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CNQX Infused Into Entorhinal Cortex Blocks Memory Expression, and AMPA Reverses the Effect

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QUILLFELDT, J. A., P. K. SCHMITZ, R. WALZ, M. BIANCHIN, M. S. ZANATTA, J. H. MEDINA AND I. IZQUIERDO. *CNQX Infused into entorhinal cortex blocks memory expression, and AMPA reverses the effect.* PHARMACOL BIOCHEM BEHAV **48**(2) 437–440, 1994.—Rats were trained in a step-down inhibitory avoidance task using a 0.8-mA foot shock and tested for retention 26 days later. Three to five days prior to the retention test they were bilaterally implanted with cannulae aimed at the entorhinal cortex. Ten minutes before testing they received an infusion, into the entorhinal cortex, of vehicle, ciano-nitro-quinoxaline-dione (CNQX; 0.5 μg), amino-hydroxy-methyl-isoxalone-propionate (AMPA; 1.0 or 2.5 μg), or AMPA (1.0 μg) plus CNQX (0.5 μg). CNQX blocked memory expression; the effect lasted less than 90 min. AMPA had no effect of its own, but at the lower dose level it counteracted the depressant influence of CNQX. It is not likely that the effect of CNQX could have been due to an influence on performance: In separate sets of experiments the bilateral intraentorhinal infusion of CNQX (0.5 μg) 10 min before training did not affect either acquisition or retention of the avoidance task or general activity during 3 min of free exploration in the training box. The results indicate that the integrity of AMPA receptors in the entorhinal cortex is necessary for memory expression.

Memory expression Glutamatergic AMPA/kainate receptors Memory, AMPA/kainate receptors in Memory, effects of CNQX on

INFUSION of ciano-nitro-quinoxaline-dione (CNQX) prior to a retention test into the hippocampus (1,5), amygdala (9,11), hippocampus and amygdala (1,5), or entorhinal cortex (7) blocks the expression of various types of memory in the rat, measured by retention test performance. CNQX is an antagonist of glutamatergic receptors of the amino-hydroxy-methyl-isoxalone-propionate (AMPA)/kainate family and blocks all glutamatergic transmission that uses these receptors, including the expression of long-term potentiation (LTP) (16). The induction of LTP occurs through *N*-methyl-D-aspartate (NMDA) glutamatergic receptors and can be blocked by the NMDA receptor antagonist D-amino-phosphono valerate (AP5) (2,16). Memories of the tasks whose expressions are blocked by CNQX can be blocked during or immediately after acquisition by AP5 infused into the amygdala (6,10,15) and/or the hippocampus (4,8) or 90–180 min after training into the entorhinal cortex (3). This has suggested that these memories involve LTP generated in synapses of these structures by the stimuli pertinent to training; reiteration of these stimuli at the time of testing would trigger expressions of this LTP and, in consequence, of the memories [(1,5,7,8); see (2,16)]. It has long been known that sensory stimuli evoke specific response patterns and converge upon neurons of the amygdala (12), hippocampus (4), and entorhinal cortex, which is interconnected with the other two structures and, through the perirhinal region, with sensory cortical areas [see (18)].

The present article investigates the effect of pretest CNQX administration into the entorhinal cortex on expression of inhibitory avoidance and the reversal of this effect by the concomitant administration of AMPA. In our previous study on the effect on retention test performance of CNQX given into the entorhinal cortex we used a training-test interval of only 1 day (7). The effect of pretest CNQX infused into the hippocampus or the hippocampus and the amygdala can be seen 20 days after training (1). In the present study, the training-test interval was 26 days. Furthermore, to investigate the possibility that the effect of CNQX on test session performance of the avoidance task could have been due to an influence on

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performance rather than on memory expression, we examined the effect of CNQX given into the entorhinal cortex on various measures of general activity in the training box.

MATERIAL AND METHODS

Subjects

Sixty-five young adult Wistar rats (three months of age, 240–300 g) were used, 45 in experiment 1 and 20 in experiments 2 and 3.

Behavioral, Surgery, and Microinfusion Procedures

In experiment 1 the animals were trained in a step-down inhibitory avoidance task using a 50 × 25 × 25-cm acrylic box with a frontal glass panel whose floor was a grid of parallel 0.1-cm-caliber stainless steel bars spaced 1.0 cm apart; on the left extreme this grid was covered by a 2.5-cm-high, 8 × 25-cm platform. The animals were placed on the platform and their latency to step down placing their four paws on the grid was measured, upon which they received a 1.0-s 0.8-mA scrambled foot shock.

Twenty-one or 22 days after training the animals were implanted under thionembutal anesthesia (30 mg · kg-1, IP) with 27-g guide cannulae aimed 2.0 mm above the entorhinal cortex, at coordinates A 7.0, L 5.0 mm, DV 2.0 mm of the atlas of Paxinos and Watson (17). Stereotaxic coordinates and surgical procedures were as in previous articles (3,7,8).

At 26 days from training, the animals were submitted to a retention test for the avoidance task. Retention tests were procedurally identical to the training sessions, except that the foot shock was omitted. Retention of this task is expressed by an increase of the step-down latency in the test session (1,3,5–8,14).

Ten minutes prior to the test sessions 30-g injection cannulae were fitted into the guide cannulae. The tips of the former protruded 1.0 mm beyond those of the latter and were therefore aimed at the entorhinal cortex. Animals received through the injection cannulae a bilateral 0.5-μl infusion of vehicle (20% dimethylsulfoxide in saline), or of CNQX (0.5 μg) dissolved in the vehicle, or AMPA (1 or 2.5 μg) or of CNQX plus AMPA (0.5 μg) into the entorhinal cortex. The dose of CNQX was chosen from previous studies (1,5,7,8). The doses of AMPA were chosen assuming that this drug had an affinity for the receptor that bound CNQX similar to that of CNQX. The animals in the vehicle and CNQX-alone groups were tested twice; the second test was 90 min after the first test session (and 100 min after the infusions). By this time it was presumed that the effect of the CNQX had faded away, as happens with various drugs infused into brain tissue (13) [see (1,5)].

Experiment 2 studied the effect of a bilateral infusion into the entorhinal cortex of CNQX (0.5 μg) or of the vehicle on general activity in the training box measured for 3 min starting 10 min after the infusions. Cannulae were implanted three to six days prior to behavioral testing. In this experiment the platform was 12.5 cm wide. The animals were placed on it and step-down latency was measured as in experiment 1. Following this, the animals were left to explore the box freely for the remainder of the 3 min. The number of rearings and the number of crossings of two imaginary lines at 12.5 cm and 25.0 cm from the border of the platform were counted. Stepping back onto or down again from the platform was counted as a crossing.

Experiment 3 studied the effect of a bilateral intraentorhinal infusion of CNQX (0.5 μg) or vehicle given 10 min prior to training on acquisition and on retention test performance of the inhibitory avoidance task. The animals were trained and tested as in experiment 1 and the width of the platform was 8 cm, as in that experiment, but here the foot shock level was 0.5 mA and the training-test interval was 24 h. The foot shock level was lower in this experiment than in experiment 1 so as to make acquisition weaker [see (14)] and thus more susceptible to an eventual deleterious effect of CNQX. The animals used in this experiment were the same that had been used on the preceding day in experiment 2.

Statistics and Cannula Placement Control

Behavioral data of experiments 1 and 3 were submitted to two-way analyses of variance (ANOVAs) followed by Newman–Keuls tests. The data of experiment 2 were analyzed by individual *t* tests.

One day after the end of the test session of the avoidance task injection cannulae were again placed and 1.0 μl of 4%

TABLE 1

EFFECT OF CNQX (0.5 μg) INFUSED INTO THE ENTORHINAL CORTEX 10 MIN PRIOR TO TESTING ON RETENTION PERFORMANCE OF STEP-DOWN INHIBITORY AVOIDANCE IN RATS, 26 DAYS AFTER TRAINING; REVERSAL BY AMPA (1.0 μg); AND LACK OF EFFECT OF AMPA (1.0 and 2.5 μg) ON ITS OWN

		Mean ± SE Step-Down Latency (s)		
Pretest Treatments	*N*	Training Session	First Test Session	Second Test Session
Vehicle	9	5.9 ± 1.3	81.7 ± 16.5	92.1 ± 13.4
CNQX	8	6.8 ± 1.3	11.4 ± 1.7*	88.8 ± 12.0
AMPA (1 μg)	10	7.1 ± 1.4	95.0 ± 13.4	–
AMPA (2.5 μg)	5	5.8 ± 1.9	88.6 ± 11.6	–
AMPA (1 μg + CNQX)	10	4.8 ± 1.2	90.6 ± 15.4	–

Differences among groups in training session latency were not significant in a 1-way ANOVA, $F(4, 37) = 1.21$. Training footshock = 0.8 mA. The second test session in the vehicle and CNQX groups was carried out 90 min after the first test session. *Significant difference from all other test session values at $p < 0.01$ level in a Newman–Keuls test.

TABLE 2

EFFECT OF CNQX (0.5 μg) INFUSED 10 MIN BEFORE INTO THE ENTORHINAL CORTEX ON GENERAL ACTIVITY DURING 3 MIN OF FREE EXPLORATION OF THE TRAINING APPARATUS

		Mean ± SE		
Treatments	*N*	Step-Down Latency (s)	Number of Crossings	Number of Rearings
Vehicle	9	8.6 ± 2.5	22.8 ± 3.8	17.5 ± 3.3
CNQX	10	9.1 ± 2.2	24.6 ± 3.6	17.4 ± 3.0

Differences between groups were not significant in *t* tests.

methylene blue in saline was infused. In 62 of the 65 animals the injections were found to invade a large area of the entorhinal cortex at least 2 mm long and 2 mm wide, as described elsewhere in detail (3,8). Only behavioral data from these animals were analyzed statistically.

RESULTS

Results of experiment 1 are shown in Table 1. The two-way ANOVA revealed a significant sessions effect, $F(9, 360) = 8.9$, $p < 0.01$; a significant drugs effect, $F(3, 360) = 9.0$, $p < 0.01$; and a significant Treatments × Drugs interaction, $F(9, 360) = 92.1$, $p < 0.01$.

As had happened when animals were tested 1 day after training (7), here CNQX blocked the expression of inhibitory avoidance 26 days after training. The effect was no longer seen when the animals were retested 90 min later, at which time retention scores recovered to normal. At this time, it is to be presumed that CNQX had diffused away from the infusion site (5,7,13). AMPA had no effect of its own at the two dose levels studied. However, its administration concomitantly with CNQX cancelled the effect of the latter.

The results of experiment 2 are shown in Table 2. The larger width of the platform probably accounted for the step-down latencies being slightly higher in this experiment than in experiments 1 or 3. CNQX infused into the entorhinal cortex 10 min before had no effect on the various measures of general performance carried out during 3 min of free exploration of the training apparatus: step-down latency, number of crossings, and number of rearings.

The results of experiment 3 are shown in Table 3. The two-way ANOVA revealed a significant sessions effect, $F(3, 34) = 46.63$, $p < 0.01$, but no significant drugs or Drugs × Sessions interactions, ($F = 0.03$ and 0.04, respectively). Retention scores were lower than in experiment 1 because of the lower foot shock intensity. CNQX given into the entorhinal cortex 10 min prior to training had no influence on training or test session step-down latency (i.e., on acquisition or retention test performance of the avoidance task).

TABLE 3

EFFECT OF CNQX (0.5 μg) INFUSED INTO THE ENTORHINAL CORTEX 10 MIN PRIOR TO TRAINING ON TRAINING AND RETENTION TEST PERFORMANCE OF STEP-DOWN INHIBITORY AVOIDANCE IN RATS

		Mean ± SE Step-Down Latency (s)	
Pretraining Treatments	*N*	Training Session	Test Session
Vehicle	9	6.2 ± 1.4	53.7 ± 11.7
CNQX	10	7.2 ± 1.4	51.9 ± 7.0

Training footshock = 0.8 mA, training-test interval = 24 h. Training-test differences were significantly different in both groups at $p < 0.01$ level in a Newman-Keuls test.

DISCUSSION

The effect of intraentorhinal CNQX on retention test performance confirms a previous report using a 24-h training-test interval (7) and is similar to that obtained using intrahippocampal and intraamygdala infusions of the same drug (1, 5). Clearly, at 26 days from training memory expression of the inhibitory avoidance task was sensitive to the administration of this drug into the entorhinal cortex. It is unlikely that the effect was due to an influence on performance rather than on memory expression: Exploratory performance (experiment 2) and acquisition of the avoidance task (experiment 3) were unaffected by CNQX. In addition, no overt signs of motor impairment (ataxia, shakes, tremors, etc.) were detected in the CNQX-treated animals, neither here nor in any previous experiment using brain infusions of this drug (1,3,5,7-9).

The effect of CNQX on retention test performance can be explained by a blockade of receptors of the AMPA/kainate family, since it was overcome by a low dose of AMPA. The present findings are thus compatible with the earlier suggestion that the entorhinal cortex (7), like the amygdala and hippocampus (1,5,6), participates in memory through LTP whose expression, as is known (2,16), occurs through AMPA receptors. The infusion of AP5 immediately after training into the amygdala or hippocampus (5,8) or shortly after training into the entorhinal cortex (3,8) hinders memory consolidation. AP5 blocks NMDA receptors and LTP induction (2,16). Since, however, AMPA receptors are involved in other processes besides the expression of LTP, the present findings, or others similar to them (1,5,7,9,11), do not prove that memory expression is LTP expression. They merely indicate that memory expression requires intact AMPA/kainate receptors in the entorhinal cortex and suggest that this may also be the case in the amygdala and hippocampus, where pretest CNQX infusion has a similar effect (1,5,9,11).

The lack of effect of AMPA on its own, even at a dose 2.5 times higher than that needed to overcome the effect of CNQX, suggests that in the present conditions AMPA did not act as a partial agonist or antagonist and that retention test performance does not involve the massive or generalized use of AMPA receptors, but instead relies on the speci-

fic activation of AMPA receptors at selected synapses—which is of course what would be expected from the LTP hypothesis [see (4-7)]. If memories were "carried" by LTP (1,2, 5-8,16) or depended in any other way on the selective activation of synapses in the entorhinal cortex, their expression should be triggered only by the specific effect, on those synapses, of the stimuli inherent to each particular training experience.

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