Pharmacology Biochemistry & Behavior, Vol. 10, pp. 643–645. Printed in the U.S.A.

Retrograde Amnesia in Chicks and Mice Induced by 3,4-Dehydro-DL-Proline, A Proline Analog

JOEL L. DAVIS

Psychobiology Research Laboratory, VA Medical Center, Sepulveda, CA 91343 and Department of Psychiatry, University of California School of Medicine, Los Angeles, CA 90024

AND

ARTHUR CHERKIN

Psychobiology Research Laboratory, VA Medical Center, Sepulveda, CA 91343 and Departments of Anesthesiology and Psychiatry, University of California School of Medicine, Los Angeles, CA 90024

(Received 4 December 1978)

DAVIS, J. L. AND A. CHERKIN. *Retrograde amnesia in chicks and mice induced by 3,4-dehydro-DL-proline, a proline analog*. PHARMAC. BIOCHEM. BEHAV. **10**:(5) 643–645, 1979.—L-proline induces retroactive amnesia without causing brain seizures or isoelectric activity. 3,4-dehydro-DL-proline, a proline analog containing a double-bond in the 5-membered ring, has similar effects at a smaller dose. Three experiments describe the amnestic qualities of 3,4-dehydro-DL-proline in a chick memory paradigm, the retrograde quality of this amnesia, and its existence in a mammalian (mouse) preparation. Finally, EEG records show that chicks injected with amnestic doses of 3,4-dehydro-DL-proline do not exhibit seizure spiking or abnormal electrical activity.

Amino acids 3,4-dehydro-DL-proline L-proline Retrograde amnesia Chicks Mice Memory

PREVIOUS studies have demonstrated the effect of proline [10] and proline-related compounds on memory. Beginning with the description of proline-induced retrograde amnesia (RA) in chicks [3] the retrograde effect has been shown to be stereospecific in both chick [1] and mouse [4]. Some proline-related compounds have been shown to be ineffective in producing RA, e.g., L-azetidine-2-carboxylic acid [2] whereas other analogs, e.g., L-baikiain (Fig. 1) produce RA at lower doses than does L-proline [5]. Neither L-proline nor L-baikiain depend for their amnestic effects on EEG seizures or depression [7,8]. These results suggest that the L-configuration and proper molecular size are essential for L-proline-produced RA. We believe that molecular geometry may also play an important role, which led us to examine the amnestic effects of 3,4 dehydro-DL-proline (DHP), an analog of both L-proline and L-baikiain.

EXPERIMENT 1

Procedure

Neonatal cockerels (N=150, Strain DeKalb XL, Pace/Setter Products, Alta Loma, Ca., 44±10 hr old) were housed individually in 1-qt disposable white cartons, 8.5 cm dia. ×18.5 cm tall. The temperature in the cartons was 32.5 to 34.5 C°, the ambient relative humidity was 40 to 46% and the masking background white noise level was 76 db re 0.0002 dynes/cm^2. Chicks were acclimated to their individual cartons for 2 hr prior to training. All training and testing occurred between 10:00 a.m. and 11:45 a.m. A transparent plastic cover with a 3-cm circular aperture was centered over each carton. Chicks remained in this environment and were not fed or watered throughout the experiment. The injection solutions were blind coded. In addition, five hours after training all chicks were individually blind coded by being placed randomly in different cartons. Thus, experimenters knew neither which solution they were injecting nor which solution the chick they were testing had received 24 hr earlier.

The training was one-trial avoidance conditioning, utilizing the chick's spontaneous tendency to peck a small, bright object. The training target was a 3-mm stainless steel bead fixed to the end of a 25 cm wire. The bead was made aversive by dipping it into liquid methyl anthranilate just prior to each training presentation. The methyl-anthranilate-coated bead was passed through the aperture of the cover and held approximately 1 cm in front of the chick's beak. A timer was started when the chick oriented towards the bead, typically within 0.5 sec. Ten sec later, the bead was withdrawn. The latency of the first peck and the total number of pecks in the 10-sec period were recorded. Chicks not responding (less

TABLE 1

AMNESTIC EFFECTS OF VARIOUS DOSES OF 3,4-DEHYDRO-DL-PROLINE, L-PROLINE, AND D-PROLINE IN CHICKS

Compound	Dose/Chick (μmols)	N	Avoidance Score (%)	Peck Score ($\sqrt{p} \pm$ SD)
DHP	1.5	57	47.3	1.29 ± 1.54
DHP	3.0	49	12.2	2.48 ± 1.35
L-PRO	3.0	39	51.3	0.93 ± 1.15
L-PRO	6.0	304	34.5	1.59 ± 1.49
D-PRO	6.0	296	56.1	0.77 ± 1.15
Uninjected	—	50	60.0	0.72 ± 1.09

than 2%) were injected to preserve the timing rhythm of the experiment but the test data were excluded from the analysis.

Chicks were injected 1 min after the start of the 10-sec training period. Each chick was removed from its carton and restrained in a headholder precalibrated to guide the 27 ga needle of a Hamilton microliter injection syringe into each forebrain hemisphere. This injection method [6] produces a high percentage of lateral ventricle distribution of drugs. Each chick received 10 μl/hemisphere of 150 mM L-Pro (N=40) or DHP (N=60), or 75 mM DHP (N=50). All solutions were buffered, if necessary, to pH 7.2±0.2 with $NaHCO_3$. Retention of the avoidance response was tested 24 hr later using the uncoated dry target; reduced avoidance scores (percentage of chicks that do not peck during the retention test) and increased peck scores indicate impaired memory retention. Peck scores represent the mean of the square root of the number of pecks in 10 sec; the square-root transformation was used to normalize distributions.

Results

The results (Table 1) demonstrate that DHP is an effective amnestic agent at a dose (3 μmols/chick) which is below the amnestic dose of L-proline (6 μmols/chick). Data for 6.0 μmols of L- and D-proline, and for chicks receiving no injection, obtained in previous experiments, are tabulated here for comparison. For DHP and L-proline at 3.0 μmols, the avoidance scores ($p<0.001$; χ^2 test) and peck scores ($p<0.0001$; t-test) differ significantly. The amnestic effect of 1.5 μmols of DHP is significantly less than that of 3.0 μmols of DHP (peck score, $p<0.0001$) and not significantly greater than that of 3.0 μmols of L-proline ($p>0.23$). D-proline injected chicks do not differ significantly from uninjected chicks (peck score, $p>0.7$; avoidance score, $p>0.7$); thus, D-proline injected chicks serve as an appropriate non-amnestic control group.

EXPERIMENT 2

Procedure

Training, injection, blind-coding and testing procedures were as described in Experiment 1, with the exception that 3.0 μmols/chick of DHP was administered at either 1, 59, or 239 min after training (N=28/group).

TABLE 2

RETROGRADE AMNESIA PRODUCED BY 3,4,-DEHYDRO-DL-PROLINE IN CHICKS (3.0μMOLS/CHICK

Training Injection Interval (min)	N	Peck Score ($\sqrt{p} \pm$ SD)
1	28	1.39 ± 1.43
59	28	0.71 ± 1.97
239	28	0.74 ± 1.18

TABLE 3

RETROACTIVE AMNESIA PRODUCED BY 3,4-DEHYDRO-DL-PROLINE IN MICE (1.5μMOLS/MOUSE)

Compound	Injection Interval (min)	N	Median Latency (sec)	U	p
DHP	1	13	20.0	26.5	<0.01
Saline	1	13	158.0		
DHP	60	9	184.0	45.0	>0.05
Saline	60	10	151.0		

Results

A t-test comparison (two-tailed) of 1 min vs 59-min and 239-min training-injection intervals (Table 2) shows injection at the 1-min interval to be significantly more amnestic than injection at the 59-min interval ($p<0.05$). The difference between the 1-min and 239-min intervals approached significance ($p<0.06$), but the difference between the 59-min and 239-min intervals was non-significant. These data suggest a retrograde effect of post-training injections of DHP. The avoidance scores at the 1-, 59-, and 239-min intervals were 39.3, 60.7 and 64.3 respectively. The difference between the 1-min score and each of the later scores did not reach significance at the 0.05 level. Previous work with this paradigm has indicated the peck score to be more sensitive than the avoidance score as a measure of retention.

EXPERIMENT 3

Procedure

Mice (Swiss HLA-sw-1CR males, 50–65 days old, 34 g body weight, Hilltop Laboratories, Chatsworth, CA) were acclimated to the laboratory for at least seven days before random assignment to one of four treatment groups when the experiments began. Bilateral cannulae were stereotaxically implanted to deliver solutions into the third ventricles. One week was allowed for recovery, and cannulae locations of all experimental animals were histologically verified at the end of the experiment. Training and testing occurred between 1 and 3 p.m., using a one-trial passive avoidance apparatus [9], comprising a small, lighted compartment connected to a larger dark compartment, both trough-shaped and covered. When the mouse enters the dark compartment, it interrupts a photobeam and receives foot shock (0.8 mA, 2 sec) through parallel plates on the floor. Twenty-four hr later, latency to enter the dark box is determined by measuring the time between placing the mouse in the light box and interruption of

the photobeam. Decreased latency of entry indicates memory disruption.

DHP or saline was injected into mice 1 or 60 min after training. Each mouse received 5 μl/hemisphere of 150 mM DHP (1.5 μmols per mouse) or of saline, then was tested 24 hr later. Data from 4 animals, in which histological verification of both cannulae could not be determined, were excluded.

Results

As shown in Table 3, DHP injected 1 min after training significantly ($p<0.01$; Mann-Whitney U-test) reduced the entry latency compared to saline, indicating an amnestic effect. A comparison of entry latencies between mice given saline or DHP 60 min after training did not show a significant difference between the two groups ($p>0.05$). That is to say, delaying the injection for 60 min abolished the amnestic effect observed with the 1 min delay suggesting the amnesia to be retrograde.

SUMMARY AND CONCLUSIONS

The major behavioral result of the chick experiments is that DHP produces retrograde amnesia similar to that produced by L-proline but at a smaller dose (i.e., 3.0 vs 6.0 μmols). DHP then appears similar to another proline analog, L-baikiain, which produces amnesia at a dose of 1.5 μmols [5]. There is a further similarity in that at amnestic doses, baikiain and DHP do not produce seizure spiking or isoelectric activity in electrophysiological recording of multiple-unit activity and raw EEG records of chicks.

DHP resembles both L-baikiain and L-proline in structure. We have not been able to acquire the L-form of DHP, which our comparisons of L-proline with D-proline indicate may be selectively efficient in producing amnesia. The presence of a double bond in the ring (either 5 or 6-membered) may be a factor in increasing the amnestic potency of these imino acids. Preliminary work indicates that pipecolic acid, which contains no double bonds (Fig. 1), is not amnestic.

Amnestic potency and anti-spreading depression potency have been correlated for L-proline and related compounds. (Van Harreveld, personal communication). Both L-proline

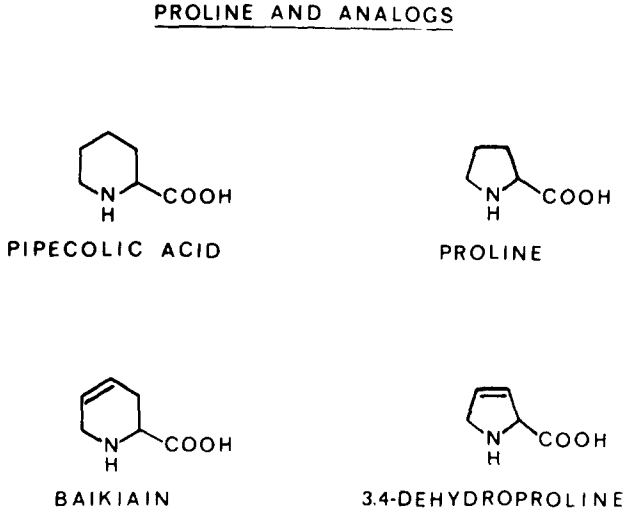

FIG. 1. Proline and its next higher (pipecolic acid) homolog. Both baikiain (4,5-dehydro-L-pipecolic acid) and 3,4 dehydro-DL-proline contain a double bond and are amnestic.

[2] and L-baikiain block glutamate-dependent spreading depression in the chick retina. The findings presented here are consistent with the Van Harreveld and Fifkova [10] model of glutamate-dependent memory formation, although other interpretations can be made. Van Harreveld and Fifkova [10] demonstrated the amnesic effect of L-proline after systemic administration. The intraperitoneal administration of 0.75 ml of 300 mM L-proline (an amnestic dose) resulted in a whole brain concentration of 0.74 mM L-proline one hr after administration. We are now involved with experiments to define further details of amnesia produced by proline analogs.

ACKNOWLEDGEMENTS

We thank Mary W. Garman for excellent technical assistance. Supported by the Medical Research Service of the Veterans Administration and by Grant NIMH 1RO1 MH30279.

We thank Professor Murray Jarvik for loan of the mouse apparatus.

REFERENCES

1. Cherkin, A. Retrograde amnesia induced in chicks by L-proline is stereospecific. *Neurosci. Abstr.* **2:** 426, 1976.
2. Cherkin, A. and A. Van Harreveld. L-proline and related compounds: Correlation of amnesic potency with antispreading depression potency. *Brain Res.* **156:** 265–273, 1978.
3. Cherkin, A., M. J. Eckardt and L. K. Gerbrandt. Memory: Proline induces retrograde amnesia in chicks. *Science* **193:** 242–244, 1976.
4. Davis J. L. and A. Cherkin. Intraventricular L-proline induces retrograde amnesia in mice. *IRCS Med Sci.* **5:** 88, 1977.
5. Davis J. L., L. K. Gerbrandt and A. Cherkin. Retroactive amnesia induced in chicks by the proline analog L-baikiain, without EEG seizures or depression. *Physiol. Behav.* **21:** 653–658, 1978.
6. Davis, J. L., D. Masuoka, L.K. Gerbrandt and A. Cherkin. Autoradiographic distribution of L-proline in chicks after intracerebral injection. *Physiol. Behav.* **22:** 693–695, 1979.
7. Gerbrandt, L. K., M. J. Eckardt, J. L. Davis and A. Cherkin. Retrograde amnesia in neonatal chicks: Induction by L-proline is not accompanied by occult seizures. *Physiol. Behav.* **19:** 723–728, 1977.
8. Gerbrandt, L. K., M. J. Eckardt, J. L. Davis and A. Cherkin. Retrograde enhancement of memory with flurothyl: Electrophysiological effects in chicks. *Physiol. Behav.* **19:** 729–734, 1977.
9. Jarvik, M. E. and R. Kopp. An improved one-trial passive avoidance learning situation. *Psychol. Rep.* **21:** 221–224, 1967.
10. Van Harreveld, A. and E. Fifkova. Involvement of glutamate in memory formation. *Brain Res.* **81:** 455–467, 1974.