Clonidine action in spontaneously hypertensive rats (SHR) depends on the GABAergic system function

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Summary. The effect of γ-aminobutyric acid (GABA)A antagonists (bicuculline, picrotoxin) on clonidine hypotension in spontaneously hypertensive (SHR) and Wistar Kyoto (WKY) rats were examined. The GABA turnover changes after clonidine injection in both strains were also studied. Administration of clonidine alone induced the stronger decrease of systolic blood pressure (SBP) in SHR. Co-dosage of clonidine with these agents reduced its hypotensive effect in dose dependent manner and the effectiveness of both antagonists was higher in SHR. We find that clonidine stimulates GABA synthesis in the hypothalamus and the pons-medulla in both strains but the GABA turnover rate is significantly slower in SHR. Therefore, the differences in inhibitory action of GABA_A receptor antagonists between WKY and SHR rats may be explained by central GABAergic system dysfunction in the hypertension. Our results indicate that the down regulation of the GABAergic system observed in hypertension may be compensated by the action of clonidine.

Keywords: Amino acids – Clonidine – GABA_A receptor antagonists – GABA turnover – SHR – WKY rats

Introduction
Clonidine (CLON) is a well know centrally acting antihypertensive drug, which produces hypotensive effect inducing by various mechanisms at the CNS level (Karege and Gaillard 1990, Weekly et al., 1985). Some authors (Pittaluga and Raiteri, 1988) suggest that GABAergic transmission plays an important role in the mechanism of clonidine action. The results obtained by Marmo et al. (1987) indicate that GABA_A receptor antagonist bicuculline decreases antihypertensive effect of clonidine in the same manner in normotensive (WKY) and hypertensive rats (SHR). It is worth noting, that our previous results (Czyżewska-Szafran and Wutkiewicz, 1986) and those of others (Wible et al., 1988; Sasaki et al., 1992; Singewald et al., 1992), have
shown that GABAergic inhibition is impaired in hypertension. This implies that GABA<sub>A</sub> receptors antagonists action should be more pronounced in hypertensive rats. Lately, our studies (Czyżewska-Szafran et al., 1991) performed only on SHR rats demonstrate that clonidine is able to evoke evident changes in GABAergic indices. Therefore, the goal of the current studies is to explore the contribution of γ-aminobutyric acid (GABA) to the clonidine hypotension in SHR in comparison with WKY rats.

**Material and methods**

**Animals**

Age-matched female 15 week-old rats weighing 230 ± 10g (WKY), 180 ± 8g (SHR) were used. Rats were maintained on a 12/12h light/dark cycle, at a constant temperature, five to cage with free access to food and water.

**Blood pressure recording**

SBP was measured in conscious rats by the indirect tail-cuff method using a pulse detector connected to a computer (IITC. Inc. Mod. 179, Woodland Hills, CA 91367.1253, USA). Animals were maintained at a temperature of 26 ± 1°C during blood pressure recording sessions. All rats were acclimated to the measuring device for 3 days prior to measurements. Mean value from at least 8 consecutive readings for computation was used. Only rats with stable basal SBP were taken for the experiment. SHR or WKY rats received intraperitoneally single clonidine doses of 5, 10 or 20μg/kg. SBP was measured immediately before and 45 min after clonidine administration. Bicuculline was given at the doses of 0.5, 1.0 and 1.5mg/kg and picrotoxin at the doses of 0.5, 1.0, 1.5, 2.0mg/kg intraperitoneally. SBP was recorded before and 15 min after GABA<sub>A</sub> antagonist administration. To study the combined effects of clonidine and GABAergic antagonists, bicuculline was injected at the doses of 0.5, 1.0 and 1.5mg/kg and picrotoxin at the doses ranging from 0.5 to 2.0mg/kg. In this experiment clonidine doses were 20μg/kg in WKY and 20 or 10μg/kg in SHR. The antagonists were given 30min after clonidine.

**Biochemical studies**

Since indirect measurement of blood pressure is stressful to the animals and may alter the amino acid content of the brain, additional group of rats were used for the study of changes in GABA chemistry after administration of drugs. Two groups of rats were injected i.p. with the GABA transaminase inhibitor aminooxyacetic acid at a dose of 50mg/kg according to Yoneda et al. (1983). 15min later one group received saline and second one clonidine. All rats were decapitated 60min after AOAA injection for determination of GABA content. The difference in GABA content between AOAA alone and AOAA with clonidine treated groups was used to determine the GABA turnover. To prevent post mortem increase in GABA content, rats before decapitation were injected with the glutamic acid decarboxylase inhibitor 3-mercaptopropionic acid (100 mg/kg i.p.), according to Carmona et al. (1980). GABA accumulation in the hypothalamus and the pons-medulla was determined. The tissues were isolated according to Balcom et al. (1975). Frozen tissues were homogenized in a Potter-Elvehjem homogenizer and centrifuged (20min, 8000g, 4°C). The clear supernatants were stored at -20°C for up to 24h. The GABA concentration was determined spectrofluorimetrically according to Lowe et al. (1958) with the modification of Uchida and O’Brien (1964). Fluorescence was determined with a Shimadzu spectrofluorometer at 380/450nm. GABA content was expressed in nmol/mg protein. The detection limit of GABA was 0.1nM. The protein