Pharmacology Biochemistry and Behavior, Vol. 45, pp. 455–464, 1993
Printed in the U.S.A.

0091-3057/93 $6.00 + .00

Role of Angiotensin II and AT_1 Receptors in Hippocampal LTP

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Received 10 December 1992

WAYNER, M. J., D. L. ARMSTRONG, J. L. POLAN-CURTAIN AND J. B. DENNY. *Role of angiotensin II and AT_1 receptors in hippocampal LTP.* PHARMACOL BIOCHEM BEHAV 45(2) 455–464, 1993.—Results of a previous study showed that angiotensin II (AII) inhibited the induction of long-term potentiation (LTP) in hippocampal granule cells in response to dorsomedial perforant path stimulation in urethane-anesthetized rats. The results of present experiments demonstrate a dose-dependent inhibition of LTP induction under the same conditions due to ethanol (EtOH) administered by stomach tube and diazepam (DZ) injected IP. The inhibition of LTP induction by EtOH and DZ can be blocked by saralasin (SAR) applied directly to the dorsal hippocampus and by lorsartan (DuP 753) administered IP. Lorsartan or a metabolite crosses the blood–brain barrier because it also blocks the inhibition of LTP induction due to AII administration directly into the dorsal hippocampus. Lorsartan is a competitive antagonist of the AT_1 subtype AII receptor. Therefore, the AII and the EtOH and DZ inhibition of LTP induction are mediated by the AII subtype receptor AT_1. AIII and the AT_2 antagonist PD123319 did not produce any significant effects. These in vivo effects can be reproduced in brain slices and therefore cannot be attributed to other factors, such as the urethane. In addition, electrical stimulation of the lateral hypothalamus (LH) inhibits LTP induction, and the inhibition can be blocked by SAR. These data on LH stimulation indicate that LH AII-containing neurons send axons into the hippocampus that inhibit the induction of LTP. These results not only provide new information on a neurotransmitter involved in the amnesic effects of benzodiazepines and ethanol-induced memory blackouts, but also testable hypotheses concerning recent observations that angiotensin converting enzyme (ACE) inhibitors elevate mood and improve certain cognitive processes in the elderly.

Hippocampus Lateral hypothalamus Long-term potentiation Angiotensin II Saralasin Lorsartan Ethanol Diazepam Aging Mood elevation Amnesia Memory

ANGIOTENSIN II (AII) and AII receptors are widely distributed in mammalian brain (38,48,56), particularly in those structures involved in the regulation of body fluids and cardiovascular functions. The hippocampus has the highest concentration of AII, even significantly more than plasma extracts (48). Receptor binding studies reveal that AII receptors are localized in the molecular layer of the dentate gyrus (DG), in the CA2 region, and subiculum (38). The moderate binding observed in the DG and CA2 region at 2 weeks of age was no longer evident at 4 weeks, and only low binding was found in the DG. Two subtype AII receptors, AT_1 and AT_2, have been characterized in rat brain by the specific antagonists lorsartan (DuP 753) and CGP42112A (56). AT_1 receptors were present at 2 weeks of age in parts of the limbic system, including the DG. The number of receptors in the DG increased by a factor of 5 at 8 weeks of age. The fact that AT_1 and AT_2 receptors developed differently suggests that they might subserve different functions. Angiotensin-positive cell bodies in the paraventricular nucleus and the perifornical region of the hypothalamus were reported in 1978 (28). However, data on the distribution of AII-immunopositive soma utilizing colchicine pretreatment in the rat hippocampus did not appear until 1980 (23), when AII-like immunoreactivity was found in CA1, CA3, and DG. Later results of a major study on the location and organization of AII-immunoreactive cells and fibers in the rat brain (32) demonstrated immunoreactive axons in all hippocampal fields; however, the existence of positively staining pyramidal and granule cells could not be confirmed.

In 1973 (59,60) we reported that AII excited certain brain cells, including neurons in the DG, lateral hypothalamus, and the zona incerta. Cells were tested directly in whole-animal anesthetized preparations by iontophoretic ejection of AII, and responses were recorded extracellularly. Some of these cells in the lateral hypothalamus were very sensitive to both AII and ethanol (61).

By means of intracellular recording in hippocampal slices (24), AII was found to excite CA1 pyramidal cells by a direct and a disinhibitory mechanism. These excitatory effects were blocked by saralasin (SAR), [Sar^1,Val^5,Ala^8]-AII, an AII antagonist. Depolarization due to pressure ejection of carbachol

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was not affected by perfusion with SAR. When cells were tested with GABA applied iontophoretically at regular intervals to apical dendrites and soma, the recurring depolarization and hyperpolarization effects were not modified by AII. These results indicate that acetylcholine and GABA might not be directly involved in the mediation of the AII effects. However, all CA1 pyramidal cells are not excited by AII (44). Cells that were inhibited by AII increased in activity in response to SAR at concentrations as low as 10^{-14} M for both peptides. The effect of SAR was consistently opposite to that of AII on spontaneous activity. Saralasin did not affect carbachol responses, and confirms previous results that acetylcholine might not be involved and that AII has a direct effect on CA1 cell membranes.

The function of hippocampal AII and AII receptors remained unknown until recently, when we discovered that AII inhibited the induction of long-term potentiation (LTP) (15). LTP, a frequency-dependent model of learning and memory, has been studied extensively in the hippocampus (31,34,55). In the rat, the use of specific receptor antagonists known to block LTP selectively impairs olfactory and spatial learning (35), indicating that LTP is related to important cognitive processes in this animal. When AII is injected above the hippocampus in the intact anesthetized rat, the induction of LTP is blocked in perforant path-stimulated dentate granule cells. The inhibition is entirely prevented when the AII antagonist SAR is coadministered with the AII. Saralasin alone has no effect on the induction of LTP.

Early studies on rats indicated an inhibitory effect of AII in certain types of learning. In a passive avoidance task, AII injected directly into the neostriatum disrupted retention of the task 24 h later and identical injections of AII administered 22 h after learning were without effect on retention (39). More recently, however, AII administered intracerebroventricularly immediately after active or passive avoidance training improved memory when retention was tested 24 h later (21), and it is interesting that the enhancement was completely blocked by baclofen, a $GABA_B$ agonist, injected IP at the same time. Baclofen alone, injected immediately after passive avoidance, impaired retention (52).

Since benzodiazepine (BZ) receptors are widely distributed in the hippocampus (16,20,26), and both $GABA_A$ and $GABA_B$ receptors are involved in granule cell LTP induction (41,42), it is not surprising that the anxiolytic BZs inhibit LTP induction (1,2). Ethanol enhances the effects of GABA-mediated functions (25,29) and also inhibits hippocampal LTP induction in very low doses in slices (7), although the $GABA_A$ Cl^- channel is not involved because the effect could not be blocked by picrotoxin. Acute ethanol application to hippocampal slices had no effect on pre- or postsynaptic $GABA_B$ inhibition in CA1 pyramidal neurons; however, in slices from EtOH-dependent rats, there was a decrease only in postsynaptic $GABA_B$ inhibition (19). In anesthetized rat preparations, dentate recurrent inhibition is enhanced by acute ethanol administration without any change in the excitability of granule cells, as measured by population spikes (63). Differential sensitivity of $GABA_A$ subunits has been determined for EtOH and DZ, and the γ2L subunit is critical for the effects of ethanol but only partially sensitive to DZ (58). Apparently, the enhancing effect of ethanol on the GABA chloride channel depends upon the addition of eight amino acids to the intracellular loop of the γ2L subunit. However, mRNA for the γ2L subunit, which is found in the rat cerebral cortex and cerebellum, is not present in the hippocampus (62).

NMDA (*N*-methyl-D-aspartate) receptors are definitely involved in the mediation of ethanol effects in the rat hippocampus. Prenatal exposure to ethanol reduces the sensitivity of adult hippocampal tissue slices to NMDA (40). This decrease in sensitivity seems to have resulted from abnormal magnesium regulation of the NMDA receptor-channel complex. Ethanol also inhibits the ion current stimulated by NMDA in voltage-clamped disassociated hippocampal neurons (33). The drug MK-801 binds to the phencyclidine (PCP) site within the ion channel of the NMDA receptor-channel complex and inhibits the response to NMDA (64). In mouse hippocampal tissue at the time of ethanol withdrawal, the number of NMDA receptor-channel complexes was increased significantly (27). In rat hippocampal slices, MK-801 attenuates the development of long-term potentiation but has no effect on the previously potentiated population spike (51). Even though ethanol displays more structural specificity than either the BZs or barbiturates in terms of $GABA_A$ subunits, the $GABA_B$ receptors and the involvement of the NMDA channel in the inhibition of LTP induction indicates that ethanol, even at low doses, has widespread neural effects, including several neurotransmitters and receptor-channel complexes.

Considerable clinical evidence exists on the effects of EtOH and the BZs in the brain. Both EtOH and the BZs have well-documented amnesic effects, particularly in humans; anterograde amnesias associated with Korsakoff's Syndrome (43) and memory blackouts (22), and the use of diazepam and, more recently, midazolam and propofol (30) in many clinical procedures to reduce the recall of associated unpleasant experiences.

The possibility that AII might be involved in mental functioning has been evident for some time and related to the use of angiotensin converting enzyme (ACE) inhibitors in treating hypertension (57). These compounds inhibit the enzyme responsible for conversion of AI to AII. Early reports (14,66) were based on anecdotal data from case studies. Two major reports (12,50) utilizing the same clinical trial data on 299 patients showed that captopril, an ACE inhibitor, as compared to methyldopa, improved cognitive performance over a 24-week treatment period and that the captopril was more effective in older, more impaired, patients. A difference between captopril and propranolol treatments was not significant. In the same studies, rats trained on a shuttle avoidance task demonstrated beneficial retention effects of captopril and two other ACE inhibitors, zofenopril and fosinopril, whereas methyldopa decreased retention. Captopril, therefore, not only elevates mood in depressed patients but improves cognitive performance independently of blood pressure, particularly in the older impaired patient. Captopril and SQ29,852 improve performance in cognitive tests in mice and rats (10) and anxiety-related behaviors in rodents and marmosets (11). [^{3}H]SQ29,852 has been used to selectively identify the ACE inhibitory recognition site in human temporal cortex (4). Additional studies utilizing this stable, high-affinity radioligand might provide important data on the location of AII-synthesizing neurons in other parts of the brain, particularly in Alzheimer's patients. ACE is one enzyme known to be elevated in the Alzheimer's diseased brain (3); most other enzymes that have been studied tend to be depressed. The human hippocampus contains low to moderate levels of ACE (8), with the highest binding occurring in the molecular layer of the dentate gyrus and none in the fascia. The CA4 area and the dentate hilus were moderately labeled. However, ACE activity (54) is highest in the hypothalamus. Many older people report that they reduced their intake of alcohol beverages because of the greater effects of the alcohol with increasing

age, especially on cognitive processes and emotionality. These reports suggest that ethanol has a greater effect in the elderly because of its facilitatory action on an already enhanced inhibitory effect of increased AII on hippocampal LTP. However, there is little evidence to suggest that alcoholism-related muscarinic and BZ receptor loss contributes to the dementia observed in Alzheimer's diseased patients (17). Alcoholism does reduce hippocampal BZ and muscarinic receptors (18). Elderly individuals in good health are very sensitive to DZ, single doses of 2.5 mg, which impaired both immediate and delayed recall, psychomotor performance, and increased sedation as compared to young individuals (46).

The evidence seems clear that inhibitory functions of brain AII, if enhanced by increasing age, alcohol, and BZs, can have detrimental effects on cognitive process in animals and humans, and these effects can be reduced by limiting the available AII either by inhibiting synthesis or blocking the appropriate AII subtype receptor. Until more information is available on the validity of hippocampal LTP, it continues to be difficult to understand what specific role it plays in cognitive processes under normal conditions. Following the development of specific AII subtype blockers that cross the blood-brain barrier (49), it is now possible to develop testable hypotheses concerning the role of AII and possibly LTP in mental functions (5). It should also be possible with further research to reconcile the differences in the results in animal studies on the effects of AII on the retention of active and passive avoidance learning.

The purpose of the present study was to determine if AII played a significant role in any of the known effects of ethanol and diazepam on hippocampal LTP induction in dentate granule cells. Results of a series of experiments are reported, which demonstrate unequivocally that the AII subtype AT_1 receptor mediates the inhibition of dentate granule cell LTP induction, in response to medial perforant path stimulation in the anesthetized rat, due to ethanol and diazepam. In addition, evidence is provided on a possible neural pathway involved in the AII mediation of the inhibition of granule cell LTP induction.

METHOD

Animals

Male Sprague-Dawley rats from Harlan Sprague-Dawley weighing between 250 and 400 g were used. Animal rooms were maintained at 21 ± 1°C with a 12L : 12D cycle, lights on at 5:30 a.m. The rats were housed in plastic cages containing hardwood chips and covered with ventilator tops. Commercial Tekland rat chow and water were available ad lib. Animals were housed as littermates until they were about 200 g, at which time they lived with one littermate. All rats were allowed a minimum of 1- to 2-weeks acclimation before being used. The rats were food deprived 24 h before surgery.

Surgery

One to 2 h prior to surgery, animals were anesthetized using a dose of 1.4 g/kg 25% urethane (Sigma), administered IP. The rats were then placed in a Narishige stereotaxic instrument with the head fixed such that the Paxinos and Watson's (45) coordinate system could be used. At the beginning of all surgeries, xylocaine (USP, Schein Pharmaceutical) was applied in small quantities to the incision area. The surface of the skull was exposed, two holes were made with a dental drill, and the dura matter was incised. To prevent drying, 0.9% saline was periodically applied to the exposed skull and brain. Throughout the surgery and experiment, core body temperatures were monitored (YSI Tele-Temperature probe) and maintained at 35 ± 1.0°C with a feedback control system (Frederick Haer).

Recording and Stimulating Electrodes

The recording electrodes were prepared from single-barrel borosilicate glass micropipettes, 1.2 mm o.d. × 0.6 mm i.d. (Frederick Haer), pulled on a Narishige vertical puller and filled with 3 M NaCl. Resistance ranged from 1–3 MΩ. The electrodes were positioned approximately 3.5 mm posterior to bregma and 2.0 mm lateral to the midline. The dentate gyrus was identified by single-unit activity characteristic of granule cells, as well as by an electrode depth of 3.0–3.5 mm below the brain surface.

The stimulating electrodes were platinum concentric bipolar electrodes with a tip diameter of 25 microns. The stereotaxic coordinates were adjusted for variation in rat ages and to maximize the monosynaptic responses of the positive-going population excitatory postsynaptic potentials (pEPSP) produced by the granular cells in response to stimulation of the dorsomedial perforant path. The average stimulating coordinates were 8.5 mm posterior to bregma and 4.4 mm lateral to the midline. At the end of the experiment, placement in the dorsomedial perforant path was verified with a 100-Hz tetanic stimulation (36).

Once both the recording and stimulating electrodes were positioned, 5% agar (Difco Laboratories) dissolved in 0.9% NaCl was applied over the exposed brain and skull to prevent surface drying and reduce movement artifacts.

Electrophysiological Recording

Stimulation consisted of 50-μs duration monophasic constant current pulses delivered once per minute. An input–output curve was obtained over the range of 32–460 μA. Stimulus intensities for actual recording ranged from 50 to 251 μA and produced average pEPSP amplitudes of 3–5 mV, which were slightly at or above threshold for evoking a negative-going waveform (population spike) on the declining phase of the pEPSP (6,37). Once determined, stimulus current remained constant throughout the experiment. After recording 30 min of baseline responses, four sets of tetanic stimulation were administered to induce LTP. Each set contained five trains, 10 pulses per train at 400 Hz, delivered at a rate of one train per second for 5 s. The pulse width in the trains was 50, 100, 150, and 200 μs, respectively. The pEPSPs were recorded every minute for 2.5 h. Percent pEPSP was calculated as follows: amplitude at each minute minus the mean baseline amplitude and then divided by the mean baseline amplitude.

The granule cell pEPSPs were amplified by a conventional amplifier at a frequency band of 1.6 Hz to 2 kHz. All potentials were monitored on an oscilloscope, digitized at a sampling interval of 20 μs for on-line computer display (RC Electronics). The first millisecond of the pEPSP amplitude was used in the analysis. All data were stored on floppy disks.

Drug Administration

Angiotensin II. One microliter of 4.78 μM AII (4.78 pmol peptide) (Sigma No. A9525) in artificial cerebral spinal fluid (ACSF) was injected stereotaxically above the hippocampus on the same side of the brain from which the recordings were obtained (15). The stereotaxic coordinates were 3.5 mm posterior to bregma, 2.0 mm lateral to the midline, and a depth of

1.75 mm below the brain surface. The 1.0-μl volume was injected in equal amounts of 200 nl/min. Results on the distribution of ^{125}I-angiotensin II in the rat brain after injection above the hippocampus revealed significantly more AII in the hippocampus on the injected side as compared to the other side of the brain, 5099 cpm vs. 331 after 1 h. Relatively small amounts were detected in the cerebellum, brain stem, and the thalamus. However, a large amount, 15,002 cpm, was found in the cortex, which might be attributed to leakage along the track of the injection cannula.

Ethanol. Room temperature 25% ethanol (EtOH; 95%, Midwest Grain Products Co.) was prepared in distilled water. Ten minutes after beginning the experiment, EtOH was administered by gavage at doses of 0.5, 1.0, 1.5, and 2.0 g/kg. Doses were selected on the basis of previous results (13). Distilled water and saline were used as controls. Blood alcohol levels were randomly analyzed using gas-liquid chromatography.

Diazepam. Injectable diazepam (Elkins-Sinn, Inc., 5 mg/ml) was administered IP 15 min prior to the onset of tetanic stimulation in doses of 1.0, 1.75, 2.5, 4.0, 6.0, and 8.0 mg/bg. Doses were based upon the results of previous research (13).

Saralasin. The specific AII antagonist peptide SAR ([Sar^1,Va^{15},Ala^8]-AII, Sigma No. A2275) was dissolved in ACSF to yield a final concentration of 1.0 mM. One microliter of this solution was injected at predetermined stereotaxic coordinates above the hippocampus 1 h prior to the onset of recording. The 1.0-μl volume was injected in equal amounts of 200 nl/min.

Lorsartan. DuPont (DuP 753) was prepared fresh in distilled water at room temperature. Fifty minutes prior to recording baseline responses, DuP 753 was injected IP, 10.0 mg/kg body weight.

Lateral Hypothalamic (LH) Stimulation

A stimulating electrode was positioned in the LH according to the following coordinates: AP −1.8 mm, L 1.5 mm, V 8.2 mm to activate possible angiotensin-containing neurons projecting to the hippocampus. Stimulation consisted of 50-μs pulses applied at 2 Hz for 30 min using 250-μA current intensity. The LH was stimulated for 30 min and then the usual 30-min baseline data were obtained before the first tetanus was applied. When the effects of SAR on LH stimulation were determined, the SAR was administered 1 h prior to LH stimulation. Following LH stimulation under these conditions, an additional 30 min was required to remove the LH stimulating electrodes and position the stimulating and recording electrodes for measuring LTP, before obtaining 30 min of baseline data.

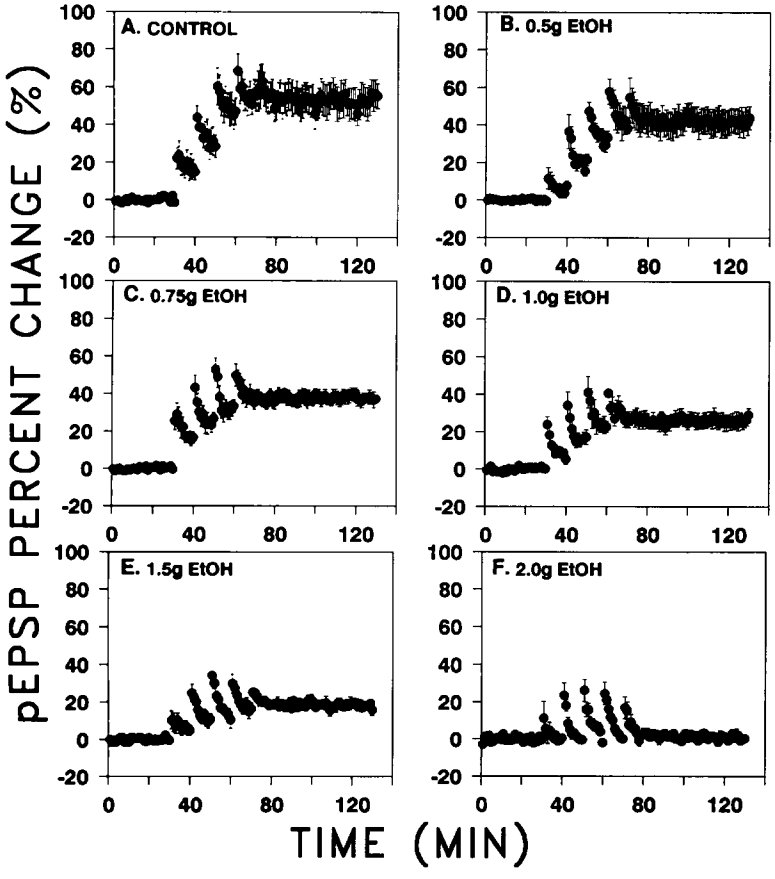

FIG. 1. Percent change in the mean pEPSPs and SEMs presented as function of time in minutes for five doses of EtOH and a control group. Tetani, which produced the LTP of the pEPSPs, were administered at 30, 40, 50, and 60 min. A dose-dependent EtOH inhibition of LTP induction can be observed.

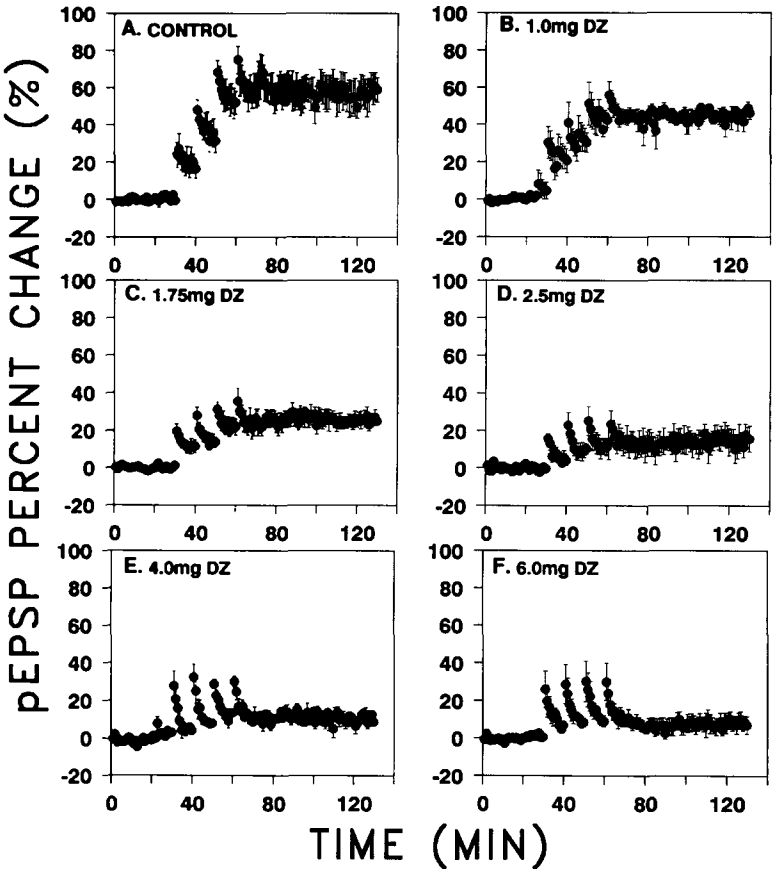

FIG. 2. Same as Fig. 1 except for five doses of DZ (diazepam). A dose-dependent inhibition of LTP induction can be observed.

Statistical Analysis

Data were expressed as percent change from baseline and are illustrated as means ± SEM as a function of time at minute intervals. A two-factor ANOVA with repeated measure on one factor (time) and Student Newman-Keuls multiple-range post hoc determinations ($p < 0.05$) were utilized to compare the last 10 min of all experiments in Figs. 1-3. There were six animals in each of the groups in Fig. 1. There were five animals in each of the groups in Figs. 2 and 3. Data were analyzed utilizing the Statistical Analysis System (SAS Institute Inc., Cary, NC) by an IBM VM/CMS computer. In Fig. 4, there were five animals in each of the experiments in panels A and B. There were four animals in each of the experiments in panel C and D. The means of the pEPSPs for the last 10 min for each group were compared by means of a *t*-test for independent samples and the difference, $t(6) = 19.15$, $p < 0.001$, was significant.

RESULTS

Ethanol

Percent change in baseline as a function of time in minutes for each of the six groups corresponding to the five doses of EtOH (0.5, 0.75, 1.0, 1.5, and 2.0 g/kg) and a control group, which received distilled water, is presented in Fig. 1. There were six animals in each group. Baseline data were collected for 30 min before the tetani were applied at 30, 40, 50, and 60 min. EtOH was administered at 10 min baseline, which was 20 min before the first tetanus was applied. The ANOVA revealed that the dose effect was significant, $F(5, 30) = 20.79$, $p < 0.0001$. There were no significant changes due to time, $F(9, 270) = 0.71$, and the interaction was also not significant $F(45, 270) = 1.22$. The post hoc Newman-Keuls analysis revealed that all doses produced significant ($p < 0.05$) decreases in LTP induction as compared to the control group, except the 0.5-g dose. The 2.0-g dose was significantly different from all the other doses and the 0.75-, 1.0-, and 1.5-g doses were not significantly different from one another. Therefore, EtOH definitely inhibits LTP induction in a dose-dependent way.

Diazepam

Percent change in baseline as a function of time in minutes for each of the six groups corresponding to five doses of DZ (1.0, 1.75, 2.5, 4.0, and 6.0 mg/kg) and the same control group as in the EtOH study, except that only five control animals were used for the comparisons with DZ, is presented in Fig. 2. Therefore, there were five animals in each of the seven groups. The results obtained with 8.0 mg/kg of DZ are included in panel A of Fig. 4. Baseline data were collected for 30 min before the tetani were applied at 30, 40, 50, and 60 min. The DZ was injected IP at 15 min baseline, which was

15 min before the first tetanus was applied. The ANOVA revealed that the dose effect was significant, $F(6, 28) = 23.65$, $p < 0.0001$. There were no significant changes in time, $F(9, 252) = 1.32$, and the interaction was also not significant, $F(54, 252) = 0.86$. A post hoc Newman-Keuls analysis revealed that all doses produced significant ($p < 0.05$) decreases in LTP induction as compared to the control group, except the 1.0-mg dose. The 2.5-, 4.0-, 6.0-, and 8.0-mg/kg doses all produced more inhibition of LTP induction than the 1.0-mg dose. The 1.75-mg dose was also significantly different from the 6.0- and 8.0-mg dose. Furthermore, the 2.5-, 4.0-, and 6.0-mg groups did not differ significantly from one another. The 2.5- and 8.0-mg groups were also different. Therefore, DZ definitely inhibits LTP induction in a dose-dependent way.

Ethanol and Saralasin

Since 2.0 g/kg of EtOH completely inhibits LTP induction, the effects of 1.0 mM of SAR on this particular dose of EtOH were determined. The 1.0 mM SAR was administered above the hippocampus 1 h prior to tetanization, 30 min before the beginning of baseline. The results, percent pEPSP for 1 mM SAR and 2 g EtOH, are shown in panel C of Fig. 3, where they can be compared directly to the control data in panel A. This is the same control group as in Fig. 2. There were no significant differences between these two groups, even though the SAR does not appear to completely eliminate the AII-mediated inhibition of LTP induction as displayed in the controls. Therefore, SAR is an effective antagonist to the inhibition of LTP induction by EtOH.

Diazepam and Saralasin

Since 4.0 mg/kg of DZ significantly reduced the induction of LTP to approximately 10%, the effects of 1.0 mM of SAR on this particular dose of DZ were determined. The SAR was administered above the dorsal hippocampus 1 h prior to tetanization, 30 min before beginning of baseline. These results are presented in panel B of Fig. 3, where they can be compared directly to the control data in panel A. Saralasin almost completely blocked the inhibition of LTP induction by the DZ, and reduced the inhibition to that produced by 1.0 mg DZ (see panel B in Fig. 2). The differences between the controls and these data in panel B were not significant. Because the SAR was administered directly into the brain just above the hippocampus, two control experiments were carried out in which only the ACSF was administered prior to DZ treatment with 4.0 mg/kg. The data on these two animals were identical to the group results in panel E of Fig. 2. Therefore, SAR by

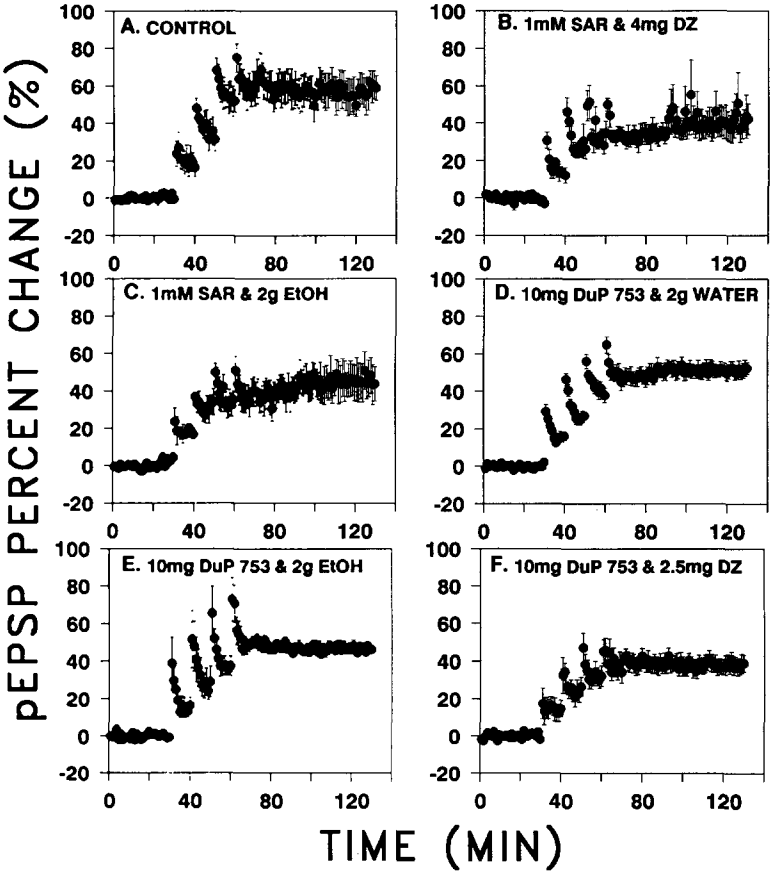

FIG. 3. Same as Fig. 1. (A) Control; (B) 4.0 mg DZ inhibition of LTP is blocked by 1.0 mM of SAR; (C) 2.0 g EtOH inhibition of LTP is blocked by 1.0 mM of SAR; (D) 10 mg of DuP 753 alone has no effect on LTP induction; (E) 10 mg DuP 753 blocks the inhibition of LTP by 2.0 of EtOH; (F) 10 mg DuP 753 blocks the inhibition of LTP by 2.5 mg DZ.

itself blocks DZ inhibition of LTP induction mediated by AII, and the effect cannot be attributed to the ACSF or brain tissue destruction.

Results indicate clearly that the inhibition of LTP induction by EtOH and DZ is mediated by AII.

Ethanol and Lorsartan

Lorsartan, DuP 753, is a specific AT_1 subtype AII antagonist that appears to cross the blood-brain barrier and can therefore be administered peripherally. When animals are pretreated with 10 mg/kg DuP 753 injected IP (50 min prior to baseline) and then administered 2.0 g/kg EtOH by stomach tube (15 min prior to tetanization), the EtOH does not inhibit LTP induction, as shown in panel E of Fig. 3. The results are almost identical to those in the controls in panel A of Fig. 3. DuP 753, when administered in another group of animals, except that these animals received 2 g/kg of distilled water in place of the EtOH, produced similar results, as can be seen in panel D of Fig. 3 and panel B in Fig. 4. An ANOVA did not reveal any significant differences between the means for the final 10 min of any of the panels in Fig. 3. For the different treatments, $F(5, 24) = 2.43$, and for the time factor $F(9, 216) = 1.17$. The interaction was also insignificant, $F(45, 216) = 0.99$. These results confirm the fact that DuP 753 or a metabolite passes the blood-brain barrier and also blocks the EtOH inhibition of LTP induction mediated by AII subtype AT_1 receptors.

Diazepam and Lorsartan

When animals are pretreated with 10 mg/kg DuP 753 injected IP and then injected 65 min later IP with 2.5 mg/kg DZ, the DZ does not inhibit LTP induction, as can be seen in panel F of Fig. 3. These data confirm the results obtained with DuP 753 and EtOH, and support the conclusion that the AT_1 subtype AII receptor is involved in the AII mediation of EtOH and DZ inhibition of LTP induction in the hippocampus.

Angiotensin and Lorsartan

When animals are pretreated with 10 mg/kg DuP 753 injected IP and then 4.78 μM AII is administered directly to the dorsal hippocampus, the inhibitory effect of AII on LTP induction is completely blocked, as illustrated in panel B of Fig. 4.

These results clearly demonstrate that the inhibition of LTP induction produced by direct application of AII to the hippocampus can be blocked by the specific AT_1 subtype competitive antagonist DuP 753. Also, DuP 753 or an active metabolite clearly crosses the blood-brain barrier.

LH Stimulation and Saralasin

Because of our earlier research, which showed that neurons in the LH and in the dentate gyrus were sensitive to AII and that the LH AII-sensitive neurons were also differentially sensitive to EtOH, the effect of LH electrical stimulation on hippocampal LTP induction was determined. The results are presented in panel C of Fig. 4 and clearly indicate that LH stimulation inhibits LTP induction. The inhibitory effect of LH stimulation can be prevented by pretreatment with 1.0 mM SAR, applied directly to the hippocampus, as in the previous experiments reported here and illustrated in panel D of Fig. 4. The effect cannot be attributed to brain tissue destruction associated with the central administration of the SAR. The data in panel C of Fig. 4 were obtained in the same way,

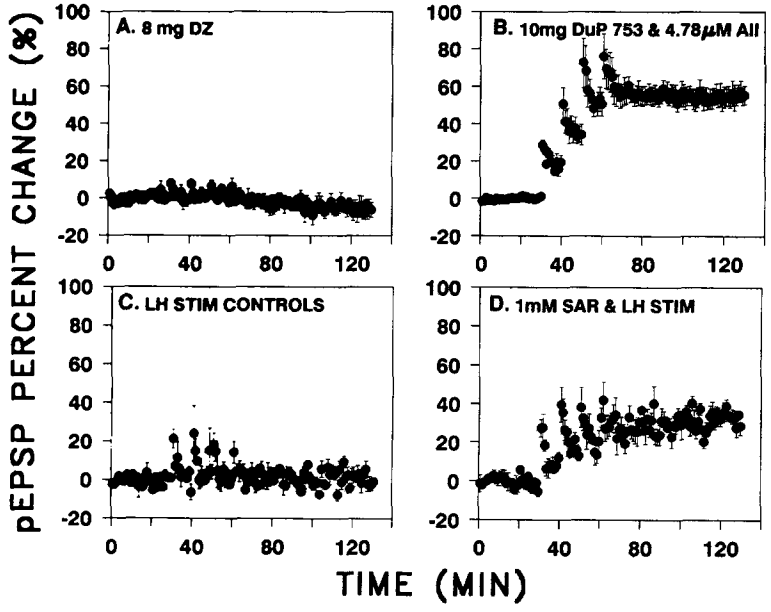

FIG. 4. Same as Fig. 1. (A) Continuation of Fig. 2, 8.0 mg of DZ; (B) 10 mg of DuP 753 blocks the 4.78 μM AII inhibition of LTP induction; (C) complete inhibition of LTP induction by electrical stimulation of the lateral hypothalamus (LH); (D) LH stimulation inhibition of LTP induction blocked by 1.0 mM SAR (SAR) applied directly to the dorsal hippocampus. Artificial cerebral spinal fluid (ACSF) was applied in place of the SAR in (C).

except ACSF was injected into the brain in place of the SAR. These results support the conclusion that LH stimulation inhibits LTP induction and that the inhibition is mediated by AII because it can be prevented by pretreatment with SAR. In addition, some of the AII axons that terminate in the hippocampus originate in cells of the LH and are possibly involved in the EtOH inhibition of LTP induction.

DISCUSSION

These results demonstrate clearly that EtOH and DZ inhibition of LTP induction is mediated by subtype AT_1 AII receptors in the hippocampus, because the effects cannot only be blocked only by SAR but also by the specific AT_1 competitive receptor antagonist lorsartan. These data also confirm our previous results (15), which demonstrated for the first time that hippocampal AII receptors have a significant physiological function in the inhibition of LTP induction. Urethane anesthesia and the fact that lorsartan can be expected to reduce blood pressure significantly (65) cannot be factors in the interpretation of these results, because the major effects can be observed in tissue slices (unpublished data from this laboratory). It should also be mentioned that, in slices, picrotoxin does not affect AII inhibition of LTP induction. These results confirm the data of a previous report in which picrotoxin also did not block EtOH inhibition of LTP in slices (7). These observations indicate that the chloride channel and $GABA_A$ receptors might not be involved in the mediation of the AII inhibition of LTP induction. AII definitely increases the Ca^{2+} efflux in smooth muscle (9). In rat aortic smooth muscle cells, 3.0×10^{-8} M AII produced a maximal efflux of $^{45}Ca^{2+}$, approximately 40% of total cell $^{45}Ca^{2+}$ under control conditions. If AII were to affect hippocampal granule cells similarly, LTP, which depends upon significant postsynaptic influx of Ca^{2+}, would be prevented from occurring. In brain minces, AII produces an inhibitory effect on phosphotidylinositide (53), and AII mobilizes intracellar Ca^{2+} (47) in NG 108-15 neuroblastoma cells, which is mediated by AT_1 receptors. Therefore, it also seems likely that the NMDA gated receptor complex and the Ca^{2+} channel might be involved in the inhibition of LTP induction in this study. Binding studies (9) utilizing DuP 753 indicate highly specific structural requirements and similar AII receptors in adrenal cortex and vascular smooth muscle. It seems reasonable to assume that brain AII receptors display similar characteristics. If this proves to be correct, AII might also have an effect on Ca^{2+}-dependent transmitter release in presynaptic endings.

Results of preliminary experiments indicate that AIII does not inhibit LTP induction and that PD123319, an AII subtype AT_2 receptor antagonist, does not block AII inhibition of LTP induction. Since the AT_1 subtype AII receptor clearly mediates AII inhibition of LTP induction, further dose-effect experiments on AT_2 receptor antagonists have not been carried out.

Even though the normal regulation of AII inhibition of LTP induction and all the AII cells of origin that might control it must still be determined, these results demonstrate a creditable basis for recent observations that ACE inhibitors elevate mood and improve certain cognitive processes, particularly in the elderly. In addition, these data provide a physiological basis in terms of a known neurotransmitter for the amnesic effects of the benzodazepines and ethanol-induced memory blackouts. Also, these results provide strong support for the implication of hippocampal LTP in the memory consolidation process. The results of current research in progress in this laboratory will further elucidate the mechanisms and neural pathways involved in the role of AII in certain cognitive processes.

ACKNOWLEDGEMENTS

We wish to acknowledge the help of John McGlothan for technical assistance and Marianne Van Wagner for typing the manuscript. We appreciate the comments and criticisms made by Dr. Clyde Phelix on an earlier draft of the manuscript. The lorsartan, DuP 753, was generously provided by Dr. Ronald D. Smith, the DuPont Merck Pharmaceutical Co., Wilmington, DE.

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