Ketamine prevents lidocaine-caused neurotoxicity in the CA3 hippocampal and basolateral amygdala regions of the brain in adult rats

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Abstract
Our objective was to prove whether blocking the action of glutamate on N-methyl-D-aspartate (NMDA) receptors could prevent the neuronal damage caused by the acute administration of lidocaine. Twenty male 2-month-old Wistar rats were randomly assigned to the following groups ($n = 5$ in each group): groups I and II received 0.9% saline i.p., and groups III and IV received 100 mg·kg^{-1} of ketamine i.p. Thirty minutes later, groups I and III were again dosed with 0.9% saline i.p., and groups II and IV received 60 mg·kg^{-1} of lidocaine i.p. During treatment, the rectal temperature of the animals was monitored and maintained at $37.5 \pm 0.5°C$. Ten days after administration of the agents, all rats were transcardially perfused, under pentobarbital anesthesia, with 10% formaldehyde. Their brains were removed and were embedded in paraffin. Coronal cuts of 7 μm were obtained from –2.3 to –3.8 mm from the bregma. Each brain section was stained with cresyl violet-eosin. The number of normal and abnormal pyramidal neurons in the CA3 hippocampal region and the number of large and medium neurons in the basolateral amygdala within an area of 10 000 μm^2 were evaluated. We found that lidocaine significantly reduced the number of normal neurons in both the CA3 hippocampal region ($F_{3,16} = 225.8$; $P < 0.001$) and the basolateral amygdala ($F_{3,16} = 253.3$; $P < 0.001$). The ketamine pretreatment attenuated the lidocaine-induced damage in the CA3 hippocampal region and the basolateral amygdala. These results demonstrate the participation of NMDA-receptor activation by lidocaine in the CA3 hippocampal and basolateral amygdala regions as a neurotoxic mechanism.

Key words Lidocaine · Neurotoxicity · Neuronal damage

Lidocaine is the most widely used local anesthetic in clinical treatment. A higher lidocaine dose than the therapeutic range (1–5 mg·kg^{-1}) can be neurotoxic [1]. Recently, we have shown that lidocaine at subconvulsive (60 mg·kg^{-1}) and convulsive (90 mg·kg^{-1}) doses caused neuronal damage in the CA3 hippocampal region and basolateral amygdala [2]. The mechanism of this toxicity is unrelated to lidocaine's primary action on voltage-gated Na$^+$ channels. Studies in vitro have revealed that lidocaine enhanced intracellular Ca^{2+} homeostasis [3], causing apoptosis and/or necrosis, depending on the dose and the exposure time to lidocaine [1,3]. In the rat brain, a hippocampal Ca^{2+} increase was found after a lidocaine-induced seizure [4]. In addition, Biella and coworkers [5] demonstrated that lidocaine increased the frequency of neuronal firing before the administration of N-methyl-D-aspartate (NMDA). It is possible that a reduction of inhibition and enhancement of NMDA-receptor activity could mediate neuronal damage. Our objective was to determine whether blocking the action of glutamate on NMDA receptors could prevent the neuronal damage caused by acute administration of lidocaine.

Twenty male 2-month-old Wistar rats (200–250 g) were used. The rats were housed in groups of 5 in a Plexiglas cage, with food and water ad libitum, in a room with constant temperature ($21 \pm 2°C$), and a 12-h light: 12-h dark cycle (lights on at 0800). All experimental procedures described in this study were in accordance with the guidelines of the Laws and Codes of Mexico in The Seventh Title of the Regulations of the General Law of Health Regarding Health Research. Five animals were randomly assigned to each of four groups. Groups I and II received 0.9% saline i.p., and groups III and IV received 100 mg·kg^{-1} of ketamine i.p. This ketamine dose was used because it is known that it does not affect hippocampal activity [6,7]. Thirty minutes after these administrations, groups I and III were given 0.9% saline i.p., and groups II and IV

received 60 mg·kg^{-1} of lidocaine i.p. During treatment, the rectal temperature of the animals was monitored and maintained at 37.5 ± 0.5°C. Ten days after administration of the agents, the animals were transcardially perfused, under pentobarbital anesthesia (35 mg·kg^{-1} i.p.), first with 0.9% saline and then with 10% formaldehyde. The brains of the rats were removed and were embedded in paraffin. Coronal cuts of 7 μm were obtained from –2.3 to –3.8 mm from the bregma [8]. Each brain section was stained with cresyl violet-eosin. The number of normal and abnormal pyramidal neurons in the CA3 hippocampal region and the number of large and medium neurons in the basolateral amygdala within an area of 10 000 μm^2 were determined. This evaluation was made with a bidimensional counting method, using ten slices per brain of each region, randomly selected from the left and the right hemispheres. All cell counts were made as previously described [2]. Briefly, we used a binocular Zeiss light microscope (Zeiss, Munich, Germany) with an ocular micrometer scale at a magnification of 500× (4× objective, 10× ocular, tube factor of 1.25). The anatomical identification of neurons was based on cytoplasmic characteristics, including the presence of a readily distinguishable nucleus, nucleolus, and neuronal shape, in accordance with the usual rules used in stereological studies to identify neurons. All the neuronal counts were made by a person blinded to the details of the experiment. Data values are expressed as means ± SEM and were analyzed using one-way analysis of variance followed by the Tukey test for all variables studied temporally. A P value of <0.05 was considered statistically significant.

After the administration of lidocaine, all rats became sedated, with cataleptic-like unresponsiveness, in 20 min. Ketamine produced the same response, but the rats were unresponsive to external stimulation within 30 min.

Lidocaine 60 mg·kg^{-1} caused abnormalities in both the CA3 pyramidal neurons and in the medium and large neurons of the basolateral amygdala. The neurons had morphological alterations such as chromatin condensation, nucleolus loss, and cell shrinkage (Fig. 1).

As shown in Table 1, lidocaine reduced the number of normal pyramidal neurons in the CA3 hippocampal region from 20.2 ± 0.3 to 5.4 ± 0.2 neurons in 10 000 μm^2 ($F_{3,16}$ = 225.8; P < 0.001). The number of normal medium and large neurons in the basolateral amygdala decreased from 16.9 ± 0.4 to 5.4 ± 0.2 neurons in 10 000 μm^2 ($F_{3,16}$ = 253.3; P < 0.001). The ketamine pretreatment attenuated the lidocaine-induced damage in the CA3 hippocampal and the basolateral amygdala regions; the numbers of normal neurons in these regions were 9.8 ± 0.6 and 13.6 ± 0.5, respectively. The total number of neurons was not modified in any group.

Previously, we reported that lidocaine caused neurotoxicity in the CA3 hippocampal and basolateral amygdala regions [2]. The mechanism of the lidocaine-induced injury in these limbic structures is still unknown. In the present study, ketamine pretreatment was effective for preventing neuronal damage in the amygdala and hippocampus, which supported the hypothesis that NMDA-receptor antagonism could prevent lidocaine-induced neurotoxicity. We think lidocaine has two effects on neurons of the limbic structures mentioned. One effect is short-term, and it causes disruption of intracellular Ca^{2+} homeostasis [3,9]. A second effect is long-term and involves the intracellular signaling pathways of NMDA-receptor activation [5]. A high intracellular Ca^{2+} concentration could activate constitutive nitric oxide synthase (NOS, EC 1.14.13.39), because the preadministration of N^{G}-vitro-L-arginine methylester (L-NAME) prevented a lidocaine-caused seizure [10]. In addition, lidocaine-caused nitrate accumulation in neuronal cultures was shown to be related to increased NOS activity and to inducible isoform iNOS overexpression [11]. Also, high intracellular Ca^{2+} concentration-induced disturbances of the mitochondrial respiratory chain cause the production of reactive oxygen species [12] and unchain the long-term activation of signaling pathways involved in neuronal death [1]. Also, protein kinase type C (PKC) activated by lidocaine decreases membrane-cytoskeleton adhesion [13]. As well, the long-term effects of lidocaine on intra-

Table 1. Number of neurons in 10 000 μm^2 of the CA3 hippocampal and basolateral amygdala regions of the brain in rats treated with 100 mg·kg^{-1} ketamine 30 min before one dose of 60 mg·kg^{-1} lidocaine

Region		Group I (control; n = 5)	Group II (0.9% s.s. + 60 mg·kg^{-1} lidocaine; n = 5)	Group III (0.9% s.s. + 100 mg·kg^{-1} ketamine; n = 5)	Group IV (100 mg·kg^{-1} ketamine + 60 mg·kg^{-1} lidocaine; n = 5)
CA3 hippocampal region	Normal	20.2 ± 0.3	5.4 ± 0.2*	20.0 ± 0.7	9.8 ± 0.6*,**
	Abnormal	4.7 ± 0.2	17.0 ± 0.1*	4.4 ± 0.09	14.4 ± 0.2*,**
Basolateral amygdala	Normal	16.9 ± 0.4	5.4 ± 0.2*	17.5 ± 0.2	13.6 ± 0.5*,**
	Abnormal	2.3 ± 0.2	15.8 ± 0.5*	3.1 ± 0.3	6.7 ± 0.4*,**

*P < 0.05 vs control group; **P < 0.05 vs 0.9% s.s. + 60 mg·kg^{-1} lidocaine
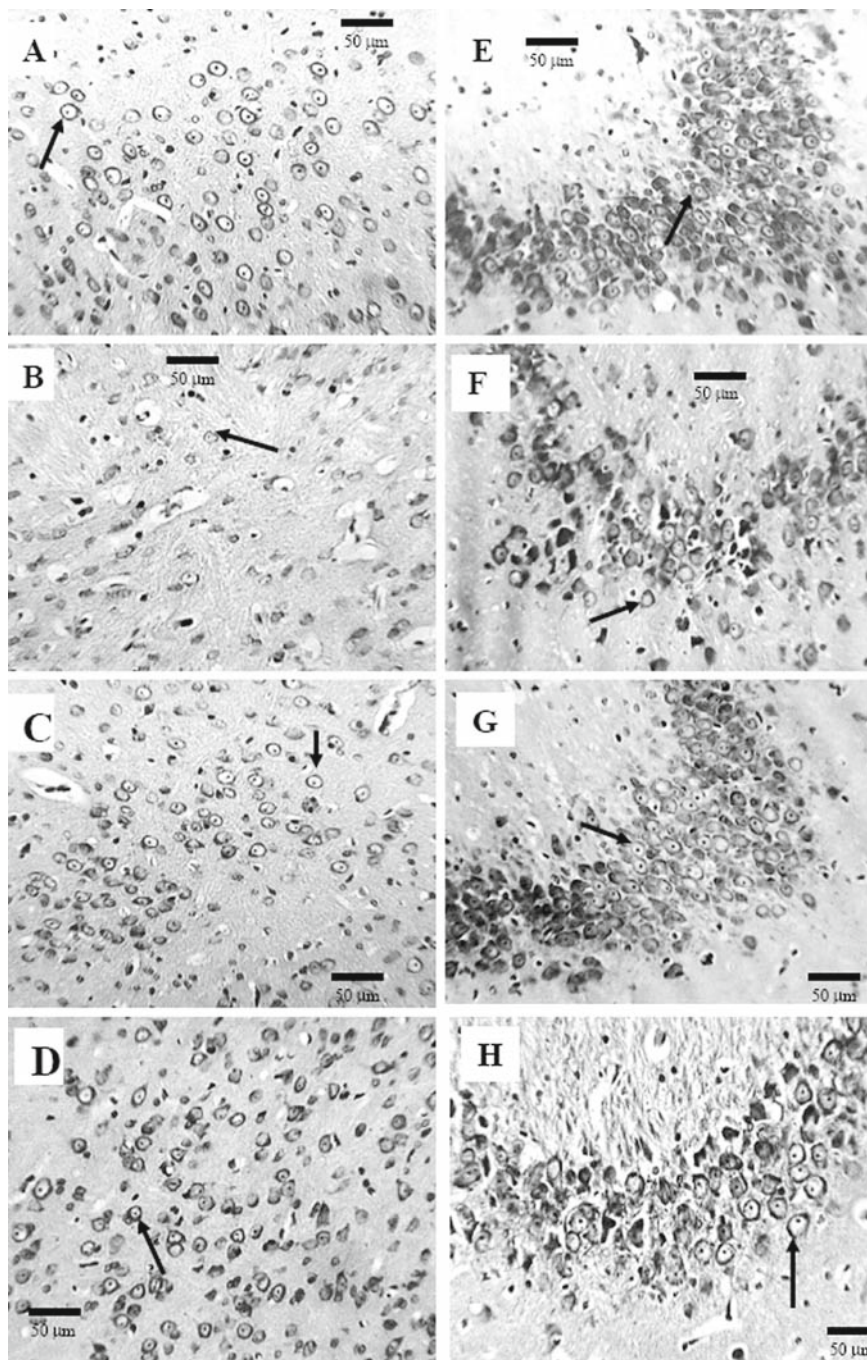Data values are means ± SEM; s.s., saline solution; n, number of animals

Fig. 1A–H. Local brain damage in baso-lateral amygdala (**A** 0.9% saline solution; **B** lidocaine 60 mg·kg^{-1}; **C** ketamine 100 mg·kg^{-1} + 0.9% saline solution; **D** ketamine 100 mg·kg^{-1} + lidocaine 60 mg·kg^{-1}) and CA3 hippocampal region (**E–H**) caused by lidocaine administration revealed by cresyl-eosin staining. *Arrows* in **A**, **E**, **C**, **G**, **D** and **H** show normal neurons; *arrows* in **B** and **F** show abnormal neurons

cellular signaling have been shown to involve activation of the intrinsic pathway of apoptosis [14], and apparently this event is related to the phosphorylation of focal adhesion kinase (FAK) [15] mediated by PKC activation [16].

The research in the present study demonstrates the participation of NMDA-receptor activation by lidocaine in the CA3 hippocampal and basolateral amygdala regions in lidocaine's neurotoxic mechanism. However, we need more behavioral, biochemical, and molecular studies of the neuronal damage caused by lidocaine. Also, the intracellular signaling pathways that are involved in the lidocaine-caused cellular damage in the above-mentioned limbic structures should be studied in more depth.

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