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Phlorizin Enhancement of Memory in Rats and Mice

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HALL, J. L., R. T. REILLY, K. L. COTTRILL, W. S. STONE AND P. E. GOLD. *Phlorizin enhancement of memory in rats and mice.* PHARMACOL BIOCHEM BEHAV 41(2) 295-299, 1992. – Glucose administration near the time of training or testing elevates blood glucose levels and enhances memory in rodents and humans. The magnitude of increases in circulating glucose levels predicts later retention performance in these and several other situations. Thus, circulating glucose levels appear to contribute to the regulation of memory storage processes. Phlorizin is an inhibitor of glucose transport, which, in view of the effects of glucose on memory, should impair memory. However, rats and mice injected with phlorizin before training in an inhibitory (passive) avoidance task demonstrated significantly enhanced memory performance compared to that of control animals. The effective dose of phlorizin did not significantly change regional brain-relative ^{3}H-2-deoxyglucose uptake or plasma glucose levels. To summarize, phlorizin is a potent memory-enhancing drug. While the mechanism of this enhancement is unknown, it does not appear to include changes in blood glucose levels or brain glucose uptake.

Phlorizin Enhancement of memory Blood glucose Brain glucose Brain ^{3}H-2-deoxyglucose uptake

WHEN injected near the time of training, glucose modulates memory processing for a variety of learned tasks in rodents and humans (for reviews, see 12,33). The enhancement of memory by glucose in both humans (27) and rodents (11,13) follows an inverted-U dose-response curve in which memory enhancement is seen at moderate doses and amnesia is evident at high doses. Enhancement of memory by glucose is also time dependent (11); glucose administered 1 h after training does not affect later retention performance.

In addition to the effects of glucose treatments on memory, circulating glucose levels measured shortly after training are related to later retention performance. Several treatments that result in good retention (e.g., epinephrine or glucose injection) are accompanied by moderate increases in circulating glucose levels (14,17). Similarly, treatments that result in relatively poor retention, such as low doses of glucose or epinephrine, do not appreciably alter blood glucose levels (14,16). Finally, treatments that impair memory are accompanied by abnormally high (14,16,17,23) or abnormally low (15) blood glucose levels. Thus, posttraining blood glucose levels are related to later retention performance in an inverted-U manner (i.e., moderate elevations in blood glucose levels predict good retention, while greater or lesser changes in blood glucose levels predict poor retention).

Intracerebroventricular injections of glucose also enhance memory in a dose- and time-dependent manner (19) and antagonize the effects of scopolamine on spontaneous alternation performance (28). Thus, the memory effects of peripheral glucose administration may be mediated by direct actions on the central nervous system. Glucose normally enters the brain from blood via a sodium-independent, carrier-mediated, facilitated diffusion mechanism (21,26,29). 1-[2-(β-D-Glucopyranosyloxy)-4,6-dihydroxy-phenyl-3-(4-hydroxyphenyl)-1-propanone (phlorizin) is a fully competitive antagonist for this glucose carrier (2,3,5,20). Phlorizin blocks glucose uptake into the brains of rats (25,30), rabbits (31), and dogs (5,34), but is not itself transported (32). If increasing the availability of glucose to the brain enhances memory (i.e., by elevating circulating glucose levels), decreasing the availability of glucose with phlorizin might impair memory. The following experiments investigated this possibility by determining the effects of phlorizin on memory performance in an inhibitory avoidance task.

EXPERIMENT 1

Here, the effects of phlorizin on memory for inhibitory avoidance training were determined in rats and mice.

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METHODS

Part 1

Male Sprague-Dawley rats (275–400 g, Dominion Labs) were individually housed (lights on 0700 h, lights off 1900 h). Food was available ad lib. The animals were water deprived to 80% of their free-drinking weights.

The training apparatus was a two-compartment Plexiglas box. A white start compartment (10 × 14 × 23 cm) was separated from a dark compartment (10 × 14 × 37 cm) by a sliding white Plexiglas door. The dark compartment had a grid floor for footshock delivery. A water spout was located at the far end of the dark compartment.

After reaching 80% of initial weights (5–12 days), rats were pretrained to drink from the water spout in the inhibitory avoidance box. During pretraining (4–6 days, 1 trial/day), each rat was placed in the white compartment, the door was opened, and the animal was allowed to approach and drink from the spout for 30 s after the first lick. By the final pretraining trial, all animals included in the experiment began drinking within 10 s of opening the door and for at least 20 of the following 30 s; 4 rats did not meet these criteria and were excluded from the experiment.

One day after completion of pretraining, the rats were injected with phlorizin (0.03–300 μg/kg, IP; N = 9–13) or saline (N = 18). Thirty minutes later, each animal was placed in the apparatus as before, but after 10 s of drinking a mild footshock was delivered (500 μA, 0.5 s), after which the rats were immediately removed from the apparatus and returned to the home cages. On the retention test, 24 h later, animals were placed in the white compartment, the door was opened, and the latency to lick for 30 s (cumulative time spent drinking) was recorded (600 s max). Group differences in the latencies to lick for 30 s were analyzed by the Mann–Whitney U-test.

To determine flinch thresholds, rats were injected with phlorizin (3, 30, or 300 μg/kg, IP; N = 5) or saline (N = 5). Thirty minutes later, each animal was placed in the dark compartment of the inhibitory avoidance chamber and ascending shocks (0.5 s duration) were delivered in 50-μA increments (30-s intertrial interval) until a flinch response was observed. Results were analyzed with Student's t-tests (two-tailed).

Part 2

Six-week-old male mice (DUB-ICR; Dominion Labs) were housed 4 per cage (lights on 0700 h, lights off 1900 h) with food and water available ad libitum.

The apparatus was a trough-shaped alley (upper width 13.5 cm, lower width 3 cm, height 11 cm) divided into a white compartment (20 cm long) and a dark compartment (40 cm long). The walls and floors of the dark compartment were stainless steel plates through which footshock could be delivered.

Thirty minutes prior to training, animals were injected with phlorizin (0.0003–30.0 μg/kg, IP, N = 10–17) or saline (N = 27). Each animal was placed in the white compartment from which the dark compartment was entered where escapable footshock (200 μA) was delivered. Forty-eight hours later, each animal was placed in the white compartment and the latency to enter the dark compartment was recorded (600 s max). Results were analyzed using the Mann–Whitney U-test.

RESULTS AND DISCUSSION

Phlorizin injection enhanced retention in an inverted-U dose–response manner (Fig. 1), with significant enhancement

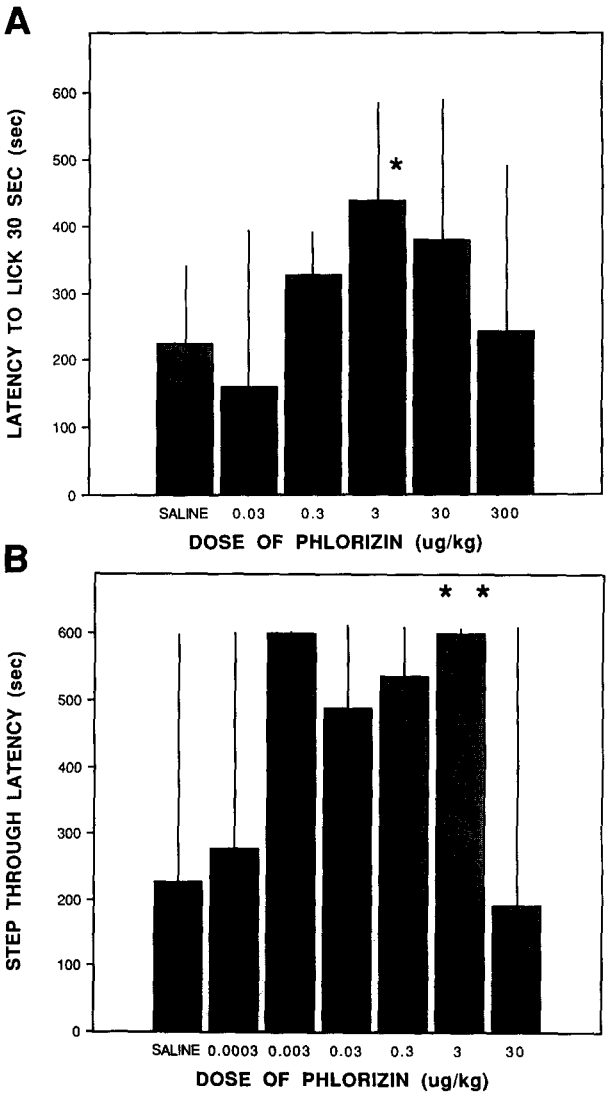

FIG. 1. Effect of phlorizin on inhibitory avoidance responses (medians ± interquartile ranges) in (A) rats and (B) mice. Note that phlorizin improved retention performance in an inverted-U dose–response manner (*$p < 0.05$ vs. saline-injected animals; **$p < 0.01$ vs. saline-injected animals).

seen at 3 μg/kg in both rats ($U_{11,18} = 53$, $p < 0.05$) and mice ($U_{17,27} = 129$, $p < 0.01$). This enhancement did not result from altered sensitivity to the shock since there were no significant between-group differences in the flinch thresholds after phlorizin injection (mean flinch threshold for saline-injected rats ± SEM = 206 ± 10 μA; for animals injected with 3, 30, or 300 μg/kg phlorizin, mean flinch thresholds = 186 ± 4, 184 ± 10, and 198 ± 10 μA, respectively).

Thus, the memory effect of phlorizin was opposite in direction to that expected on the basis of previous findings with glucose treatments. Increasing the availability of glucose to the brain through glucose injection or consumption enhances memory, so decreasing the availability of glucose to the brain with phlorizin should have impaired memory. However, rather than impairing memory, phlorizin enhanced performance in the inhibitory avoidance task. Other glucose antago-

nists such as 3-*O*-methyl glucose and 2-deoxyglucose also enhance the performance of a learned conditioned emotional response (23). Thus, both glucose and glucose antagonists appear to enhance memory with inverted-U dose–response curves.

EXPERIMENT 2

In an effort to explain these apparently paradoxical findings, we first wished to verify that the memory-enhancing dose of phlorizin effectively blocked brain glucose transport. Studies describing phlorizin-induced blockade of glucose transport into the brain assessed transport 1 min after intravenous infusion of phlorizin (25). Since phlorizin was injected 30 min prior to training in the present study, its inhibitory effect on brain glucose transport may have subsided or even produced a reflexive increase in brain glucose transport by the time the training experience occurred and the new information was being processed into memory.

Alternatively, the effects of glucose and phlorizin might be indirectly mediated by peripheral mechanisms responsive to elevations in circulating glucose levels, rather than by increasing the availability of glucose to the brain. In this regard, several treatments that produce optimal levels of memory performance are accompanied by moderate increases in circulating glucose levels (15–40 mg/dl), while treatments that result in poor memory performance either do not appreciably alter or drastically increase glucose levels well beyond normal physiological levels (14–17). Thus, phlorizin might enhance memory by producing moderate increases in circulating glucose levels.

The following experiments investigated the possibilities that phlorizin enhanced memory by 1) altering brain glucose transport at the time of training, or 2) elevating circulating glucose levels. The first part of this experiment was conducted in mice to reduce the required quantity of the costly radioactive tracer. The second part of this experiment was conducted in rats because blood samples can be collected from rats after arterial catheterization without disturbing the animals, an important consideration since blood glucose levels are sensitive to mild stressors including handling.

METHODS

Part 1

Mice received intraperitoneal injections of the memory-enhancing dose of phlorizin (3.0 μg/kg; $N = 6$) or saline ($N = 6$). Twenty minutes later, the animals were injected with ^{3}H-2-deoxyglucose (10 μCi, SC) that had been lyophilized and reconstituted in physiological saline to a final injection volume of 0.3 ml. Forty minutes later, animals were decapitated and the brains were removed and immediately placed on ice. Brains were dissected into eight regions including cortex, striatum, septal area, hippocampus, upper brainstem (thalamus plus colliculi), cerebellum, lower brainstem (pons and medulla), and hypothalamus (10). Brain regions were weighed and solubilized in 2 ml TS-1 tissue solubilizer (Research Products International), after which 15 ml Aquasol were added for liquid scintillation counting. Relative glucose transport was determined from these ^{3}H-2-deoxyglucose levels. The smallest change in ^{3}H-2-deoxyglucose uptake between the saline and phlorizin groups occurred in the striatum. Relative changes in ^{3}H-2-deoxyglucose uptake were then assessed by obtaining ratios of ^{3}H-2-deoxyglucose uptake values in other brain regions to ^{3}H-2-deoxyglucose uptake values in the striatum (22). These ratios were statistically compared between groups using Student's *t*-tests (two-tailed).

TABLE 1

RELATIVE REGIONAL BRAIN UPTAKE OF ^{3}H-2-DEOXYGLUCOSE AFTER PHLORIZIN INJECTION (3.0 μg/kg) NORMALIZED TO STRIATAL ^{3}H-2-DEOXYGLUCOSE UPTAKE LEVELS (MEANS ± SEM)

	Saline	Phlorizin
Cortex	1.44 ± 0.33	0.92 ± 0.02
Septum	2.06 ± 0.39*	1.34 ± 0.10
Hippocampus	1.34 ± 0.23	1.08 ± 0.05
Thalamus	1.24 ± 0.19	0.99 ± 0.06
Hypothalamus	1.34 ± 0.19	1.22 ± 0.10
Cerebellum	0.95 ± 0.08	0.96 ± 0.02
Brainstem	1.21 ± 0.30	0.84 ± 0.04
Total	1.25 ± 0.19	0.96 ± 0.02

*$N = 5$.

Note that there were no significant differences in relative ^{3}H-2-deoxyglucose uptake in any brain area examined ($N = 6$).

Part 2

In preparation for catheterization, rats received atropine sulfate (0.13 mg/animal, IP) and were anesthetized with sodium pentobarbital (48 mg/kg, IP). A catheter was implanted in the ventral tail artery. The catheter exited from the neck and allowed blood samples to be collected later without disturbing the animals. This method has been described in detail elsewhere (8). Catheters were flushed once daily with 0.5 ml heparinized saline (500 IU/ml).

Twenty-four hours after surgery, rats were injected with phlorizin (3 or 300 μg/kg, IP) or saline ($N = 6$–7). Plasma glucose levels were determined prior to (basal) and 15, 30, 45, and 60 min after injection. Blood samples were drawn into iced, heparinized tubes in 0.5-ml aliquots. After collection of

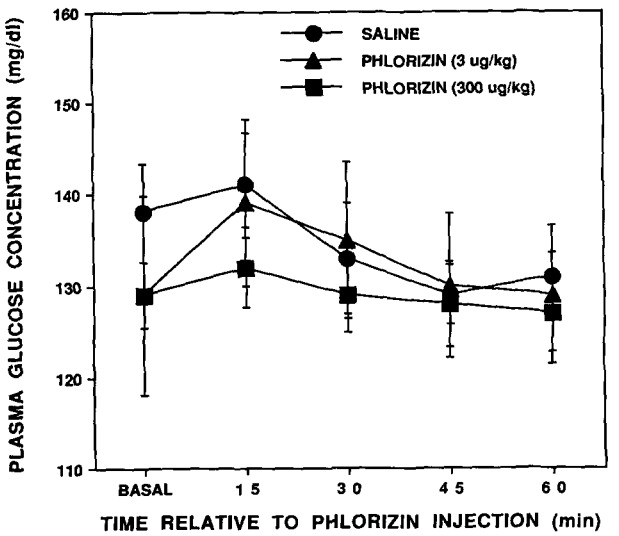

FIG. 2. Plasma glucose levels after phlorizin injection (means ± SEM). Note that phlorizin had no substantial effect on circulating glucose levels.

each sample, blood volume was restored with an equal volume of heparinized saline (50 IU/ml). Plasma was separated within 30 min of withdrawal by centrifugation (3000 rpm, 10 min, 4°C) and frozen in 20-μl aliquots for analysis. Determinations of plasma glucose levels were made using a colorimetric hexokinase assay (Sigma diagnostic kit 115). Briefly, samples were thawed and incubated for 5–10 min in glucose assay reagent. The reaction was stopped with 10 ml 0.1 N HCl and the absorbency at 520 nm was recorded. Plasma glucose levels were calculated by normalization to 100 mg/dl standards. This assay provides a linear relationship between glucose concentration and absorbency from at least 50–300 mg/dl (i.e., well beyond the range of expected values). Data were analyzed with the Student's and matched *t*-tests (two-tailed).

RESULTS AND DISCUSSION

Table 1 shows regional brain relative ^{3}H-2-deoxyglucose uptake levels after injection of a memory-enhancing dose of phlorizin. Relative ^{3}H-2-deoxyglucose uptake was not significantly affected by the memory-enhancing dose of phlorizin in any brain area examined. Assuming that the ^{3}H-2-deoxyglucose required 10 min to be fully absorbed into the circulatory system, these values should reflect brain glucose uptake during the 30 min after training in experiment 1 (i.e., during memory storage). Thus, the absence of an effect on relative ^{3}H-2-deoxyglucose uptake suggests that phlorizin does not enhance memory by altering glucose transport across the blood–brain barrier.

Changes in plasma glucose levels from individual basal values of rats injected with saline or phlorizin are illustrated in Fig. 2. Plasma glucose levels of saline-injected animals were unchanged with time relative to individual basal values. Similarly, plasma glucose levels of animals injected with either the memory-enhancing dose or the higher dose of phlorizin were not significantly different from individual basal values or those of saline-injected animals at any time examined. Thus, it appears unlikely that the memory enhancement accompanying phlorizin treatment can be attributed to elevations in circulating glucose levels.

GENERAL DISCUSSION

These studies demonstrated that pretraining administration of phlorizin enhanced memory in both rats and mice. The dose–response curves for the memory enhancement with phlorizin treatment were inverted-U in shape, as is common with numerous other memory-modulating treatments (18). Although a potent inhibitor of brain glucose transport (5, 25,30,31,34), the memory-enhancing effect of phlorizin did not appear to be related to an effect on brain glucose transport. Similarly, while numerous memory-enhancing treatments produce modest elevations in blood glucose levels (14–16), phlorizin did not affect circulating glucose levels in this fashion. Thus, while the results do not clearly suggest how phlorizin affects memory, the findings suggest a mechanism alternate to those underlying the effects of peripheral or central glucose administration on memory.

In view of the well-documented inhibitory effects of phlorizin on glucose transport in several model systems (1,4,5,6, 9,24,25,30,31,34), it was somewhat surprising that phlorizin did not inhibit regional brain-relative ^{3}H-2-deoxyglucose uptake. There are several possible explanations for this finding. First, the dose of phlorizin used to produce memory enhancement may have been insufficient to produce measurable effects on brain-relative ^{3}H-2-deoxyglucose uptake. Alternatively, the effects of phlorizin on glucose transport might have been rapid and transient. Studies describing phlorizin-induced blockade of glucose transport into the brain assessed transport 1 min after intravenous infusion of phlorizin (25), but the time course of phlorizin-induced decreases in brain glucose transport after intraperitoneal injection, as in the present study, is unknown. Thus, phlorizin may have inhibited brain-relative ^{3}H-2-deoxyglucose uptake, but the effect might have been washed out by a rapid return to normal levels of glucose transport during the relatively long (30 min) period examined.

Another possible mechanism for the enhancement of memory by phlorizin, and possibly glucose, might include interactions with hypothalamic glucoreceptors. Evidence supporting this view includes demonstrations that phlorizin treatment prevents gold thioglucose-induced ventromedial hypothalamic necrosis (7) and that intracerebroventricular glucose injections enhance memory (19). Thus, it may be useful to investigate the role of hypothalamic glucoreceptors in the effects of phlorizin and perhaps other memory-modulating treatments to determine a mechanism for these effects.

In summary, the results of the present experiments indicate that phlorizin is a potent memory-enhancing treatment. Enhancement of memory by phlorizin did not result from altered perception of footshock, changes in regional brain-relative ^{3}H-2-deoxyglucose uptake, or elevations in circulating glucose levels. Thus, the mechanism of this enhancement remains unknown, but may include interactions with hypothalamic glucoreceptors.

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