Tonin and Kallikrein in the Brain of Transgenic Rat Line Expressing Human Tissue Kallikrein

Eliane S.L. Lomez, Ronaldo C. Araujo, Michael Bader, Joao B. Pesquero, Jorge L. Pesquero

Abstract—A transgenic rat line harboring the human tissue kallikrein gene was investigated for expression and activity of tonin and kallikrein in different regions of the brain. The introduction of the transgene into the rat genome produced a significant augmentation of the expression levels and activity of rat tissue kallikrein. The possibility that human kallikrein does not hydrolyze the rat substrate is probably responsible for the augmented expression of the rat enzyme. On the other hand, although expression of tonin was significantly reduced, tonin activity was not altered in most brain structures, except for cerebellum and neurohypophysis. (Hypertension. 2002;39:229-232.)

Key Words: tonin ■ kallikrein ■ angiotensin II ■ rats ■ brain

The coexistence of angiotensin (Ang) II–generating and kallikrein-kinin (KKS) systems in different sites suggests diverse physiological effects for these systems in the local control of blood flow and in the pathophysiology of hypertension and cardiovascular diseases. In the literature, there is a growing body of evidence for alternative pathways in the formation of Ang II in many tissues, including the brain. It has been suggested that proteinases other than renin and ACE may be involved in the local liberation of Ang II directly from angiotensinogen. An alternative route that produces Ang II directly from angiotensinogen involves the enzyme tonin. Tonin activity has been found in several rat tissues, including the brain. The liberation of Ang II by tonin in the brain may be important for the control of blood pressure and body fluid volumes. There is evidence that the KKS is also involved with the modulation of the brain circulation and central control of the blood pressure. To elucidate the functions of the KKS, transgenic techniques have been employed to either overexpress or ablate pertinent genes. This has led to new animal models and has substantially increased our understanding of the physiological functions of this peptide system.

A transgenic rat (TGR) harboring the human kallikrein gene (hKLK1) was recently established. The TGR(hKLK1) model is hypotensive. Kallikreins are present in common sites to tonin such as the submandibular gland, kidney, and brain. Recently, we determined the regional distribution of tonin- and kallikrein-similar activities in the brain of Wistar rats. We showed that the highest values of tonin activity is in the neurohypophysis and archicerebellum, whereas the kallikrein activity is not present in the neurohypophysis and is homogeneous for other brain regions. In this study, we used reverse transcription–polymerase chain reaction (RT-PCR) to determine the regional expression pattern of tonin and kallikrein in the brain of the TGR(hKLK1). The activities of the enzymes were also determined in the same tissues of the brain and cerebellum of the rat.

Methods

The Methods section can be found in an online data supplement available at http://www.hypertensionaha.org.

Results

Our results show that the human transgene hKLK1 is expressed in all tested brain structures from the TGR (Figure 1) but is not expressed in the structures of the control Sprague-Dawley rat (data not shown). Figures 2 and 3 show the results of expression of the rat kallikrein gene in the Sprague-Dawley rat and TGR(hKLK1), respectively. Low levels of rat kallikrein expression were observed in the regions neurohypophysis, adenohypophysis, hypothalamus, and choroid plexus of the Sprague-Dawley rat. No expression of the rat kallikrein gene was observed in the other regions (Figure 2). Unlike the Sprague-Dawley rat, it was possible to detect expression of the rat kallikrein gene in almost all of the structures of TGR(hKLK1), except in the medulla oblongata (Figure 3). When compared with Sprague-Dawley rat, the expression levels in neurohypophysis and adenohypophysis are strongly higher.
In the Sprague-Dawley rat, tonin is expressed in all structures tested with the highest level in the midbrain (Figure 4). In the transgenic rat, the expression levels are diminished in all structures, becoming undetectable in the midbrain and neurohypophysis (Figure 5). The results of ribonuclease protection assay (RPA) for tonin expression in the TGR(h-KLK1) are in agreement with those of RT-PCR. By RPA, it was possible to detect tonin expression in the cerebellar cortex, medulla oblongata, and thalamus (Figure 6). With regard to the kallikrein activity, the results are in agreement to the observed for gene expression as determined by RT-PCR. Kallikrein activity is higher in several brain structures of the TGR(hKLK1) than of the Sprague-Dawley rat (Figure 7A). The most altered levels of kallikrein activity were observed in the neurohypophysis, choroid plexus, medulla oblongata, and archicerebellum. In general, the levels of tonin activity are not significantly different for both animals. Significant augment of tonin activity by the introduction of the transgene was observed for archicerebellum and cerebellar cortex and the reduction of activity in neurohypophysis, cerebellar nuclei, and thalamus (Figure 7B).

**Discussion**

Recently we elucidated some aspects related to the distribution of the tonin- and kallikrein-like activities in the brain and cerebellum of the Wistar rat. We verified that in the rat, kallikrein activity is homogeneously distributed in the brain, except for the neurohypophysis, where it is not present. On the other hand, tonin activity is concentrated in the neurohypophysis and archicerebellum. A probable function of tonin in the hypophysis-hypothalamus axis is to process precursor proteins into active forms and peptides, as it can do with β-lipoprotein and adrenocorticotropic hormone. In this study, we show the levels of these enzymes, determined by quantitation of the specific mRNA and activity, in the same regions of the brain of a rat expressing human kallikrein. Because this rat is hypotensive, we speculate that tonin and kallikrein are involved in this phenotype. As we determined the activity of the enzymes in crude extracts and the finding that the D-Pro-Phe-Arg-p-nitroanilide is a selective substrate for kallikrein rather than a specific one, we performed an assay in the presence of 5 μmol/L soybean trypsin inhibitor (SBTI) in an attempt to selectively inhibit interfering proteinases while still retaining tissue kallikrein activity. From the kallikrein superfamily, tissue kallikrein is the major protease that shows relative resistance to inhibition by SBTI. Our results showed that the introduction of the human kallikrein gene into rat genome produced alterations in the activity and gene expression of the enzymes. For kallikrein, in general, we observed augmented activity and mRNA levels. In the presence of SBTI, the activity of enzyme in the thalamus of TGR(hKLK1) was inhibited by 50%; however, no inhibition was observed for other regions. This means that in the thalamus, plasma kallikrein may be interfering in our assay.
How can the expressed human transgene upregulate the expression levels of the endogenous rat enzyme? One possible explanation involves the specificity of the substrate-enzyme interactions. We postulate that the product of the human transgene can interact with the rat substrate but cannot hydrolyze it well. Indeed, we verified that human urinary kallikrein is not able to hydrolyze partially purified rat kininogen. In this way, less substrate is available for the endogenous kallikrein, and as a result, less kinin is produced. The lower levels of kinins may represent a stimulus for rat kallikrein synthesis and release. This higher level of kallikrein would be able to keep the same levels or an increased level of kinins compared with that of genetically unmodified rats. However, another interesting aspect that could also explain the hypotensive phenotype in the TGR(hKLK1) is the decrease in the levels of activity and expression of tonin. The expression of tonin diminished in all structures studied, and the activity decreased in the neurohypophysis, cerebellar nuclei, and thalamus. Even though we had been not able to detect tonin mRNA in the midbrain and in the neurohypophysis of the transgenic rat, tonin activity is present in these structures. Perhaps the enzyme is made somewhere in the brain and transported to these structures by a mechanism that, for neurohypophysis, is facilitated in the transgenic animal. To determine the specificity of our assay, tonin activity was performed in the absence and in the presence of an antibody. The distinct gene and tissue-specific expression of activity and expression of tonin. The expression of tonin would be able to keep the same levels or an increased level of kinins compared with that of genetically unmodified rats. However, another interesting aspect that could also explain the hypotensive phenotype in the TGR(hKLK1) is the decrease in the levels of activity and expression of tonin.

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