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Protein Kinase Inhibitors Disrupt Memory Formation in Two Chick Brain Regions

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SERRANO, P. A., W. A. RODRIGUEZ, E. L. BENNETT AND M. R. ROSENZWEIG. *Protein kinase inhibitors disrupt memory formation in two chick brain regions.* PHARMACOL BIOCHEM BEHAV **52**(3) 547–554, 1995.—The amnesic effects of protein kinase inhibitors (H-7, HA-156, TFP, W-9, and W-13) on memory formation for a one trial peck-avoidance task in chicks were investigated with bilateral and unilateral injections into either the left or the right intermediate medial hyperstriatum ventrale (IMHV) or the left or right lobus parolfactorius (LPO). All five inhibitors injected bilaterally 5 min pretraining into either the IMHV or LPO or unilaterally into the left IMHV produced amnesia. Unilateral injections into the right IMHV did not produce amnesia. Unilateral injections of W-9 or W-13 into the left but not the right LPO produced amnesia. H-7, HA-156, or TFP did not produce amnesia when injected unilaterally into either the left or right LPO. The time of onset of amnesia produced by injecting TFP and W-13 into the LPO occurred 45 min after training, whereas H-7 produced amnesia significantly later (90 min after training). Amnesia induced by TFP, W-13, and H-7 injected into the LPO occurred significantly later than amnesia produced when these agents were injected into the IMHV. Together these data suggest that the IMHV and LPO process memory sequentially using various protein kinase activities.

Amnesia Cerebral lateralization Chick CAM kinase Protein kinase C PKA PKG
Learning Memory formation Protein kinase inhibitors

THE ROLE of protein kinases during memory formation has been investigated using various models of learning in both invertebrates and vertebrates. These involve phototaxic behavior in *Hermissenda* (18), classical conditioning of *Aplysia* (26), the nictitating membrane reflex in rabbits (1), spatial learning in rats (19,20), and long-term potentiation (LTP) in rats (7). The one trial peck-avoidance task in chicks is also a useful behavioral paradigm in which to investigate the cellular correlates of memory formation (5,21,23,25). Research investigating the biochemical correlates of learning and memory formation in chicks shows that the inhibition of protein kinase C (PKC) in the intermediate medial hyperstriatum ventrale (IMHV) produces amnesia for the task, the amnesia starting about 60 min after training (27,28). The inhibition of PKC also disrupts the phosphorylation of the GAP-43/F1 protein by PKC (4,28). The cascade of PKC related molecular events associated with LTP (8) coincides with the functions of PKC suggested by these behavioral pharmacological studies.

The chick as a behavioral model for memory is also used to study lateralized memory processing (9). Evidence of lateralized memory processing as a consequence of imprinting comes from both the increased metabolic activity in the left IMHV (14) and increased levels of phosphorylation for the myristoylated alanine-rich C-kinase substrate (MARCKS) protein in the left IMHV but not the right IMHV (31). Similarly, pretraining lesions to the left, but not the right IMHV disrupt imprinting (6). Chicks trained in the one trial peck-avoidance task also show increased neural activities in the left IMHV [metabolic activity (22), glycoprotein synthesis (2,3), protein phosphorylation of GAP-43/F1 (4)]. In addition, lesioning the left IMHV pretraining results in amnesia for peck-avoidance learning but lesioning the right IMHV does not (12).

Another brain region important for peck-avoidance learning in chicks is the lobus parolfactorius (LPO). However, this brain region, unlike the IMHV, shows no evidence of lateralized memory processing. Glycoprotein synthesis and synapse number significantly increase in both the left and the right LPO after peck-avoidance learning (3,15), and unilateral lesions of the LPO posttraining are not effective at disrupting

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memory for peck-avoidance learning while bilateral lesions do produce amnesia (12).

METHODS

Animals

XL White-Leghorn cockerels were purchased from Lamont Featherland Farms, Inc. (Coburg, OR). The housing conditions are the same as those described in more detail elsewhere (24). The 1-day-old cockerels arrived at the laboratory between 0800 and 0900 h. They were left in their shipping boxes overnight, provided with water, and placed in a dark behavioral testing room at 35° ± 2°C and 40% ± 5% relative humidity. The following morning at 0800 h the lights were turned on and the chicks were transferred to individual containers 2 hours before the experiment began.

Training and Drug Administration

The chicks were trained on a one trial peck-avoidance task and administered drugs using the protocol described in more detail elsewhere (24). Each chick was presented with a stainless steel 3-mm bead for 10 s. The bead used during training was dipped in an aversive substance, methyl anthranilate (MeA). Chicks pecking at the coated bead showed a typical disgust response characterized by head shaking, beak wiping, and distress peeping. During testing chicks were given another exposure to a similar but dry bead for 10 s. Those chicks that remembered the aversive training stimulus withheld the peck response during the test trial. Those chicks that forgot the training stimulus pecked during the test trial. The percent avoidance score for chicks was calculated by dividing the number of chicks that avoided pecking (remembered) by the total number of chicks trained in a given experimental condition.

A Plexiglas headholder was used to direct injections into a particular brain region. The headholder is designed to position the chick's head so that designated brain regions match the small holes in the top plate of the headholder (10). To control the depth of the injection, a plastic sleeve was fitted over the upper part of the shaft of the 30-ga injection needle. Visual confirmation of the injection site on the brain surface and also within the brain revealed that 90% of the injections were within 1.0 mm of the target area. The injections were delivered using a 500-μl Hamilton repeating syringe. Five min before training, each chick received a 10-μl injection either 3-mm deep into the IMHV or 4.5-mm deep into the LPO of each hemisphere. To investigate lateralized amnesic effects in the IMHV (Experiment 1) or the LPO (Experiment 2), unilateral injections of a protein kinase inhibitor (PKI) coupled with a saline injection into the contralateral brain region were delivered. Because the skull of the 2-day-old chick is unossified, intracranial injections delivered into the unanesthetized chick evoked no signs of discomfort. The injection protocol allowed 15 chicks to be injected in 5 min.

During training we recorded the latency to peck, the number of pecks, and whether a disgust response occurred. Chicks that did not peck the MeA-coated bead during the 10-s training trial or did not show a disgust response were eliminated from the experiment; typically, about 5%–10% of the chicks were eliminated for this reason. To control for the variability among batches of chicks (150–200 animals), chicks were assigned to all conditions of an experiment, and several replications were run to accumulate a minimum of 25 chicks per treatment condition. All agents were administered blind and the codes were revealed only at the conclusion of each replication. The number of chicks in each drug treatment condition that pecked or did not peck the test bead served as the outcome measure. A chi-square test and a log-linear multicontingency table analysis were used for statistical tests.

Materials

The following agents were purchased from Toronto Research Chemicals: *N*-(6-aminohexyl)-5-chloro-2-naphthalenesulfonamide hydrochloride (W-9); *N*-(4-aminobutyl)-5-chloro-2-naphthalenesulfonamide hydrochloride (W-13); 1-(5-isoquinolinesulfonyl)-2-methylpiperazine hydrochloride (H-7); and 1-(8-chloro-5 isoquinoline-sulfonyl) piperazine (HA-156). Trifluoperazine (TFP) was purchased from Sigma Chemicals (St. Louis, MO). Agents were dissolved in 0.9% isotonic saline solution and adjusted to pH 7.0 on the day of the experiment.

EXPERIMENT 1

Investigating Hemispheric Asymmetry of Effects of Injections Into the IMHV

Hemispheric asymmetry in the chick IMHV was investigated by comparing the memory disrupting effects of unilateral and bilateral injections of the following PKIs: H-7, HA-156, TFP, W-9, and W-13. We have shown that these PKIs disrupt protein kinase activities in chick brain and that amnesia produced by PKIs can be attenuated by protein kinase activators (28). Table 1 shows the concentration of each agent necessary to produce 50% inhibition of various enzymes (K_i values).

Groups of chicks were injected bilaterally or unilaterally into the IMHV 5-min before training with H-7 (6.0 Mm), HA-156 (2.6 Mm), TFP (3.0 mM), W-9 (2.6 mM), W-13 (7.5

TABLE 1

K_i VALUES IN μM FOR THE INHIBITION OF CALMODULIN, CA^{2+}/CALMODULIN KINASE (CAMK), PKA, PKC, AND PKG BY SEVERAL AGENTS

Agent (ref)	Calmodulin	CAMK	PKA	PKC	PKG
H-7 (13)	–	97.0	*3.0*	*6.0*	*5.8*
HA-156 (17)	–	–	–	*7.2*	–
TFP (33,30)	*10.0*	–	NE	38–50	NE
W-9 (16)	–	*18.0*	–	–	–
W-13 (16)	–	*25.0*	*24.0*	120.0	44.0

The K_i values shown were determined in mammalian tissue. The main effects of each agent are in italics. The number in parentheses refers to the reference number. NE indicates no effect; (–) indicates no data found.

mM), or saline. The concentration of each PKI when injected into the IMHV (bilaterally) was found to produce significantly lower avoidance scores than avoidance scores of saline-injected chicks at a test 24 h after training (28). These concentrations were chosen because they produced significant amnesia with minimal behavioral side effects and did not cause chicks to exceed the following values typical of control groups: < 10% of the chicks failed to train, and < 30% of the chicks' bead peck latencies lasted longer than 3 s.

Results

Figure 1 shows the percent avoidance scores for saline-treated chicks and for chicks injected unilaterally or bilaterally into the IMHV with each of the five PKIs. A chi-square test for independent samples was used to determine the significance of the differences between the treatment conditions. PKIs (H-7, HA-156, TFP, W-9, and W-13) injected either bilaterally or unilaterally into the left IMHV produced significantly lower avoidance scores (poorer memory) than avoidance scores of saline injected control chicks (p's < 0.01). Unilateral left IMHV injections of H-7 ($n = 53$), TFP ($n = 47$), W-9 ($n = 50$), and W-13 ($n = 31$) produced significantly lower avoidance scores compared to avoidances scores of chicks injected unilaterally into the right with these agents ($n = 41, 47, 50, 35$ respectively) ($p < 0.05$). Chicks injected bilaterally with H-7 ($n = 47$), TFP ($n = 47$), W-9 ($n = 58$), or W-13 ($n = 34$) into the IMHV showed significantly lower avoidance scores than chicks injected unilaterally into the right IMHV with these agents ($p < 0.01$). Chicks injected with HA-156 bilaterally ($n = 55$) or unilaterally into the left ($n = 54$) or the right ($n = 54$) IMHV did not differ significantly from each other in avoidance scores ($p > 0.05$), but all three groups differed significantly from the saline controls.

EXPERIMENT 2

Investigating Hemispheric Asymmetry of Effects of Injections Into the LPO

In Experiment 2 the same experimental design, PKIs, and concentrations were used as in Experiment 1 on the IMHV, except for a higher concentration of W-9 (4.0 mM). In this experiment all injections were into the LPO.

Results

Figure 2 shows the percent avoidance scores for saline-treated chicks ($n = 35$) and for each group of chicks injected

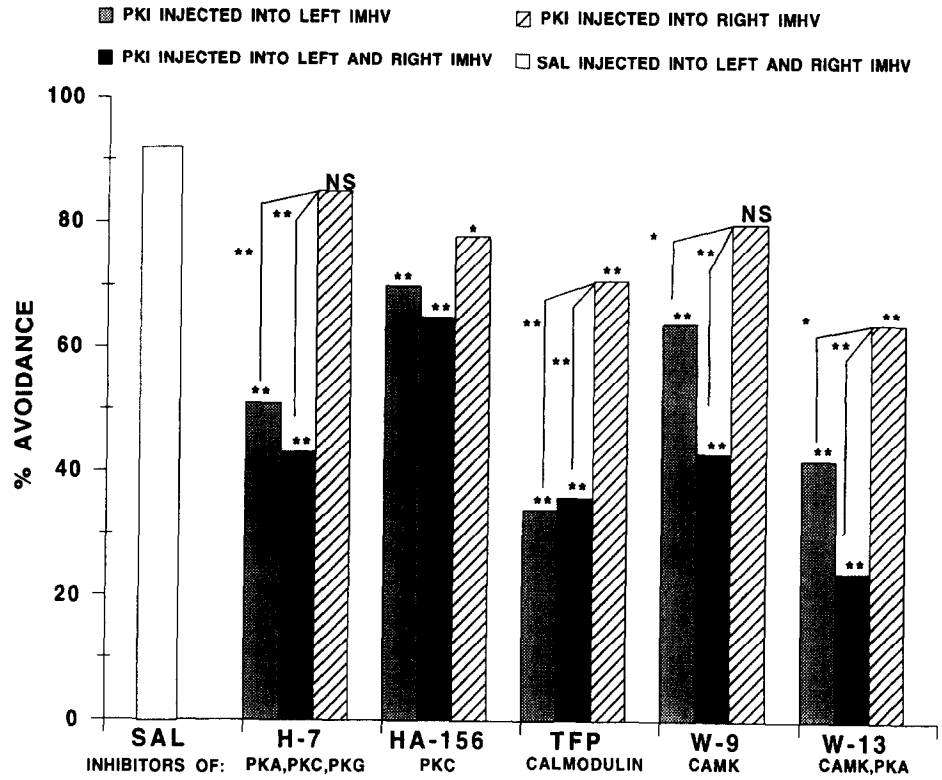

FIG. 1. Amnesic effects produced by protein kinase inhibitors (PKIs) H-7 (6.0 mM), HA-156 (2.6 mM), TFP (3.0 mM) W-9 (2.6 mM), and W-13 (7.5 mM) injected into the IMHV 5 min pretraining; tests at 24 h. For each agent, there were four groups of chicks: (a) injected bilaterally with saline; (b) injected bilaterally with a PKI; (c) injected unilaterally with a PKI into the left IMHV with a corresponding saline injection into the right IMHV; or (d) injected unilaterally with a PKI into the right IMHV with a corresponding saline injection into the left IMHV. Asterisks or NS (not significant) directly above the columns represent comparisons to saline-injected controls. Between group comparisons for each amnestic agent are indicated by lines connecting the columns of the compared groups. Between group comparisons not significantly different do not have connecting lines. There were 33–55 chicks per group. $*p < 0.05$; $**p < 0.01$.

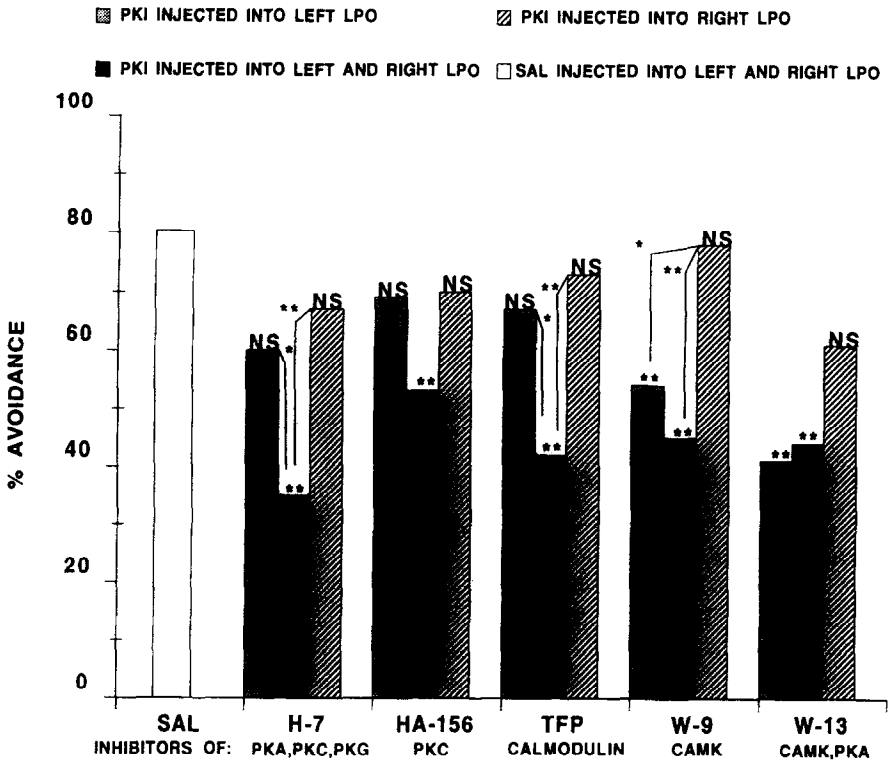

FIG. 2. Amnesic effects produced by protein kinase inhibitors (PKIs) H-7 (6.0 mM), HA-156 (2.6 mM), TFP (3.0 mM) W-9 (4.0 mM), and W-13 (7.5 mM) injected into the LPO 5 min pretraining; tests at 24 h. For each agent, there were four groups of chicks: (a) injected bilaterally with saline; (b) injected bilaterally with a PKI; (c) injected unilaterally with a PKI into the left LPO with a corresponding saline injection into the right LPO; or (d) injected unilaterally with a PKI into the right LPO with a corresponding saline injection into the left LPO. Asterisks or NS (not significant) directly above the columns represent comparisons to saline injected controls. Between-group comparisons of each amnestic agent are indicated by the lines connecting the compared groups. Between group comparisons not significantly different do not have connecting lines. There were 29–50 chicks per group. $*p < 0.05$; $**p < 0.01$.

unilaterally or bilaterally into the LPO. A chi-square test for independent samples was used to determine the significance of the differences between the four treatment conditions. W-9 (2.6 mM) injected bilaterally ($n = 29$) or unilaterally into the left ($n = 33$) or the right ($n = 33$) produced avoidance scores that were not significantly different from avoidance scores of saline injected chicks ($p > 0.05$) (data not shown). Because of this, subsequent studies used 4.0 mM W-9, which inhibited memory formation but did not cause any visible side effects. All five PKIs [H-7 ($n = 34$), HA-156 ($n = 36$), TFP ($n = 33$), W-9 ($n = 34$), and W-13 ($n = 29$)], when injected bilaterally into the LPO, produced significantly lower avoidance scores than those of chicks injected unilaterally into the right LPO [($n = 48, 50, 44, 29, 36$, respectively) $p < 0.01$].

Injecting H-7 ($n = 48$), HA-156 ($n = 36$), or TFP ($n = 36$) unilaterally into the left or the right LPO did not produce avoidance scores significantly different from those of chicks injected bilaterally with saline ($p > 0.05$). Chicks injected bilaterally into the LPO with H-7 and TFP produced significantly lower avoidance scores than those of chicks injected unilaterally into either the left or the right LPO ($p < 0.05$).

Chicks injected unilaterally into the left LPO with W-9 ($n = 29$) or W-13 ($n = 32$) showed significantly lower avoidance scores compared to avoidance scores of saline injected chicks ($p < 0.01$). W-9 (4.0 mM) injected bilaterally or unilaterally into the left showed significantly lower avoidance scores than those of chicks injected unilaterally into the right with (4.0 mM) ($p < 0.05$).

EXPERIMENT 3

The Time of Onset of Amnesia Produced by W-13, TFP, or H-7 Injected Into the LPO

We showed previously that inhibitors of CAM-kinase or calmodulin (i.e., W-13, TFP) injected into the IMHV disrupt memory formation of peck-avoidance learning with amnesia starting about 30 min posttraining, and that the PKA, PKC and/or PKG inhibitors (i.e., H-7) disrupt peck-avoidance learning, with amnesia starting 60 min posttraining (28). In the present study the onset of amnesia produced by injections of H-7, TFP, and W-13 into the LPO was investigated. Groups of chicks were injected bilaterally into the LPO 5 min before training with H-7 (6.0 mM), TFP (3.0 mM), W-13 (7.5 mM), or saline. Groups of chicks were tested for their memory of the peck-avoidance task at each of the following test times after training: 5, 10, 15, 30, 45, 60, 90, or 120 min.

Results

The results of this experiment were analyzed using a log-linear multiway contingency table analysis (33). A $3 \times 8 \times 2$

analysis of the 3 PKIs (TFP, W-13, and H-7), 8 test times (5, 10, 15, 30, 45, 60, 90, and 120), and 2 outcomes measures (forget or remember) were conducted to answer two questions: (A) For each PKI, are there significant differences among the percentages of subjects forgetting and remembering the peck-avoidance task over the eight test times? These results will indicate any differences in the time of development of amnesia and will be useful in determining the sequence of memory-related protein kinase activities in the LPO. (B) Are the time courses for the onset of amnesia produced by TFP, W-13, and H-7 when injected into the LPO different from the time courses for the onset of amnesia when these agents were injected into the IMHV? To answer question B, we compared the present results with data from a previous study with the IMHV that used identical training and testing conditions (28).

Figures 3A, 3B, and 3C show the percentages of chicks in each treatment condition that avoided pecking the test bead (remembered) at each of the eight training test intervals. An analysis of the time courses in Fig. 3 shows that H-7, TFP, and W-13 produced significantly lower avoidance scores at the longer posttraining test times, compared to memory performance of saline injected controls (G^2 [1] = 29 [TFP], 23.04 [W-13], 21.17 [H-7]; all p's < 0.001). Saline injected control groups at all time points had peck-avoidance scores of 80% or more, except for small dips in avoidance scores at the 5- and 60-min test times, which showed 69% and 79% avoidance, respectively.

Further analysis of these data shows that TFP impaired memory significantly earlier, and W-13 impaired memory marginally earlier than H-7; (TFP, G^2 [7] = 16.54, $p = 0.02$; W-13, G^2 [7] = 13.99, $p = 0.0514$). This result is consistent with our previous finding that TFP and W-13 injected into the IMHV produce amnesia significantly earlier than H-7 (26). The amnesias produced by injecting TFP and W-13 into the LPO did not differ significantly from each other (G^2 [1] = 0.49, $p = 0.48$). Both of these agents produced significantly lower avoidance scores by 45 min posttraining and at all subsequent test times compared to saline controls (χ^2's [1] > 6.64, p's < 0.01). This result is also consistent with our previous finding that TFP and W-13 when injected into the IMHV do not differ significantly in the time of amnesia onset (28).

The avoidance scores of H-7 injected chicks was not significantly different from those of saline injected controls at the 5–60-min test times. However, at the 90-min and the 120-min test times, H-7 produced significantly lower avoidance scores than those of saline-treated animals (χ^2's [1] > 6.64, p's < 0.01).

It is important to note that this experiment used the same experimental design, procedure, supplier of chicks, and experimenter as our previous investigation of the time-course of amnesia produced by PKIs injected into the IMHV (28). Also, saline injected controls from these two experiments do not differ significantly ($p > 0.05$).

Another log-linear multiway contingency table analysis was conducted of the 2 memory measures × 3 PKIs × 8 training-test intervals × 2 drug injection sites (LPO or IMHV). This overall analysis yielded only marginally significant differences (G^2 [37] = 51.87; $p = 0.053$). Focused planned contrasts restricted to the PKIs TFP and W-13 still did not show significant differences in amnesia from injections into the LPO compared to the IMHV when all eight time points were included (TFP: G^2 [7] = 3.39; $p = 0.84$; W-13: G^2 [7] = 7.66, $p = 0.36$). This result was expected because these agents appear to produce differences in the development of amnesia only during the 15–30 min training-test intervals. Restricting the analysis to the 15–30 min training-test interval yielded significant

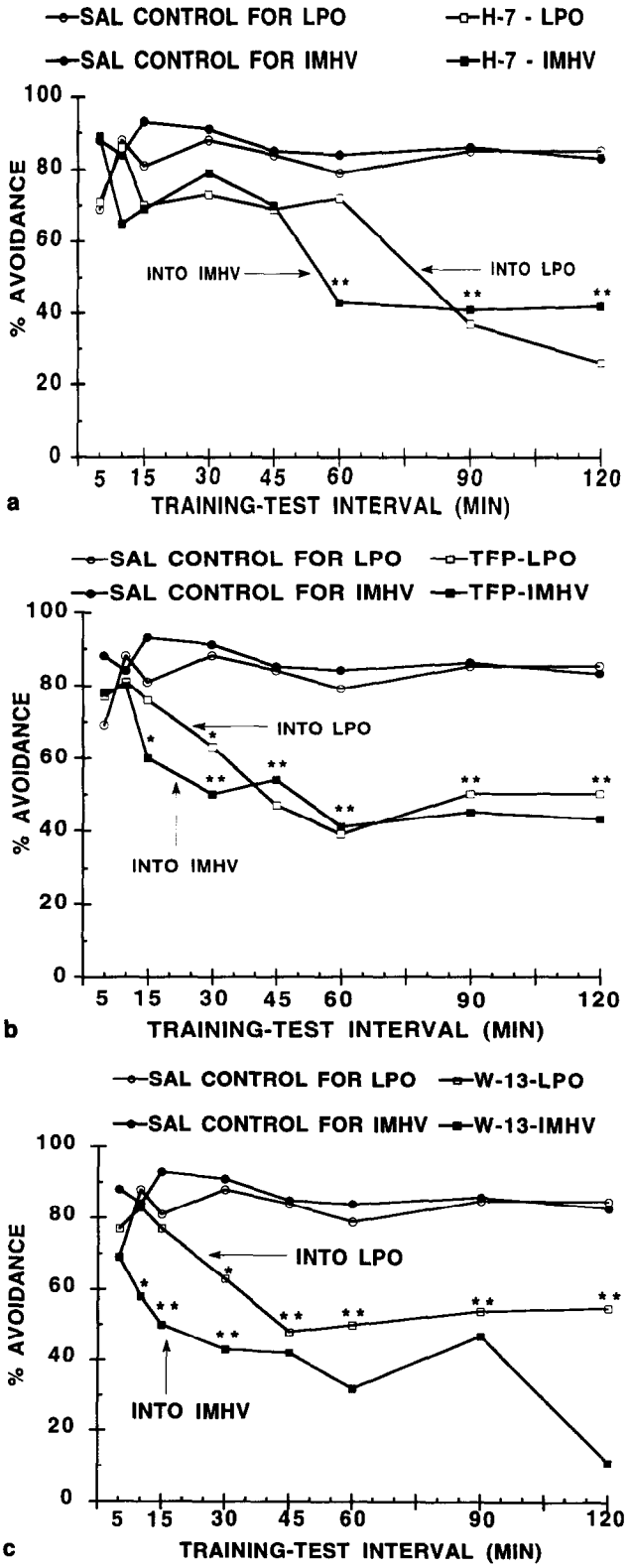

FIG. 3. Time course of memory strength (measured by percent avoidance scores) for peck-avoidance learning after an injection of (a) H-7 (6.0 mM); (b) TFP (3.0 mM); (c) W-13 (7.5 mM) or saline into the IMHV or LPO. Injections were given 5 min pretraining. Groups of chicks were tested at the designated test times. There were 25–43 chicks per group. Data points below the asterisks are significant (*$p < 0.05$; **$p < 0.01$).

differences for injecting TFP or W-13 into the IMHV compared to injecting either of these agents into the LPO (TFP: G^2 [1] = 3.91, p = 0.047; W-13: G^2 [1] = 9.24, p = 0.002). Injections of H-7 produced memory disruption at an earlier time posttraining when injected into the IMHV than when injected into the LPO and this difference was significant (G^2 [7] = 14.97; p = 0.036). At 45 min posttraining, H-7 has not yet produced amnesia in either brain region, whereas TFP and W-13 has already produced amnesia in both regions. These results show that in both the IMHV and the LPO, TFP and W-13 disrupt memory formation at a significantly earlier time posttraining than H-7. Furthermore, the development of amnesia produced by injections of each of the three agents into the LPO occurs significantly later than the development of amnesia after injections into the IMHV.

DISCUSSION

The results of Experiments 1 and 2 show that all five PKIs (H-7, HA-156, TFP, W-9, and W-13) disrupt memory formation for the peck-avoidance task when injected bilaterally into either the IMHV or the LPO. The results of Experiment 1 replicate the memory impairment effects of these agents injected into the IMHV (28), and the results of Experiment 2 extend these findings to the LPO. In Experiment 1, the memory disruption effects of H-7, HA-156, TFP, W-9, and W-13 all indicate that memory processing is lateralized to the left IMHV. This result suggests that many protein kinase activities are involved in memory formation, and that these agents do not diffuse between hemispheres in effective amnestic concentrations. If the agents had diffused between hemispheres in effective amnestic concentrations, amnesia would have occurred after injection into either hemispheric structure, but this was not the case. Furthermore, hemispheric asymmetry suggests that these PKIs are not simply affecting nonmemorial aspects of the response, but that these PKIs disrupt memory formation in discrete brain regions. We did find some puzzling amnesic effects with HA-156. Although this agent, when injected bilaterally, produced significantly lower avoidance scores than found in saline-treated subjects, this amnesic effect was not large. Thus, we failed to observe differences between unilateral and bilateral injections of HA-156.

It might be asked whether the PKIs may cause amnesia by inducing cell death. Evidence against this hypothesis is that there is a narrow time window for amnestic effectiveness of PKIs: injected at 5 min pretraining they are amnestic, but injected 15 or 10 min pretraining or 10 min posttraining they are not. If the PKIs caused amnesia by inducing cell death, then injections at 10 or 15 min pretraining should be as effective in preventing formation of long-term memory as injections at 5 min, but they are not. We have published similar results for a selective inhibitor of PKC—chelerythrine (30). In the present series of experiments, we have found that H-7 and W-13 cause amnesia when injected either 5 min pre- or posttraining, but are not amnestic when injected 10 min pre- or posttraining.

In Experiment 2, we showed that H-7, HA-156, TFP, and W-13 all produced amnesia when injected bilaterally into the LPO at the same concentration used to produce amnesia in the IMHV. However, W-9 (2.0 mM) produced amnesia when injected into the IMHV but not when injected into the LPO at this concentration. This result is surprising given the consistency of amnestic concentrations and effects produced by H-7, HA-156, TFP, and W-13 when injected bilaterally into either the IMHV or LPO. We did find that a higher dose (4.0 mM) of W-9 injected bilaterally into the LPO produces amnesia for peck-avoidance learning.

H-7, HA-156, or TFP injected unilaterally into the LPO did not disrupt memory formation. This result suggests that both the left and the right LPO are involved in memory formation, and so unilateral injections result in less forgetting than bilateral injections of these agents. These findings are consistent with other reports indicating concurrent memory processing in the left and the right LPO (3,12,29).

W-9 or W-13 injected unilaterally into the left LPO, but not into the right LPO, produced amnesia. This result suggests that the inhibition of CAM-kinase by W-9 or the inhibition of CAM-kinase and PKA by W-13 disrupts memory-related protein kinase activity occurring in the left, but not the right LPO. It is possible that PKA activity is also a lateralized memory process occurring in the left LPO because it too is inhibited by W-13. However, the inhibition of PKA by H-7 did not result in lateralized amnesic effects, and so supports our assumption that PKA is not a lateralized memory process in the LPO. Therefore, we propose that lateralized memory processing in the left LPO involves CAM-kinase activity. We believe this is the first report to suggest that lateralized memory processing in the LPO involves CAM-kinase activity.

In Experiment 3, we found that TFP and W-13 when injected 5-min pretraining into the LPO produced amnesia 45-min posttraining, while H-7 injected into this brain region produced amnesia 90 min after training.

It is possible that these time courses reflect distinct stages of memory formation, a phenomenon that was first reported in the chick by Gibbs and Ng (11). Since then, similar time courses for the onset of amnesia induced in the chick by either IC injections [for reviews see (23,25)] or systemic injections (32) of a variety of agents have been reported.

Although the administration of the PKIs was given pretraining, we believe the amnesic effects represent the disruption of memory formation and not of acquisition. This is shown by the fact that the memory for peck-avoidance learning was at saline control levels for considerable periods posttraining, at least 15 min for TFP and W-13, and at least 45 min for H-7.

It is possible that the pharmacokinetic properties of these agents may contribute to the delay in onset of amnesia, however, given that these agents appear to induce their neurochemical inhibition within minutes (in-vitro) (13,34) we believe that the short time to induce PK inhibition in vivo is similar.

These results suggest that the LPO, like the IMHV, is involved in the sequential processing of memories, and that CAM-kinase and calmodulin activities occur prior to memory-related PKA, PKC, and/or PKG activities. When these PKIs (TFP, W-13, and H-7) were injected into the LPO they produced amnesia at significantly later times posttraining than when these agents were injected into the IMHV. Both TFP and W-13 when injected into the LPO produced significant forgetting 45 min after training, and when they were injected into the IMHV produced significant forgetting 30 min after training (28). Similarly, H-7 injected into the LPO produced significant forgetting 90 min posttraining, and when it was injected into the IMHV produced significant forgetting 60 min after training (28). Together these data suggest that the LPO is a brain region in which memory-related protein kinase activity occurs at a later time than in the IMHV. Thus our reports using pharmacological interventions are consistent with reports using lesion techniques to investigate the relationship between memory processing in the IMHV and LPO (12).

It is unlikely that the later onset of amnesia produced by

PKIs injected into the LPO is a result of the agent diffusing into other brain regions, such as the IMHV. The lateralized amnesic effects shown in the IMHV indicate that these PKIs do not diffuse 1 mm from the injection site and into the contralateral IMHV. PKIs injected into the LPO would have to diffuse 2.5 mm from the LPO to reach the IMHV. If these agents were diffusing from the LPO to the IMHV, one would predict that a higher concentration of the inhibitor would be required in the LPO for the agent to reach the IMHV in an amnestic concentration. We did not find a higher concentration to be required in the LPO than in the IMHV to produce amnesia for four of the five inhibitors tested.

The data on hemispheric asymmetry involving PKIs together with the data on the time course for the onset of amnesia in the IMHV and LPO produced by PKIs are useful for elucidating a neurochemical sequence of protein kinase activities during memory formation in the chick. Our data suggests that the neurochemical cascade for peck-avoidance learning involves CAM-kinase about 30 min after peck-avoidance training in the left IMHV and about 45 min after peck-avoidance learning in the left LPO. About 60 min after training, the left IMHV begins memory-related PKA, PKC, and/or PKG activities. About 90 min after training both the left and the right LPO are involved in PKA, PKC, and/or PKG activities. Disrupting any of these protein kinase activities prevents the further formation of memory. Thus, our data can be modeled as a cascading series of protein kinase activities that result in the development of a memory trace for the learned experience.

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