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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 anatgonists 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 $GABA_A$ 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 ± 10 g (WKY), 180 ± 8 g (SHR) were used. Rats were maintained on a 12/12 h 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 20 μg/kg. SBP was measured immediately before and 45 min after clonidine administration. Bicuculline was given at the doses of 0.5, 1.0 and 1.5 mg/kg and picrotoxin at the doses of 0.5, 1.0, 1.5, 2.0 mg/kg intraperitoneally. SBP was recorded before and 15 min after $GABA_A$ antagonist administration. To study the combined effects of clonidine and GABAergic antagonists, bicuculline was injected at the doses of 0.5, 1,0 and 1.5 mg/kg and picrotoxin at the doses ranging from 0.5 to 2.0 mg/kg. In this experiment clonidine doses were 20 μg/kg in WKY and 20 or 10 μg/kg in SHR. The antagonists were given 30 min 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 50 mg/kg according to Yoneda et al. (1983). 15 min later one group received saline and second one clonidine. All rats were decapitated 60 min 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 (20 min, 8000 g, 4°C). The clear supernatants were stored at -20°C for up to 24 h. 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 spectrofluorotometer at 380/450 nm. GABA content was expressed in nmol/mg protein. The detection limit of GABA was 0.1 n/M. The protein

concentration was determined by the method of Lowry et al. (1951) using bovine serum albumin as standard.

Chemicals

Clonidine hydrochloride (CLON) and bicuculline methobromide (BIC) were purchased from Sigma Chemical Co (USA) and picrotoxin (PIC) from Aldrich chemical Co (FRG). All drugs were dissolved in saline. Picrotoxin solution was adjusted to pH 7.4 with 0.5 N NaOH and the final volume made up of 0.9% NaCL. The solutions were injected intraperitoneally in a volume of 1 ml/kg. All other compounds were of the highest quality available commercially.

Data analysis

All values are expressed as means ± S.E.M. The statistical significance was calculated by using Student's t-test.

Results

Basal SBP was significantly higher in SHR as compared to WKY rats (Table 1.) In both strains clonidine induced a dose-dependent decrease of systolic blood pressure. Nonetheless, the drug was more effective in SHR than in WKY rats. Thus, to achieve the similar decreases of blood pressure in WKY as in SHR it was necessary to double the dosage. There was no influence of picrotoxin or bicuculline on SBP at the doses ranging from 0.5 to 2.0 mg/kg in both strains (Table 1). The $GABA_A$ receptor antagonists counteracted dose dependently the hypotension of clonidine. The effectiveness of picrotoxin in reducing the clonidine action is much higher in SHR than in WKY rats. The antihypertensive effect of clonidine administered at a dose 20 μg/kg was re-

Table 1. Systolic blood pressure response to clonidine and $GABA_A$ receptor antagonists administration. The data are presented as mean ± SEM (n = 10)

Treatment		SBP (mmHg)	
		WKY	SHR
Control	1 ml/kg	138 ± 3	196 ± 5
Clonidine	5 μg/kg	117 ± 4*	160 ± 3**
	10 μg/kg	105 ± 5*	149 ± 3**
	20 μg/kg	96 ± 9**	131 ± 7**
Control	1 ml/kg	137 ± 6	188 ± 4
Picrotoxin	0.5 mg/kg	–	177 ± 6
	1.0 mg/kg	126 ± 6	185 ± 5
	1.5 mg/kg	141 ± 3	181 ± 7
	2.0 mg/kg	142 ± 4	177 ± 6
Control	1 ml/kg	135 ± 5	187 ± 7
Bicuculline	0.5 mg/kg	124 ± 6	182 ± 4
	1.0 mg/kg	134 ± 4	185 ± 2
	1.5 mg/kg	131 ± 3	186 ± 4

*$P < 0.01$; **$P < 0.001$ compared with vehicle group.

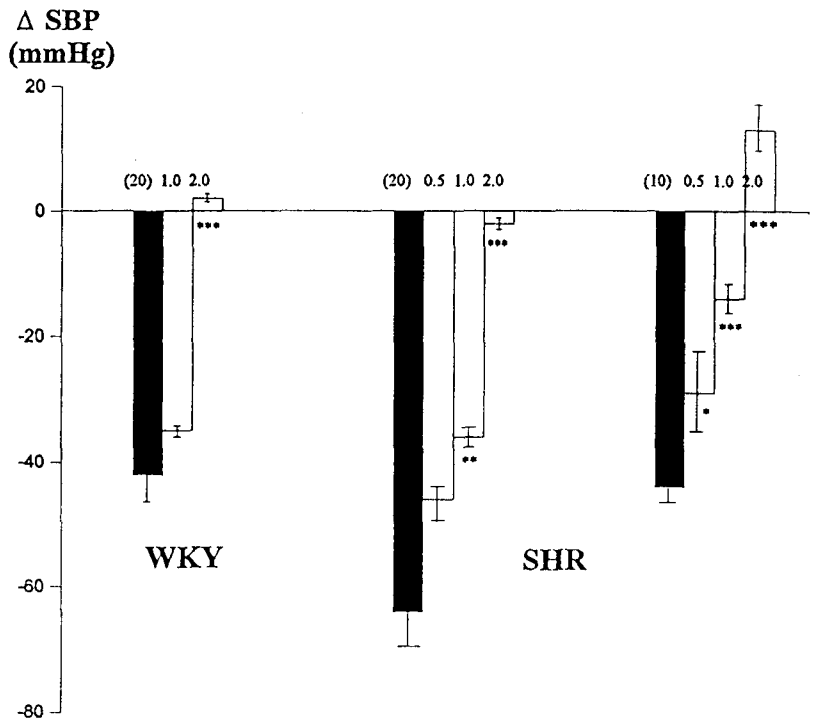

Fig. 1. Effect of picrotoxin on hypotension induced by clonidine in conscious SHR and WKY rats. ■ CLON; □ CLON plus PIC. Number in parentheses indicates the dose of clonidine alone, others show doses of picrotoxin combined with clonidine. SBP was measured 45 min after CLON administration. Each value is the mean ± SEM of 7–9 rats. Statistical significance: * $P < 0.05$, ** $P < 0.001$, *** $P < 0.0001$ vs. controls

duced by picrotoxin at a dose 1 mg/kg to 44% and 17% of its original value in SHR and WKY, respectively. This phenomenon occured also when equivalent hypotensive doses of clonidine were used, that is 10 μg/kg in SHR and 20 μg/kg in WKY (Fig. 1). Similar effect was observed after administration of bicuculline in combination with clonidine. Clonidine hypotension was diminished by bicuculline in a dose related fashion. The hypertensive rats were more sensitive to antagonistic effects of bicuculline than normotensives (Fig. 2). It was found that clonidine elevated the GABA turnover in the studied areas in SHR and WKY rats (Fig. 3). Increase of GABA turnover was significantly higher in the hypothalamus than in the pons-medulla, in both strains. Although, the effect of clonidine was weaker in SHR than in WKY rats. Initial GABA content was also lower in hypothalamus and the pons-medulla in SHR in comparison with WKY i.e. 44 and 35 nmol GABA/mg protein and 78 and 71 nmol GABA/mg protein, respectively.

Discussion

The present data demonstrate the differences between SHR and WKY rats either in the $GABA_A$ receptor antagonists action on clonidine hypotension or

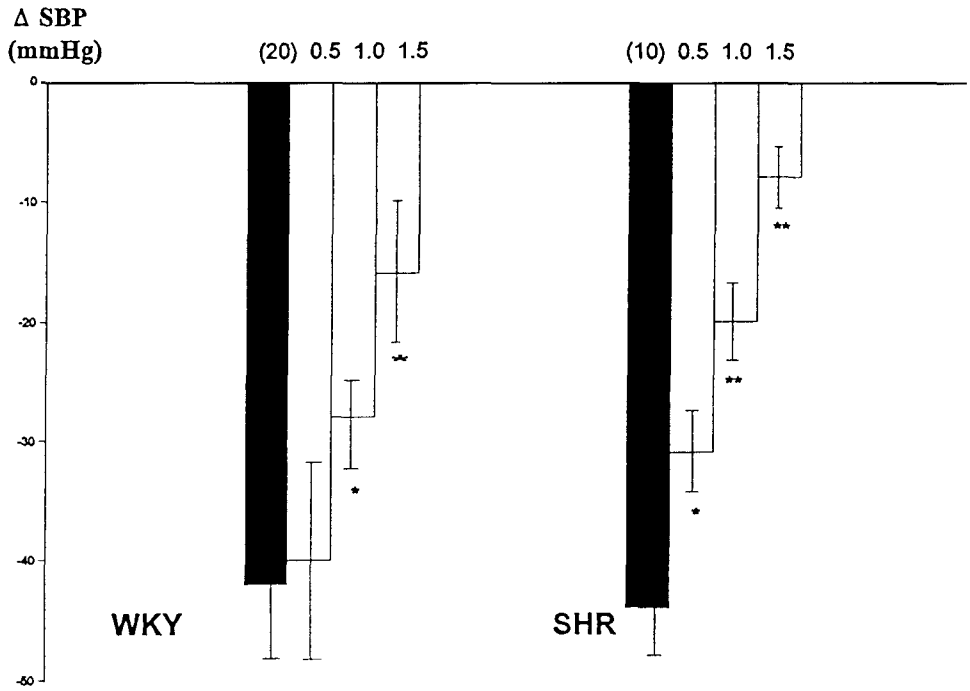

Fig. 2. Effect of bicuculline on clonidine-induced hypotension in conscious SHR and WKY rats. ■ CLON; □ CLON plus BIC. *P < 0.05, **P < 0.0001. Other details as in Fig. 1

in GABA turnover rate after drug injection. It should be kept in mind that the both $GABA_A$ antagonists counteract the clonidine hypotension more effectively in SHR. Since bicuculline and picrotoxin given alone did not affect systolic blood pressure, this antagonism cannot be explained by a direct pharmacodynamic interaction. The same observation was made in our previous studies with muscimol (Jastrzębski et al., 1993). It is well established that these GABAergic antagonists interfere with $GABA_A$ receptor complex i.e. as picrotoxin by blockade of the chloride ion channels or as bicuculline by competition with GABA for the recognition site (Soltis and DiMicco, 1991). It is possible that antihypertensive action of clonidine relies on the $GABA_A$ receptor stimulation. Thus, the reduction of the clonidine antihypertensive effectiveness by picrotoxin or bicuculline could be related to the antagonistic action of these agents at the level of the $GABA_A$ receptor complex. Presently, there is a wide body of evidence showing the impairment of central GABAergic system in the hypertension (Czyżewska-Szafran et al., 1989; Sasaki et al., 1990; Singewald et al., 1992). This may indicate that the stronger impact of GABA antagonists on clonidine hypotension in SHR can be due to the downregulation of GABAergic system. It should be noted, that our results are in contrast with those found by Marmo et al. (1987). We believe that a lack of differences between SHR and WKY rats in their studies may simple be dependent on the fact that the time of observation was not well selected.

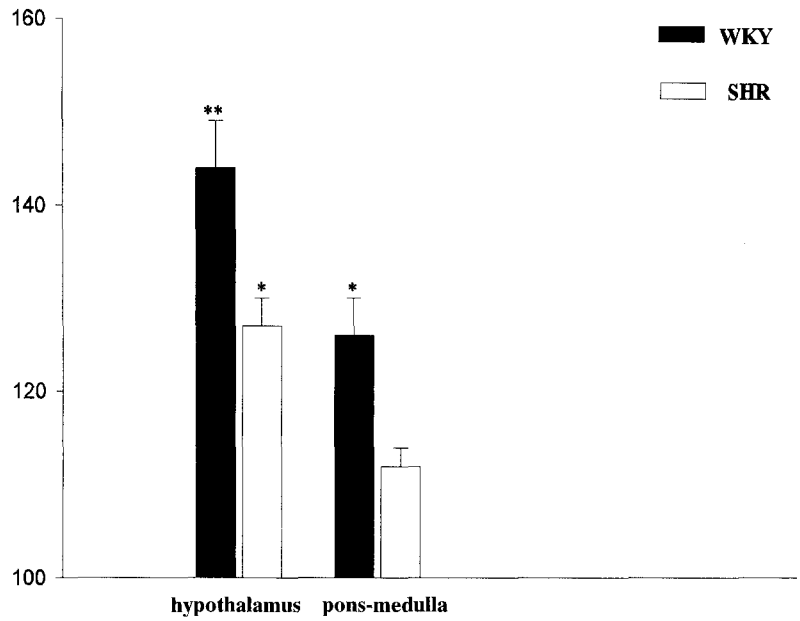

Fig. 3. AOAA-induced GABA accumulation after clonidine 10μg/kg (SHR) and 20μg/kg (WKY) (AOAA alone = 100%) Effect of clonidine on hypothalamus and pons-medulla GABA accumulation induced by AOAA in rats. Clonidine was injected 15 min after AOAA, 50 mg/kg i.p. GABA accumulation was determined 60 min after injection of AOAA. The effects of clonidine on AOAA-induced GABAaccumulation are shown in percent compared to the individual control group which was treated with AOAA alone together with clonidine treated group. For these calculations, the GABA content determined after AOAA alone in the respective control group was used as 100%. Data are means ± SEM of 9–11 rats per time. Significant differences between GABA accumulation in groups treated with clonidine plus AOAA compared to the individual time-matched group treated with AOAA alone are marked by asterisk * $P < 0.05$ ** $P < 0.001$. Average GABA contents in rats treated with AOAA alone (mean ± SEM of 9–11 rats) were as follows (all values in nmol/mg protein): hypothalamus 125 ± 7 (WKY), 73 ± 4 (SHR), pons-medulla 91 ± 6 (WKY), 49 ± 3 (SHR) AOAA-induced GABA accumulation after clonidine 10μg/kg (SHR) and 20μg/kg (WKY) (AOAA alone = 100%)

The present study argues the weaker effect of clonidine on GABA turnover in the hypothalamus and pons-medulla in SHR than in WKY rats. Some reports (Hambley et al., 1984; Wible et al., 1989) provide evidence that particularly in these areas abnormalities occur in GABAergic inhibition. In our previous experiments we also indicated that the basal rate of GABA turnover in the hypothalamus was significantly lower in hypertensive rats than in normotensive ones (Czyżewska-Szafran and Wutkiewicz, 1986). Similar results for the hypothalamus were reported by Sasaki et al. (1992). Therefore, it could be explained that the less potent clonidine effect in SHR is connected with the impairment of the GABAergic system in hypertension.

Our results indicate that the spontaneously hypertensive rats are more sensitive to the antihypertensive action of clonidine than the normotensive rats. It is consistent with the results reported previously by other authors (Dalas et al., 1986; Tibrica et al., 1988) by using similar doses and routes of

drug administration. From our data it can be seen that clonidine stimulates GABAergic system function. Thus, it is very likely that one of the possible mechanisms of the antihypertensive action of clonidine consists of restoration of the normal GABAergic neurotransmission. It is well known that in the essential hypertension the function of many neurotransmitter systems controlling arterial blood pressure is changed (Schultz et al., 1988; Lindhorst et al., 1993; Yang et al., 1994). As mentioned in the introduction hypotensive effect of clonidine is also induced by various neurotransmitter systems. Thus, with respect to the higher effectiveness of clonidine in hypertensive animals and simultaneously with the weaker drug effect on the GABA turnover we confirm that not only the GABAergic system is involved in the antihypertensive action of clonidine.

In summary, we postulate that the downregulation of the GABAergic system observed in hypertension may be compensated by the regulatory action of clonidine.

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