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INCREASED ACTIVITY OF THE GABAERGIC SYSTEM IN SELECTED BRAIN AREAS AFTER CHRONIC PROPRANOLOL TREATMENT IN SPONTANEOUSLY HYPERTENSIVE RATS

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Abstract—The influence of chronically administered propranolol on the functional state of the γ-aminobutyric acid-ergic (GABAergic) system in spontaneously hypertensive rats was studied and compared with the effect of dihydralazine. GABA content, synthesis and turnover rate in selected brain areas were assessed. Hypotensive activity of propranolol and dihydralazine after injection of GABA antagonist pictrotoxin was examined in acute experiment. Prolonged administration of propranolol increased GABA content, synthesis and turnover rate in the hypothalamus and the pons-medulla. After chronic injections of dihydralazine there was no change in GABA indices. Antihypertensive activity of dihydralazine in picrotoxin-treated animals remained unchanged. On the contrary, picrotoxin suppressed the propranolol-induced decrease in blood pressure. Our results indicate that propranolol increases GABAergic system activity. Therefore, we conclude that down-regulation of the GABAergic system in hypertension may be compensated by the regulatory action of propranolol.

γ-Aminobutyric acid (GABA†), the primary inhibitory neurotransmitter in the central nervous system, participates in cardiovascular regulation [1]. Many investigations imply that central GABAergic dysfunction may contribute to the development of spontaneous hypertension [2–4]. The mechanism of genetic hypertension is still not fully elucidated. However, there is evidence that increased peripheral sympathetic nerve activity originating from the central nervous system is of great importance [5–8]. Wible *et al.* [9] proved that in spontaneously hypertensive rats (SHR) and Wistar Kyoto rats (WKY) the hypothalamus contains a sympatho-excitatory mechanism that is inhibited by GABA. Abnormalities in GABAergic inhibition have been found in several regions of the brain, including the hypothalamus. It has been reported that GABA indices are significantly lower in SHR than in Wistar Kyoto rats [3, 4, 10, 11]. These studies clearly demonstrate that GABAergic inhibition is impaired in hypertension.

Previous data from our laboratory have indicated that the hypotensive action of clonidine is related to the stimulation of GABAergic function in SHR [12]. Our results are in agreement with those reported by other authors, who showed that clonidine enhances GABA release in some brain structures [13–15]. Therefore, it is possible that the GABAergic system is involved in the antihypertensive mechanism of other centrally acting drugs. β-Adrenoceptor blockers, including propranolol, are widely used as antihypertensive agents. Despite many existing hypotheses and much research the mechanism of the hypotensive action of propranolol remains unclear. At the beginning, it was suggested that the drug lowers blood pressure exclusively via peripheral action [16, 17]. Recently however, several findings demonstrated apparent central hypotensive effects of propranolol and determined putative brain areas involved in this action [18, 19]. The present experiments were carried out to establish whether the postulated impairment of GABAergic transmission in hypertension can be attenuated by propranolol.

MATERIALS AND METHODS

Animals and drugs

Male 14–16-week-old SHR were used. The rats were housed five to a cage, given food and water *ad lib.* and maintained on a 12 hr dark–light cycle under standard conditions.

DL-Propranolol HCl and dihydralazine HCl were obtained from Polfa (Warsaw, Poland). Picrotoxin and aminooxyacetic acid (AOAA) were purchased from the Sigma Chemical Co. (St Louis, MO, U.S.A.). Other chemicals used were reagent grade. All drugs were dissolved in saline except propranolol which was dissolved in water. Solutions of propranolol and AOAA were adjusted to pH 7.4 by the addition of 0.01 N sodium hydroxide.

Experimental design

In the first series of experiments two groups of rats were administered chronically propranolol or dihydralazine, and the third group, being a control,

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† Abbreviations: AOAA aminooxyacetic acid; GABA, γ-aminobutyric acid; MAP, mean blood pressure; SHR, spontaneously hypertensive rats.

received saline. Propranolol was administered orally at a dose of 30 mg/kg in a volume of 10 mL/kg three times daily for 8 days. Dihydralazine was injected subcutaneously at a dose of 20 mg/kg in a volume of 1 mL/kg once daily for 8 days.

Mean blood pressure (MAP) was recorded indirectly in conscious rats with a photoelectric system using tail pulsations at 25–28° (IITC Inc., Mod. 59, NJ, U.S.A.). Measurements were done prior to propranolol administration and on the 3rd, 7th and 8th day of the experiment. In the dihydralazine group blood pressure was recorded on the 1st, 3rd and 8th day after drug injection. The mean value of four consecutive readings was used for calculations.

Since indirect measurement of blood pressure is stressful to the animals and may alter the amino acid content of the brain, three additional groups of rats were used for the study of changes in GABA chemistry after repetitive administration of drugs or saline as described. For biochemical analysis the rats were killed by decapitation on the 8th day of the experiment, 90 min after the last administration of drug. The brain was rapidly removed and frozen on dry ice. Four brain structures were isolated according to Balcom *et al.* [20]: hypothalmus, hippocampus, striatum and pons-medulla. GABA synthesis and turnover rate were determined after chronic administration of propranolol and dihydralazine. In addition, GABA content was also measured in rats receiving propranolol.

In the second series of experiments the influence of GABA antagonist picrotoxin on the hypotensive activity of propranolol and dihydralazine was examined. Animals received an acute administration of propranolol at a dose of 60 mg/kg p.o. or dihydralazine at a dose of 1 mg/kg s.c. Then, they were given picrotoxin in a volume of 5 mL/kg at a dose of 0.2, 0.6 or 1.0 mg/kg i.p. 75 min after propranolol and 15 min after dihydralazine. Blood pressure was recorded 15 min after GABA antagonist administration. Control groups received propranolol or dihydralazine only. Effect of picrotoxin was evaluated by comparing drug-treated groups with controls.

Biochemical analysis

GABA assay. All rats used for the determination of GABA were injected with the glutamic acid decarboxylase inhibitor 3-mercaptoproprionic acid (100 mg/kg i.p.) to prevent post mortem increase in GABA content, according to Carmona *et al.* [21]. Frozen tissues were homogenized in a Potter–Elvehjem homogenizer and centrifuged (20 min, 8000 *g*, 4°). The clear supernatants were stored at −20° for up to 24 hr. The GABA concentration was determined spectrofluorimetrically according to Lowe *et al.* [22] with the modification of Uchida and O'Brien [23]. Fluorescence was determined with a Shimatzu spectrofluorometer at 380/450 nm. GABA content was expressed in nmol/mg protein. The detection limit of GABA was 0.1 nM. The protein concentration was determined by the method of Lowry *et al.* [24] using bovine serum albumin as standard.

GABA synthesis. In order to increase the intracellular concentration of GABA rats were injected i.p. with the GABA transaminase inhibitor aminooxyacetic acid at a dose of 100 mg/kg 30 min before decapitation, according to Löscher [25]. GABA accumulation within the areas studied was determined. Turnover rate was estimated from the difference in the GABA content of AOAA-injected and non-injected animals.

Statistics

The statistical significance of the difference between means was evaluated using Student's *t*-test for independent samples. All values given are means ± SEM.

RESULTS

Effects of prolonged propranolol or dihydralazine administration on blood pressure

The basal blood pressure of SHR before administration of drugs was 204 ± 5 mmHg and was not significantly altered by solvents. Chronic administration of propranolol for 8 days at a total daily dose of 90 mg/kg p.o. produced a significant ($P < 0.01$) fall in blood pressure on the third day. The effect was intensified on the consecutive days of the experiment. The maximum decrease in blood pressure of 37 mmHg was reached on the 7th day. Prolongation of the treatment for up to 15 days did not change this effect (unpublished data). In dihydralazine-treated animals significant reduction of blood pressure ($P < 0.01$) occurred after the first injection. The effect was maintained at almost the same level throughout the whole experiment (Fig. 1).

GABA content after propranolol administration

Chronic administration of propranolol increased

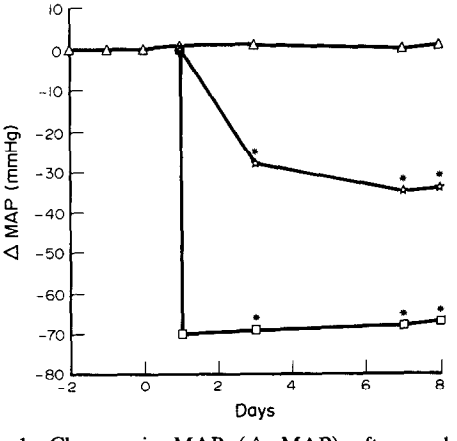

Fig. 1. Changes in MAP (△ MAP) after prolonged administration of propranolol (☆), dihydralazine (□) or saline (△) in conscious SHR. The drugs and saline were administered for 8 consecutive days. The initial blood pressure in the groups was similar: 204 ± 5 mmHg (N = 16). Values are expressed as means ± SEM of 7–10 rats. The significance of differences as compared with the current control group is indicated by asterisks: *$P < 0.01$.

Table 1. The effect of prolonged propranolol treatment on the GABA content of selected brain areas removed from rats 90 min after the last oral dose

	GABA (nmol/mg protein)	
	Control	Propranolol
Hypothalamus	31.6 ± 1.5	36.7 ± 0.7*
Hippocampus	31.9 ± 1.3	37.3 ± 0.8†
Striatum	27.9 ± 0.8	29.2 ± 0.6
Pons-medulla	30.1 ± 0.7	34.2 ± 0.8†

Values are means ± SEM obtained from 5–6 separate experiments in quadruplicate. *$P < 0.01$, †$P < 0.001$.

the GABA content of all regions examined except the striatum. The differences in the GABA content of these regions as compared with the control group were highly significant (Table 1).

GABA synthesis and turnover rate after chronic administration of propranolol or dihydralazine

AOAA increased significantly ($P < 0.01$) the GABA content of all brain regions. Propranolol augmented significantly ($P < 0.01$) GABA accumulation in the hypothalamus and the pons-medulla. The concentration of GABA did not change in the hippocampus or the striatum (Fig. 2).

It was found that the GABA turnover rate was very similar in all regions except the hippocampus. It ranged from 16.4 to 21.0 nmol/mg protein/hr. GABA turnover was slowest in the hippocampus. Chronic pretreatment with propranolol elevated significantly the GABA turnover rate in the hypothalamus by 155% ($P < 0.001$). GABA turnover increased also in the pons-medulla by 65% ($P < 0.001$). In contrast, chronic administration of dihydralazine did not alter the GABA turnover in any brain region (Fig. 2).

Hypotensive effect of propranolol and dihydralazine after treatment with picrotoxin

It was found that administration of picrotoxin suppressed the propranolol-induced decrease in MAP and it even caused an increase in MAP after the higher doses of the GABA antagonist. The effect was dose-related. Picrotoxin at a dose of 0.2 mg/kg reduced the hypotensive effect of propranolol from 34 to 10 mmHg. At the higher picrotoxin doses, i.e. 0.6 and 1.0 mg/kg, the propranolol effect was abolished. The antihypertensive activity of dihydralazine remained unchanged in picrotoxin-treated animals (Fig. 3).

DISCUSSION

Clinical studies have shown that propranolol is an effective hypotensive agent when used in the long-term treatment of patients with essential hypertension [26]. On the contrary, in spontaneously hypertensive rats the hypotensive effect of propranolol is questionable. Weiss *et al.* [27] reported that chronic treatment of SHR with propranolol (100 mg/kg) prevented the development of hypertension in young animals but it was ineffective against already established hypertension in adult animals. Other authors [28] found that propranolol decreased the blood pressure of adult SHR but only after 48 days of treatment. The results of the subsequent studies confirmed the fact that propranolol exerts its

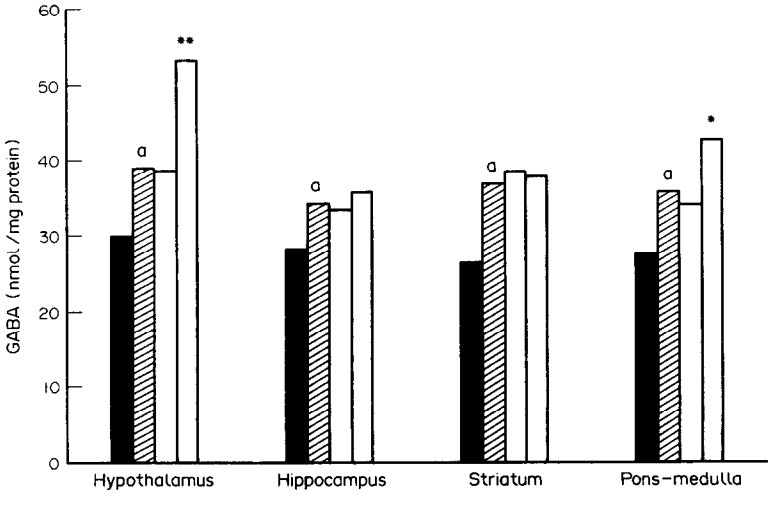

Fig. 2. Comparison of the effects of propranolol (P) and dihydralazine (DIH) on regional GABA accumulation induced by AOAA. (■) Control group, (▨) AOAA-treated group, (□) AOAA + DIH-treated group, (□) AOAA + P-treated group. Data are means ± SEM from 7–10 rats in each drug-treated group. Control data are from 12 animals. Differences between controls and AOAA-treated rats are marked by a ($P < 0.001$), and between groups treated with P plus AOAA and AOAA alone by asterisks: *$P < 0.01$, **$P < 0.001$.

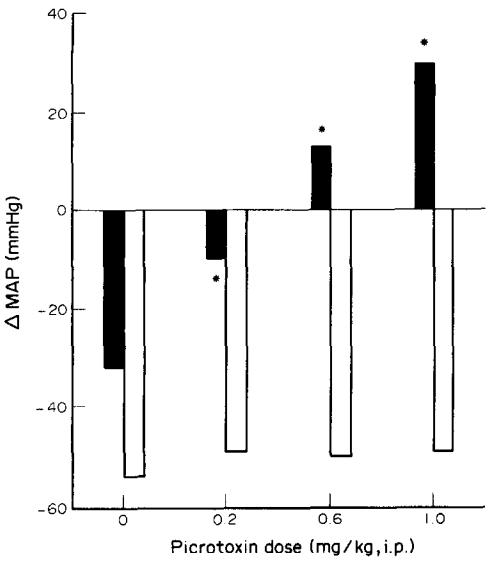

Fig. 3. Comparison of the effects of propranolol (P) and dihydralazine (DIH) on MAP (△ MAP) after pictrotoxin injection. (■) P-treated group with or without picrotoxin, (□) DIH-treated group with or without picrotoxin. Data are means ± SEM from 7–9 rats in each group. Differences between P-treated rats and rats treated with P plus picrotoxin are marked by asterisks: *$P < 0.01$.

antihypertensive effect in young rats and that its effectiveness in adult SHR is uncertain [29, 30]. The present study demonstrates that repetitive administration of propranolol lowers the blood pressure of adult SHR on the 3rd day of treatment. The observed discrepancy in rat reactivity to propranolol between our results and those of others [27, 29] might be due to differences in the development of hypertension in the animals used.

Recently, much interest has been focused on the possible mechanism of propranolol action. The exact mode and site of action remains unclear and both peripheral and central mechanisms have been postulated. Some authors [31, 32] suggested that the reduction of cardiac output and renin release are the primary effects of propranolol action. However, others had an opposite view as they could not demonstrate any significant correlation between propranolol-induced hypotension and reduction of plasma renin activity [33, 34]. More recent evidence indicates that the central nervous system plays an important role in the hypotensive effect of propranolol. Matsunaga *et al.* [35] and Privitera *et al.* [19] reported that the central administration of propranolol into various brain areas produced a significant fall in blood pressure. The effect was attributed to the inhibition of sympathetic outflow from the central nervous system.

There are many reports on the cerebral areas involved in the hypotensive action of propranolol [19, 36–39]. The most important ones are the hypothalamus, hippocampus, C_1 area of rostral ventrolateral medulla, striatum and septum. Accordingly, we assayed GABA indices in the following brain regions: hypothalamus, pons-medulla, striatum, and hippocampus. In the present study, prolonged administration of propranolol activated the GABAergic system in the hypothalamus and the pons medulla, structures closely related to cardiovascular control [9, 19]. However, it cannot be excluded that other brain areas are also involved in the effect of propranolol. In contrast, multiple dihydralazine injections did not alter the GABA chemistry in any of the studied structures. Our results indicate that dihydralazine hypotension cannot be attributed to changes in GABAergic activity. They are inconsistent with the results of Kihara and Kubo [40] and Kubo *et al.* [41] showing a decreased GABA content of the medullary regions of normotensive rats receiving dihydralazine. It seems very likely that in those experiments the effects on GABA content were due mainly to the decrease in blood pressure and not to any specific central effect of dihydralazine and they could be explained most probably by a feedback mechanism.

Recent evidence suggests that in hypertension the effect of centrally acting hypotensive drugs is based—besides other factors—on stimulation of the GABAergic neurons [2, 3, 12]. Lack of such effect after dihydralazine administration indicates that this drug does not lower the blood pressure through any influence on the central nervous system. Furthermore, the present study demonstrated that the blood pressure-lowering effect of propranolol was reversed in SHR treated with the GABA antagonist picrotoxin. In contrast, the hypotensive response to dihydralazine was not antagonized by this antagonist. The data obtained support the hypothesis that the GABAergic system is involved in the hypotensive action of propranolol.

Previous studies [19, 36, 38, 39] proved that the hypotensive effect of propranolol is evoked by the alteration of noradrenergic neurotransmission. It has been postulated that the hypotensive action of propranolol results from a drug-induced release of norepinephrine from central noradrenergic neurons which subsequently activates postsynaptic neuronal α-2 adrenergic receptors producing a fall in systemic arterial pressure [19]. Regardless of these observations, our study indicates the importance of the GABAergic system in the hypotensive effect of propranolol. Taking into account the similar distribution of noradrenergic and GABAergic neurons in all brain structures, related to blood pressure control, a direct interaction between them cannot be excluded [42, 43]. It was found that the central GABAergic dysfunction may result in reduced inhibition of the sympathetic outflow and therefore facilitate the development of hypertension [3, 4, 9]. Thus, it may be assumed that the interaction of both neurotransmitter systems determines the hypotensive effects and may represent a crucial mechanism of propranolol action.

In conclusion, the present study demonstrates that propranolol-induced hypotension is accompanied by an enhancement of GABA content, synthesis and turnover rate, predominantly in the hypothalamus and the pons-medulla. GABA antagonist attenuates the hypotensive effect of propranolol. These data strongly suggest that propranolol exerts its

hypotensive effect also through the activation of GABAergic neurons.

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