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Brain Cyclic AMP and Memory in Mice[1]

CLARK T. RANDT, MARTIN E. JUDGE,[2] KENNETH A. BONNET[3] AND DAVID QUARTERMAIN

Division of Behavioral Neurology, Department of Neurology, New York University Medical Center New York, NY 10016

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RANDT, C. T., M. E. JUDGE, K. A. BONNET AND D. QUARTERMAIN. *Brain cyclic AMP and memory in mice.* PHARMAC. BIOCHEM. BEHAV. 17(4) 677–680, 1982.—A phosphodiesterase inhibitor 4-(3-cyclopentyloxy-4-methoxyphenyl)-2-pyrrolidone (Rolipram, 10 mg/kg IP) administered immediately, but not 3 hr post-training, reversed an amnesia for an inhibitory avoidance response induced by the protein synthesis inhibitor anisomycin. Immediate post-training administration of Rolipram also enhanced retention for a weakly learned avoidance response. Unshocked animals did not show increased test latencies thus ruling out conditioned aversion as an explanation for the enhanced avoidance. Mice treated with Rolipram (10 mg/kg after training showed elevated cyclic AMP but not cyclic GMP in frontal cortex, thalamus, and hypothalamus. These results support the suggestion that cyclic AMP may play a role in memory processes.

Memory Brain cyclic AMP Passive avoidance Mice

MEMORY enhancement in rodents incident to administration of catecholamines [11] and neuropeptides [4] has been demonstrated in a variety of behavioral paradigms. Both groups of compounds have been shown to utilize cyclic AMP as a second messenger in the brain, activating protein kinases for phosphorylation of proteins which may be involved in memory processes [7]. Cholinergic compounds have been shown to be involved with cyclic GMP but may act through noradrenergic systems in vivo to elevate cyclic AMP as well [9,10]. It has been hypothesized that brain cyclic AMP may act as the final common pathway of several classes of pharmacologic agents which facilitate memory in animals.

Post-training parenteral administration of the phosphodiesterase inhibitors papaverine, RO 20-1724, and isobutylmethylxanthine have been reported to facilitate retention in mice for: maze learning [3,12]; passive avoidance [3, 15, 16]; and latent learning [3]. Intracerebroventricular injection of dibutyryl cyclic AMP immediately following one trial passive avoidance training has also been reported to enhance memory [3]. These results suggest that memory formation may be mediated by elevation of brain cyclic AMP, although measurement of brain cyclic AMP has not been correlated previously with the memory facilitation findings.

The present study was designed to determine whether post-training administration of the phosphodiesterase inhibitor 4-(3-cyclopentyloxy-4-methoxyphenyl)-2-pyrrolidone (Rolipram) could reverse an amnesia induced by a protein synthesis inhibitor (anisomysin) and facilitate a weakly learned avoidance response. In addition, the effect of Rolipram on regional cyclic AMP levels in brain was determined.

EXPERIMENT 1

Memory was measured using an approach-avoidance task. Thirsty mice were adapted to drink from a water tube in one session and, in the next, they were given brief electric shocks for drinking in a training session. Memory was evaluated by the latency to complete a specified amount of drinking in a subsequent test session. The aim of this experiment was to determine whether post-training administration of Rolipram would reverse an amnesia induced by pretraining treatment with a protein synthesis inhibitor, anisomycin (Ani).

METHOD

Subjects

Naive male Swiss-Webster mice (West Jersey Biological Supply) approximately eleven weeks of age weighing from 30 to 38 grams were housed in groups of 15 with free access to food and water for one week before beginning the experiment.

Mice were adapted, trained, and tested in a small black lucite chamber (10×10×5 cm) with an aluminum plate floor. In the center of one wall, 2 cm above the floor, a stainless steel water tube was accessible through a hole 1.3 cm in diameter. The floor and water tube served as electrodes which could be automatically connected to either a contact sensor or a constant current shock source (Grason Stadler). Closure of a clear lucite lid activated solid state control and data collection circuitry (BRS/LVE) establishing zero time for a response latency timer. The test chamber was housed in a ventilated, dimly lit, sound attenuating enclosure to reduce extraneous stimulation.

[1]Supported in part by Schering AG, Berlin. Correspondence should be sent to C. T. Randt, 550 First Avenue, New York, NY 10016.
[2]Salary from The Rubin Foundation.
[3]Department of Psychiatry, N.Y.U. Medical Center.

Procedure

Approach-avoidance training was conducted in three sessions: adaptation; training 24 hours later; and testing 48 hours after the training session. Mice were deprived of water in their group cages for one day before transfer to small cages (30×15×15 cm), in pairs, two hours before the first session. During the adaptation period, an approach response was established by placing an animal in the chamber permitting drinking until 5 seconds of contact with the tube was completed. Following this session, mice were given access to water in their home cages for 1.5 hours after which water was again withdrawn. Twenty-four hours after adaptation, mice were replaced in the apparatus and allowed 5 seconds contact with the water tube. Once this contact was completed, control circuitry responded to subsequent contacts by switching the tube to a source of 2.0 mA shock. This produced an immediate withdrawal response following which the apparatus was reset to detect and shock contacts. Shock initiation started a timer and, when the mouse suppressed drinking for 60 seconds, avoidance training was completed. A maximum of five shocked responses were permitted. If only one shock was received, mice were given up to three minutes to make a second contact. Animals were eliminated from the study if they failed to drink within 300 seconds, resulting in discard of approximately 2% of the subjects. After training, mice were replaced in pairs in their original cages, given water ad lib for 24 hours and then water deprived for 24 hours. In the test session, 48 hours after training, the latency to make five seconds contact with the water tube was measured. A latency of 1000 seconds was assigned as a subject's score if the required responses were not completed within that period of time. No shock was administered. Long latency indicated good memory for the avoidance conditioning established in the training session.

Drugs

All injections were administered parenterally in volume of 0.01 ml/g of body weight. Thirty minutes before training, groups of mice were injected subcutaneously with a protein synthesis inhibitor, anisomycin, 150 mg/kg, to produce amnesia for training. Control groups received saline injections. Immediately post-training, or, three hours after training, groups of mice were injected intraperitoneally with the phosphodiesterase inhibitor, Rolipram, in doses of 5 mg/kg or 10 mg/kg suspended in Cremophor EL® 10% w/v in saline or with the solvent-saline mix alone.

RESULTS

Mean test latencies for the Rolipram and solvent saline control animals are summarized in Fig. 1. A two-way analysis of variance (ANOVA) comparing the effects of Rolipram 10 mg/kg, 5 mg/kg, and saline-solvent, on shocked and unshocked groups, shows significantly longer latencies for both drug, $F(2,52)=11.25$, $p<0.001$, and shock, $F(1,52)=30.85$, $p<0.001$, treatments as well as a significant interaction effect, $F(2,52)=7.73$, $p<0.001$. These findings indicate that Rolipram 10 mg/kg given immediately after training significantly enhanced latencies of Ani-treated shocked mice at a test 48 hour post-training as compared to solvent-saline treated mice, $t=4.49$, $p<0.0003$. No significant effect on the latencies of mice not shocked on the training day was apparent. This demonstrates that Rolipram 10 mg/kg enhanced subsequent avoidance behavior compared

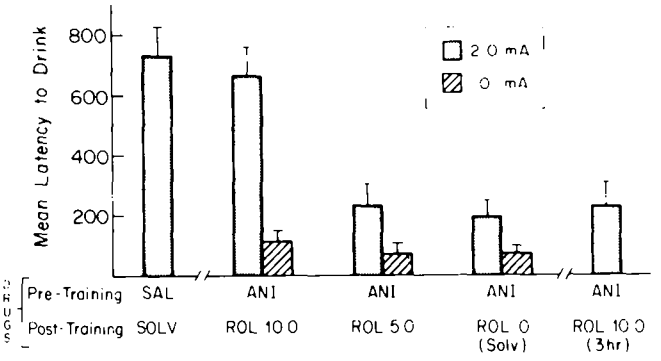

FIG. 1. Mean test latencies (±SEM) of mice injected with saline (SAL) or anisomycin (ANI) before training, trained with 2.0 mA shock or no shock, and injected immediately after training with saline-solvent (SOLV) or Rolipram (ROL) at 10.0 mg/kg, 5.0 mg/kg or 0 mg/kg (SOLV). One group was injected with ROL 10.0 mg/kg 3 hours after training.

to that seen with Rolipram 5 mg/kg or solvent and that facilitation of memory with the former dose was not due to conditioned aversion on other non-shock associated effects of the drug. Rolipram 10 mg/kg given 3 hours after training did not significantly increase the latencies to drink as compared to solvent-saline injected controls.

The demonstration that Rolipram is effective when given immediately but not 3 hours after training is consistent with many other observations which indicate the time dependent effects of post-training treatments. In addition, the time dependent action of Rolipram rules out pro-active drug effects at the time of testing as an explanation for the observed memory enhancement.

EXPERIMENT 2

The objective of this experiment was to determine possible memory facilitation with Rolipram for a weakly trained avoidance response. This was tested with reduced intensity training shock without anisomycin. Regional brain levels of cyclic AMP were determined just after administration of training shock in another group of animals.

METHOD

Mice were adapted and trained as in Experiment 1 except that the intensity of shock was reduced from 2.0 mA to 0.8 mA. Rolipram 10 mg/kg or solvent-saline was given IP immediately after training or three hours later.

A group of 30 mice were killed at 30, 60 and 120 minute intervals after training by immersion in liquid nitrogen for 30 seconds, half having been given Rolipram 10 mg/kg and the remainder solvent-saline immediately after training.

Neurochemical Determinations

Brains were removed for cyclic AMP and cyclic GMP estimations, in frontal cortex, hippocampus, thalamus, hypothalamus and cerebellum. Specific regions were freeze punched from each brain using anatomical landmarks [1]. Frozen samples were individually homogenized in 6% trichloroacetic acid and the supernatant extensively washed with diethyl ether. Aliquots of each sample were evenly di-

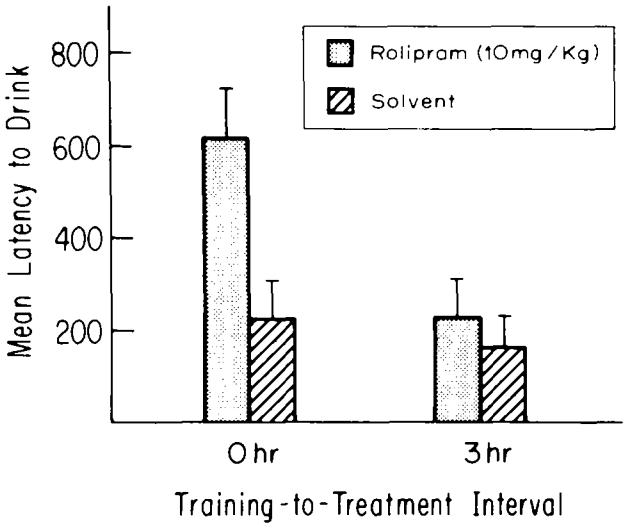

FIG. 2. Mean test latencies (±SEM) of mice trained with weak shock (0.8 mA) and injected with saline-solvent or Rolipram 10.0 mg/kg either immediately or 3 hours after training.

vided for radioimmunoassay for cyclic AMP and for cyclic GMP by the method of Gilman [6].

RESULTS

The latencies to lick for 5 seconds in 24 hour water deprived mice are summarized in Fig. 2. A 2×2 ANOVA on this data indicated a significant interaction between drug and training-to-treatment interval, $F(1,30)=5.53$, $p<0.03$. Rolipram 10 mg/kg significantly increased the latencies to drink when administered immediately after 0.8 mA shock as compared to solvent-saline injections, $t=2.950$, $p<0.008$, but failed to affect performance when administration of the drug was delayed for three hours.

Regional cyclic AMP determinations are shown in Table 1. Elevations in brain cyclic AMP after Rolipram, 10 mg/kg as compared to saline-solvent injections immediately post-training were found in all areas measured (hippocampus not available for assay), but were only significant in frontal cortex, $t=4.50$, $p<0.001$, at 30 minutes, 60 minutes, $t=5.85$, $p<0.001$, and 120 minutes, $t=2.21$, $p<0.04$. Determinations of cyclic AMP in the brain regions under study showed a trend toward returning to control levels at 120 minutes. Cyclic GMP determinations from the same brain regions revealed no consistent alterations following administration of Rolipram.

TABLE 1

REGIONAL BRAIN CYCLIC AMP LEVELS*

Time (min after injection)	Rolipram 10 mg/kg	Saline Solvent	Percent Increase
	Frontal Cortex (means ± SEM)		
30	11.08 ± 0.56	6.41 ± 0.88	73
60	14.19 ± 0.48	8.83 ± 0.78	60
120	10.78 ± 0.52	9.33 ± 0.40	16
	Hypothalamus (means ± SEM)		
30	7.58 ± 0.64	5.15 ± 0.77	47
60	7.59 ± 0.38	5.48 ± 0.24	38
120	6.23 ± 0.43	5.36 ± 0.15	16
	Thalamus (means ± SEM)		
30	6.90 ± 0.57	5.02 ± 0.45	37
60	10.02 ± 0.72	7.26 ± 0.62	38
120	8.13 ± 0.52	6.92 ± 0.66	17
	Cerebellum (means ± SEM)		
30	8.83 ± 1.09	6.96 ± 0.56	27
60	8.10 ± 0.71	7.28 ± 0.88	11
120	5.60 ± 0.71	6.03 ± 0.34	−7

*(Picomoles/mg of tissue) measured 30, 60, or 120 minutes after mice were trained with 0.8 mA shock and then immediately injected with saline-solvent or Rolipram 10 mg/kg.

GENERAL DISCUSSION

The phosphodiesterase inhibitor, Rolipram, given immediately after training reverses an experimentally produced amnesia and facilitates memory for a weak habit in an inhibitory avoidance test in mice. These results are similar to those previously reported for other phosphodiesterase inhibitors.

The relative elevations of brain cyclic AMP compared to cyclic GMP confirms the selectivity of Rolipram for cyclic AMP.

The regional levels of cyclic AMP following administration of Rolipram 10 mg/kg after training in the approach-avoidance paradigm used in this series of experiments showed increases in frontal cortex, and diencephalic structures and, to a lesser extent, in cerebellum. Similar results with Rolipram have been obtained in rats in vivo [13]. The lesser effect on cerebellum in addition to the Rolipram specificity for cyclic AMP is consistent with the findings of Kant *et al.* [8] with respect to RO 20-1724. It may be that phosphodiesterase inhibitors such as papaverine and the alkylxanthines (isobutylmethylxanthine, caffeine, theophylline) would be less effective in producing memory enhancement since they are not as selective for cyclic AMP as the data indicates for Rolipram.

Rolipram at low concentrations has been reported to be 100 times more effective than RO 20-1724 with respect to inhibition of calcium dependent cyclic AMP phosphodiesterase. In rat brain slices, Rolipram is 100 times more potent than RO 20-1724 in enhancing accumulations of cyclic AMP elicited by norepinephrine [14]. Studies of behavioral parameters associated with increases in brain cyclic AMP have shown Rolipram to be 5 to 20 times more potent than RO 20-1724 and about 30 times more potent than isobutylmethylxanthine [17].

The evidence available to date suggests that the memory facilitation documented in animals with phosphodiesterase inhibitors is not a result of increased release of norepinephrine immediately following training and administration of phosphodiesterase inhibitors [3,16] but, rather, is the result

of increased brain cyclic AMP acting as a second messenger for a variety of monaminergic neurotransmitters [10] and peptides [4,18].

Clinical trials using the phosphodiesterase inhibitor papaverine for possible memory enhancement have suggested favorable results [19] on test scores in long-term treatment of the milder but not the more severe forms of dementia. Lack of memory enhancement with papaverine has been reported also [2]. Further trials in humans with more effective or more highly selective phosphodiesterase inhibitors seem to be in order on the basis of results with animal models.

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