Pharmacology Biochemistry and Behavior, Vol. 62, No. 1, pp. 39–43, 1999

0091-3057/99 $–see front matter

PII S0091-3057(98)00091-4

Baclofen and AII 3–7 on Learning and Memory Processes in Rats Chronically Treated With Ethanol

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Received 16 September 1997; Revised 11 May 1998; Accepted 11 May 1998

KUZIEMKA-ŁĘSKA, M., H. CAR, AND K. WIŚNIEWSKI. *The effect of baclofen and AII 3–7 on learning and memory processes in rats chronically treated with ethanol.* PHARMACOL BIOCHEM BEHAV **62**(1) 39–43, 1999.—The aim of this study was to determine the possible influence of baclofen, an agonist of the $GABA_B$ receptor on behavioral activity (recall, acquisition of conditioned reflexes) of angiotensin II fragment 3–7 (AII 3–7) in rats chronically treated with ethanol. Long-term (9 weeks) ethanol intoxication profoundly impaired learning and memory processes in all tests used. The $GABA_B$ receptor agonist baclofen (0.75 mg/kg IP) did not influence exploratory and motor activity in the control rats, but we observed a tendency (without significance) to decrease the psychomotor activity in the alcohol-intoxicated groups of animals, when it was injected together with AII 3–7 (2 μg ICV). Baclofen did not influence the retrieval process in the passive avoidance recall, and when it was given together with AII 3–7 did not change the positive action of this fragment in control groups, but significantly enhanced its action in the animals chronically treated with ethanol. Baclofen showed significant improvement of acquisition in the active avoidance test only in the alcohol-intoxicated groups. Baclofen, injected together with AII 3–7, yielded important attenuation action of AII 3–7 in the control groups in the first 3 days of test, but did not produce any changes during the fourth and fifth day of the experiment. Baclofen did not provoke any changes in activity of AII 3–7 (when it was injected together) in the acquisition of the active avoidance test in the alcohol-intoxicated groups of animals. © 1998 Elsevier Science Inc.

Baclofen AII 3–7 Learning Memory Alcohol Behavior

PREVIOUS studies related to the central action of AII and its fragment 3–7 have shown a beneficial influence of these peptides on learning and memory processes (5,6). It was established that AII and its fragment 3–7 improve the recall of passive and active avoidance responses in control groups and in animals with postalcoholic memory impairment as a result of long-term exposure to alcohol. AII 3–7 (16) is the strongest agonist of the AT_4 receptor localizated in the brain structures connected with the cognitive processes (29). It is known that chronic administration of ethanol causes damage to higher functions of the CNS (13,17). AII (2,8,28) and AII 3–7 eliminated the toxic effect of ethanol on these processes. GABA is the most potent inhibitory neurotransmitter in the central nervous system. The central action of GABA is mediated by specific receptors classified as: $GABA_A$ and $GABA_B$ (9, 14,18). Our previous studies suggest the participation of GABA in the central activity of AII (8). Baclofen is regarded as the selective agonist of $GABA_B$ receptors insensitive to bicuculline (20). The structure of baclofen is similar to GABA and influences post- and presynaptic $GABA_B$ receptors in the brain stem and other places in the CNS (21). There are changes in liberation of neurotransmitters as the result of stimulation of the presynaptic receptors by baclofen. The stimulation of the postsynaptic receptors increase the permeability of the postsynaptic membrane for K^+ions, and decrease the permeability for Ca^+ions (18). The aim of our work was to estimate the influence of baclofen on the central effects of fragment 3–7 of AII in rats chronically treated with ethanol.

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METHOD

Animals

White male Wistar rats weighing 50–60 g at the time of selection for ethanol treatment were used. The mean weight of the rats during the experiments was between 180–200 g. The animals were fed a standard diet and housed in group cages in an air-conditioned room with a 12 h L:12 D cycle beginning at 0700 h. The rats had free access to water (control groups) or alcohol (alcohol- intoxicated groups). The experiments were always carried out at the same time of the day, between 1100 and 1500 h, in a sound-isolated compartment of constant temperature and illumination. The experimental procedures carried out in this study were in compliance with the Board for Ethic Affairs and Supervision over Research on Animals and Individuals, Medical University of Bialystok.

Drug Administration

AII 3–7 (2 μg ICV) was administered into the lateral ventricle of the brain (4). Under ether anesthesia, a burr hole of 0.5 mm in diameter was drilled in the rat's skull, 2.5 mm laterally and 1 mm caudal from the point of intersection of bregma and the superior sagittal suture on the right side of the head. After 48 h an intracerebroventricular (ICV) injection was made to a depth of 4.5 mm with a Hamilton microsyringe. The solution was given in a volume of 10 μl. After termination of each experiment, all animals under ether anesthesia were killed by decapitation, their brains were removed, and the site of injection was verified microscopically. The animals with an inappropriate site of injection were deleted from the calculations.

Chronic administration of alcohol. The method described by Wajda (25) was employed in the experiments with chronic (9 weeks) administration of ethanol. Young rats, weighting 50–60 g, initially received alcohol in a concentration of 1.2% in their drinking water for 4 days. After that, the concentration of ethanol was gradually increased by 0.3% every day to reach the final concentration of 16.2% maintained for 9–12 days. During that time the rats received from 2.5 to 3.0 g of ethanol daily. Their final weight was between 180 and 200 g. At the end, 12 h before the first series of passive, active avoidance training and the open-field test, ethanol was withdrawn and rats received only tap water to drink. The controls drank water for the whole period.

Baclofen-Polfa Starogard was administered intraperitoneally (IP) in a dose (0.75 mg/kg IP). AII 3–7 was synthesized by the Department of Chemistry of Łódź.

Behavioral Tests

Passive-avoidance procedure. Passive-avoidance behavior was studied in a one-trial learning procedure, making use of the natural preference for a dark environment (1). The apparatus consisted of a 6 × 25 cm platform illuminated with a 25-W electric bulb, connected through a 6 × 6 cm opening with a dark compartment (40 × 40 × 40 cm). The floor of the box was made of metal rods of 3 mm in diameter, spaced 1 cm apart. The investigation took advantage of the natural preference of the rats to stay in the dark compartments. The test lasted for 3 days. On the first day, after 2 min of habituation in the dark compartment, the rats were placed on the illuminated platform and allowed to enter the dark compartment,

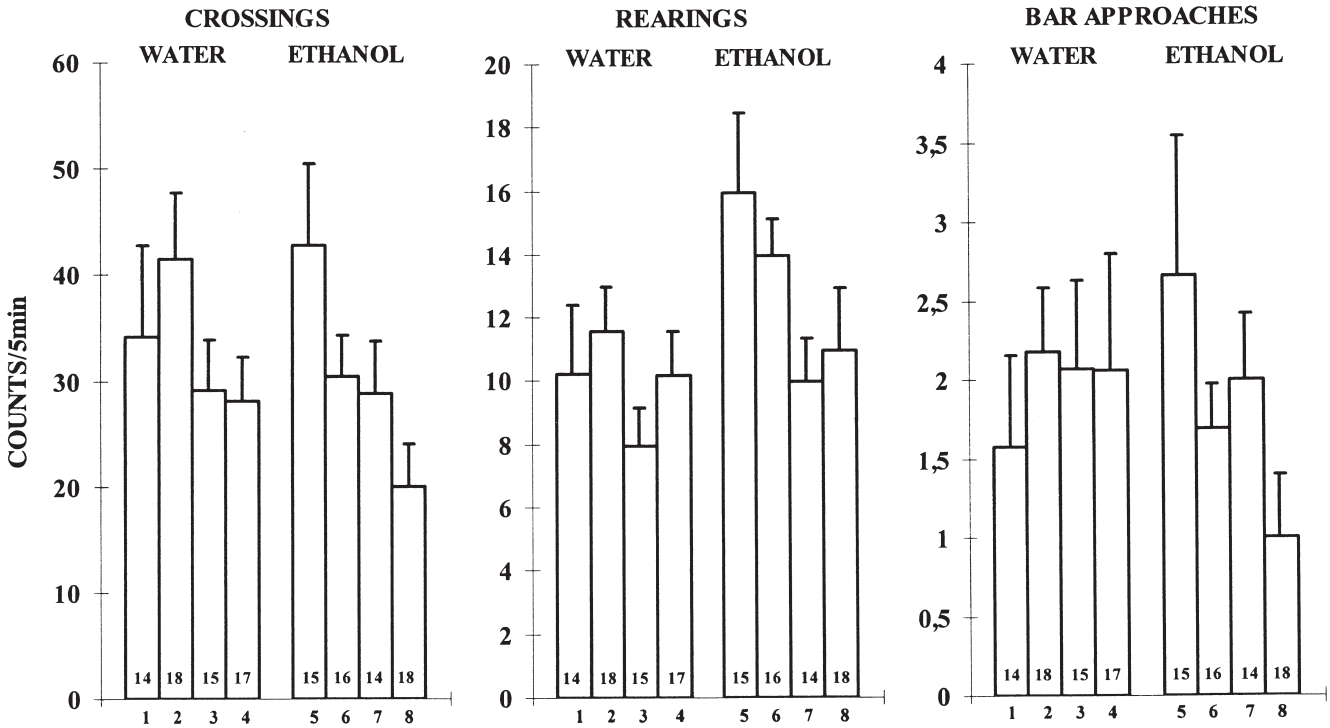

FIG. 1. The effect of AII fragment 3–7, baclofen, and chronic administration of ethanol on the number of crossings, rearings, bar approaches in the open field in control (groups 1–4), and alcohol-intoxicated rats (groups 5–8). Columns represent means ± SEM of the number of animals indicated in the columns; 1.5–0.1 ml of 0.9% NaCl/100 g IP + 10 μl of 0.9% NaCl ICV; 2.6–0.9% NaCl IP + 2 μg of AII 3–7 ICV; 3.7 baclofen 0.75 mg/kg IP + 0.9% NaCl ICV: 4.8 baclofen IP + AII 3–7 ICV.

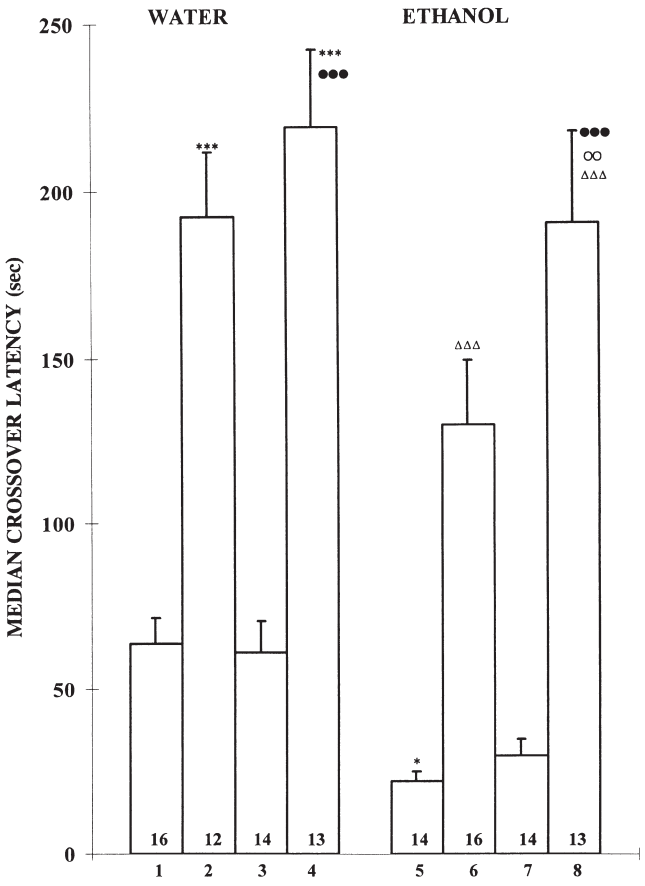

FIG. 2. The effect of AII fragment 3–7 and baclofen on recall in the passive avoidance test in control (1–4 group) and alcohol-intoxicated rats (5–8 group). Columns represent means ± SEM of the number of animals indicated in the columns. 1.5–0.9% NACl 0.1 ml/100 g IP + 0.9% NaCl 10 μl ICV; 2.6–0.9% NaCl IP + AII 3–7 2 μg ICV; 3.7 baclofen 0.75 mg/kg IP + 0.9% NaCl ICV; 4.8 baclofen IP + AII 3–7 ICV $^{*}p(1–5) < 0.02$, $^{***}p(1–2, 4) < 0.001$, $^{oo}p(6–8) < 0.01$, $^{●●●}p(3–4, 7–8) < 0.001$, $^{ΔΔΔ}p(5–6, 8) < 0.001$.

from which they were immediately removed. Two similar trials, at an interval of 2 min, were carried out on the second day. After the first trial the rats were allowed to stay in the dark compartment for 10–15 s. In the second trial when the rat entered the dark compartment it received a foot shock (0.25 mA, 3 s) delivered through the metal rods. The entrance of the box was blocked during the foot shock. The presence of passive avoidance was checked 24 h later. The rats were placed on the illuminated platform once more, and the latency to enter the dark compartment was measured, with a maximum time of 300 s. According to the protocol proposed by Matthies (20), to determine the effect of baclofen on activity AII 3–7 in retrieval, on the third day, they were administered baclofen 15 min before AII 3–7 and AII 3–7 15 min before the retention test of the passive-avoidance response.

Active avoidance procedure. The conditioned-avoidance responses (CARs) procedure was based on that described by Ferster and Skinner (10). The apparatus consisted of a box (60 × 30 × 35 cm) divided into two equal compartments with a wall having in its middle a small window (7 × 9 cm) at the floor level. The conditioned stimulus (CS) was a bell (85 dB), which was switched off immediately after the avoidance response of going from one compartment to another. If the rats did not speedily react within 5 s, the unconditioned stimulus (US) electric current (1 mA) delivered through metal rods (5 mm in diameter, spaced by 1.3 mm apart) forming the floor of the apparatus was applied. Only the compartment in which the rat stayed was electrified. The intertrial interval was 10 s. CAR acquisition training consisted of 20 trial sessions daily for 5 days. The number of positive (+)CARs was recorded every day and expressed as a percentage of the total number of trials. The grill floor was kept clean throughout training sessions. The extinction procedure was essentially the same except that the US was not applied. The duration of CS was always 5 s, and when the animal did not go to the other side during that time, another CS was delivered after a 10 s interval. The manipulation was repeated until a total of 20 trials was completed. The AII 3–7 was injected into the right cerebral ventricle on the first day of learning, 15 min before the training session. Baclofen was injected IP 15 min before AII 3–7. The presession injections of drugs were designed to influence the acquisition (20).

Locomotor and exploratory activity. The open-field test was used for estimation of locomotor and exploratory activity in rats. It was a square of 100 × 100 cm, with a white floor divided by eight lines into 25 equal squares, and surrounded by a white wall, 47 cm high. Four vertical plastic bars 20 cm high were located in four different line crossings in the central area of the floor. Single rats were placed inside the apparatus for 1 min for adaptation. Subsequently, crossings, rearings, and bar approaches were manually counted for 5 min. The AII 3–7 was administrated ICV 15 min before the observation, and baclofen was injected IP 15 min before AII 3–7 administration.

Statistical analysis. The statistical comparison of the results was carried out by analysis of variance (ANOVA) followed by modified *t*-statistics and Benferroni's procedure (27) when multiple means were compared.

RESULTS

These results were noted.

1) The effect of fragment 3–7 of AII and baclofen on locomotor and exploratory activity in rats chronically treated with ethanol (Fig. 1). The number of crossed fields, rearings, and bar approaches indicated a tendency to diminishing the mobility of the animals chronically treated with ethanol.

2) The effect of AII 3–7 and baclofen on recall in the passive- avoidance test in rats chronically treated with ethanol (Fig. 2). In rats chronically treated with ethanol, the latency to enter the dark compartment was significantly shortened. Fragment 3–7 of AII enhanced the recall of passive-avoidance responses in the control group and eliminated the unprofitable influence of ethanol on these processes in animals chronically treated with ethanol. Baclofen did not influence recalling in both groups, and given with AII 3–7 did not change the action of these fragment in control animals. In the alcohol-intoxicated group of rats baclofen significantly enhanced the action of AII 3–7, $F(7, 124) = 29.13$.

3) The effect of AII 3–7 and baclofen on acquisition in the active avoidance test in rats chronically treated with ethanol (Fig. 3). Rats that were given ethanol showed significant impairment of the active avoidance acquisition. Fragment 3–7 of AII significantly intensified this process in control and alcoholized groups. Baclofen enhanced acquisition, which was statistically significant just in the alcoholized groups. Joint administration of baclofen and AII 3–7 causes in the first 3 days

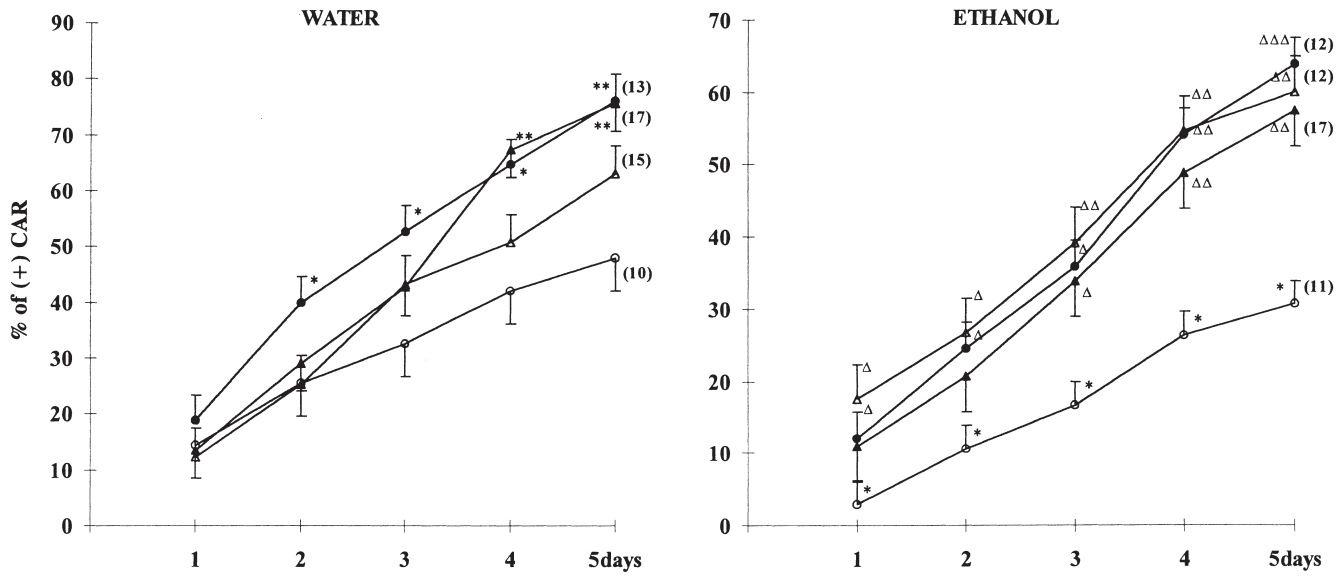

FIG. 3. The effect of AII fragment 3–7 and baclofen and chronic administration of ethanol on acquisition of the conditioned avoidance response (CAR) in control (1–4 group) and alcohol-intoxicated rats (5–8 group). Symbols represent means ± SEM of positive (+)CARs. Each group consisted of 10–17 rats. 1.5 ○ 0.9% NaCl 0.1 ml/100 g IP + 0.9% NaCl 10 μl ICV; 2.6 ● 0.9% NaCl IP + AII 3–7 2 μg ICV; 3.7 Δ baclofen 0.75 mg/kg IP + 0.9% NaCl ICV; 4.8 ▲ baclofen IP + AII 3–7 ICV. $*p(1–2, 5) < 0.02$, $**p(1–2, 4) < 0.01$, $^{\Delta}p(5–6, 7, 8) < 0.02$, $^{\Delta\Delta}p(5–6,7,8) < 0.01$, $^{\Delta\Delta\Delta}p(5–6)$ 0.001.

of estimation of acquisition reduction of action of AII 3–7, without changes in the activity of AII 3–7 after its injections with baclofen in alcohol-intoxicated group of rats, $F(7, 107) = 2.53$ day 1; $F(7, 107) = 3.89$ day 2; $F(7, 107) = 3.64$ day 3; $F(7, 107) = 5.75$ day 4; $F(7, 107) = 7.00$ day 5.

DISCUSSION

The studies carried out suggest that fragment 3–7 of AII eliminates postalcoholic damage of the recall processes in passive and active avoidance situations. Baclofen, the $GABA_B$ receptor agonist did not change the beneficial action of AII 3–7 in recalling a passive avoidance situation in the control group, but enhanced its action in the alcohol-intoxicated group. Some differences in the activity of AII 3–7 have been observed in the active avoidance test. Baclofen changed the activity of AII 3–7 in the acquisition of active avoidance responses in the control group in the first 3 days of the experiment without changes in the alcohol-intoxicated group. In the open-field test we observed a tendency to reduce the mobility of alcohol-intoxicated animals after administration of both baclofen and AII 3–7. The administration of baclofen and AII 3–7 did not change the mobility of the control group. In connection with the reduction of the locomotor and exploratory activity of animals in the open-field test, we can suggest that it may cause prolonged recall processes in animals chronically treated with ethanol in the passive-avoidance test.

Ethanol administered chronically leads to damage of the CNS, which appears as memory and learning disturbances (12,17,22,26). The specific action of ethanol can be related to its direct influence on neurotransmitter receptors in the CNS. Ethanol reduces the activity of the GABAergic system by inhibition of synthesis of decarboxylase GAD (7,13,19). The point of interaction of ethanol on this system is $GABA_A$ receptor connected with a chloride ion channel (3). Through the influence of ethanol its structure and activity are changed (23,24). The differences in the effects of baclofen on AII 3–7 activity may be a result of different mechanisms of action of these compounds on memory processes.

Literature data suggest that fragments of AII 3–8, 3–7 bind at different sites than AII (15) but we cannot exclude the possibility that angiotensin receptors are involved in the action of AII 3–7. The potentiation of ethanol action similarly is not connected with its action on the $GABA_B$ receptor (24). The hypothesis exist that enhancing of GABAergic transmission after alcohol is mediated by increasing of the $GABA_B$ receptor activity (11). The chronic administration of ethanol reduces the postsynaptic effect of baclofen by the reduction of the number of $GABA_B$ receptors. It may also lead to decreasing of regulatory function of some types of $GABA_B$ receptors. The activation of postsynaptic $GABA_B$ receptors causes inhibition of postsynaptic EPSP evoked by NMDA receptors in pyramidal cells, but does not change neurotransmitter release. The chronic administration of ethanol does not influence changes in presynaptic $GABA_B$ receptors (11). These results suggest the interaction of the GABAergic system with the central action of AII 3–7.

ACKNOWLEDGEMENTS

The authors thank J. Ropelewska and L. Stalenczyk for technical help.

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