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Effects of angiotensin II and IV on geniculate activity in nontransgenic and transgenic rats

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Abstract

Microiontophoretic ejection of angiotensin II and angiotensin IV in the vicinity of geniculate neurons was used to study the effects of these peptides on the discharge rate and the discharge pattern of extracellularly recorded activity. The main aim of the experiments was to study the effects of angiotensins in different strains of rats anesthetized with urethane (normotensive Wistar, normotensive Sprague–Dawley and hypertensive, transgenic (TGR(mREN2)27) rats). Both angiotensins mostly increased the spontaneous activity of angiotensin-sensitive geniculate neurons in all strains. Angiotensin II reduced the number of bursts in most neurons, whereas angiotensin IV significantly enhanced it. Inhibitory effects of angiotensins on spontaneous as well as on light-evoked activity could be effectively blocked by $GABA_A$ or $GABA_B$ receptor antagonists. Therefore, it can be supposed that angiotensin-containing afferent fibers innervate both projection and local circuit neurons of the dorsal lateral geniculate nucleus. In addition, angiotensin II suppressed excitation induced by glutamate receptor agonists in most neurons tested. Angiotensin-induced effects could be blocked by specific receptor antagonists. There were no significant differences in the effects of angiotensins in the various strains of rats, except for the latencies of the neuronal responses to the iontophoretic ejection of angiotensins. © 1997 Elsevier Science B.V.

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1. Introduction

Angiotensin II, the primary active hormone of the renin–angiotensin system, plays a crucial role in blood pressure regulation as well as in fluid volume and electrolyte balance. A renin–angiotensin system is present in the mammalian brain, complete with the precursors and enzymes required for the formation and metabolism of the biologically active forms of angiotensin (Phillips, 1987; Saavedra, 1992; Sumners et al., 1994; Wright and Harding, 1994). At least angiotensin II has many of the characteristics of a neurotransmitter (Phillips, 1987). The recent development of specific angiotensin II receptor ligands has allowed the identification of at least two different angiotensin II receptor subtypes whose actions are mediated through different signal transduction mechanisms. Angiotensin II has equivalent affinity for both major subtypes of the angiotensin II receptor, AT_1 and AT_2 (Sumners et al., 1994; Wright and Harding, 1994). The angiotensin AT_1 receptor mediates most of the known physiological effects of angiotensin II, among the most prominent being those associated with the cardiovascular system and kidney. The physiological role of the angiotensin AT_2 receptor has yet to be clearly defined. While an angiotensin AT_3 site has been identified in mouse neuroblastoma cells (Chaki and Inagami, 1992), no functions have so far been identified. Angiotensin IV binds selectively to the angiotensin AT_4 receptor which has been associated with a memory function and the regulation of blood flow (reviewed in Wright et al., 1995).

The thalamic nuclei contain angiotensin AT_1-, AT_2-, and AT_4-receptors (Millan et al., 1991; Song et al., 1992; Rowe et al., 1992; Wright et al., 1995). The presence of angiotensin AT_2 and AT_4 receptor sites in the thalamus, and in areas of the brain that process sensory information have suggested a novel modulatory role for angiotensins in information processing. The rat thalamus possesses high

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amounts of angiotensin II specific binding (Sirett et al., 1977; Harding et al., 1981). The dorsal lateral geniculate nucleus contains a moderate density of angiotensin II receptor binding (Coveñas et al., 1989; Gehlert et al., 1991; Song et al., 1992). Thalamic nuclei also contain moderate amounts of angiotensin converting enzyme (Chai et al., 1987).

Studies with either Sprague–Dawley or Wistar rats showed a similar distribution of angiotensin II receptor subtypes, therefore, there seem to be no strain differences. These rats can be used as control groups. The brain of hypertensive, transgenic rats (TGR(mREN-2)27) (Mullins et al., 1990) contains a concentration of angiotensin II higher than that in Sprague–Dawley rats (Senanayake et al., 1994). The transgenic rats over express renin in the adrenal but not in the kidney (Bader et al., 1992). The physiological effect of prolonged unopposed stimulation of angiotensin receptors, with presumably rising levels of brain-circulating angiotensin II in the transgenic rats, is unknown. This model may also be useful for studying the function of angiotensin in the thalamus.

Therefore, we investigated the effects of angiotensin II and angiotensin IV on spontaneous and light-evoked geniculate activity in various strains of rats: in Wistar and Sprague–Dawley rats, as well as in transgenic animals with an elevated level of angiotensin II at least in some regions of the brain. In addition, as there seems to be an interaction of angiotensins with classical neurotransmitters (Felix, 1976; Xiong and Marshall, 1994), the coadministration of angiotensins with acetylcholine and/or glutamate receptor agonists was tested in some neurons. A possible involvement of local-circuit geniculate neurons in the mediation of the effects of angiotensins was investigated by using $GABA_A$- and $GABA_B$-receptor antagonists.

2. Materials and methods

2.1. Maintenance and preparation of animals

All procedures were carried out according to the Institutional standards of animal welfare and approved by the Berlin regional animal ethics committee. The experiments were performed in three groups of male adult rats: normotensive Wistar and Sprague–Dawley, and hypertensive, transgenic rats (TGR(mRen2)27). Heterozygous transgenic rats were obtained by cross breeding male homozygous transgenic rats with female Sprague–Dawley rats. Wistar rats were purchased from Bundesgesundheitsamt (Berlin), and Sprague–Dawley as well as transgenic rats from Møellegard Breeding and Research Centre (Denmark). When experiments were started, the heterozygous TGR(mRen2)27 rats were 10–12 weeks old and hypertension was already established. All rats were kept under the same conditions without treatment. The rats were kept 5 to a cage with food and water available ad libitum and on a light–dark diurnal cycle. All testing took place during daylight hours.

Before the experiment the systolic pressure was determined by tail-cuff plethysmography (Stoelting, USA). The rats were anesthetized with urethane (1.2 g/kg, i.p.) and placed in a stereotaxic instrument. Subsequent injections of urethane were administered as needed (for detail see Albrecht and Davidowa, 1989). Rectal temperature was maintained at 37–38°C with a heating pad. Both electrocardiogram and EEG from the visual cortex were monitored. In some experiments the tail pulse rate was recorded. No changes were observed in either nontransgenic or transgenic rats during iontophoretic ejection of angiotensins.

A small hole was drilled into the skull at a site 3.5 mm lateral to the midline suture and 5.0 mm anterior to the lambdoid suture. An electrode was lowered 3.5 to 5 mm with a microdrive through the hole to the level of the dorsal lateral geniculate nucleus.

2.2. Recording and visual stimulation

Glass microelectrodes for extracellular recording were filled with saturated Trypan blue solution (tip resistance 10–30 MΩ).

The recorded action potentials were amplified and displayed on an oscilloscope and, after passing a window discriminator (World Precision Instruments, USA) were analyzed with custom-made software (spike2 from Cambridge Electronic Design, UK) running on a personal computer. Standardized pulses corresponding to individual action potentials were used for computing frequency–time histograms or peristimulus time histograms, which were displayed on-line during sampling. Data were stored on disc for subsequent analysis.

The experiments were carried out in a dimly illuminated room (brightness about 0.01 cd m^{-2}). For visual stimulation a light-emitting diode (LED, Fairchild Semiconductor FVL 352, 560 nm, 7 mcd, 500 ms) was placed at a distance of 5 mm in front of the eye contralateral to the recording site. Spontaneous activity and/or responses of geniculate cells to diffuse light stimuli were recorded before, during and after termination of drug administration.

2.3. Drugs and iontophoresis

Electrodes for iontophoresis were prepared from 5- or 7-barrel micropipettes (World Precision Instruments, USA) with a horizontal puller (P-87, Sutter Instrument). The tips were broken under microscopic visualization (tip diameter 5–7 μm). The micropipette assembly was affixed to the recording electrode with a tip separation of 20–40 μm.

The following drugs were used: angiotensin II (100 μM, pH 4.5; Research Biochemicals International, USA, or synthetized by Dr. P. Henklein, Institute of Biochemistry, Charité) and angiotensin IV (100 μM, pH 4.5;

synthetized by Dr. P. Henklein, Institute of Biochemistry), saralasin (unspecific angiotensin II receptor antagonist; 1 mM, pH 5.0; Research Biochemicals International), losartan (angiotensin AT_1 receptor antagonist; 100 μM, pH 8.0; a gift of Dr. R.D. Smith, DuPont Merck, USA), CGP 42112A and PD 123,319 ditrifluoroacetate (AT_2 receptor antagonists; 100 μM, pH 4.5; Research Biochemicals International), divalanal (AT_4 receptor antagonist; 100 μM, pH 4.5; Pacific Northwest Biotechnology, USA), (−)-bicuculline methiodide ($GABA_A$ receptor antagonist; 5 mM, pH 3; Research Biochemicals International), phaclofen ($GABA_B$ receptor antagonist; 5 mM, pH 3, Research Biochemicals International), CGP 35348 ($GABA_B$ receptor antagonist, 1 mM, pH 3.0; a gift of Ciba–Geigy), acetylcholine chloride (0.5 M, pH 4.0), *N*-methyl-D-aspartate (NMDA) and kainic acid (glutamate receptor agonists; 100 mM, pH 8.0; Research Biochemicals International). Retaining currents (4–10 nA) were applied to the pipettes between drug ejections. In a number of experiments a barrel filled with sodium chloride (165 mM, pH 4.5 or pH 8) was used for current balance. No significant contribution from current or pH was detected in control experiments.

2.4. Experimental program

In the first series of experiments after determination of the neuronal response to light, iontophoretic ejection of angiotensin II and angiotensin IV was delivered repeatedly during continuous recording of baseline activity. Currents from 10 to 50 nA were used, depending on the magnitude of the angiotensin response elicited. As tachyphylaxis to angiotensin II is known to occur (Kanashiro et al., 1995), and recovery of the surface receptor after removal of the angiotensin agonist occurs with a half-life of 15 min (Anderson et al., 1993) we repeatedly ejected angiotensin II at intervals of at least 20 min (in most cases, longer intervals) to make sure that the blocking effect of the coadministration of angiotensin II and selective receptor antagonists was not due to desensitization of receptors. Angiotensin receptor antagonists, GABA receptor antagonists and acetylcholine were applied alone and together with angiotensins.

The effects of angiotensin II on NMDA- or kainate-induced excitations could be proved in some neurons. The glutamate receptor agonists were regularly pulsed (10 to 15 s ejection time with 50 or 60 s intervals without ejection). The ejecting current was usually adjusted to produce a stable response of the neuron to the repeated ejection of glutamate receptor agonists.

In addition, the effects of angiotensin II and IV on light-evoked activity were studied. Angiotensins were applied during 30 trials of light stimuli with an intertrial interval of 8 s. After termination of angiotensin II administration recording was continued until both baseline firing and light-evoked responses recovered to their pre-angiotensin II levels. After recovery, angiotensin IV was applied iontophoretically. In some cases the blocking potency of angiotensin receptor antagonists and/or GABA receptor antagonists was investigated. These tests were started with the administration of one of the angiotensin receptor antagonists (currents between 30 and 70 nA) or of one of the GABA receptor antagonists and then continued with concomitant ejection of angiotensin. After recovery, the angiotensin response was tested and the second angiotensin or the GABA receptor antagonist, was then applied. Since not all neurons could be studied with a full experimental program, the number of neurons involved differ in Section 3.

2.5. Analysis of neuronal responses

Drug responses were compared with control firing frequency recorded immediately before drug application. Based on the continuously recorded rate-meter counts, the average discharge rate of each neuron was evaluated for 120 s prior to the iontophoresis. This value (referred to as 'control') was subtracted from all subsequent changes in firing rate and the results were expressed as '% change of control'. If the average change of discharge rate during the entire response time was greater than 40%, the neuron was considered as being sensitive to the substance applied. This criterion was used to divide the 'responders' from the 'nonresponders', taking into account that the variability of the spontaneous discharge frequency in geniculate neurons usually had not exceeded 20% change in our previous investigations. In addition, we analyzed the discharge pattern of the spontaneous activity in predrug conditions, during and after drug effects (at a time of 120 s).

The response to light was evaluated from the peristimulus time histograms which summarized activity recorded during 30 repetitions of the stimulus. The cells were grouped according to the response to light, into ON-like and OFF-like neurons. The number of spikes in the prestimulus (500 ms), the ON- (500 ms), and OFF-phases (500 ms) of the peristimulus time histograms were determined.

In addition, the discharge pattern of spontaneous as well as of flash-evoked activity was determined. Since the effects of angiotensins often developed very slowly and continued beyond termination of the iontophoresis, we analyzed the unit activity recorded during the last 120 s of each light stimulation period (15 light stimuli). We determined the number of spikes in this period (expressed as the discharge rate, imp./s), and analyzed how many of the spikes occurred individually or in groups. Sequences of spikes with an interspike interval ≤ 4 ms were regarded as bursts as recommended in the literature (Lo et al., 1991; Lu et al., 1992). As in our previous investigation (Davidowa et al., 1995) several parameters were measured: the absolute number of bursts/120 s, the percentage of spikes involved in bursts, and the number of spikes in a burst.

The paired Wilcoxon Rank sum test (two tailed), the Mann–Whitney test and the χ^2 test were used for statistical analysis.

2.6. Localization of recording sites

At the end of recording, a small amount of Trypan blue was iontophoretically deposited in the brain by passing a 10 μA negative current through the recording electrode for approximately 10 min. The rat was killed with an overdose of urethane, decapitated and the brain was fixed with 10% formaldehyde. Frontal frozen sections were stained with nuclear red. The location of blue spots within the dorsal lateral geniculate nucleus was determined.

3. Results

3.1. Effects of angiotensins on geniculate spontaneous activity in different strains of rats

The spontaneous activity of 175 geniculate neurons was analyzed. All these neurons responded to the diffuse light stimulation. Iontophoretic ejection of angiotensins increased the spontaneous activity of most geniculate neurons that were responsive to angiotensin. Examples are shown in Fig. 1A and B. Considering only neurons whose activity had been changed by more than 40%, an angiotensin-induced increase in geniculate activity was obtained in nearly one third of all neurons investigated, whereas a decrease in spontaneous activity was found in about 10–20% of the neurons in the different strains of rats (see Fig. 2). Phasic changes in the discharge rate, first excitation and then inhibition, were observed in seven neurons out of all angiotensin-sensitive neurons ($N = 132$).

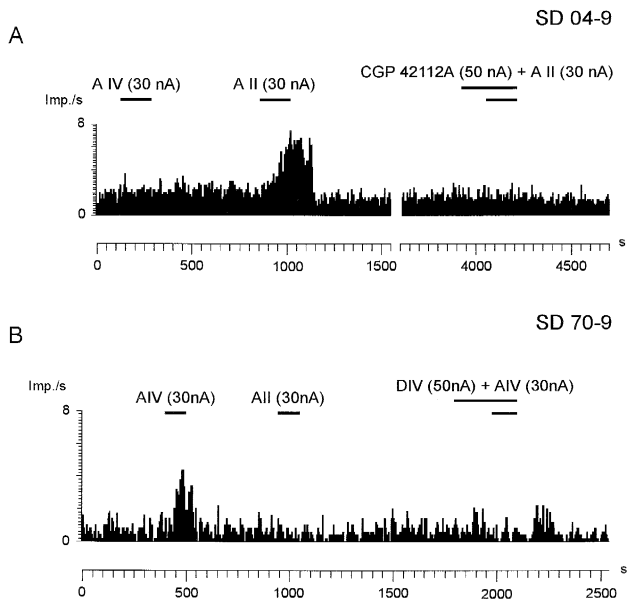

Fig. 1. Excitatory effects of angiotensin II (AII) (A) and angiotensin IV (AIV) (B) on spontaneous activity of two geniculate neurons in Sprague–Dawley rats (SD). The frequency–time histogram at the top shows that angiotensin IV did not change the discharge rate, whereas the iontophoretic ejection of angiotensin II induced acceleration of the discharges. Increases in the discharge rates could be blocked by specific angiotensin AT_2 (CGP 42112A) and AT_4 (divalanal, DIV) receptor antagonists, respectively. The *y*-axes indicate the number of spikes/s and the *x*-axes indicate the time in s (bin width 5 s). The bars represent time and duration of ejection, the numbers show current intensity.

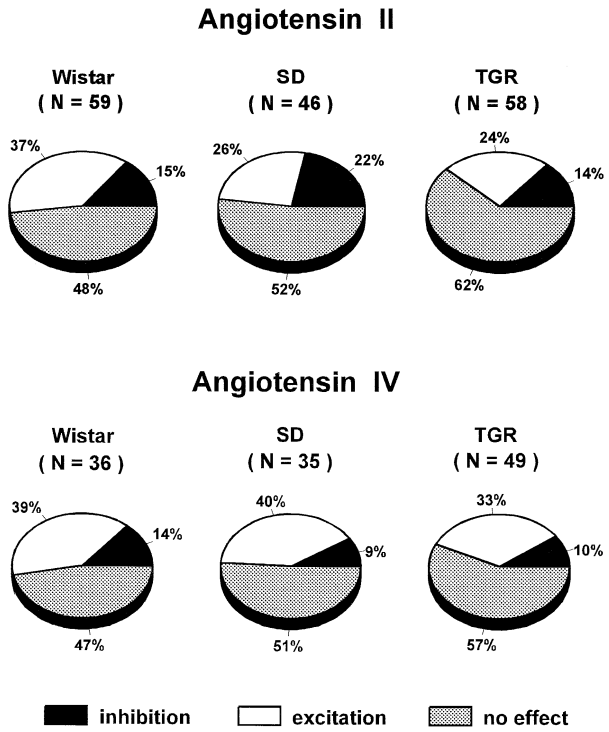

Fig. 2. Percentage of excitatory and inhibitory effects in geniculate neurons of Wistar (W), Sprague–Dawley (SD) and transgenic (TGR(mRen2)27) rats induced by the iontophoretic ejection of angiotensin II (AII) and angiotensin IV (AIV). N = number of neurons.

In general, the effects induced by angiotensins lasted for several minutes after the end of the iontophoretic ejection. However, in some cases there was a long-lasting action of both angiotensin II and angiotensin IV i.e., one that lasted about 30 min. Such long-lasting effects induced by angiotensin II were frequently found in transgenic animals. It is interesting that there was a tendency, albeit not statistically significant, for the geniculate neurons from transgenic rats to manifest responses to angiotensin II ejected with the same duration and the same mean current more prolonged than those of neurons from the Sprague–Dawley rats (974 ± 946 s (transgenic rats) versus 762 ± 520 s (Sprague–Dawley rats)). In addition, the latencies of the angiotensin II-induced effects were significantly shorter in geniculate neurons of transgenic animals than in those of the control Sprague–Dawley group ($P = 0.03$, Mann–Whitney U test). The latencies of angiotensin effects were comparable to those obtained with ejection of other peptides (Albrecht et al., 1994).

The responsiveness of geniculate neurons to angiotensins did not differ significantly in the three strains of

Table 1
Influence of angiotensin II on spontaneous activity of geniculate neurons (more than 40% changes in discharge rates) whose activity was recorded in various strains of rats

	Pre-drug conditions	Angiotensin II administration	Recovery
Neurons excited by angiotensin II (mean ± S.E.M.)			
Wistar rats (22/59)	2.58 ± 1.02	6.22 ± 1.64	2.82 ± 1.10
Sprague–Dawley rats (12/46)	1.78 ± 0.67	4.92 ± 1.19	2.16 ± 0.75
TGR rats (14/58)	1.62 ± 0.53	4.55 ± 1.43	3.14 ± 1.53
Neurons inhibited by angiotensin II (means ± S.E.M.)			
Wistar rats (9/59)	2.57 ± 1.05	0.94 ± 0.40	2.26 ± 1.12
Sprague–Dawley rats (10/46)	6.68 ± 2.16	1.62 ± 0.51	7.33 ± 2.59
TGR rats (8/58)	7.50 ± 2.21	2.05 ± 0.83	8.62 ± 2.95

rats ($P > 0.05$, χ^2 test), although in transgenic animals fewer neurons responded to iontophoretic administration of angiotensins (Fig. 2). The effects of angiotensin II on geniculate spontaneous activity are shown in Table 1. The table only includes neurons whose activity was changed by more than 40%. Concerning the control as well as the transgenic Sprague–Dawley rats it is noteworthy that excitatory effects of angiotensin II were mainly induced in slowly discharging units, whereas inhibitory effects were more frequently obtained in neurons with higher spontaneous discharge rates. However as demonstrated, the changes were similar in the different strains of rats. Comparable results were obtained with angiotensin IV.

After repeated exposure to one of the peptides within short time intervals (about 5–10 min) the neuronal response to angiotensin II or angiotensin IV was frequently lost. This desensitization mainly occurred during testing of spontaneous activity. We tested the responses to angiotensin II in 12 angiotensin-responsive neurons with time intervals of 7, 14 and 21 min. With the first two intervals, but three all of the neurons tested reduced their response or did not show a response to angiotensin II. The same response as the first was evoked after an interejection time interval of 21 min in 11 neurons. In tests of the flash-evoked activity or the effects of angiotensins on glutamate receptor agonist excitation, repeated ejection of the peptides did not cause fading of the response.

However, we could not obtain cross-desensitization between angiotensin II and angiotensin IV. Taking all strains together, 30 out of 114 of the geniculate neurons (26%) were responsive to both angiotensin II and IV. In most cases both angiotensins induced similar effects, i.e., changes of activity being excitatory or inhibitory in response to both angiotensins. There were no significant differences in the responsiveness of neurons located in different regions of the dorsal lateral geniculate nucleus.

3.2. Angiotensin receptor types in the dorsal lateral geniculate nucleus

The specific angiotensin antagonists were able to block the angiotensin-induced effects in most neurons (Fig. 1A and B). Increases as well as decreases in the discharge rate induced by angiotensin II could be blocked by angiotensin AT_1 as well as by angiotensin AT_2 receptor antagonists. Losartan antagonized the action of angiotensin II in 52% (12/23) of the neurons tested, and CGP 42112A and PD 123,319 had antagonistic effects in 67% (10/15) and 55% (11/20), respectively. The unspecific antagonist, saralasin, blocked 50% of angiotensin II-induced excitations (3/6). The blockade of the action of angiotensin IV by divalanal was specific because both angiotensin AT_1 and angiotensin AT_2 receptor antagonists failed to block the excitatory or inhibitory effects produced by the iontophoretic administration of angiotensin IV ($N = 5$). Changes in the discharge rate induced by angiotensin IV were blocked by the coadministration of divalanal and angiotensin IV in 14 out of 17 neurons tested. In transgenic rats both the AT_1 and the AT_2 angiotensin receptor antagonists caused changes in the discharge rate, when ejected alone in six neurons and 3 neurons, respectively.

3.3. Interaction of angiotensins with other transmitters

Powerful activation of geniculate neurons was obtained by ejecting glutamate receptor agonists from multibarreled electrodes. Angiotensin II caused a strong inhibition of NMDA- or kainate-induced excitation in most neurons (13/17) (Fig. 3). The suppressing effects of angiotensin II on excitatory amino acid-induced excitation were stronger than the effects of angiotensin II on the spontaneous activity recorded in the same neuron. This suppression of

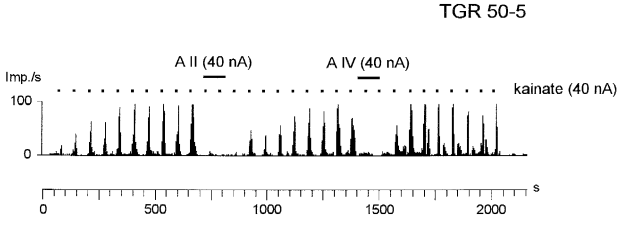

Fig. 3. Suppressive effects of angiotensin II (AII) and angiotensin IV (AIV) on kainate-induced increases of the geniculate discharge rate. Kainate was regularly pulsed by iontophoresis (15 s ejection time with 50 s intervals without ejection).

NMDA- and kainate-induced excitation could be blocked by losartan ($N = 3$), but not by PD 123, 319 ($N = 1$). However, an additive increase in the discharge frequency induced by the glutamate receptor agonist and by angiotensin II was also found ($N = 4$).

Angiotensin IV also induced suppression of glutamate receptor agonist-induced excitation in three neurons, whereas the excitatory effects were enhanced in two neurons.

The influence of angiotensins on the flash-evoked activity was investigated in 44 of the 175 geniculate neurons. Neurons responded to the diffuse light stimulation with either a primary excitation to light on and a decrease of the discharge rate in response to light off ($N = 23$, ON-neurons) or with primary inhibition of the discharge rate to light on ($N = 21$, OFF-neurons). No differences between rat strains were found for responsivity and direction of effects. Angiotensin II and angiotensin IV caused a change of the light-evoked activity in half the neurons (angiotensin II: 17/33 (51%); angiotensin IV: 17/32 (53%)) recorded in Sprague–Dawley and transgenic rats. Examples of suppressive influences on flash-evoked activity induced by angiotensin II and angiotensin IV are shown in Figs. 4 and 5. The iontophoretic ejection of angiotensin II induced suppression of the response to light in 9 neurons, whereas the light-evoked activity of eight neurons was facilitated by angiotensin II. After the administration of angiotensin IV the responses to light were facilitated in ten and suppressed in seven neurons. Angiotensin-mediated effects could be blocked by specific AT_1, AT_2 and AT_4 angiotensin receptor antagonists (see also Figs. 4 and 5).

The inhibitory effects of angiotensin II and angiotensin IV on spontaneous as well as on flash-evoked activity could be effectively antagonized by GABA receptor antagonists. Fig. 4 shows the responses to light of an OFF-like geniculate neuron whose activity was recorded for six hours. The spontaneous activity as well as the light-evoked activity was strongly inhibited by the iontophoretic ejection of angiotensin II. This inhibition of activity was mediated by the AT_2-receptor, as coadministration of the specific AT_2 receptor antagonist, PD 123,319, and angiotensin II did not change the light-evoked activity significantly. The $GABA_A$ receptor antagonist, bicuculline, showed similar blocking potency. In contrast, the angiotensin AT_1 receptor antagonist, losartan, failed to block the angiotensin II-mediated effect on neuronal activity. Bicuculline antagonized the angiotensin II or angiotensin IV-induced suppression of neuronal activity in 9/12 neurons, whereas the $GABA_B$-antagonists, CGP 35348 or phaclofen, blocked the angiotensin-induced inhibition in three neurons ($N = 8$). In two geniculate neurons neither bicuculline nor CGP 35348 were able to antagonize the inhibition of the flash-evoked activity induced by angiotensin II, at least by ejection with currents below 50 nA. The different peristimulus time histograms of an ON-like neuron are shown in Fig. 5. In this neuron angiotensin

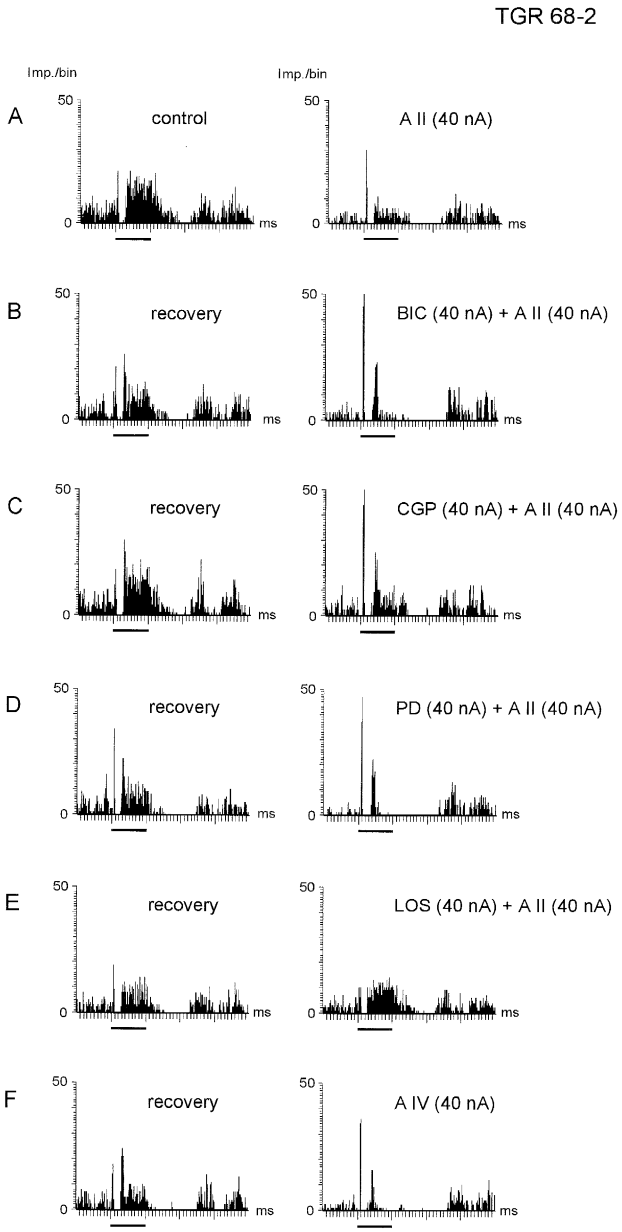

Fig. 4. Peristimulus time histograms of an OFF-like geniculate neuron. Angiotensin II (AII) induced suppression of the spontaneous as well as of the light-evoked activity (A). This effect could be blocked by an AT_2 receptor antagonist (PD 123,319) (B). Half an hour later angiotensin II repeatedly induced suppression of light-evoked activity (C) which could not be blocked by the angiotensin AT_1 receptor antagonist, losartan (D). The $GABA_A$ receptor antagonist, bicuculline, was able to block the angiotensin II-induced inhibition of the response to light (E). The *y*-axes indicate number of spikes per bin (bin width 10 ms), and the *x*-axes indicate the time in ms. The bars represent the time and the duration of light stimulation (500 ms).

II induced suppression of spontaneous activity as well as of the tonic response component to light stimulation. This suppression could not be blocked by the AT_2 receptor antagonist, whereas losartan antagonized the action of the iontophoretic ejection of angiotensin II. Bicuculline did not block the suppressing action of angiotensin II, while CGP

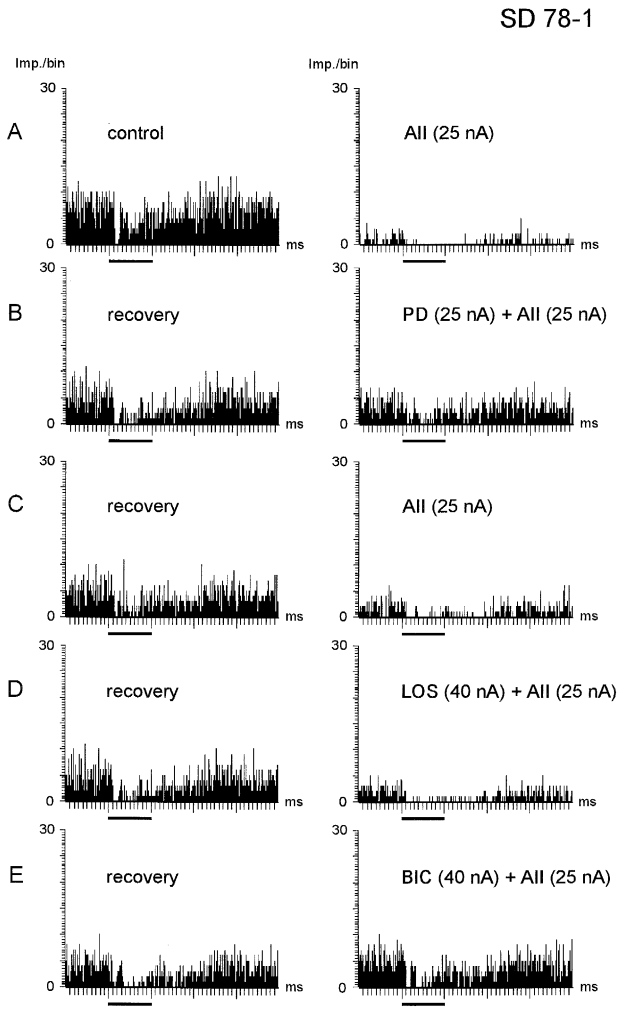

Fig. 5. Peristimulus time histograms of an ON-like geniculate neuron. Angiotensin II induced suppression of both spontaneous and light-evoked activity (A) which could not be blocked fully by either the $GABA_A$ receptor antagonist bicuculline (B), or by the $GABA_B$ receptor antagonist, CGP 35348 (C). This suppression of light-evoked activity was angiotensin AT_1 receptor- mediated because losartan effectively blocked it (E), whereas PD 123,319 did not antagonize the inhibitory effect of angiotensin II (D). The iontophoretic ejection of angiotensin IV also caused suppression of the response to light (F). The *y*-axes indicate number of spikes per bin (bin width 10 ms), and the *x*-axes indicate the time in ms. The bars represent the time and the duration of light stimulation (500 ms).

35348 had only a partial antagonizing effect. Interestingly, suppression of the tonic response by angiotensin II was accompanied by an increase of the first excitation in response to light. This facilitation of the primary response phase was blocked only by the angiotensin AT_1 receptor antagonist. Therefore, different phases of the response to light might be influenced differently by angiotensins. This assumption was confirmed by the analysis of ON- and OFF-components of the response to light. The figure also shows that angiotensin IV acts on the AT_4 receptor, as the iontophoretic ejection of angiotensin IV also induced suppression of the response to light immediately after the blockade of angiotensin AT_1 receptors by losartan.

The effect of acetylcholine on geniculate activity was tested in 50 neurons. In 15 (30%), acetylcholine caused an increase of the spontaneous discharge rate. Coadministration with angiotensins induced additive effects in nearly all cases.

3.4. Analysis of the discharge pattern

Finally, spontaneous or light-induced burst discharges were obtained in 70–80% of the geniculate neurons. These bursts consisted of 2–3 spikes. The activity of a geniculate neuron illustrated in Fig. 6 shows that angiotensin IV induced the development of bursts. The bursts disappeared in the recovery period. The iontophoretic ejection of angiotensin IV induced a significant increase of the absolute number of bursts ($P = 0.004$, Wilcoxon test, $N = 120$). Similarly to the effect of angiotensin IV on the discharge rate, the percentage of spikes involved in bursts also increased, i.e., the number of bursts increased coincidently with the enhancement of activity, whereas a decrease of the discharge frequency was accompanied by a reduction in the number of bursts.

In contrast to the effects of angiotensin IV, angiotensin II induced a decrease of the bursts in excited neurons and an increase in the occurrence of bursts in neurons inhibited by the peptide.

The number of spikes within an individual spontaneous or light-induced burst did not change significantly. In addition, in most cases silent periods prior to the first spike of a burst were mostly found to be greater than 100 ms.

Concerning the light-induced burst discharges, a similar decrease of the number of burst discharges mediated by angiotensin II was obtained in 8 out of 11 neurons which developed burst discharges, as was a change of more than 40% in the discharge rate. However, the iontophoretic ejection of angiotensin IV only induced an increase of light-evoked burst discharges in three neurons, whereas, in six neurons, the decrease in the light-evoked discharge rate was accompanied by an increase in the percentage of spikes involved in bursts. It should be mentioned that the

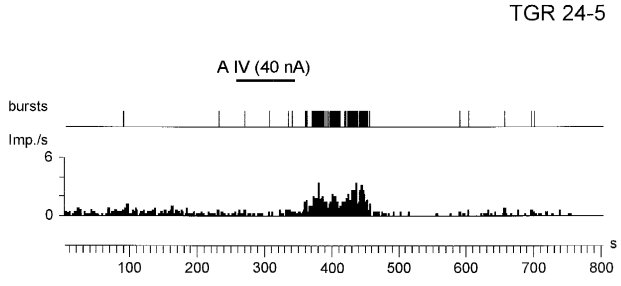

Fig. 6. Effect of angiotensin IV on burst activity. Angiotensin IV augmented the occurrence of bursts in this geniculate neuron. Effect appeared after cessation of iontophoretic ejection. Groups of potentials with interspike intervals ≤ 4 ms are marked as bursts (row above the frequency time histogram). The bars represent time and duration of the ejection, the numbers show current intensity.

discharge pattern was changed essentially with no clear changes in the discharge rate in some neurons.

4. Discussion

Our results showed that angiotensin II as well as angiotensin IV mainly increased the spontaneous activity of geniculate neurons that were responsive to angiotensins. This is consistent with results obtained in other brain structures where angiotensin II induced excitatory effects on neuronal activity (Felix, 1976; Ishibashi et al., 1985; Harding and Felix, 1987a; Jeulin and Nicolaidis, 1988; Bains et al., 1992; Barnes et al., 1993). With regard to visual structures, no angiotensin II-induced changes in the spontaneous neuronal activity were found in the superior colliculus (Mooney et al., 1994). However, in freshly dissociated retinal ganglion cells, increases as well as decreases in the high voltage-gated calcium current induced by angiotensin II were demonstrated (Guenther et al., 1996).

Angiotensin II causes direct inhibition of several K^+ channels in smooth muscle (Toro et al., 1990; Miyoshi and Nakaya, 1991). Elsewhere, angiotensin AT_1 receptors are coupled to numerous other types of ion channels, including Ca^{2+}, Cl^- and nonselective cationic ion channels (Baker et al., 1992; Yang et al., 1992; Bescond et al., 1994; Shapiro et al., 1994; Nagatomo et al., 1995). These results show that at least the angiotensin AT_1 receptor-mediated effects of angiotensin II should cause depolarization and, therefore, an increase in the discharge rate. Thus, we also found an enhancement of the activity mediated by angiotensin AT_1 receptor stimulation in geniculate neurons. The decrease in discharge frequency which also could be blocked by losartan, might have been due to the involvement of local circuit geniculate neurons. In our experiments, both $GABA_A$ and $GABA_B$ receptor antagonists blocked nearly all the inhibitory effects of angiotensins on spontaneous as well as on light-evoked activity. It can be supposed that iontophoretically ejected angiotensin from the multibarreled electrodes reached the interneurons in some cases and excited them and thus indirectly caused inhibition of the activity of the neuron which was investigated by the recording electrode. Moreover, as angiotensins are released from the multibarrel electrode like other transmitters (Harding and Felix, 1987b), it can be assumed that effects which were presumed to be direct and which arose from local drug diffusion were recorded within a zone with 50 μm radius around the microiontophoretic electrode there was a distance of 20 to 40 μm between our recording and the multibarrel electrode. Alterations in activity recorded at the most distal sites were found between 100 and 500 μm, whereas synaptic activity induced by remote drug ejection was seen most frequently within a zone 100–200 μm from the iontophoretic site (Hicks, 1984). A recently published model of the quantitative analysis of the various parameters relevant during iontophoresis (Nicholson, 1995) confirms these results.

In contrast to the angiotensin AT_1 receptors, angiotensin II elicited an AT_2 receptor-mediated increase in outward K^+ current in neurons cocultured from rat hypothalamus and brainstem (Kang et al., 1994). The resulting hyperpolarization is probably the cause of an angiotensin AT_2-mediated decrease of the discharge rate. If a similar signal transduction mechanism in the dorsal lateral geniculate nucleus is postulated, inhibitory effects of angiotensin II which could be blocked by AT_2 receptor antagonists might be due to opening of K^+ channels. As angiotensin AT_2 receptor antagonists also blocked excitatory effects induced by angiotensin II, other signal transduction mechanisms could exist in the dorsal lateral geniculate nucleus. On the other hand, angiotensin II could inhibit the activity of local circuit neurons and, in this way, result in excitation of projection neurons. It has been shown that acetylcholine excites the projection neurons and inhibits the local circuit neurons (Pape and McCormick, 1995). As we only recorded excitatory effects of acetylcholine, and as the percentage of smaller local circuit neurons within the dorsal lateral geniculate nucleus is lower than that of projection neurons (Gabbott et al., 1986), it can be supposed that we almost exclusively recorded the activity of projection neurons. The low responsivity of geniculate neurons to acetylcholine might have been due to an inhibitory influence of urethane anesthesia on cholinergic transmission (Crawford, 1970).

One may speculate that at least one of the roles of the angiotensins in the brain may be to modulate the frequency of appearance of burst discharges in the thalamus. Buisson et al. (1992) have postulated that the angiotensin AT_2 receptor subtype mediates a decrease in a T-type Ca^{2+} current. Consistent with these results we observed mainly a decrease of burst discharges induced by the iontophoretic ejection of angiotensin II, although increases of bursts were also obtained. This increase of burst discharges might be mediated by the angiotensin AT_1 receptor. In contrast to the main effect of angiotensin II, angiotensin IV predominantly induced an increase of the number of bursts which also coincided with an increase in the discharge rate of geniculate neurons.

In contrast to the finding of a predominant excitatory effect of angiotensins on spontaneous geniculate activity, the NMDA- and/or the kainate-induced increases in geniculate discharge rate were strongly suppressed by angiotensin II in most neurons. In the dorsal lateral geniculate nucleus this effect seems to be mediated by the angiotensin AT_1 receptor site, although it has to be taken into account that only few neurons were tested with antagonists. Comparable to our results with glutamate receptor agonists, angiotensin AT_1-mediated reduction of visual-evoked potentials has been found in the superior colliculus (Merabet et al., 1994). Mooney et al. (1994) observed that angiotensin II decreases the visual collicular responses and

the glutamate-elicited responses. These latter inhibitory effects were mediated by angiotensin AT_1 and AT_2 receptors and seemed to be postsynaptic. An angiotensin AT_2-mediated depression of glutamate excitations by angiotensin II has been found in the locus coeruleus (Xiong and Marshall, 1994).

If the depression of NMDA- and kainate actions by angiotensin II reflects a physiological role of this peptide, it should also be evident on synaptically released excitatory amino acids. This condition appears to be satisfied by the parallel action of angiotensin II on the effects exogenously applied excitatory amino acids and on light-evoked activity in our experiments. Investigations in rats (Crunelli et al., 1987) and cats (Sillito et al., 1990a,b) have provided strong support for a glutamate-like substance as transmitter of the optic nerve. The flash-evoked activity of many geniculate neurons was inhibited by angiotensin II. Light-evoked responses were inhibited by angiotensin II and angiotensin IV more frequently than spontaneous activity. Moreover, the angiotensin-induced effect on light responses in a few geniculate cells could not be blocked by GABA receptor antagonists. Therefore, it might be that not all inhibitory actions of angiotensins were mediated by excitation of local-circuit interneurons.

In our experiments, we found a change in the discharge rate induced by angiotensin II as well as by angiotensin IV in 26% of the investigated neurons. Therefore, it can be supposed that various angiotensin receptors may be colocalized in some thalamic neurons. The presumed coexistence of angiotensin AT_1 and AT_2 receptors on the same neuron should be taken into account since, after blockade of angiotensin AT_1 receptors, angiotensin II levels increase several-fold, possibly overstimulating the unblocked, unprotected angiotensin AT_2 receptor (Johnston and Burrel, 1995).

Angiotensin II has long been shown to down-regulate its own receptor. We used angiotensin concentrations $10\times$ lower than those usually administered in in vivo experiments. Nevertheless, the repeated ejection of angiotensin II at short intervals caused reduction or loss of the neuronal response, although the release rate should have been lower than 17.9 fmol/min per nA in our experiments. This release rate was determined for 1 mM solutions of angiotensin II (pH 4.5) ejected from 5-barrel glass micropipettes with tip diameters of 4 μm (Harding and Felix, 1987b). As desensitization was found to be lower during stimulation of glutamate receptors, this mechanism might depend on the actual membrane potential of the neuron.

It can be supposed that the changed level, at least of angiotensin II, and perhaps of angiotensin IV, in transgenic animals might upregulate the angiotensin receptors, thus increasing sensitivity to angiotensin. With the exception of the prolonged duration and the shortened latency of the angiotensin effects in transgenic rats, the present results provide no clear evidence for differences in the responsiveness of different strains of rats. Similar results were found for the somatosensory thalamus, but we have observed that the treatment with angiotensin converting enzyme inhibitors evidenced a significantly reduced neuronal responsiveness to angiotensins in Sprague–Dawley rats compared with that in the untreated Sprague–Dawley controls. Moreover, the responsiveness of somatosensory neurons was significantly greater in transgenic than in Sprague–Dawley rats while both were treated with angiotensin converting enzyme inhibitors (Albrecht et al., unpublished observation). Results for the neuronal responsiveness to angiotensin II in spontaneously hypertensive rats are contradictory. The neurons of the rostral ventrolateral medulla showed a higher sensitivity and responsiveness to the iontophoretic administration of angiotensin II in spontaneously hypertensive rats than in normotensive Wistar Kyoto rats (Chan et al., 1991). However, neurons in the subfornical organ of spontaneously hypertensive rats tended to have a lower and shorter lasting response to angiotensin than those from the Wistar Kyoto controls (Yang et al., 1994). The various angiotensin receptors may be regulated differently in hypertensive rats, as shown for kidney of spontaneously hypertensive rats (Haddad and Garcia, 1996). Angiotensin II receptors of the renal vasculature (AT_{1B}) were upregulated, while those of the glomeruli tended to be downregulated (AT_{1A}).

In summary, the high degree of correlation between the distribution of angiotensin receptor binding sites, angiotensin-responsive neurons, and angiotensin-immunoreactive terminal fields supports the hypothesis that angiotensin II as well as angiotensin IV act as neurotransmitters or neuromodulators in the dorsal lateral geniculate nucleus.

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