Interaction Between *Mas* and the Angiotensin AT1 Receptor in the Amygdala

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Von Bohlen und Halbach, Oliver, Thomas Walther, Michael Bader, and Doris Albrecht. Interaction between *Mas* and the angiotensin AT1 receptor in the amygdala. *J. Neurophysiol.* 83: 2012–2021, 2000. The *Mas*-protooncogene is a maternally imprinted gene encoding an orphan G protein–coupled receptor expressed mainly in limbic structures of the rodent CNS. Because *Mas* and the product of the *Mas*-related gene enhance the effects of angiotensins on cells expressing angiotensin receptors of the AT1 subtype, we first compared the distribution of cells expressing AT1 receptors in different limbic and thalamic brain structures in *Mas*-knockout mice and in wildtype mice by an immunohistochemical approach. No significant differences could be found between the two strains. The *Mas*-protooncogene seems to be implicated in the signal transduction of angiotensin receptors and is expressed in the amygdala. Therefore we then analyzed whether field potentials are altered by angiotensin II in brain slices of the basolateral amygdala. An opposite action of angiotensin II was obtained in mice lacking the *Mas*-protooncogene in comparison to wildtype mice. The use of different angiotensin receptor antagonists provides the first in vitro evidence for a functional interaction between the *Mas*-protooncogene and the AT1 receptor.

**INTRODUCTION**

It is known that constituents of the renin-angiotensin system (RAS), including precursors and enzymes, are also localized in the brain. Angiotensin II (Ang II) represents the classic effector peptide of the RAS. Central administration of Ang II produces a stimulation of thirst and salt appetite and modulates the release of hormones (Phillips 1987). Ang II also seems to influence cognitive processing, especially acquisition and recall of newly learned tasks (DeNoble et al. 1991; Wright and Harding 1994). Ang II is able to bind at two different receptor subtypes (AT1 and AT2). The AT1 receptor has been implicated in most of the peripheral and cerebral actions of Ang II (Wright and Harding 1994, 1995). The AT2 receptor subtype seems to be involved in apoptosis and is expressed in high levels during development (Tutsuimi and Saavedra 1991). The distribution of these receptors has been well examined for rats (Gehlert et al. 1986, 1991; Jöhren et al. 1995, 1996; Lenkei et al. 1996; Phillips et al. 1993; Reagan et al. 1994; Sirett et al. 1977; Song et al. 1992), but only a few studies are available, indicating that AT1 receptors are present in the mouse brain (Hauser et al. 1998; Jöhren et al. 1997).

The *Mas*-protooncogene has been shown to be primarily expressed in the brain (Bunnemann et al. 1990). *Mas*-mRNA was shown to be present in limbic, thalamic and cortical structures (Bunnemann et al. 1990; Martin et al. 1992). Ang II has been reported to be a functional ligand for *Mas*-encoded receptor in *Xenopus* oocytes (Ambroz et al. 1991; Jackson et al. 1988). Subsequent studies that have failed to substantiate this notion, however, have shown that *Mas*-transfection potentiates responses to Ang II in cells already expressing the AT1 receptor (Ambroz et al. 1991). Recently, we have generated mice lacking the *Mas*-gene by gene-targeting technology (Walther et al. 1998). These animals are viable and fertile and showed a sustained long-term potentiation in the hippocampus as well as alterations in anxiety behavior.

The first aim of this study was the determination of brain distribution of the AT1 receptor in these knockout mice in comparison to controls by an immunohistochemical approach. Considering the well-known function of the basolateral amygdala in fear conditioning (LeDoux 1994) as well as our results concerning the inhibitory influence of Ang II on the induction of long-term potentiation in the lateral nucleus of the amygdala (von Bohlen und Halbach and Albrecht 1998d), the second aim was to analyze the influence of Ang II on electrophysiological responses within the amygdala in *Mas*-knockout and in wildtype mice.

**METHODS**

This study was performed on adult, normal mice and mice with a targeted inactivation (“knockout”) of the *Mas*-protooncogene (Walther et al. 1998). The animals were housed on a 12 h light-dark cycle with food and water ad lib.

**Immunohistochemistry**

The animals (*n* = 11) were anesthetized with ether and decapitated. The brains were removed and fixed in Zambonis-fixative for 2 days. For a further day they were placed in 20% sucrose. Horizontal and coronal sections (30 μm) were made using a freezing microtome (Frigomat; Jung). Each second slice was stained with cresyl violet (Fig. 1), helping to identify the brain regions by a stereotaxic atlas (Paxinos and Watson 1986). The other sections were rinsed in phosphate-buffered saline (PBS) and incubated in 0.3% H₂O₂ to block endogenous peroxidase. Then we used a staining protocol, which has been introduced for mapping AT1 receptors (von Bohlen and Halbach 1998c). In brief, sections were rinsed in PBS, washed in 5 mM Tris-buffered saline, and incubated for 72 h with an AT1-antibody (AP1525, Chemicon) in the presence of 2% NGS, 0.1% NaN₃, and 0.1% Triton X-100 at 4°C. After rinsing in PBS, the slices...
were incubated with the secondary antibody (IgG A132, Chemicon) for 2 h. Then, after rinsing, they were incubated with a peroxidase antiperoxidase complex (PAP18, Chemicon) for 2 h. Visualization was done by the use of 3'-3'-diaminobenzidine (DAB, Sigma). Slices were mounted onto gelatin-coated slices and coverslipped with DePeX (Serva). The sections were examined under a microscope (Leica, Axioskop) and photographed (Ectachrome 400, Kodak).

The specificity of this antibody has been proved, because preabsorption of the antiserum with 0.5 mM homologous peptide completely eliminates reactivity. The specificity has already been demonstrated (Phillips et al. 1993). In additional controls, the nonspecific immunoreactivity was assessed by incubation without the primary antibody (von Bohlen und Halbach and Albrecht 1998c). These negative controls showed no staining.

Counts of stained cells were determined by Camera lucida projection onto a sheet of paper. On this sheet a grid was copied, representing a window of 40,000 mm² by using a magnification of 400. The restriction to a visual field was chosen to allow a quantitative examination of immunoreactive neurons. The anatomic localization for each brain nucleus was verified in adjacent sections that were Nissl-stained. Slices from different animals (n = 6 to 11) were counted within this grid was estimated for each brain region in a blind study. Slices from different animals (n = 6 to 11) were evaluated to minimize errors. The data were analyzed (mean, SD, SE). ANOVA was used to test significant differences in the two groups (wildtype vs. knockout). The location of the brain nuclei was verified by comparison with the cresyl violet–stained sections. All data were collected from horizontal sections, except for the thalamic data, which were obtained from coronal sections.

**Electrophysiology**

To evaluate the distribution of brain nuclei in the horizontal plane, one mouse brain was sectioned in 30-μm sections. These horizontal sections were counterstained with cresyl violet and compared with the results of an earlier study, introducing the horizontal plane as a suitable tool for electrophysiological investigation of the basolateral amygdala (von Bohlen und Halbach and Albrecht 1998b).

The animals were decapitated under deep ether anesthesia, and horizontal 400-μm brain slices were then prepared, as described earlier for rats (von Bohlen und Halbach and Albrecht 1998a,b,d). Glass microelectrodes for extracellular recording were filled with 154 mM NaCl and placed in the basolateral amygdala (bA), which included the basolateral and lateral nuclei of the amygdala. A bipolar stimulation electrode was also placed within this region (Fig. 1). Single stimuli (duration: 0.1 ms) were applied at intervals of 10 s. Stimulus intensities varied between 2.5 and 7 V.

Ang II (10 μM) was added in accordance with our previous employed protocol for rats (von Bohlen und Halbach and Albrecht 1998a,d) and data from the literature (Kang et al. 1992; Martens et al. 1996; Schmid et al. 1995). To determine the best concentration to use in the Mas-knockout mice, Ang II was applied in 1, 5, 7.5, 10, and 20 μM, and the resulting amplitude of field potentials was measured. On the basis of these results, we selected a concentration of 10 μM to be used in subsequent experiments.

Because the substances were washed in by perfusion, they were transported over a certain distance in tubes before they reached the incubation chamber. Therefore a certain concentration of the substance may adhere to the tubes or will be degraded during transport. In addition, applied concentrations may be very different from those encountered in vivo (Schmitz et al. 1995). Previous studies have shown that equilibration would take a long time and that after 30 min only 30% of the substance could be measured within the slice (Müller et al. 1988). In this paper it is argued, that “In slice studies, concentrations quite high in comparison to the binding constants of the particular drug are typically used. The likely reason is that by using high drug concentrations, apparent diffusional barriers described in
the present paper are overcome.” Additionally, in the study by Müller et al., 300-μm-thick slices were used. We have used 400-μm-thick slices, which will eventually decrease the concentrations within the slices further. The nominal concentration of the peptide as likely considerably more than actually “seen” by the neurons, because of the degradation mechanisms. However, even higher concentrations of Ang II (ranging from 100 μM to 1 M) were used in some in vitro studies (Guenther et al. 1996, 1997; Martial et al. 1994).

To test the specificity of the measured changes in field potentials, Ang II was washed in together with saralasin (SAR; 0.1 μM), an unspecific Ang II antagonist. To determine whether both Ang II receptor subtypes (AT1 and AT2) or only one of them are involved in observed changes in the amplitude of field potentials, losartan (LOS; 0.1 μM), an AT1 antagonist, or PD 123,319 (PD; 0.1 μM), an AT2 antagonist, were added to the bath solution together with Ang II. All drugs were obtained from Research Biochemical International (Natick, MA) except losartan, which was generously provided by Dr. Ronald D. Smith (DuPont Merck Pharmaceutical, Wilmington, DE).

**FIG. 2.** Concentration-dependent changes in field potential amplitudes in *Mas*-knockout mice. All measurements were carried out in the presence of 10 μM Ang II. A: changes in the amplitude under the influence of saralasin. B: changes in the amplitude under the influence of losartan. C: changes in the amplitude under the influence of PD123.319.
Additionally, the effect of saralasin (0.1 μM) alone was tested in wildtype and Mas-knockout mice. The used concentration (0.1 μM for each antagonist) was determined by experiments where Ang II (10 μM) was applied together with one of the different antagonists at different concentrations (0.01, 0.1, 1, and 10 μM), and the resulting amplitudes of field potentials were measured (Fig. 2).

Field potentials were recorded for a 6-min control period before the different drugs were added to the incubation solution (control measurement). Field potentials were measured over a period of 18 min, 30 min after perfusing the slices. Data were collected, averaged (in each case 6 sweeps) with the custom-made software Signal-averager. The mean, the SD, and the SE were calculated. Statistical analyses (Wilcoxon-, Mann-Whitney-U test) were performed using Winstat.

### RESULTS

**Immunohistochemical detection of AT1 expressing cells in the mouse brain**

Stained cells occurred lightly brownish stained due to the peroxidase-antiperoxidase (PAP) method. AT1 expressing cells could be found in normal as well as in Mas-knockout mice. No significant differences in the distribution of stained cells in the different brain nuclei between these two groups were found by an ANOVAs (Table 1). In detail, in both groups a labeling of cells could be found in various brain regions.

Within the entorhinal cortex and the perirhinal cortex, high amounts of cells expressing the receptor subtype AT1 could be found. The hippocampal formation was also a region that contained many cells expressing this receptor subtype. High amounts of marked cells could be detected in the dentate gyrus (Fig. 3A), in the hippocampal regions CA1, CA2, and CA3 (Fig. 3, A and B) as well as in the subiculum (Fig. 3, C and D, and Table 1). Moderately dense staining was found in the amygdala. Generally, this staining occurred in somewhat lower rates as in cortical or hippocampal regions. In the central, medial, and basolateral subdivision of the amygdala, a moderate staining was obvious (Table 1). Cells expressing the AT1 receptor subtype were also present in thalamic brain regions. The density of staining varied among the nuclei of the thalamus. In detail, cells carrying the AT1 receptor subtype could be found in the anterodorsal nucleus, the laterodorsal nucleus, the ventral posterior nucleus, as well as in the lateral posterior nucleus and in the dorsal and ventral parts of the lateral geniculate nucleus, in the reticular thalamic nucleus, and in the zona incerta (Table 1).

### Table 1. Distribution of anti-AT1–stained cells

<table>
<thead>
<tr>
<th>Brain Region</th>
<th>Normal mouse</th>
<th>Mas-knockout</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>pir</td>
<td>54.9 ± 2.9</td>
<td>40.3 ± 2.0</td>
<td>1.073</td>
<td>0.315</td>
</tr>
<tr>
<td>ent</td>
<td>35.9 ± 1.8</td>
<td>35.6 ± 2.0</td>
<td>2.629</td>
<td>0.119</td>
</tr>
<tr>
<td>sub</td>
<td>40.1 ± 2.1</td>
<td>47.4 ± 2.2</td>
<td>2.337</td>
<td>0.141</td>
</tr>
<tr>
<td>CA1</td>
<td>51.2 ± 2.8</td>
<td>38.9 ± 2.3</td>
<td>1.117</td>
<td>0.302</td>
</tr>
<tr>
<td>CA2</td>
<td>35.9 ± 1.9</td>
<td>49.1 ± 2.8</td>
<td>1.017</td>
<td>0.324</td>
</tr>
<tr>
<td>CA3</td>
<td>51.9 ± 2.7</td>
<td>57.8 ± 1.7</td>
<td>0.542</td>
<td>0.469</td>
</tr>
<tr>
<td>DG</td>
<td>54.7 ± 4.4</td>
<td>58.7 ± 2.1</td>
<td>0.453</td>
<td>0.508</td>
</tr>
<tr>
<td>bA</td>
<td>27.2 ± 1.6</td>
<td>30.2 ± 1.7</td>
<td>1.518</td>
<td>0.231</td>
</tr>
<tr>
<td>mA</td>
<td>33.2 ± 1.6</td>
<td>37.9 ± 1.4</td>
<td>4.191</td>
<td>0.053</td>
</tr>
<tr>
<td>cA</td>
<td>30.4 ± 1.6</td>
<td>33.7 ± 1.9</td>
<td>1.859</td>
<td>0.187</td>
</tr>
<tr>
<td>AD</td>
<td>26.7 ± 1.9</td>
<td>30.7 ± 1.7</td>
<td>2.413</td>
<td>0.135</td>
</tr>
<tr>
<td>LD</td>
<td>22.6 ± 1.4</td>
<td>23.2 ± 2.3</td>
<td>0.058</td>
<td>0.811</td>
</tr>
<tr>
<td>VP</td>
<td>22.7 ± 1.3</td>
<td>20.6 ± 1.0</td>
<td>1.576</td>
<td>0.223</td>
</tr>
<tr>
<td>LP</td>
<td>23.4 ± 1.9</td>
<td>25.3 ± 1.2</td>
<td>0.722</td>
<td>0.405</td>
</tr>
<tr>
<td>DLG</td>
<td>25.2 ± 1.3</td>
<td>27.4 ± 1.7</td>
<td>1.109</td>
<td>0.304</td>
</tr>
<tr>
<td>VLG</td>
<td>25.0 ± 1.4</td>
<td>23.9 ± 0.9</td>
<td>0.432</td>
<td>0.518</td>
</tr>
<tr>
<td>Rt</td>
<td>18.0 ± 1.5</td>
<td>22.2 ± 1.9</td>
<td>3.161</td>
<td>0.089</td>
</tr>
<tr>
<td>Zi</td>
<td>22.8 ± 1.3</td>
<td>24.0 ± 1.6</td>
<td>0.312</td>
<td>0.582</td>
</tr>
</tbody>
</table>

Values in Stained Cells are means ± SE. pir, piriform cortex; ent, entorhinal cortex; sub, subiculum; CA1–3, Cornus ammonis, regions 1–3; DG, gyrus dentatus; bA, basolateral amygdala; mA, medial amygdala; cA, central amygdala; AD, anterodorsal thalamic nucleus; LD, laterodorsal thalamic nucleus; VP, ventral posterior thalamic nucleus; LP, lateral posterior thalamic nucleus; DLG, dorsal part of the lateral geniculate nucleus; VLG, ventral part of the lateral geniculate nucleus; Rt, reticular thalamic nucleus; Zi, zona incerta. * In an area of 40,000 μm².
amplitude of field potentials in the bA of the wildtype mouse and that this effect is mediated by AT1 as well as by AT2 receptors (Fig. 5A).

Application of 0.1 μM saralasin (without Ang II) did not lead to significant changes in the amplitude (administration of saralasin vs. control: \(n = 6, P = 0.251\)).

Because the \(\textit{Mas}\)-protooncogene seems to be involved in Ang II signaling, we studied the electrophysiological parameters of \(\textit{Mas}\)-knockout mice in the bA. Ang II caused a significant reduction in the amplitude of evoked field potentials. This reduction was concentration dependent in that the greater the concentration of Ang II, the greater the reduction in amplitude (Fig. 6). In subsequent experiments we used a concentration of 10 μM.

In contrast to the above-described effect of Ang II in wildtype mice, we could observe a significant decrease in the amplitude of field potentials of \(-10.48\%\) in the bA of \(\textit{Mas}\)-knockout mice [effect on the amplitude of field potentials by administration of 10 μM Ang II vs. amplitudes in \(\textit{Mas}\)-knockout mice without influence of drugs (\(\textit{Mas}\)-controls); \(n = 9, P = 0.007\); Fig. 4B]. Saralasin was able to block this decrease in the amplitude, indicating that these effects were receptor specific (coadministration of Ang II and saralasin vs. \(\textit{Mas}\)-controls; \(n = 7, P = 0.176\)). The coadministration of Ang II and losartan caused an increase in the amplitude of 4.31\% compared with \(\textit{Mas}\)-controls (coadministration of Ang II and losartan vs. \(\textit{Mas}\)-controls; \(n = 7, P = 0.018\); Fig. 5B). Therefore by blocking the AT1 receptor, this observed increase of field potentials...
The superfusion of slices with PD123.319, an AT2-receptor antagonist, together with Ang II resulted in a significant decrease in the amplitude of 19.04% (coadministration of Ang II and PD vs. controls; \( n = 7, \ P = 0.018 \); Fig. 5B).

These results show that Ang II induces a decrease in the amplitude of field potentials in the bA of the Mas-knockout mouse. Coadministration of Ang II and PD123.319 leads to a strong reduction of the field potential amplitude, whereas the increase in the amplitudes by coadministration of Ang II and losartan in the wildtype and the Mas-knockout mice did not differ significantly (Fig. 5). Application of 0.1 \( \mu \)M saralasin (without Ang II) in the Mas-knockout neither leads to significant changes in the amplitude in comparison to control measurements (administration of saralasin vs. Mas-control; \( n = 6, \ P = 0.343 \)), nor to significant changes in the amplitude in comparison to the amplitude wildtype mice under the influence of saralasin alone (administration of saralasin: wildtype vs. Mas-knockout; \( n = 6, \ P = 0.446 \)).

**DISCUSSION**

The results of the present study show that the distribution of brain angiotensin AT1 receptors in Mas-knockout mice did not significantly differ from the wildtype. In contrast, significant differences were found in the electrophysiological experiments. In the wildtype mouse, Ang II induced an increase in the field potentials in the bA, whereas Ang II reduced them in the Mas-knockout mice.

Angiotensin binding and angiotensin receptors are not only restricted to brain areas related to cardiovascular control, but were also found in structures involved in learning and memory mechanisms, and in structures processing sensory information. This study shows a specific mapping of angiotensin AT1 receptors within the limbic system and the thalamus of normal and Mas-knockout mice by the use of immunohistochemistry. It could be demonstrated that the distribution of cells expressing the AT1 receptor in the mouse brain is very similar to that obtained in rats (Gehlert et al. 1986, 1991; Phillips et al. 1993; Sirett et al. 1977; Song et al. 1992; von Bohlen und Halbach and Albrecht 1998c).

The use of polyclonal antibodies selective for the AT1 receptors allowed the examination of individual stained cells. The antibody against a sequence of the AT1 receptor showed a high specificity. Antibodies of that type were also used in a previous study (Phillips et al. 1993; von Bohlen und Halbach and Albrecht 1998c) revealing excellent staining of specific cell populations. Staining was not restricted to the cell membrane but also occurred in the cytoplasm, which is probably due to internalized AT1 receptors (Inagami et al. 1994; Kanaashiro et al. 1995).

Besides AT1-mediated functions of Ang II in cardiovascular control, other functions of Ang II has been supposed (Fitzsimons 1998). We could show that Ang II changed the sensory transmission in lateral geniculate neurons (Albrecht et al. 1997). Moreover, recent experiments have demonstrated that Ang II inhibits the induction of long-term potentiation within the hippocampus (Denny et al. 1991), the dentate gyrus (Wayner et al. 1996), and the amygdala (von Bohlen und Halbach and Albrecht 1998d), and that this inhibition of long-term potentiation is mediated through the AT1 receptor. In contrast, the induction and the maintenance of hippocampal long-term potentiation is enhanced in Mas-deficient mice (Walther et al. 1998). Comparing these results the hypothesis of an interaction between Mas-gene product and the angiotensin AT1 receptor is supported.

Ang II was administered in a concentration of 10 \( \mu \)M in accordance with our experiences made in rats (von Bohlen und Halbach and Albrecht 1998a,d). Moreover, the dose-response

**FIG. 4.** Examples of extracellular recordings of field potentials in the basolateral amygdala. Amplitudes were averaged (in each case 6 sweeps). A: in a slice preparation of a wildtype mouse. B: in a slice preparation of a Mas-knockout mouse.
Our electrophysiological results show that Ang II induces an increase in the amplitude of field potentials in the bA of the wildtype mouse. This effect is mediated by AT1 as well as AT2 receptors. An Ang II-induced increase of field potentials in the lateral nucleus of the amygdala was also obtained in normal rats. Moreover, this increase in the amplitude was also blocked by both AT1 and AT2 antagonists to the same extent (von Bohlen und Halbach and Albrecht 1998a,d), revealing that both receptor types were involved in the mediation of Ang II-induced effects in the lateral nucleus of the amygdala.

Similar results could be found in single-unit studies of the rat lateral nucleus of the amygdala (Albrecht et al. 2000).
contrast to the Ang II–caused increase in the field potentials in the wildtype, in Mas-knockout mice an Ang II–mediated decrease in the amplitude of field potentials was obvious. This response was concentration dependent in that the greater the concentration of Ang II, the greater the reduction in amplitude (Fig. 6).

The blockage of the AT1 receptor caused an increase in field potentials, which was comparable to that seen in the wildtype mice by coadministration of Ang II and losartan (Fig. 5). Because the effects in the wildtype mice and in the Mas-knockout mice were nearly the same, it can be assumed that Mas did not affect the AT2 receptor.

The Ang II–induced AT1 receptor stimulation in the Mas-knockout mice resulted in a strong reduction of the field potentials. In contrast, the stimulation of the AT1 receptor in the wildtype mice induces an increase in the amplitude similar to that obtained in normal rats (von Bohlen und Halbach and Albrecht 1998d).

The slight increase in amplitude of field potential which is induced by the AT2 receptor and the strong decrease in the amplitude, induced by the AT1 receptor, leads to an Ang II–induced decrease in the amplitude in the field potentials in Mas-knockout mice.

Administration of Ang II leads to an AT1 receptor–mediated decrease in the amplitude of field potentials in Mas-knockout mice and an increase in the amplitude of field potentials in normal mice and rats. However, application of Ang II together with saralasin caused no alteration in the amplitude of field potentials (compared with controls). In addition, application of saralasin alone neither significantly changed the amplitude in wildtype nor in Mas-knockout mice. Therefore the hypothesis that the Mas-protooncogene encodes for a new receptor, capable to bind angiotensin III and angiotensin II (Jackson et al. 1988; Wolf and Neilson 1992) does not find support by our results. If the Mas-protooncogene encodes for an angiotensin receptor, differences in the field potentials in normal and Mas-knockout mice should occur by blocking AT1 receptors or AT2 receptors or both angiotensin receptor subtypes. The opposite action of Ang II in the amygdala (increase of field potentials in controls and decrease of field potentials in Mas-knockout mice) supports different hypotheses.

First, differences in the distribution of AT1 receptors on pyramidal cells and/or GABAergic interneurons within the basolateral amygdala might occur between the two mouse strains. In iontophoretic studies in urethan-anesthetized rats we have shown that Ang II–induced inhibitory effects on single-unit activity in the basolateral amygdala can be blocked by the GABA_A receptor antagonist bicuculline (Albrecht et al. 2000). Second, it could be hypothesized that Mas is involved in the signal pathway of angiotensin receptors (Monnot et al. 1991). Because the excitatory effect of Ang II via the AT2 receptor is unaltered in Mas-deficient animals, the signaling of the AT1 receptor might be affected by Mas. Further experiments using patch-clamp techniques are needed to clarify whether the Ang II–induced opening or closing of different ion channels is also altered in the knockout model. Ang II has been shown to affect

\[ y = -0.0168x^2 + 1.0753x \]
various ionic channels. An Ang II–induced action on different K⁺ currents, such as transient outward currents, Ca²⁺-activated K⁺ currents, or inward and outward rectifiers have been studied. In addition, it has been examined a possible modulation of Ca²⁺ entry via voltage-dependent Ca²⁺ channels (Chorvatova et al. 1996;Summoners et al. 1996).

Interestingly, a similar effect of Mas in rat kidney proximal tubular cells has been reported (Wolf and Neilson 1992). Transfection of these cells in culture with a Mas-expression construct alters their response to Ang II completely. Although the parent cells are induced to hypertrophy, the Mas-transfected cells start to proliferate after Ang II stimulation.

How may Mas interact with the signal transduction pathways of the AT1 receptor? As shown in this study and by others (Wolf and Neilson 1992), Mas does not change the expression of the AT1 receptor itself. However, it may alter the repertoire of expressed genes in a cell via pathways recently described (Zohn et al. 1998), which could affect also genes involved in Ang II signaling as G-proteins or the above-mentioned ion channels. Alternatively, the function of these proteins could be altered by phosphorylation as Mas has been shown to modulate the activity of several kinases, like JNK and p38 (Zohn et al. 1998). Finally, Mas could interact directly with the AT1 receptor on the plasma membrane. This hypothesis is based on the assumption that Mas encodes for a receptor-like product with seven transmembrane segments (Jackson et al. 1988;Monnot et al. 1991). A heterodimeric receptor between Mas and the AT1 receptor may elicit the effects seen in the wildtype mice, whereas the effects of Ang II on the AT1 receptor in the p53-knockout mice may be mediated by the homeric AT1 receptor.

In conclusion, this study demonstrated that the angiotensin AT1 receptor distribution is nearly the same in the wildtype and in the p53-knockout mouse brain. The AT1-mediated signal transduction is changed in mice lacking the Mas-protooncogene, supporting an important role of Mas in Ang II–mediated effects at least in the mouse. These findings may provide a key to understand the molecular basis of the behavioral alterations seen in Mas-deficient mice and the functions of angiotensin in the brain.

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