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Memory Enhancement Induced in Chicks by L-Prolyl-L-Leucyl-Glycineamide[1]

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DAVIS, J. L., R. M. PICO AND A. CHERKIN. *Memory enhancement induced in chicks by L-prolyl-L-leucyl-glycineamide*. PHARMAC. BIOCHEM. BEHAV. 17(5) 893–896, 1982.—Two-day-old chicks were injected either intraventricularly or intraperitoneally with saline or a L-prolyl-L-leucyl-glycineamide solution. This C-terminal tripeptide of oxytocin produced retrograde enhancement when injected centrally but not peripherally. Possible memory mechanisms are discussed in light of this peptide's relationship to oxytocin, MSH, and dopaminergic systems.

L-prolyl-L-leucyl-glycineamide Memory Chicks MSH Oxytocin

VARIOUS studies in the last 15 years have demonstrated that pituitary peptides may influence mechanisms of learning and memory, especially in the elderly [12]. Our interest in aging and memory has led us, in the course of examining various proline oligopeptides as amnestic agents, to an observed result in chicks which suggested that the tripeptide L-prolyl-L-leucyl-glycineamide (PLG) could produce enhanced memory retention [5]. PLG is the C-terminal tripeptide of the pituitary neuropeptide oxytocin. Earlier studies in rats [1] had shown that oxytocin injected intracerebroventicularly in rats could produce a loss of memory as measured by attenuation of a passive avoidance response. In contrast, our preliminary studies have shown oxytocin to have memory enhancing properties in our paradigm.

Studies of the function of PLG are especially relevant because of the possibility that this tripeptide is released from oxytocin by a hypothalamic enzyme system [2]. Furthermore, PLG has been shown not to degrade upon incubation with human or chick plasma or serum for 1 hr at 37°. The results of experiments [10] using human plasma as a standard found 4% degradation of PLG using chick plasma, whereas it was found that rat or carp plasma produced 100% and 30% degradation, respectively. The two aforementioned studies suggest the possibility that PLG might be found undegraded in chick CNS. These present experiments are designed to establish a possible function for the oligopeptide.

EXPERIMENT 1

METHODS

The subjects were White Leghorn cockerels (strain B-300, LakeView Hatcheries, Lakeview, CA) 44±12 hr old. The chick memory procedure has been described in detail [3] and is only summarized here. Each chick was allowed to peck 10 sec at a 3 mm stainless steel bead made aversive by a coating of ethanol. The ethanol was moderately aversive causing marked peck avoidance for several hours, with return to nonavoidance within 24 hr [3].

Intracranial injections were given 1 min after the start of the training period. Each chick, restrained in a headholder [7] precalibrated to guide the 27-ga needle of a Hamilton microliter syringe into each forebrain hemisphere, received 10 μl/hemisphere of a 150 mM solution containing 3 μmols or 0.75 μmols PLG or of isotonic saline. This injection method has been demonstrated autoradiographically to place the injected solution into the chick lateral ventricle [7].

Retention of the passive avoidance behavior, i.e., peck suppression, was test 24 hr after training by presenting the peck target again but without the aversive coating, for a 10-sec trial (PII). One min thereafter each chick was presented a novel peck target (a lit microminiature lamp at the end of a plastic rod) for an additional 10-sec trial (PIII).

During 24-hr retention testing (PII), and final testing with the novel target (PIII), the number of pecks within the 10-sec

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TABLE 1

MEMORY ENHANCEMENT EFFECTS OF L-PROLYL-L-LEUCYL-GLYCINEAMIDE (PLG) IN CHICKS AT TWO DOSE LEVELS USING INTRACEREBROVENTRICULAR INJECTION

		Retention Test Peck Score						
		Training Target				Novel Target		
Compound	Dose/Chick (μmols)	N	$\bar{P}$	SD	p values	$\bar{P}$	SD	$p$values
PLG	3.0	25	2.52	3.84	<0.01*, <0.05†	9.40	6.33	NS*
PLG	0.75	25	8.16	6.10	NS*	7.24	6.72	NS*
Saline	—	25	10.04	7.94		6.36	7.00	NS*

*Significance levels obtained by comparison with Saline Control Group.
†Significance level obtained by comparison of the two experimental groups.

TABLE 2

MEMORY ENHANCEMENT EFFECTS OF L-PROLYL-L-LEUCYL-GLYCINEAMIDE (PLG) IN CHICKS USING INTRAPERITONEAL INJECTION

		Retention Test Peck Score						
		Training Target				Novel Target		
Compound	Dose/Chick (μmols)	N	$\bar{P}$	SD	p values	$\bar{P}$	SD	p values
PLG	3.0	25	9.36	7.75	NS*	9.68	7.90	<0.01*
PLG	6.0	23	9.09	6.68	NS*	6.61	6.29	NS*
PLG	9.0	23	10.39	7.24	NS*	6.22	5.38	NS*
Saline	—	25	8.52	8.21		4.92	4.54	

*Significance levels obtained by comparison with Saline Control Group.

trial served as the dependent variable. Effective enhancement treatment is indicated by lower PII peck rates (more avoidance) as compared to saline controls. Previous amnestic studies using methyl anthranilate as a strong aversant have yielded highly skewed distributions of peck scores which has necessitated the use of data transformations prior to analysis. Using ethanol in these memory enhancement studies produces a PII peck score distribution nearly always normal in skewness and kurtosis as tested by the Condescriptive subprogram of the Statistical Package for the Social Sciences (SPSS) [8]. One way ANOVA was performed on all data and comparisons of the experimental groups with the relevant control group were accomplished using the Dunnett test. Contrasts between treatment groups were examined for significance by the Tukey-Honest Significant Difference (HSD) statistic, also supported by SPSS.

RESULTS

The statistical results of Experiment 1 are displayed in Table 1. The reduced mean peck rates at PII for the groups receiving PLG, as compared to the saline control group, characterized enhanced retention and resulted in significant differences between the group means, $F(2,72)=9.99$, $p<0.0001$. Comparisons made by the Dunnett statistic revealed that the high dose (3 μmols/chick) PLG group pecked significantly less than did the saline controls, $p<0.01$. Tukey's-HSD test showed that the high dose group also pecked less at the target than did the low dose group (0.75 μmols/chick), $p<0.05$. The low dose group was not significantly different from the saline group. All groups showed similar mean peck scores for the PIII, i.e., novel target, session; thus, no significant statistical differences were found indicating that peck performance *per se* was not altered by PLG.

EXPERIMENT 2

METHOD

All methods duplicated those described in Experiment 1, except for the following: Chicks were injected intraperitoneally with either 3.0, 6.0, or 9.0 μmols/animal dissolved in either 10, 20 or 30 μliters of solution, respectively. As in the previous experiment, all injections took place 1 min after training.

RESULTS

Table 2 lists the means and standard deviations for the peck scores measured at PII and PIII after intraperitoneal injections of PLG or saline. For PII all groups displayed similar mean peck rates, with no significant differences found between treatment groups. The data collected at PIII resulted in a significant F ratio, $F(3,92)=2.66$, $p<0.05$, over all groups. The application of Dunnett's test revealed a significant comparison between the saline and the PLG group that received a 3 μmol injection, $p<0.01$. As the results show that the PLG group pecked much more than did the saline

TABLE 3

RETROACTIVE MEMORY ENHANCEMENT EFFECTS OF L-PROLYL-L-LEUCYL-GLYCINEAMIDE (PLG) IN CHICKS USING INTRACEREBROVENTRICULAR INJECTION

Injection Interval (min)	Compound	Dose/Chick (μmoles)	Retention Test Peck Score: Training Target N	$\bar{P}$	SD	p values	Novel Target $\bar{P}$	SD	p values
1	PLG	3.0	25	2.68	4.29	<0.01*, <0.05†	5.32	3.83	NS*, <0.05‡
9	PLG	3.0	25	6.84	6.13	NS*	5.48	5.01	NS*, <0.05‡
59	PLG	3.0	25	10.20	7.02	NS*	10.04	7.25	NS*
1	Saline	—	25	8.24	5.44		8.80	7.23	

*Significance levels obtained by comparison with Saline Control group.
†Significance levels obtained by comparison of the 1 min PLG group to the 59 min PLG group.
‡Significance levels obtained by comparison with the 59 min PLG group.

controls, this is again indicative of non-impairment of this behavior after PLG administration.

EXPERIMENT 3

METHOD

All methods duplicated those described in Experiment 1, except for the following: The training-to-injection interval (TTI) included 1, 9, and 59 min groups. Saline controls were injected one min after training.

RESULTS

The means and standard deviations derived from peck rates for all groups at PII and PIII are represented in Table 3. The TTI intervals are also given for each group. An ANOVA performed on the PII data revealed a significant omnibus F ratio, $F(3,96)=7.533$, $p<0.0001$. The control-experimental comparisons made using the Dunnett test showed that the PLG group receiving injections 1 min after training pecked significantly less than the saline group, $p<0.01$. The 1-min PLG group was also found to be significantly different from the 59-min PLG group (lower mean peck rate) using the Tukey-HSD procedure, $p<0.05$. The PIII data analysis revealed a significant difference among the treatment groups, $F(3,96)=3.903$, $p<0.01$. The Tukey-HSD statistic showed that the 1-min and 9-min PLG groups pecked significantly less than the 59-min PLG group, $p<0.05$. No treatment group differed significantly from the saline controls.

DISCUSSION

Our results indicate that PLG can enhance memory in our preparation, when the oligopeptide is injected intraventricularly and soon after training. Peripheral injections do not produce the enhancement effect. In additon, the retrograde quality of the memory enhancement is evidenced by the failure of PLG to have the effect when injection is delayed for 59 min after training.

In a behavioral paradigm such as ours, which seeks to measure memory enhancement by a decreased response, it is incumbent to show that those response decreases are not the result of debilitation in the animal's general condition. Although our PIII data are not absolutely clear on this point, both Experiment 1 and Experiment 3 yielded data indicative that decreased responding to the test stimulus (PII) is not accompanied by a general decrease in peck activity as measured by PIII. Experiment 2, which shows no peripheral effect of PLG, is confusing in that the responses to the novel target (PIII) give an indication of "dose dependency." We have no good explanation for this result.

Recently, researchers [4] have investigated the desensitizing effect of PLG on dopaminergic receptor function as measured by (^{3}H) spiroperidol binding rat structures, and suggested the possibility that there may be a pharmacologically distinct receptor for PLG functionally linked to the dopamine receptor. In addition, they demonstrated that PLG administered together with chlorpromazine, prevents dopamine supersensitivity from developing. These data are interesting in light of our previous finding that chlorpromazine in the same chick memory model has been shown to be memory enhancing [6], albeit with very high doses. The binding data would indicate that PLG would antagonize the enhancement effects of chlorpromazine, but as of yet we have no data.

Finally, the amino acid sequence L-prolyl-L-leucyl-glycineamide is also thought to be an MSH inhibitory factor (MSH-IF), although this finding is not universally accepted. MSH has been shown to restore normal rates of extinction of an avoidance response in posterior lobectomized rats [11] and increases visual retention in humans [9]. We are not aware of any reports of MSH-induced memory effects in birds. Such findings would be interesting in light of the PLG, i.e., MSH-IF-induced memory enhancement described in the paper.

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