removed and placed in a 30% sucrose/formalin solution. The brains were frozen and cut in coronal sections (40 μm) on a sliding microtome. The sections were mounted onto slides, dried, and stained with

cresyl violet. Following staining, the sections were examined to determine location of the dialysis probe.

Statistical Analysis. For analysis of the microdialysis data, the raw values were converted to percentages from each subjects' baseline output. The baseline output was calculated from the mean of the first five samples for each subject. The percent values from all of the groups were analyzed by a two-way ANOVA with one-repeated measure. Simple main-effect tests were applied to compare groups at specific times and Dunnett's test for comparison to baseline levels.

A one-way ANOVA was used to analyze the percent alternation scores and number of arm entries. Newman–Keuls post-hoc tests were used to compare the data between the different drug groups.

Student *t* tests were used to compare differences in the percent alternation scores of unoperated controls versus microdialysis controls and of the no-delay condition versus the 60-sec delay condition.

RESULTS

Spontaneous Alternation Condition. The biochemical results during spontaneous alternation testing are illustrated in Figs. 1 and 2. Saline-treated rats exhibited an increase in hippocampal ACh output (+60%) during spontaneous alternation testing. In saline controls, ACh levels remained elevated above basal levels 12 min after testing but returned to basal levels by 24 min following the behavioral test. Systemic administration of glucose potentiated the increase in hippocampal ACh release (+121%) during behavioral testing in a dose-dependent manner. As shown in Figs. 1 and 2, saline controls exhibited a significant increase in hippocampal ACh release during behavioral testing compared to basal levels ($P < 0.01$). ACh output in saline controls remained significantly enhanced (+28.5%) for the sample immediately following behavioral testing ($P < 0.01$) but was not significantly different from baseline levels 24 min after testing ($P > 0.05$).

During spontaneous alternation performance, administration of glucose (250 mg/kg) significantly potentiated ACh output compared to that of saline controls, $F_{1,17} = 8.17$, $P < 0.02$. Twelve minutes following testing, hippocampal ACh output of rats receiving glucose (250 mg/kg) was still significantly augmented compared to that of saline-treated rats, $F_{1,17} = 7.48$, $P < 0.02$. By the last sample, ACh output of glucose (250 mg/kg)-treated rats was not significantly different from that of saline controls or basal levels ($P > 0.05$).

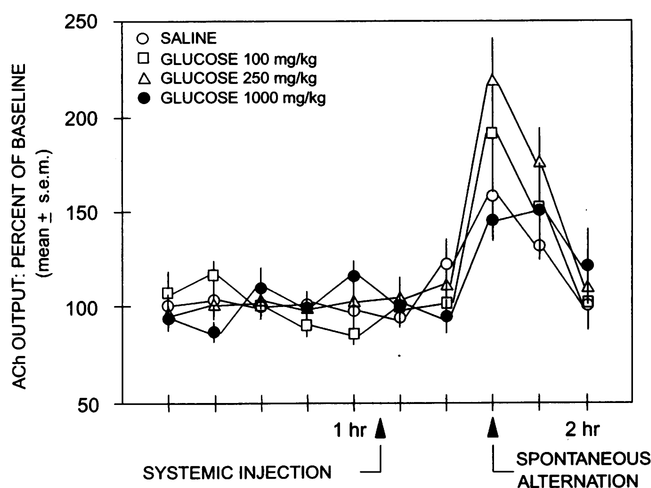

FIG. 1. Hippocampal ACh output in the behavioral condition following injections of saline or glucose. ACh output was significantly enhanced during spontaneous alternation and for the sample immediately following testing in saline controls. Glucose at 250 mg/kg significantly increased ACh output during behavioral testing and for the sample following testing compared to that of saline controls. Glucose treatment at 100 and 1000 mg/kg significantly raised ACh output above their respective basal levels during the time of spontaneous alternation testing and fourth postinjection sample. The ACh output increases with glucose at 100 and 1000 mg/kg was not significantly different from that of saline controls.

At the time of spontaneous alternation testing, rats that received glucose injections at 100 or 1000 mg/kg had significant increases in ACh output compared to their respective basal levels ($P < 0.01$); however, these increases were not significantly different from the increase in ACh output seen in saline controls ($P > 0.05$). In the sample following behavioral testing, rats that received glucose at 100 or 1000 mg/kg exhibited significant increases in ACh output above their basal levels ($P < 0.01$) but, again, this increase in ACh output was not significantly different from the increase seen in saline-treated rats ($P > 0.05$). During the fifth postinjection sample, ACh release in the groups receiving glucose 100 and 1000 mg/kg was not significantly different from ACh output of their respective baseline values ($P > 0.05$).

There were some baseline samples that varied between groups. At the second baseline sample, glucose (1000 mg/kg)-treated rats had significantly lower levels of ACh output than those of glucose (100 mg/kg)- and saline-treated rats ($P < 0.01$). At the fifth baseline sample, ACh output of glucose (100 mg/kg)-treated rats was significantly different than that of rats who received glucose 250 or 1000 mg/kg ($P < 0.02$). While statistically different, the magnitudes of these changes were small and their significance unclear.

The spontaneous alternation scores and corresponding changes in hippocampal ACh output are illustrated in Fig. 2. Analysis of the percent alternation scores indicated a significant group effect ($F_{3,31} = 4.11$; $P < 0.02$). Saline controls had a mean percent alternation score of 44.7 ± 5.3 SEM, a value significantly above chance (22%) levels ($P < 0.01$). Glucose treatment at 250 mg/kg (69.5 ± 3.1) significantly enhanced spontaneous alternation performance compared to saline controls ($P < 0.05$). In contrast, glucose treatment with 100 or 1000 mg/kg did not significantly modify spontaneous alternation scores compared to those of saline controls ($P > 0.05$). Unoperated controls injected with saline (44.2 ± 3.1) had alternation scores that were not significantly different from those of operated-saline controls ($P > 0.05$).

As observed in previous experiments (14, 24), there were minor differences in the number of arm choices between the groups (Fig. 3). A one-way ANOVA on the number of arm choices revealed there was not a significant group effect ($F_{3,31} = 1.26$; $P > 0.05$). The mean number of arms entered for the groups was as followed: saline = 19.0 ± 1.7 SEM; glucose at 100 mg/kg = 15.5 ± 1.3; glucose at 250 mg/kg = 20.8 ± 2.4; and glucose at 1000 mg/kg = 20.5 ± 3.1. Post-hoc analyses indicated that the number of arms entered was not significantly different between any of the groups ($P > 0.05$).

Resting Condition. The results of the treatments in the resting condition are illustrated in Fig. 4. In contrast to the findings in the spontaneous alternation condition, neither saline nor glucose injections modified hippocampal ACh output in the resting condition. The ANOVA revealed that there were no significant differences between groups ($F_{3,19} = 0.27$; $P > 0.05$) or across time ($F_{9,180} = 0.53$; $P > 0.05$) nor was there a significant interaction between treatment and time ($F_{27,144} = 0.61$; $P > 0.05$).

Delay Spontaneous Alternation. Inserting a 60-sec delay between arm choices reduced spontaneous alternation scores to values near chance (22%). A Student *t* test indicated that the percent alternation scores of rats in the delay condition (mean = 27.1 ± 2.7 SEM, $n = 6$) compared to those of rats in the no-delay condition (45.5 ± 6.5, $n = 6$) were significantly different ($P < 0.05$).

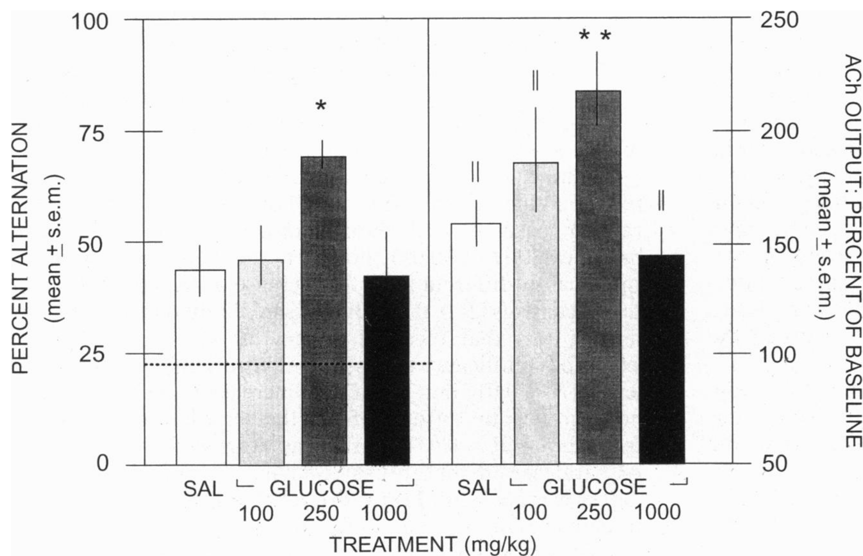

FIG. 2. Intraperitoneal injections of glucose enhance spontaneous alternation performance and potentiate an increase in hippocampal ACh output in a dose-dependent manner. Saline treatment significantly enhanced ACh output during behavioral testing compared to baseline levels. Glucose at 250 mg/kg significantly improved spontaneous alternation performance and significantly augmented ACh output above that of saline controls. ACh output was significantly enhanced following glucose treatment at 100 and 1000 mg/kg above their respective basal levels. Glucose at 100 and 1000 mg/kg did not modify alternation scores or ACh output compare to the scores and output of saline controls. Chance alternation performance is indicated by the dotted line on the left panel. Key to symbols: *, $P < 0.05$ versus SAL (saline); ||, $P < 0.01$ versus baseline levels; **, $P < 0.02$ versus SAL.

DISCUSSION

This experiment demonstrates that ACh output in the hippocampal formation of rats is increased during spontaneous alternation testing in a cross maze, that glucose augments ACh output in the hippocampal formation under behavioral testing conditions, and that glucose enhances spontaneous alternation performance in a four-arm maze; glucose effects on both ACh output and spontaneous alternation had similar dose-response characteristics.

In saline controls, ACh levels remained elevated for at least 12 min after testing and returned to basal levels thereafter. These findings are consistent with and extend previous results demonstrating an increase in high-affinity choline uptake in the hippocampal formation of rodents immediately following memory testing (27, 28, 30) and lasting for ≈15 min (27). In a recent experiment using *in vivo* microdialysis, rats trained to anticipate a liquid reward also exhibited enhanced hippocampal ACh release (31). In the present study, a 60-sec delay between arm choices significantly reduced percent alternation scores. Furthermore, removal of extramaze cues also decreased spontaneous alternation scores (unpublished observations). These findings suggest that this task has spatial and working memory components. Thus, the present results demonstrating an enhancement of hippocampal ACh output during a spatial memory task agree with previous findings suggesting that activation of the septohippocampal cholinergic system accompanies learning and memory.

Activation of the septohippocampal cholinergic system also occurs under other conditions—e.g., immobilization (32). However, the possibility that the increase in ACh release observed in the present experiment is due solely to stress or novelty is unlikely because similar increases in ACh output were evident after up to three to four repeated spontaneous alternation test sessions (unpublished observations). Similarly, repeated training produces changes in hippocampal high-affinity choline uptake when rats learn to swim to an escape platform but not when trained in a swimming-only condition (33). Again, these findings suggest that an increase in hippocampal ACh output may be important in the acquisition and/or storage of information.

When injected during behavioral testing, glucose significantly enhanced hippocampal ACh output. Glucose augmented ACh output in an inverted-U dose-response manner with peak effects evident at 250 mg/kg. In contrast to the effect of glucose on hippocampal ACh output during the behavioral condition, glucose did not alter ACh release in rats kept in their home cages. These findings indicate that glucose does not modulate ACh release under all conditions and suggest that glucose may augment ACh output only when cholinergic neurons are activated. Consistent with this idea, glucose itself

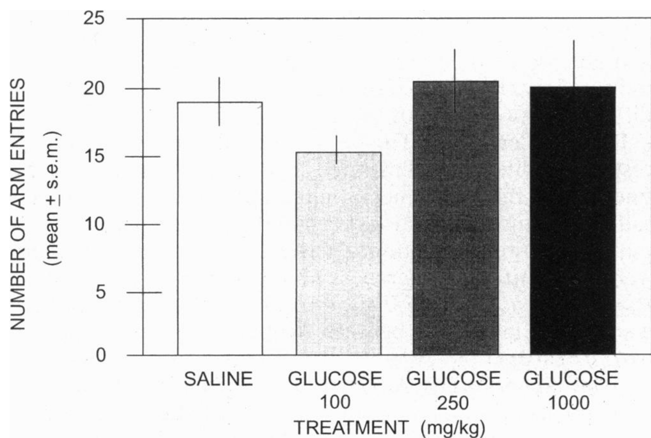

FIG. 3. The number of arm choices during spontaneous alternation performance following saline or glucose treatment. There were no significant differences in the number of arm choices made between the groups.

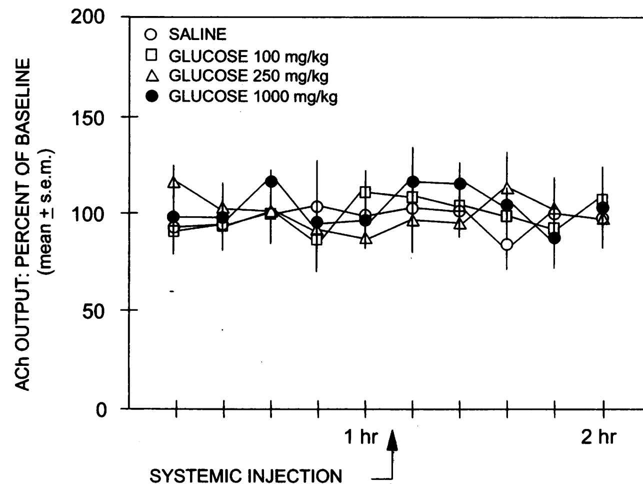

FIG. 4. Hippocampal ACh output following injections of saline or glucose in the resting condition. Saline and glucose treatment did not modify ACh output in the resting condition.

does not initiate tremors but, when coadministered with physostigmine, potentiates the onset and severity of tremors (22). Also, administration of an α-noradrenergic antagonist has no effect on septohippocampal cholinergic neurons in a resting condition but attenuates the activation of septohippocampal cholinergic cells and memory performance during testing in a radial-arm maze (30). Such findings highlight the importance of using behavioral testing in combination with drug treatments and biochemical measurements when investigating the neural bases of memory; when not studied in the context of behavioral testing, such experiments may yield potentially misleading results.

In a dose-response manner similar to that seen for glucose effects on ACh output, glucose (250 mg/kg) also enhanced spontaneous alternation performance as compared to saline controls at a dose that also improves memory under other conditions (19). Previous findings indicate that injections of glucose and other memory-enhancing treatments do not increase spontaneous alternation performance in a Y-maze, although glucose attenuates deficits produced by several drug treatments (13, 14, 18, 19). Therefore, results obtained with the four-arm cross maze provide, to our knowledge, the first demonstration that glucose itself enhances spontaneous alternation performance and that glucose itself enhances memory in a nonappetitive and nonaversive task. Moreover, saline and unoperated controls have similar spontaneous alternation scores, indicating that the glucose effect on spontaneous alternation reflects enhancement of baseline performance and not attenuation of a deficit that might have been caused by the implantation of the probe and/or microdialysis procedure.

The glucose effects on hippocampal ACh output and spontaneous alternation behavior were not secondary to changes in locomotor activity. There were no statistically significant differences between the groups on the number of arms entered. This finding is comparable to past evidence that glucose treatment does not alter the number of arm choices made (14, 24) and is consistent with previous findings indicating that locomotor activity does not predict changes in high-affinity choline uptake in the hippocampal formation of rats (28, 33). In contrast, Day *et al.* (34) found that ACh release in the hippocampal formation is correlated with locomotor activity. The reason for the discrepancy between these findings is unclear. The differences may result from the use of different apparatuses or task demands in the two experiments.

Glucose injected at 100 or 1000 mg/kg did not significantly augment ACh release or enhance spontaneous alternation performance above control levels. During behavioral testing, glucose at 100 mg/kg produced a nonsignificant increase in ACh output compared to controls. Interestingly in this group, four rats showed more than a 100% change in ACh output, comparable to the change found in the glucose (250 mg/kg) group. These four rats also had higher alternation scores (mean = 55%) than did the four rats who displayed an ACh increase of less than 100% (mean = 38%). Although there is not a significant correlation between change in ACh output during behavioral testing and alternation scores within any group tested (data not shown), hippocampal ACh output may still be related to spontaneous alternation performance. For example, an increase in hippocampal ACh output may have to reach a threshold (i.e. increase $\geq$100%) to render an improvement in spontaneous alternation scores.

Interestingly, glucose not only improves memory in an inverted-U dose-response manner but also increases hippocampal ACh release in a similar fashion, raising the possibility that the inverted-U dose-response curve for ACh release may underlie the inverted-U for memory. Numerous studies in humans and rodents found that memory-facilitating doses of glucose elevate circulating glucose levels within a specific range (1). Glucose injections at 1000 mg/kg, which had no effect on memory or ACh release in the present study, increase circulating glucose levels outside the range correlated with memory improvement (35). The higher doses of glucose may not affect ACh release because of a greater insulin response and/or alteration in the transport mechanisms across the blood–brain barrier. Lower doses of glucose may not alter ACh release simply because circulating glucose levels are not increased enough to provide sufficient substrate to increase the synthesis and release of ACh.

The results of the present study suggest that increased release of ACh in the hippocampal formation may contribute to glucose enhancement of memory on the cross maze, either by direct or indirect effects of glucose. One possible explanation for the glucose potentiation of ACh release is by direct actions at cholinergic neurons to increase the availability of acetyl-CoA for synthesis of ACh. Although high-affinity choline uptake is considered to be the rate-limiting step for ACh synthesis (36), the availability of acetyl-CoA becomes rate-limiting under some conditions (17, 37)–e.g., in chronically depolarized brain synaptosomes (37). Thus, under conditions in which cholinergic neurons are activated, glucose treatment may modulate synthesis and release by increasing the availability of acetyl-CoA.

Another possibility is that glucose may enhance ACh release by blocking the opiate inhibition of cholinergic cells. Activation of opiate receptors in the hippocampal formation decreases ACh release (38). Glucose reverses decreases in memory performance and hippocampal ACh output induced by morphine, an opiate agonist (19, 23, 24). Furthermore, an increase in blood glucose levels reduces the affinity of opiate-receptor binding (39), suggesting that glucose may act functionally as an opioid antagonist. These results suggest that the increase in ACh release following glucose treatment may be through indirect actions.

An alternative explanation is that augmentation of ACh release is not critical for glucose improvement of spontaneous alternation performance. Glucose enhancement of memory may be through other mechanisms in which an increase in ACh release is a secondary consequence but not involved in glucose effects on memory. For example, glucose metabolism is also important for the synthesis of glutamate and aspartate (40). Glutamate, which facilitates memory (41), appears to regulate ACh output in several brain regions (42). Therefore, the change in ACh release following glucose treatment could be secondary to glucose directly enhancing glutamate release. Alternatively, the change and/or interaction of both the glutamatergic and cholinergic systems may be critical in certain forms of learning and memory–i.e., spontaneous alternation behavior. In either case, whether glucose enhancement of memory is through direct actions on one or more neurotransmitter systems and/or other biochemical processes remains to be determined.

If glucose enhances memory by increasing hippocampal ACh release, then it raises the question: What does a greater release of ACh do to improve memory? Recent studies on rats with selective lesions of septohippocampal cholinergic neurons found memory impairments on delayed non-matching-to-sample tasks (43, 44). In addition, excitotoxic lesions of the medial septum or intraseptal drug infusions that reduce activity of cholinergic neurons impair working memory on temporal discrimination tasks (45, 46). Furthermore, findings from a temporal discrimination task suggest that physostigmine treatment accelerates memory storage speed (47). Thus, an increase in the activation of septohippocampal cholinergic neurons may facilitate the processing of temporal information.

In summary, the present results indicate that spontaneous alternation performance in a four-arm cross maze increases hippocampal ACh output, which remains elevated for at least 12 min but less than 24 min after testing. Systemic glucose injections potentiate the testing-related increase in ACh output and also enhance spontaneous alternation performance in

a similar dose-dependent manner. In contrast, glucose administered under a resting condition does not modify hippocampal ACh output. These findings suggest that increases in hippocampal ACh release may participate in glucose enhancement of memory; the increases in ACh release may be mediated by either direct or indirect actions of glucose. Moreover, because glucose modifies ACh output under the behavioral but not resting conditions, glucose may only modulate ACh output when cholinergic neurons are activated.

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