

Effects of angiotensin II on visual evoked potentials in the superior colliculus of juvenile rats

G. Coudé, A. Marois, C. Casanova

Laboratoire des Neurosciences de la Vision, École d'optométrie, Université de Montréal, Montréal, Québec, Canada

Summary There are age-related changes in the relative expression of the AT₁ and AT₂ receptors of angiotensin II (Ang II) in brain regions such as the superior colliculus, a midbrain visual structure where both receptor subtypes are found. We investigated the effects of Ang II on gross visual activity in the colliculus of anesthetized rats aged between 15 and 35 post-natal days. Microinjection of Ang II in the superficial layers yielded a strong reduction in the amplitude of visual evoked potentials in a dose-related manner. Injection of the peptide in more ventral collicular layers did not modify the potential confirming the discrete localization of the angiotensinergic receptors in the superficial layers. Preliminary data indicated that the co-injection of Ang II with Losartan or PD 123319 yielded a partial blockade of Ang II suppressive effects, indicating that both AT₁ and AT₂ receptors are likely to be involved in mediating these responses. Overall, this study shows that the inhibitory nature of Ang II action is similar in juvenile and adult animals (Merabet et al. 1994 and Merabet et al. 1997) © 2000 Harcourt Publishers Ltd

INTRODUCTION

It is now clearly established that central angiotensin II (Ang II) is not solely associated with fluid homeostasis and blood pressure control because it can modulate neuronal activity in regions of the brain involved in cognition (Wayner et al., 1995; Walther et al., 1999; Winnicka and Wisniewski, 1999), motor control (Ambühl et al., 1992; Raghavendra et al., 1998), and sensory integration (Jacobi et al., 1994; Albrecht et al., 1997). Consistent with these findings, we reported that local intra-cerebral administration of Ang II reduces the amplitude of visually evoked potentials in the superficial layers of the superior colliculus (SC) in adult rats (Merabet et al., 1994). Comparable findings were reported in the SC of adult hamsters (Mooney et al., 1994). The SC contains both AT₁ and AT₂ receptors subtypes (Michels et al., 1994; Wright and Harding, 1994) and the inhibitory action of Ang II on adult rat collicular gross visual activity was shown for the

most part to be mediated by activation of AT₁ receptors (Merabet et al., 1997). This observation is in agreement with the general view that Ang II exerts its physiological action through AT₁ receptor sub-types. Only a few *in vitro* studies have revealed a clear physiological role of the AT₂ receptors in the neuromodulatory activity of Ang II (Ambühl et al., 1992; Xiong and Marshall, 1994). AT₂ receptors have been associated with repair processes and neuronal development and maturation, depending on the experimental model (Wright et al., 1995; Shenoy et al., 1999). This conclusion is derived in part from the fact that in some structures (e.g. the lateral geniculate nucleus [Michels et al., 1994], AT₂ receptors are only found during the fetal and post-natal developmental period and that in other structures (such as the SC), the density of this receptor sub-type declines with age. (Cook et al., 1991; Millan et al., 1991; Rowe et al., 1991; Tsutsumi and Saavedra, 1991a; Tsutsumi and Saavedra, 1991b; Tsutsumi et al., 1993).

Baxter et al. (1980) showed that there is an increase in the density of angiotensinergic receptors in the brain during the first 2 weeks after birth. In the midbrain, the number of receptors peaks at post-natal day 14 (PND 14) which corresponds to the time of opening of the eyelids and the onset of visual activity in the SC (Molotchnikoff and Itaya, 1993). The Ang II receptor density then decreases and reaches near adult values after PND 40. To

Received 14 February 2000

Accepted 2 June 2000

Correspondence to: Christian Casanova, Laboratoire des Neurosciences de la Vision, École d'optométrie, Université de Montréal, CP 6128, Succ. Centre-Ville, Montréal, Québec, Canada H3C 3J7. Tel.: +1514 343 2407 Fax: +1514 343 2382; E-mail: casanovc@ere.umontreal.ca

our knowledge, no studies have investigated *in vivo* the physiological action of Ang II in young animals. It is possible, given the changes in receptor density and the relative proportions of AT₁-AT₂ receptors during brain development (Millan et al., 1991; Tsutsumi and Saavedra, 1991a; Tsutsumi and Saavedra, 1991b), that Ang II may have different physiological effects in young and adult animals. In the present study we investigated whether Ang II can modulate visual evoked activity in the SC of juvenile rats using an experimental approach similar to that previously utilized in adult animals (Merabet et al., 1994; Merabet et al., 1997). Parts of these findings have been presented in abstract form (Marois et al., 1996; Patry et al., 1997).

METHODS

Animal Preparation

Experiments were performed on normotensive Long Evans rats aged between 14–35 days and weighing between 30 and 130 g. All animals were treated according to the guidelines of the Canadian Council on Animal Care. Anesthesia was induced by intra-peritoneal (i.p.) injection of Urethane 25% (2.5 g/kg). Atropine (0.04 mg/kg) was added to the solution for rats weighing more than 50 g to reduce tracheal secretions. The level of anesthesia was monitored throughout the experiment (i.e., using a leg stretch or pinch reflex) and supplemental doses of anesthetic were administered when needed. The pups were placed in a stereotaxic head holder designed to secure small animals (Stoelting). The electrocardiogram (ECG) was continuously monitored and the core temperature was maintained near 37°C by a custom made small heating pad (FHC) placed under the animal. Atropine sulfate (1%) eye drops were used to dilate the pupils and the corneas were protected by application of artificial tears. Lidocaine hydrochloride (2%) was administered at all points of pressure and incision. A bilateral craniotomy was performed anterior to *lambda* to expose the cerebral cortex overlying the SC of each hemisphere. The dura was incised and reflected only for animals older than PND 30. A surgical microscope was used to lower a glass microelectrode until the tip just penetrated the cortex. The skull opening was filled with agar to protect the tissues from dessication. Using a micromanipulator, the microelectrode was advanced vertically through the cortex and into the superficial layers of the SC on the basis of visual response cues, and for older animals, adult stereotaxic coordinates. At the end of all experiments, a solution of artificial cerebrospinal fluid (aCSF) stained with Chicago Sky Blue (0.2–0.5%) was injected to confirm that the electrode was located in the superficial layers of the colliculus (see Fig. 1).

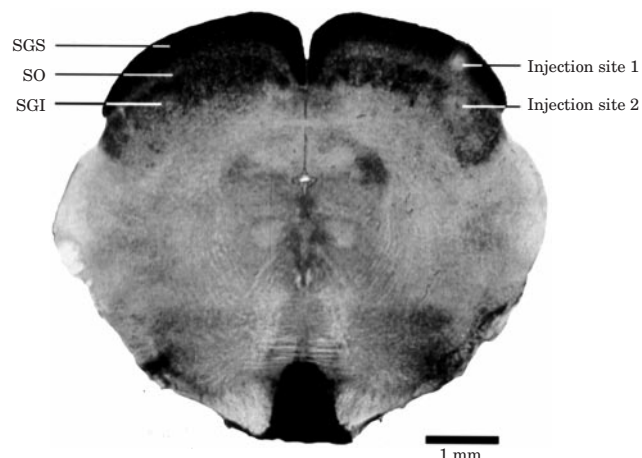


Fig. 1 Photograph of a coronal section of a juvenile rat brain stained to reveal acetylcholinesterase (AChE) activity. The Ang II injections and recordings sites appear pale when compared to neighboring regions. Note that a first injection was located in the stratum griseum superficiale (SGS) while a second one was made in the stratum griseum intermediale (SGI). Abbreviation: (SO); stratum opticum.

Recording and Visual Stimulation

Glass microelectrodes with a tip opening of ~25 µm were used to record visual evoked potentials (VEP) in the SC (see Merabet et al., 1997 for details). The same microelectrode was used to deliver the peptide or its antagonists (see below). The signals were amplified and bandpass filtered between 10 and 1000 Hz. The recorded signals were also passed through an audio monitor and an oscilloscope. They were also fed to a PC compatible computer via an analogue/digital interface (CED 1401, CED Cambridge, UK). Triggered evoked potentials were averaged over 35 successive presentations using the SIGAVG software and later on, its MS Windows® version (SIGNAL v.1.x; CED, Cambridge, UK). Gross visual stimulation was provided by a diffuse flash (Grass photostimulator, intensity level 4; duration 10 µs) placed 30 cm away from the contralateral eye of the hemisphere being recorded from. The stimulus frequency was set at 1 Hz.

Delivery of Pharmacological Agents

To permit a direct comparison between the effects of Ang II in juvenile and mature animals, we followed the same injection technique as that used in adult rats (Merabet et al., 1994; Merabet et al., 1997). The glass microelectrode filled with the drug was inserted in the head of a nanopump (WPI A1400 nanoliter injector) modified to allow the simultaneous recording of neuronal activity from the region immediately beneath the tip of the electrode. In the first experiments, Ang II (val⁵ Ang II: Ciba-Geigy Ltd) was dissolved in NaCl (0.9%). It was later

diluted in a more physiological medium, artificial cerebrospinal fluid (aCSF: in mM; NaCl 124, KCl 3, KH₂PO₄ 1.25, MgSO₄ 2.5, NaHCO₃ 26, d-glucose 10, 1-ascorbate 2, and CaCl₂ 3.4; osmolarity: 297 mOsm; pH = 7.2–7.3). The peptide was administered by micropressure at concentrations varying from 10⁻³ M to 10⁻¹² M at a constant rate of 10 nl/min for a total of 40 nl for 4 min. Studies of the effect of the specific AT₁ receptor antagonist Losartan (DUP 753) were carried out at 10⁻³ or 10⁻⁶ M and injected in conjunction with Ang II at equal concentrations ratios (1:1) but at an increased antagonist ratio (1:10 Ang II:Losartan) (Merabet et al., 1997). A similar procedure was used to study the effect of the AT₂ receptor antagonist PD 123319.

Experimental Protocol and Data Analysis

The electrode was lowered and positioned at the surface of the colliculus (determined by the first appearance of a visual response to the flash presentation). The electrode was then slowly advanced and positioned in the superficial layers of the SC on the basis of the VEP waveform. The 'multi-unit' receptive field at the recording site was mapped. Recordings of the VEP were taken at approximately 1–2 min intervals until the waveform and amplitude appeared stable. Then, three successive control recordings were taken and the pharmacological agent was injected (the injection onset represents time at zero). The VEP was recorded every minute throughout the period of injection. Once injection was completed, recordings were taken 1, 3 and 6 min after injection and then every 5 min until a partial or total recovery of the VEP amplitude was observed. The area, amplitude and latency of the evoked potential waveform were calculated before, during, and after injection of the peptide. There was an intimate relationship between the measurements based on both the amplitude of the potential and the area under the curve ($r(45)=0.85$, $P < 0.001$), and the former was used to quantify the effects as was the case in our previous studies of adult animals (Merabet et al., 1994; Merabet et al., 1997). The calculated values were all normalized and the magnitude of the effect was expressed as a percentage (of the initial amplitude control measurements) to facilitate comparison across experiments. Overall results are expressed as means \pm SEM and statistical significance of the observed effects was determined using one-way ANOVA with Dunnett's post hoc test or ANOVA on ranks (Kruskal–Wallis) with Dunn's multiple comparison test depending on the distribution of variables ($*P < 0.05$; $**P < 0.01$). Percentage data were subjected to arcsin-square root transformation before analysis. The injection was considered as successful only when: 1) control recordings were stable; 2) there was a clear time relationship between the injection period and

the potential changes; 3) the electrode was not obstructed (as confirmed by the characteristic sound of the pump during injection and by the fact that the solution could be released freely when the electrode was removed from the brain); 4) there was a partial or total recovery of the signal (to at least 75% of the control recordings); 5) the stained solution administered at the end of the experiment was located in the *stratum griseum superficiale*.

Histology

Each animal was given an overdose of Halothane by inhalation. The brain was then removed and fixed in buffered formalin (10%) for a period of 1 week. The fixed tissue was then cut in 100 μ m coronal sections and stained to reveal acetylcholinesterase (AChE) activity. For each experiment, histological observation was carried out to confirm the position of the Chicago Sky Blue stained solution. Figure 1 depicts a photograph of a coronal plane through a rat SC showing the injection of Ang II in the superficial layers and a second more ventral injection in the *stratum griseum intermediale* (SGI).

RESULTS

General observations

Out of 216 collicular injections, 134 were considered successful based upon the criteria described in the Methods section. At effective concentrations, Ang II always reduced the amplitude of the VEP with a maximum effect generally observed during or immediately after the end of injection. There was no change of the VEP latency. Representative examples are depicted in Figure 2. The averaged control potentials shown in the top panels are decreased by 49% and 44% respectively after the administration of 10⁻³ and 10⁻⁶ M Ang II. An almost complete recovery of the signal was observed 15 min after the end of injection (bottom panels). Panels A and B of Figure 3 show the profiles of action of the peptide for two different concentrations and are represented by the normalized amplitude of the VEP as a function of time. The reduction in the amplitude of the potentials occurred shortly after the onset of injection and the time course of the effect was much more abrupt than the recovery period. These last examples further suggest that the magnitude of the inhibition depends on the peptide concentration.

Dose Response Relationship

Ang II was injected at four different concentrations in order to reveal any concentration-dependent effect of the

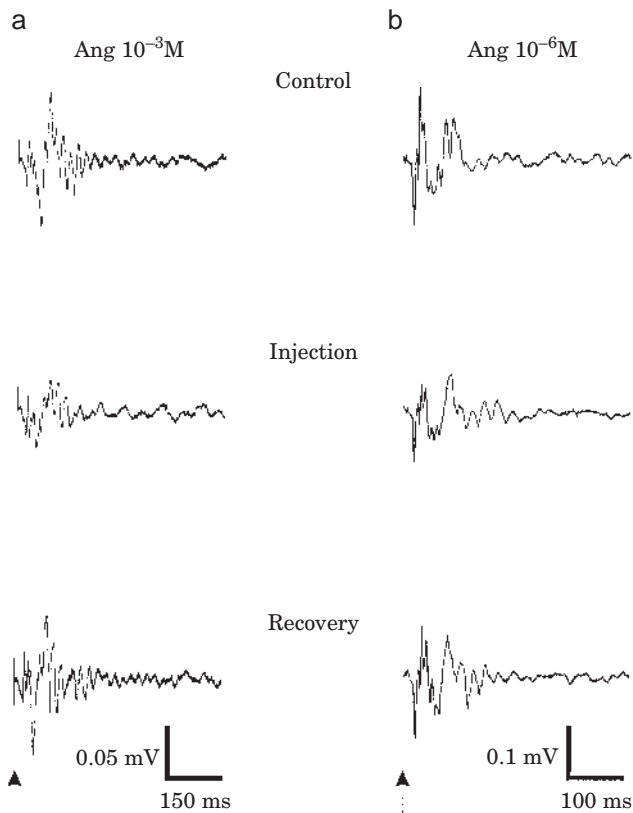


Fig. 2 Representative examples showing the effect of the injection of Ang II at two different concentrations (10^{-3} and 10^{-6} M in columns A and B, respectively) on VEPs recorded from the SGS of the SC. The peptide clearly reduced the amplitude of the VEP. An almost complete recovery of the potential was evident approximately 15 min after injection. The arrows represent stimulus onset. Injections in A and B were made at PND 25 and 22, respectively.

peptide. Overall, the strength of the inhibitory effect of Ang II tended to decrease as the concentration of the peptide was lowered (Fig. 4A). The concentrations of 10^{-3} , 10^{-6} , 10^{-9} , 10^{-12} M yielded mean (\pm SEM) reductions in amplitude of the VEP of 45.4 ± 2.2 , 42.2 ± 3.0 , 34.5 ± 4 and $38.7 \pm 2.9\%$, respectively. ANOVA showed a significant effect of Ang II concentration on the VEP amplitude ($F(4,106)=5.05$; $P < 0.001$). Dunnett's comparisons between each Ang II concentration and control aCSF injections (mean reduction of $25.5 \pm 3.3\%$) indicated that the mean reductions observed at concentrations of 10^{-3} M and 10^{-6} M were significant ($P < 0.01$). There was also a relationship between the Ang II concentration and the mean recovery time ($H(4)=13.53$; $P < 0.01$). The VEP reached 75% of its initial amplitude after 25 and 21 min for, respectively, Ang II concentrations of 10^{-3} M and 10^{-6} M. The injections of Ang II at lower concentrations (10^{-9} and 10^{-12} M) were characterized by shorter recovery periods (14 and 10 min respectively). The duration of recovery at any concentration was not significantly different

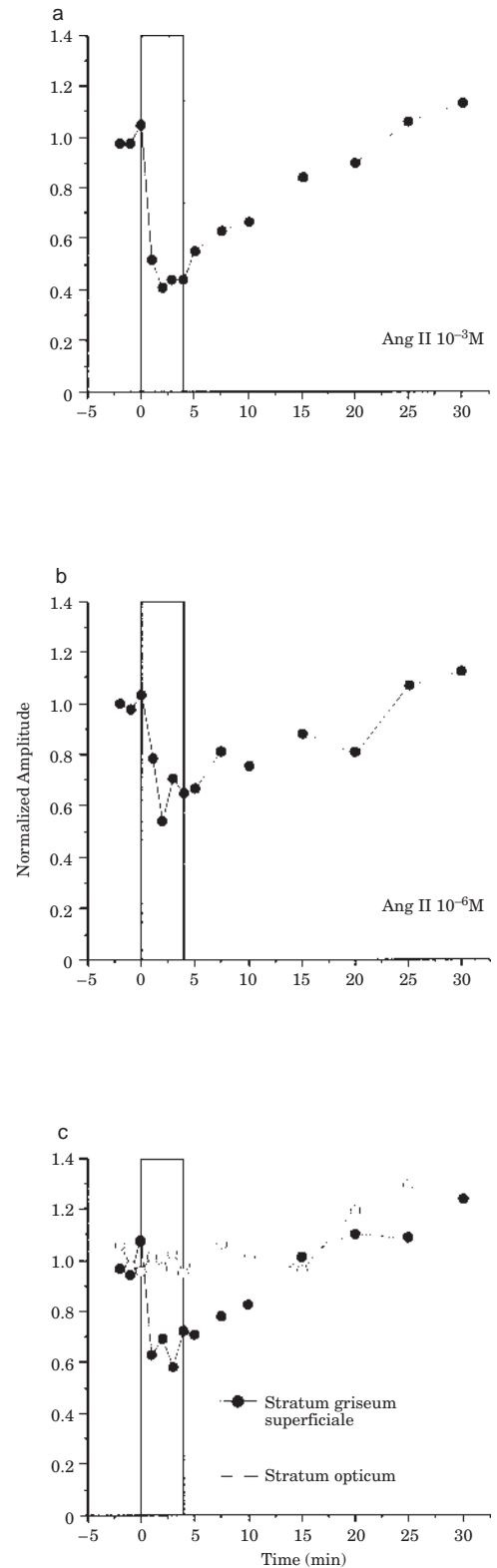


Fig. 3 Panels A and B show representative examples of the profile of action of Ang II injected at two different concentrations. Normalized amplitude is plotted as a function of time. The vertically oriented rectangle represents the period of injection. The onset of injection is at time 0. Panel C illustrates the effects of Ang II (10^{-3} M) as a function of depth in the colliculus. Injections in A, B and C were made at PND 24, 20 and 25, respectively.

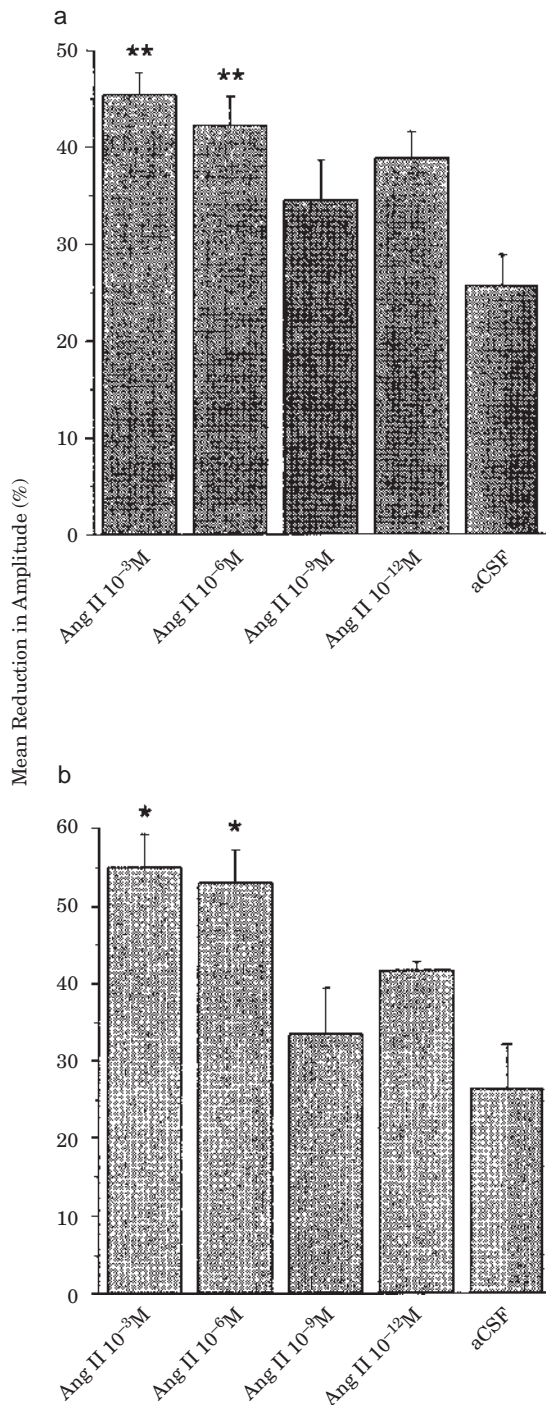


Fig. 4 Dose dependent inhibitory effect of Ang II as a function of the peptide concentration. In panel A, injections in all animal groups were pooled. The effects are compared to aCSF control injections, and were significant (Dunn's test, $P < 0.01$) for concentrations $>10^{-9}$ M. Panel B shows the mean reduction in amplitude observed for the youngest animal group (PND 15–19). Note that the maximum effect of Ang II was noted for juvenile rats aged between 15 and 19 days. The mean reduction in amplitude observed at 10^{-3} and 10^{-6} M was significantly different from that computed after administration of the vehicle (Dunn's test, $P < 0.05$). Number of injections for Ang II 10^{-3} , 10^{-6} , 10^{-9} , 10^{-12} M and aCSF are 58, 26, 8, 6 and 17, respectively for panel A and 15, 9, 3, 4 and 6 respectively for panel B.

from that observed with aCSF ($P > 0.05$, Dunn's test). In summary, the magnitude of the inhibitory action of Ang II in juvenile rats and the duration of the associated recovery period tended to increase as the concentration of the peptide increased.

Localization of effects

We verified the laminar localization of the Ang II receptors by injecting the peptide in different layers of the colliculus. In all six cases, Ang II exerted its inhibitory action only when the injection was confined to the SGS. Injection below this visual layer yielded no significant change in the latency and magnitude of the VEP. An example is shown in panel C of Figure 3. As expected, the administration of Ang II in the SGS decreased the strength of the visual response (unfilled circles). A second injection, made more ventrally (600 μ m below the first site, in the *stratum optimum*) did not modify the signal.

Influence of age

We investigated the possibility that the action of Ang II may differ during the visual development of juvenile rats. In particular, we were interested in determining whether the effect of Ang II during the first week following the onset of visual activity in the colliculus was analogous or not to the effect observed in adulthood. Figure 4B shows that the effect in the youngest animals (PND 15–19) was similar to that observed for our complete sample (Fig. 4A) and that previously reported for the adult (Merabet et al., 1997). It is of note that Ang II tended to be more effective in the younger group of juvenile rats (PND 15–19), these are the visually active animals in which Ang II receptors are the most common (Baxter et al., 1980). Despite this latter observation, our study did not reveal significant differential effects of Ang II, neither at a concentration of 10^{-3} M ($F(3,57) = 2.04$; $P > 0.05$) nor at 10^{-6} M ($H(2) = 4.90$, $P > 0.05$) throughout the targeted development period.

Receptor subtypes

In order to compare the contribution of the two receptors in the inhibitory action of Ang II between juvenile and adult rats, we investigated the effect of Ang II with the presence of selective AT_1 and AT_2 antagonists for animals aged between 15 and 24 days which is a period characterized by a high density of Ang II receptors in the mid-brain (Baxter et al., 1980). In one series of experiments, Ang II was injected with the AT_1 receptor antagonist Losartan (DUP 753). Co-injection of Losartan and Ang II at equal concentrations (10^{-3} or 10^{-6} M) yielded a partial

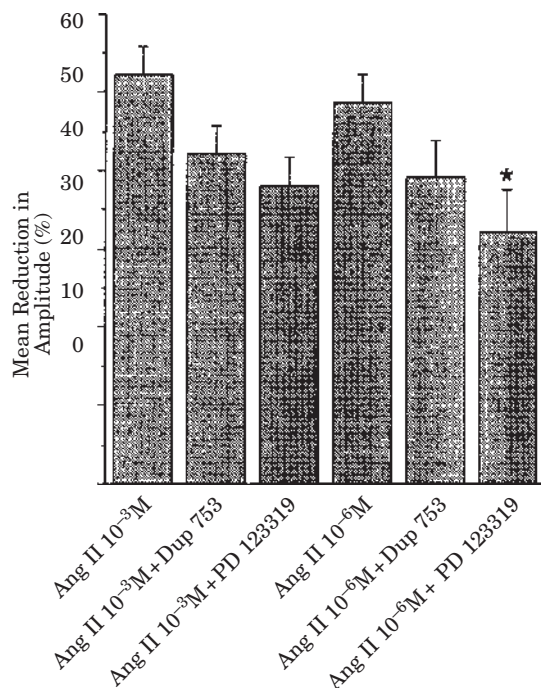


Fig. 5 Injection of Ang II with AT_1 and AT_2 antagonists on the VEP amplitude at two different concentrations. Each bar represents the mean \pm SEM. Injection of Ang II with the AT_1 antagonist shows a slight blockade of the Ang II inhibitory effect. Similarly, injection of Ang II with the AT_2 receptor antagonist PD 123319 partially blocked the inhibitory action of the peptide (Dunnett's test, $P < 0.05$). Number of injections from left to right is 25, 15, 13, 13, 10 and 8.

blockade of the inhibitory effect of Ang II (Fig. 5). In a second set of experiments, the effect of PD 123319, which specifically binds to AT_2 receptors, was studied. PD 123319 also reduced the effect of Ang II at the two concentrations used (10^{-3} and 10^{-6} M). This effect was more pronounced than that observed for the presence of Losartan, particularly at 10^{-6} M where it reached statistical significance ($F(2,28) = 3.67$, $P < 0.05$; Dunnett's test, $P < 0.05$). Despite the relatively small number of injections, these observations suggest that both AT_1 and AT_2 receptors are likely to be involved in mediating the suppressive effect of Ang II in juvenile animals, with a greater effect on AT_2 receptors. In eight cases, Ang II was injected with both Losartan and PD 123319. The overall degree of blockade when both antagonists were present was not significantly greater than that observed with a single antagonist ($F(2,49) = 0.24$, $P > 0.05$).

Indirect peripheral effects

For 14 successful Ang II injections, we also quantitatively measured the ECG frequency throughout the control and injection periods to determine whether the inhibitory effect of Ang II in the colliculus of juvenile rats was associated with peripheral changes. In our

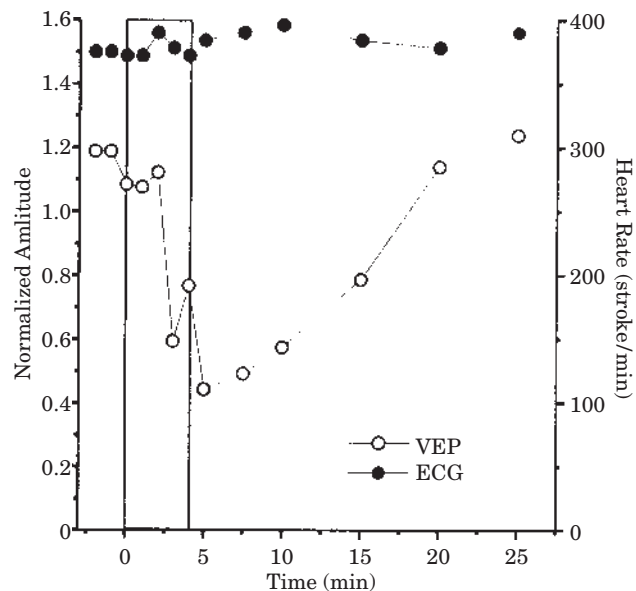


Fig. 6 Injection of Ang II at a high concentration (10^{-3} M) did not significantly modify the frequency of the cardiac rhythm but strongly reduced the amplitude of the visual evoked potential.

experimental conditions, we did not observe any significant changes in heart rate frequency at any of the tested Ang II concentrations (repeated ANOVA, $F(4,49) = 0.36$, $P > 0.05$). As shown in Figure 6, the injection of Ang II yielded a decrease of collicular activity (empty symbols), whereas the ECG frequency was not affected (filled symbols).

DISCUSSION

To our knowledge, this study is the first to demonstrate in vivo physiological effects of Ang II in brain structures of juvenile animals. Our results indicate that Ang II exerts an inhibitory action on collicular evoked potentials, an effect that is comparable to that observed in adult rats (Merabet et al., 1997). These effects were confined to the superficial layers of the SC in agreement with the anatomical location of angiotensinergic receptors revealed by autoradiographic studies (Michels et al., 1994). This last observation also rules out the possibility that the action of Ang II reported in the present study arises from direct action of the peptide on the vascular circuitry within the colliculus (see Merabet et al., 1997). By injecting Ang II at various concentrations (10^{-3} M to 10^{-12} M), we demonstrated that the effects of the peptide express a dose-related dependency. In contrast to adult animals (Merabet et al., 1994; Merabet et al., 1997), we did not observe a clear dose-response relationship for concentrations less than 10^{-6} M, as shown in Figure 4. There are several possible explanations for this finding.

1. A smaller range of concentrations was tested in the juvenile rats compared to adult animals, so we did not get an effect. In our previous study, the action of Ang II at eight concentrations ranging from 10^{-3} to 10^{-10} M was studied (Merabet et al., 1997). 2. The effects at the lowest concentrations, and particularly at 10^{-9} M, could have been masked by the perturbation caused by the injection per se. Comparing the results from this study and that of Merabet et al's study 1997 demonstrated that injection of the vehicle created a more pronounced transient perturbation in young than in adult animals (mean transient reduction of approximately 7% and 25% respectively). It is likely this difference occurs because the nervous tissue of immature animals is more sensitive to mechanical stress (Akaoka et al., 1992). 3. It is also possible that the receptors in young animals are less sensitive to exogenous Ang II or that the cascade of cellular events triggered by Ang II are not fully developed. Co-administration of the aminopeptidase inhibitor amastatin with Ang II did not increase the strength or duration of the Ang II effects on collicular potentials (data not shown) ruling out, to some extent, the possibility that the activity of the peptidases may be different in juvenile and adult animals. 4. Finally, the lack of effect at lower Ang II concentration may reflect the functional immaturity of the nervous tissue of juvenile animals. We know that only a limited number of synaptic connections in the young will be consolidated and maintained in the adult (Blue and Parnaveles, 1983; Miller et al., 1983), so the greater concentration of Ang II receptors found in juvenile animals (Baxter et al., 1980) may be associated with non-functional synapses.

Preliminary data also suggest that the inhibitory action of Ang II in animals aged between 14 and 25 days involves activation of both AT_1 and AT_2 receptors. Again the data are different from those observed in adults (Merabet et al., 1997) as 1. the strength of the blockade was more pronounced in mature rats and; 2. in young animals, the blocking effect of the AT_2 receptors was more effective than that of the AT_1 receptors. The latter observation is interesting because it is consistent with the finding that the AT_2 receptor sub-type is more common in young than in adult animals (Wright and Harding, 1994; Millan et al., 1991; Tsutsumi et al., 1991a). The fact that we could not observe a strong blockade of the inhibitory action of Ang II with either losartan or PD 123319 in juvenile rats was surprising. Several explanations can be proposed. 1. Again, this may be related to the changes induced by the injection per se that may have masked the more subtle effects of the antagonists. 2. It is also possible that the receptors' affinity to the antagonists is lower in immature animals, or that these antagonists bind to other receptors thereby reducing their efficacy (unspecific binding). 3. Moreover,

it is possible that the antagonists failed to effectively block the Ang II mediated effects because of an immediate action of Ang II. We used the same administration techniques as in adult animals (Merabet et al., 1997), which could indicate that the kinetics of the diffusion or action of Ang II differs between juvenile and mature rats. 4. A further possibility is that there is extensive inhibitory cross-talk (Gelband et al., 1997) between these two major receptors (AT_1 and AT_2) during development. For example, blocking the AT_2 receptors would reduce the inhibitory action on the AT_1 receptors, and increase the affinity of AT_1 receptors to Ang II, yielding a reduction of the VEP despite the presence of the antagonist. However, our observation that the blocking of both receptor sub-types did not fully block the Ang II mediated effects does not support this last explanation. However, these same observations are consistent with an alternative explanation regarding the weak effects of the Ang II antagonists in immature animals. It is possible that part of the action of Ang II was indirectly mediated through AT_4 receptors which are also expressed in the SC (Wright et al., 1995).

Finally, we did not observe any significant changes in cardiac rhythm during the administration of Ang II in the superficial layers of the SC of young animals. This observation is at odds with the data of D'Amico et al. (1998) which indicated that Ang II yielded cardiovascular changes in adult rats. This discrepancy may be related to the difference in the age of the animals used in both studies. Unfortunately, we did not quantitatively analyze the ECG changes in our previous study using mature animals; (Merabet et al., 1994; Merabet et al., 1997) therefore it is possible that such changes occurred during the administration of the peptide but that they were not evident based on informal qualitative observations by those experimenters. Another factor that may have contributed to this disparity between the present study and that of D'Amico et al. (1998) is that in our investigation, only 40 nl were injected over a period of 4 min while D'Amico and collaborators administered a larger volume (100 nl) over a very brief time period (5 sec).

In conclusion, we have shown that Ang II induces a reduction of VEPs in the superficial layers of the SC of juvenile rats. This is the first in vivo demonstration that Ang II has a physiological action on the activity of neurons of immature animals. Therefore, Ang II can modulate the activity of the SC, a structure involved in visuomotor functions, and consequently may influence the exploratory and avoidance behavior of the animals throughout their development. Preliminary data further suggest that the Ang II effects are mediated only in part by AT_1 and AT_2 receptors, with a greater contribution from the latter receptor sub-type.

ACKNOWLEDGMENTS

We are grateful to François Jolicoeur for his comments and suggestions and to O. Patry for his help in some experiments. Part of the salary of CC came from the 'Fonds de la Recherche en Santé du Québec' (FRSQ). GC and AM were respectively supported by 'FCAR centre' and 'FRSQ-FCAR santé' scholarships.

This work was supported by a team grant (#MT-13391) from the Medical Research Council of Canada held in collaboration with Nicole Gallo-Payet and Marcel Daniel Payet of the Université de Sherbrooke.

REFERENCES

- Akaoka H, Saunier CF, Chergui K, Charlety P, Buda M, Chouvet G. Combining in vivo volume-controlled pressure microinjection with extracellular unit recording. *Journal of Neuroscience Methods* 1992; 42: 119–128.
- Albrecht D, Broser M, Kruger H, Bader M. Effects of angiotensin II and IV on geniculate activity in nontransgenic and transgenic rats. *European Journal of Pharmacology* 1997; 332: 53–63.
- Ambühl P, Felix D, Imboden H, Khosla MC, Ferrario CM. Effect of angiotensin II and its selective antagonists on inferior olivary neurones. *Regulatory Peptides* 1992; 41: 19–26.
- Baxter CR, Horvath JS, Duggin GG, Tiller DJ. Effect of age on specific angiotensin II-binding sites in rat brain. *Endocrinology* 1980; 106: 995–999.
- Blue ME, Parnavelas JG. The formation and maturation of synapses in the visual cortex of the rat. II. Quantitative analysis. *Journal of Neurocytology* 1983; 12: 697–712.
- Cook VI, Grove KI, McMennamin KM, Carter MR, Harding JW, Speth RC. The AT₂ angiotensin receptor subtype predominates in the 18 day gestation fetal rat brain. *Brain Research* 1991; 560: 334–336.
- D'Amico M, Di Filippo C, Rossi F, Warner TD. Role of AT₂ receptors in the cardiovascular events following microinjection of angiotensin II into the superior colliculus of anaesthetized rats. *Naunyn-Schmiedeberg's Archives of Pharmacology* 1998; 357: 121–125.
- Gelband CH, Zhu M, Lu D et al. Functional interactions between neuronal AT₁ and AT₂ receptors. *Endocrinology* 1997; 138: 2195–2198.
- Jacobi PC, Osswald H, Jurklics B, Zrenner E. Neuromodulatory effects of the renin-angiotensin system on the cat electroretinogram. *Investigative Ophthalmology and Visual Science* 1994; 35: 973–980.
- Marois A, Darveau S, Casanova C. Effects of angiotensin II on the visual responses of the rat's superior colliculus during neonatal development. *Society for Neuroscience abstract* 1996; 22: 635.
- Merabet L, de Gasparo M, Casanova C. Neuromodulatory effects of angiotensin II in the visual layers of the rat superior colliculus. *Neuroreport* 1994; 5: 2649–2652.
- Merabet L, de Gasparo M, Casanova C. Dose-dependent inhibitory effects of angiotensin II on visual responses of the rat superior colliculus: AT₁ and AT₂ receptor contributions. *Neuropeptides* 1997; 31: 469–481.
- Michels KM, Heemskerk FM, Saavedra JM. Selective changes in angiotensin II AT₁ and AT₂ receptor subtypes in the rat superior colliculus following eye enucleation. *Neuroscience* 1994; 58: 835–844.
- Millan MA, Jacobowitz DM, Aguilera G, Catt KJ. Differential distribution of AT₁ and AT₂ angiotensin II receptor subtypes in the rat brain during development. *Proceedings of the National Academy of Sciences of the USA* 1991; 88: 11440–11444.
- Miller AJ, McKoon M, Pinneau M, Silverstein R. Postnatal synaptic development of the rat. *Brain Research* 1983; 284: 205–213.
- Molotchnikoff S, Itaya SK. Functional development of the neonatal rat retinotectal pathway. *Brain Research Developmental Brain Research* 1993; 72: 300–304.
- Mooney RD, Zhang Y, Rhoades RW. Effects of angiotensin II on visual neurons in the superficial laminae of the hamster's superior colliculus. *Visual Neuroscience* 1994; 11: 1163–1173.
- Patry O, Marois A, Casanova C. Effects of angiotensin II on the visual activity of the rat's superior colliculus during neonatal development: implication of AT₁ and AT₂ receptors. *Journal of the American Academy of Optometry* 1997; 74: 121.
- Raghavendra V, Chopra K, Kulkarni SK. Modulation of motor functions involving the dopaminergic system by AT₁ receptor antagonist Losartan. *Neuropeptides* 1998; 32: 275–280.
- Rowe BP, Grove KL, Saylor DL, Speth RC. Discrimination of angiotensin II receptor subtype distribution in the rat brain using non-peptidic receptor antagonists. *Regulatory Peptides* 1991; 33: 45–53.
- Shenoy UV, Richards EM, Huang XC, Sumners C. Angiotensin II type 2 receptor-mediated apoptosis of cultured neurons from newborn rat brain. *Endocrinology* 1999; 140: 500–509.
- Tsutsumi K, Saavedra JM. Characterization and development of angiotensin II receptor subtypes (AT₁ and AT₂) in rat brain. *American Journal of Physiology* 1991a; 261: R209–R216.
- Tsutsumi K, Saavedra JM. Differential development of angiotensin II receptor subtypes in the rat brain. *Endocrinology* 1991b; 128: 630–632.
- Tsutsumi K, Seltzer A, Saavedra JM. Angiotensin II receptor subtypes and angiotensin-converting enzyme in the fetal rat brain. *Brain Research* 1993; 631: 212–220.
- Walther T, Voigt JP, Fukamizu A, Fink H, Bader M. Learning and anxiety in angiotensin-deficient mice. *Behavioural Brain Research* 1999; 100: 1–4.
- Wayner MJ, Polan-Curtain J, Armstrong DL. Dose and time dependency of angiotensin II inhibition of hippocampal long-term potentiation. *Peptides* 1995; 16: 1079–1082.
- Winnicka MM, Wisniewski K. Disruption of temporo-entorhinal connections abolishes the facilitatory effect of angiotensins on memory in rats. *Pharmacological Research* 1999; 40: 53–59.
- Wright JW, Harding JW. Brain angiotensin receptor subtypes in the control of physiological and behavioral responses. *Neuroscience and Biobehavioural Review* 1994; 18: 21–53.
- Wright JW, Harding JW. Brain angiotensin receptor subtypes AT₁, AT₂, and AT₄ and their functions. *Regulatory Peptides* 1995; 59: 269–295.
- Wright JW, Krebs LT, Stobb JW, Harding JW. The angiotensin IV system: functional implications. *Frontiers in Neuroendocrinology* 1995; 16: 23–52.
- Xiong H, Marshall KC. Angiotensin II depresses glutamate depolarizations and excitatory postsynaptic potentials in locus coeruleus through angiotensin II subtype 2 receptors. *Neuroscience* 1994; 62: 163–175.