Clonidine decreases kynurenic acid production in rat brain cortex in vitro – a novel antihypertensive mechanism of action?

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Abstract

Introduction and objective. Clonidine, an antihypertensive agent, is known to activate presynaptic α2-adrenoreceptors and imidazoline receptors in the central nervous system. Clonidine may also have influence on glutamatergic neurotransmission. Kynurenic acid (KYNA) is an endogenous antagonist of excitatory amino acid receptors. Cerebral KYNA synthesis from its bioprecursor L-kynurenine is regulated by aminotransferases localized preferentially within astrocytes. Isoforms KAT I and KAT II are mainly responsible for the formation of KYNA in the brain. KYNA was shown to display potent neuroprotective properties and its impaired production was implicated in central nervous system diseases [11]. Additionally, it has been reported that KYNA could play a role in the regulation of blood pressure since a significant reduction of blood pressure has been achieved by KYNA administration to the RVLM in an animal model of hypertension [12]. However, KYNA did not change basal blood pressure levels in freely moving [13] or anesthetized normotensive animals after intrathecal [14] or intra-RVLM administration [12], but reversed cardiovascular stimulation caused by the RVLM activation.

Conclusion. The study revealed that clonidine decreases KYNA production in rat brain cortex in vitro. The obtained results suggest that augmentation of glutamatergic transmission may play an important role in the antihypertensive action of clonidine.

Key words
kynurenic acid, clonidine, arterial hypertension, central nervous system, brain cortex

INTRODUCTION

Clonidine is a centrally-acting antihypertensive agent which activates both imidazoline receptors (I,) and α1-adrenergic receptors in the central nervous system [1, 2]. The exact mechanism by which clonidine decreases blood pressure is not fully understood.

It is known that clonidine may have an influence on glutamatergic neurotransmission [3]. Glutamate (GLU) is the main excitatory mediator leading to neuronal fibers activation in the RVLM (rostral ventrolateral medulla) [4]. It is well documented that both N-methyl-D-aspartate (NMDA) and α-amino-3-hydroxy-5-methyl-4-isoazolopropionic acid (AMPA)/kainate receptors influence blood pressure level [5]. Furthermore, it was found that GLU administered intracerebrally leads to pressor cardiovascular responses [6]. Interestingly, NMDA or AMPA receptor antagonists were shown to abolish the decrease in blood pressure and heart rate evoked by intracerebroventricular administration of clonidine [7]. Similar effects were observed when clonidine was given intravenously [8, 9].

Kynurenic acid (KYNA) is the only known endogenous antagonist of GLU and α7-nicotinic receptors [10]. Cerebral synthesis of KYNA from its bioprecursor L-kynurenine is catalyzed by kynurenine aminotransferases (KAT) localized preferentially within astrocytes. Isoforms KAT I and KAT II are mainly responsible for the formation of KYNA in the brain. KYNA was shown to display potent neuroprotective properties and its impaired production was implicated in central nervous system diseases [11]. Additionally, it has been reported that KYNA could play a role in the regulation of blood pressure since a significant reduction of blood pressure has been achieved by KYNA administration to the RVLM in an animal model of hypertension [12]. However, KYNA did not change basal blood pressure levels in freely moving [13] or anesthetized normotensive animals after intrathecal [14] or intra-RVLM administration [12], but reversed cardiovascular stimulation caused by the RVLM activation.

OBJECTIVES

The aim of the study was to examine whether clonidine affects KYNA synthesis in rat brain cortex in vitro.

MATERIALS AND METHOD

Experiments were conducted on male Wistar rats (weight 150–200 g). Animals were kept under standard laboratory conditions at 20 °C temperature, 12-hour light-dark cycles, with food and water available ad libitum. Experiments were performed between 07:00–13:00. All utilized procedures were approved by the I Local Ethics Committee for Animal Experiments and are in agreement with Directive.
Statistical significance was set at P<0.05. All calculations of variance (ANOVA) with post-hoc Tukey-Kramer test. Minimum of 3 times.

2 hours at 37 °C, the samples were placed into an ice cold Krebs-Ringer buffer. Each experiment was conducted at least 3 times to achieve comparable results.

To prepare fresh cortical slices, the rats were decapitated, their brains removed from the skulls and placed into ice cold Krebs-Ringer buffer, as described previously [15]. Dissected brain cortex was immediately cut with a McIlwain tissue slicer. Cortical slices (1mm × 1mm) were placed into incubation wells (10 slices in each well) filled with oxygenated Krebs-Ringer buffer at pH 7.4. Each well contained 1 ml of Krebs-Ringer buffer. Incubation was carried out for 2 hours at 37 °C in the presence of 10 μM of L-kynurenine and 7 different clonidine concentrations (0.001 mM; 0.01 mM; 0.1 mM; 0.5 mM; 1 mM; 3 mM or 5 mM). A minimum of 6 wells were used to examine each clonidine concentration. After incubation, the obtained supernatants were centrifuged and applied to ion exchange resin Dowex 50 W+ column. Eluted KYNA was analyzed by HPLC and quantified fluorometrically (ThermoFisher Scientific HPLC system; ESA catecholamine HR-80, 3 μm, C18 reverse-phase column). Experiments were conducted at least 3 times to achieve comparable results.

To assess KAT I and KAT II activity, rat brain cortex obtained after decapitation were homogenized in the dialysate buffer containing 5 mM Tris-acetate buffer at pH 8.0, 50 μM pyridoxal 5'-phosphate and 10 mM 2-mercaptoethanol. Prepared brain cortical homogenate was centrifuged, then the supernatant was dialyzed for 12 hours at 8 °C with the use of cellulose membrane dialysis tubing in 4 l of the dialysate buffer, prepared as described previously. Later, homogenate was incubated in the mixture containing the incubation solution (different for KAT I and KAT II), L-kynurenine and clonidine’s solutions (7 tested concentrations). The reaction pH was 9.5 for KAT I activity and 7.0 for KAT II activity. At the end of sample preparation, L-glutamine was added to achieve activation of imidazoline (I1) receptors and α2-adrenergic receptors [4]. It was also shown that clonidine may influence glutamatergic neurotransmission. Clonidine decreases GLU release in the spinal cord and brain structures [17, 18, 19, 20]. However, a hypothetical decrease of GLU release induced by clonidine cannot explain the inhibition of KYNA production in cortical slices. On the contrary, an increase should be expected, since GLU dose-dependently reduces KYNA synthesis in the brain and spinal cord slices [21].

Interestingly, suggestions of a common binding site of I1 and NMDA or AMPA/kainate receptors have been presented. An electrophysiological study showed that 8 imidazolines, including clonidine, dose-dependently and non-competitively inhibited NMDA currents [22]. Furthermore, it was proposed that a binding site for imidazolines is located within the NMDA channel pore [23, 24].

There is also strong evidence of a functional interaction between GLU and imidazolines within the RVLM influencing cardiovascular regulation. It has been reported that intra-RVLM injection of 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), a potent AMPA/kainate receptor antagonist, attenuated the reduction in blood pressure, heart rate, and
renal sympathetic nerve activity elicited by intra-RVLM or intravenous clonidine [8]. Similarly, it was demonstrated that direct intra-RVLM injection of NMDA receptor antagonist dizocilpine significantly attenuated the decrease in blood pressure and heart rate induced by intracerebral clonidine, and antagonized the bradycardia and hypotension produced by intravenously administered clonidine [9]. Importantly, KNYA injected intracebroventricularly was shown to reverse cardiovascular response to clonidine [7]. Thus, taking into account that KNYA is a broad spectrum GLU antagonist acting on both NMDA and AMPA/kainate receptors, it can be speculated that reduction of KNYA synthesis elicited by clonidine may result in amelioration of the antihypertensive action of the drug.

CONCLUSIONS

In conclusion, the obtained findings prompt the suggestion of a novel mechanism of action of clonidine, and probably other antihypertensive imidazolines as well.

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REFERENCES


