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D-Glutamine Produces Seizures and Retrograde Amnesia in the Chick

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DAVIS, J. L. AND A. CHERKIN. *D-Glutamine produces seizures and retrograde amnesia in the chick.* PHARMAC. BIOCHEM. BEHAV. 15(3) 367–369, 1981.—D-glutamine, 6 μmols/chick, injected intracranially one min after training, produces retrograde amnesia when tested 24 and 48 hr post training on an avoidance task. D-glutamine also produces ectostriatal seizures that correlate with amnestic activity.

D-Glutamine Retrograde amnesia EEG Seizures Chicks

THE retrograde amnesia induced by proline in chick [3] and mouse [4] is stereospecific; L-proline causes amnesia whereas D-proline has no effect on memory. We have also reported [2] that the sodium chloride dependence of lethal convulsant activity in the chick is stereospecific, D-proline producing a much higher mortality. During a study of the positive correlation of the amnestic potency of proline analogs with their anti-spreading depression potency [6] we observed L-glutamine to produce neither amnesia nor seizures. D-Glutamine caused ectostriatal seizures and was therefore not suitable for the correlation study which excluded agents found to produce seizures. We now report our findings on the retrograde amnestic potency of D-glutamine and provide more detail regarding D-glutamine related seizure activity, in addition to its amnestic potency.

EXPERMENT 1

METHOD

The chicks were White Leghorn cockerels (Pace/Setters, Alta Loma, CA) aged 44±12 hr at the start of training. L-Glutamine, D-glutamine and D-proline (all from Sigma Chemical) and methyl anthranilate (Givaudan) were used as received. One min after training, all chicks were given bilateral intraventricular 10 μl injections of 300 mM solution (pH 6.9) of the amino acids made up in sterile water. Injections were made with 26 gauge needles without anesthesia since previous work showed chicks to be unaffected by such injections.

The chick memory procedure has been described in detail [1] and is only summarized here. Chicks peck spontaneously at an attractive target, e.g., a 3-mm stainless steel bead. Suppression of the peck response is conditioned by a single 10-sec training trial wherein the bead is coated with an aversive liquid (methyl anthranilate). Retention of peck suppression is tested 24 hr after training, by presenting the peck target again but without the aversive coating. Two measures of peck suppression are recorded: (1) percentage of chicks which avoid pecking for 10 sec, the cut-off limit; and (2) total number of pecks emitted during the 10-sec presentation of the target. Amnestic treatments reverse the learned peck-suppression, as indicated by lower avoidance and higher peck rate compared to controls. The two measures are interdependent and highly correlated but previous work with this paradigm has indicated the peck score to be more sensitive than the avoidance score.

RESULTS

The results (Table 1) demonstrate that D-glutamine (6.0 μmols/chick) injected 1 min after training is an effective amnestic agent. Data for 6.0 μmols of D-glutamine, L-glutamine, and D-proline, and data in control chicks receiving D-proline or no injection, obtained in previous experiments, are tabulated for comparison. Comparisons using peck scores show the D-glutamine group to be significantly different from the L-glutamine group ($p<0.005$; t-test), and from the D-proline ($p<0.0001$) and uninjected control groups ($p<0.0001$). The avoidance score of the D-glutamine group also significantly differs from that of the L-glutamine group ($p<0.05$; χ^2 test) and those of the D-proline and uninjected control groups ($p<0.0001$ in each case; χ^2 test). D-proline injected chicks do not differ significantly from uninjected but trained chicks; thus D-proline injected chicks serve as an appropriate nonamnestic control group. In addition, D-proline and L-glutamine do not significantly differ from each other by either measure in this experiment.

TABLE 1

AMNESTIC EFFECT OF D-GLUTAMINE INJECTED ONE MIN POST-TRAINING AND TESTED 24 HR LATER

Compound	N	Peck Score ($\sqrt{p}$±S.D.)	Avoidance Score (%)
D-glutamine	29	2.00 ± 1.55	27.6
L-glutamine	28	0.81 ± 1.22	60.7
D-proline	28	0.37 ± 0.97	82.1
D-proline	296*	0.77 ± 1.15	56.1
Uninjected	50	0.72 ± 1.09	60.0

*Pooled data, previously published [6].

TABLE 2

RETROGRADE AMNESIA PRODUCED BY D-GLUTAMINE

Training-Injection Interval (min)	N	Peck Score ($\sqrt{p}$±S.D.) 24 hr	48 hr
1	28	2.27 ± 1.55	—
59	28	1.96 ± 1.68	—
239	29	1.05 ± 1.14	—
1	42	—	2.18 ± 1.60
59	44	—	1.21 ± 1.31
239	45	—	1.19 ± 1.32

EXPERIMENT 2

METHOD

Procedures were as described in Experiment 1, with the exception that 6.0 μmols/chick of D-glutamine was administered at 59 or 239 min after training, in addition to replicating the 1-min interval. (We assume an arbitrary delay of 1 min for uptake of injected compounds at the unknown site of action, so that the intervals between training and onset of effect are 2, 60 and 240 min, respectively).

RESULTS

A *t*-test comparison (two-tailed) of the peck score of 1-min vs 59-min and 239-min training-injection intervals shows (Table 2) injection at the 1-min interval to be significantly more amnestic than injection at the 239-min interval ($p<0.002$). The difference between peck rates at the 59-min and 239-min intervals was also significant ($p<0.02$) but the difference between the 1-min and 59-min interval scores was non-significant. These data suggest a retrograde effect of post-training injections of D-glutamine. The avoidance scores at the 1-, 59-, and 239-min intervals were 21.4%, 32.1%, and 44.8% respectively. Although these scores suggest a gradient, the differences among them do not reach significance at the 0.05 level.

EXPERIMENT 3

METHOD

All procedures duplicated Experiment 2, except that testing was delayed for 48 hr after testing, instead of 24 hr.

RESULTS

t-Test comparisons (two-tailed) of the peck scores show the 1-min training-injection interval produced significantly more amnesia than the 59-min ($p<0.005$) or 239-min ($p<0.005$) intervals (Table 2). The 59-min vs 239-min intervals did not differ significantly. These data suggest a retrograde effect of post-training injections of D-glutamine even when testing is delayed for 48 hr. As in Experiment 2, the avoidance scores of 31.0%, 43.2%, and 48.9% reinforce the suggestion of a gradient, but do not approach significance at the 0.05 level.

EXPERIMENT 4

METHOD

The implant and recording procedures have been described in detail elsewhere [5]. Briefly, 11 White Leghorn cockerels, 29 hr old, had active bilateral recording electrodes stereotaxically positioned in right and left ectostriata, under the comb (indifferent) and in dorsal neck muscle (ground). Under halothane anesthesia, the electrode assembly, with miniature amphenol recording connections, was embedded and fixed to the skull with Grip dental acrylic. Active recording electrodes were insulated to 0.5 mm of the tip. Recording was begun approximately 24 hr after surgery, always between 1300-1600 hr. Electrode locations were histologically verified.

Electrophysiological screening and amplifier adjustments to be used in the experiment were determined during the adaptation period. During this period recording leads were fastened and each chick was placed in a 8.5-cm dia.×21.0-cm deep carton. The electrical activity was recorded on 6 channels of a Grass Model 73 polygraph. Two of the polygraph channels were used to monitor monopolar-EEG activity from each ectostriatum referenced to the comb, and a third channel recorded bipolar activity between hemispheres. Three EEG amplifiers were set at a band pass of 1-75 Hz (−3 dB points). The fourth channel recorded the same bipolar activity at a band pass of 10–75 Hz.

Upon completion of a 10-min adaptation period, each chick was removed from the recording carton and manually restrained while a 26 gauge needle of a Hamilton microliter injection syringe was used to direct 10 μl/hemisphere of the 300 mM D-glutamine solution into each lateral ventricle. Within 5 sec after the injections a 10-min post injection recording period commenced. EEG records were analyzed by counting the very high-amplitude (200–400 μV) spikes which typically occurred in trains of 2–4/sec.

RESULTS AND DISCUSSION

In only 3 of the 11 chicks were seizure spikes observed less than 60 sec post-injection. However, by 4 min post-injection all animals displayed spiking activity. Although the primary purpose of the present study was to investigate the amnestic properties of D-glutamine, it is also of interest to

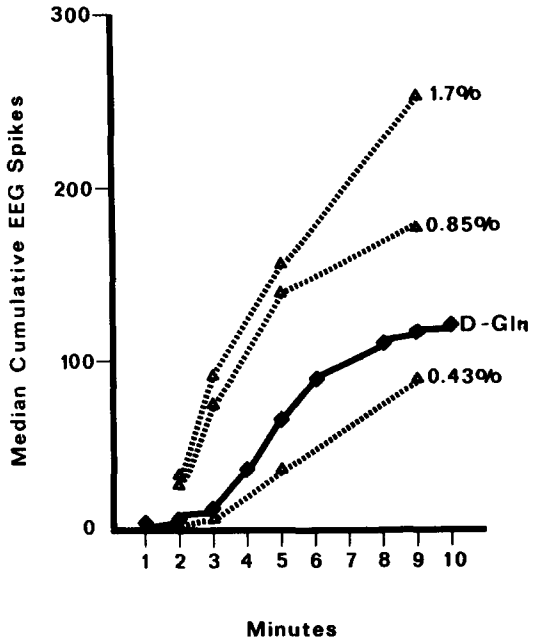

FIG. 1. Comparison of median cumulative number of striatal EEG spikes produced by 3.0 mM D-glutamine with non-amnestic (0.43% v/v) and amnestic vapor concentrations (0.85% and 1.7% v/v) of flurothyl in the chick.

relate these EEG findings to those obtained with other amnestic agents. Figure 1 compares the median cumulative number of striatal EEG spikes with non-amnestic (0.43% v/v) and amnestic doses (0.85% and 1.7% v/v) of flurothyl in the chick [5]. Although strict comparisons are not possible, note that the curve representing the median cumulative number of EEG spikes lies above the curve displaying the non-amnestic dose of flurothyl. This result reinforces the suggestion [5] that the spike frequency of EEG is a potentially useful correlate of retrograde amnesia.

GENERAL DISCUSSION

The major behavioral result of these experiments is that D-glutamine, but not L-glutamine, produces retrograde amnesia at a dose of 6.0 μmols per chick. In contrast, previous reports of amnesia induced by proline in this model have described the L- rather than the D-isomer to be amnestic [3,6]. In the chick model, the presence of severe brain seizures induced by agents such as flurothyl correlate with amnesia when concentrations are sufficient to produce a large number of seizure spikes [5]. We report here electrophysiological recordings of EEG activity showed D-glutamine to also produce ectostriatal seizures at a rate comparable to that of the amnestic dose of flurothyl. We conclude, therefore, that the amnesia produced by D-glutamine is seizure-related, but note that the production of ectostriatal spiking represents only one possible mechanism for interrupting storage or recall.

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