Blood Pressure Reduction Induced by Chronic Intracerebroventricular or Peroral Clonidine Administration in Rats with Salt-Dependent or Angiotensin II-Dependent Hypertension

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Summary

The agonists of α_2-adrenergic receptors such as clonidine, rilmenidine or monoxidine are known to lower blood pressure (BP) through a reduction of brain sympathetic outflow but their chronic antihypertensive effects in rats with low-renin or high-renin forms of experimental hypertension were not studied yet. Moreover, there is no comparison of mechanisms underlying BP reduction elicited by chronic peroral (po) or intracerebroventricular (icv) clonidine treatment. Male salt-sensitive Dahl rats fed a high-salt (4% NaCl) diet and Ren-2 transgenic rats were treated with clonidine administered either in the drinking fluid (0.5 mg/kg/day po) or as the infusion into lateral brain ventricle (0.1 mg/kg/day icv) for 4 weeks. Basal BP and the contributions of renin-angiotensin system (captopril 10 mg/kg iv) or sympathetic nervous system (pentolinium 5 mg/kg iv) to BP maintenance were determined in conscious cannulated rats at the end of the study. Both peroral and intracerebroventricular clonidine treatment lowered BP to the same extent in either rat model. However, in both models chronic clonidine treatment reduced sympathetic BP component only in rats treated intracerebroventricularly but not in perorally treated animals. In contrast, peroral clonidine treatment reduced angiotensin II-dependent vasoconstriction in Ren-2 transgenic rats, whereas it lowered residual blood pressure in Dahl rats. In conclusions, our results indicate different mechanisms of antihypertensive action of clonidine when administered centrally or systemically.

Keywords

Central α_2-adrenergic receptors • Sympathetic tone • Dahl salt-sensitive rats • Ren-2 transgenic rats • Vasoactive systems • Renin-angiotensin system • Sympathetic nervous system • Nitric oxide

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Introduction

The activation of brain α_2-adrenoceptors and/or I_1 imidazoline receptors has sympatholytic effects leading to the reduction of blood pressure (BP) [1,2]. The acute administration of clonidine, rilmenidine or moxonidine elicited a substantial BP decrease in conscious spontaneously hypertensive rats (SHR) [3,4]. On the other hand, chronic treatment of SHR with these drugs caused a less pronounced BP reduction [5-7]. There are few data on BP effects induced by intracerebroventricular (icv) administration of the above drugs. It seems that the acute icv injection of clonidine or rilmenidine caused BP reduction only in pentobarbital-anesthetized SHR but not in conscious animals [8]. However, Nurminen *et al.* [9] reported that acute icv administration of clonidine or moxonidine lowered BP, heart rate (HR) and sympathetic nerve activity (SNA) in conscious SHR. The improved baroreflex sensitivity and enhanced control of sympathetic tone was described in SHR subjected to acute icv injection of clonidine or moxonidine [10,11].

Most of the data on BP effects of chronic stimulation of α_2-adrenoceptors or I_1 imidazoline receptors were obtained in SHR, whereas the information on other models of experimental hypertension is rather scarce. As far as salt-dependent hypertension is concerned, Fujita *et al.* [12] reported that chronic oral

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moxonidine treatment reduced BP and sympathetic tone in uninephrectomized Sprague Dawley rats fed 8 % NaCl diet. On the other hand, chronic icv moxonidine administration, which substantially decreased urinary norepinephrine excretion, caused a considerable HR reduction but only minimal BP changes in salt-sensitive Dahl rats with established salt hypertension [13]. It should be noted that the chronic stimulation of hypothalamic α_2-adrenoceptors with clonidine lowered plasma norepinephrine levels, BP and heart hypertrophy in SHR fed 8 % NaCl diet, whereas intravenous clonidine infusion had no such effects [14]. The acute icv injection of α_2-adrenoceptor agonist guanabenz caused a more pronounced decrease of BP, HR and renal SNA in salt hypertensive Dahl rats than in normotensive animals of this strain [15,16].

Hypertension in heterozygous Ren-2 transgenic rats (TGR) is characterized not only by enhanced angiotensin II-dependent vasoconstriction but it has also a very important sympathetic BP component. Our earlier study [17] demonstrated that the contribution of the sympathetic nervous system (SNS) to BP maintenance is increasing in heterozygous TGR with age. Another our study [18] indicated that chronic intracerebroventricular administration of losartan (angiotensin type 1 receptor blocker) or aliskiren (direct renin inhibitor) lowered BP of heterozygous TGR through the reduction of sympathetic tone. Finally, we have reported that central sympathoexcitation in TGR is highly susceptible to the inhibition by low doses of icv administered losartan [19].

The aim of the present study was to evaluate the effects of chronic treatment with clonidine (agonist of α_2-adrenoceptors) on blood pressure in two different models – salt hypertension in Dahl rats and Ren-2 transgenic rats with angiotensin II-dependent hypertension. We also compared cardiovascular effects of peroral clonidine administration with those elicited by chronic icv clonidine infusion. A special attention was paid to sympathetic tone changes (evaluated on the basis of BP response to acute ganglionic blockade) elicited by these two different modes of clonidine treatment.

Methods

Animals

Male inbred salt-sensitive (SS/Jr) Dahl rats aged 8 weeks were obtained from the breeding facility of the Institute of Physiology CAS in Prague. This colony was established from the breeding pairs kindly provided by Prof. John R. Rapp in 1985. The animals were housed at 23 °C under a 12 h light/dark cycle, given tap water *ad libitum* and fed a low-salt diet (0.3 % NaCl) prior to the experiment. During the experiment they were switched to a high-salt diet (4 % NaCl) for four weeks.

Male heterozygous (mRen-2)27 transgenic (TGR) rats aged 10 weeks were housed at 23 °C under a 12 h light/dark cycle, given Altromin diet (0.45 % NaCl) and tap water *ad libitum*. All rats used in this study were bred at the Institute of Clinical and Experimental Medicine (Prague) from stock animals supplied from Max Delbrück Center for Molecular Medicine in Berlin, Germany.

All procedures and experimental protocols were approved by the *Ethical Committee of the Institute of Physiology, Czech Academy of Sciences* (Protocol Nr. 90/2019) and conform to the *European Convention on Animal Protection and Guidelines on Research Animal Use* (Directive 2010/63/EU).

Experimental strategy

In chronic experiments, adult Dahl and TGR animals were subjected to either systemic (peroral, po) or central (intracerebroventricular, icv) administration of α_2-adrenoceptor agonist clonidine for 4 weeks. At the end of the experiment we determined the participation of major vasoactive systems (RAS, SNS and NO) in BP maintenance of conscious cannulated animals.

Chronic systemic and intracerebroventricular administration of clonidine

Chronic systemic administration of clonidine (0.5 mg/kg/day in the drinking fluid) was compared with chronic icv application of clonidine (0.1 mg/kg/day). The used clonidine doses were selected on the basis of our preliminary experiments.

For chronic icv clonidine application the osmotic minipumps (Alzet, type 2004) were placed on the back of the animals and connected by polyethylene catheter with the fixed application icv cannula which was implanted into the lateral cerebral ventricle 24 h before the experiments [18]. Briefly, animals were anesthetized with 2.5 % isoflurane. Permanent cannula was placed in a position for later injections into the right lateral ventricle using stereotactic apparatus (AP = -1.0, L = 1.8, V = 4.2; according to Paxinos and Watson [20]) and fixed to the skull with synthetic resin (Duracrol, Pentron, Czech Republic). Clonidine infusion into lateral cerebral ventricle lasted 4 weeks.

In acute experiments, a separate group of Dahl rats fed 4 % NaCl diet received clonidine (100 μg in a volume of 2 μl) through the application icv cannula. Thirty min later, ganglionic blocker pentolinium (5 mg/kg iv) was given to evaluate the actual contribution of sympathetic nervous system to BP maintenance. At the end of acute experiments hypertonic saline (2 μl of 3 M NaCl) was injected into the lateral ventricle to verify the patency of the implanted icv cannula.

Participation of major vasoactive systems in blood pressure maintenance

The changes of mean arterial pressure (MAP) elicited by the sequential blockade of major vasoconstrictor and vasodilator systems (RAS, SNS and NO) were determined in conscious rats cannulated one day prior to the experiment. Two polyethylene cannulas which were implanted under 2.5 % isoflurane anesthesia (PE 50 for BP measurement in the left carotid artery, PE 10 for the infusion of drugs to the jugular vein), were exteriorized in the interscapular region. Blood pressure and heart rate were recorded using a pressure transducer and a multichannel recorder (ADInstruments, Bella Vista, Australia) in conscious animals placed in small plastic cages. All measurements were carried out between 8:00 - 12:30 to reduce the circadian variations in BP levels.

We used a modified protocol of Minami *et al.* [21] which was adapted in our laboratory [17, 22]. Briefly, baseline MAP levels were recorded in conscious rats after a 30-min adaptation period in transparent measuring plastic cages. Then, we started with RAS blockade (captopril 10 mg/kg iv) which was followed by ganglionic blockade of SNS (pentolinium 5 mg/kg iv) 15 min later. Five min later, NO synthase inhibitor (30 mg/kg L-NAME iv) was injected and BP was monitored for further 20 min. Finally, sodium nitroprusside (20 μg/kg iv) was given to induce maximal dilatation and to record so-called residual MAP. BP levels before and after particular blockades were determined and the respective BP changes were calculated.

Drugs

All drugs were obtained from Sigma (St Louis, Missouri, USA). Captopril, pentolinium and L-NAME were dissolved in saline and given as intravenous bolus injections (1 ml/kg). Clonidine was administered either in the drinking water (0.5 mg/kg/day) or intracerebroventricularly in saline (0.1 mg/kg/day).

Statistics

The data are presented as the means ± SEM. One-way ANOVA was used to analyze BP and HR as well as their response to the sequential blockade of above mentioned vasoactive systems. The differences were considered to be significant at $p<0.05$ level.

Results

Salt-sensitive Dahl rats

Chronic clonidine treatment lasting four weeks attenuated the development of salt hypertension in salt-sensitive Dahl rats fed 4 % NaCl diet from the age of 8 weeks (Fig. 1). Both peroral and intracerebroventricular clonidine administration lowered BP to the same extent but the mechanisms of clonidine-induced BP reduction seemed to be different in animals subjected to peroral or icv clonidine treatment. The expected attenuation of sympathetic tone (decreased BP response to acute ganglionic blockade by pentolinium) was found only in rats receiving clonidine through icv infusion, whereas no significant change was observed in animals given clonidine in the drinking fluid. Chronic clonidine administration did not affect angiotensin II-dependent vasoconstriction (BP response to acute captopril injection) in either clonidine-treated group. NO-dependent vasodilation (evaluated as BP response to acute L-NAME injection) was diminished in both clonidine-treated groups of Dahl rats proportionally to clonidine-induced BP reduction (Fig. 1).

Peroral clonidine treatment (0.5 mg/kg/day) decreased body weight of salt hypertensive Dahl rats but this was not the case of animals treated with icv clonidine (0.1 mg/kg/day) (Table 1). Relative heart weight was greater in rats treated with peroral clonidine as compared to those treated by icv clonidine infusion. On the other hand, peroral but not icv clonidine treatment reduced relative kidney weight of salt hypertensive Dahl rats. Both clonidine-treated groups differed substantially in residual MAP recorded after the sequential RAS and SNS blockade. Residual MAP was significantly decreased in rats treated with peroral clonidine, while it was increased in rats treated with icv clonidine infusion (Table 1).

The acute administration of clonidine (100 μg) into the lateral brain ventricle of salt hypertensive Dahl rats lowered their BP by 13±3 mm Hg through the attenuation of sympathetic tone because this acute clonidine pretreatment diminished pentolinium-induced

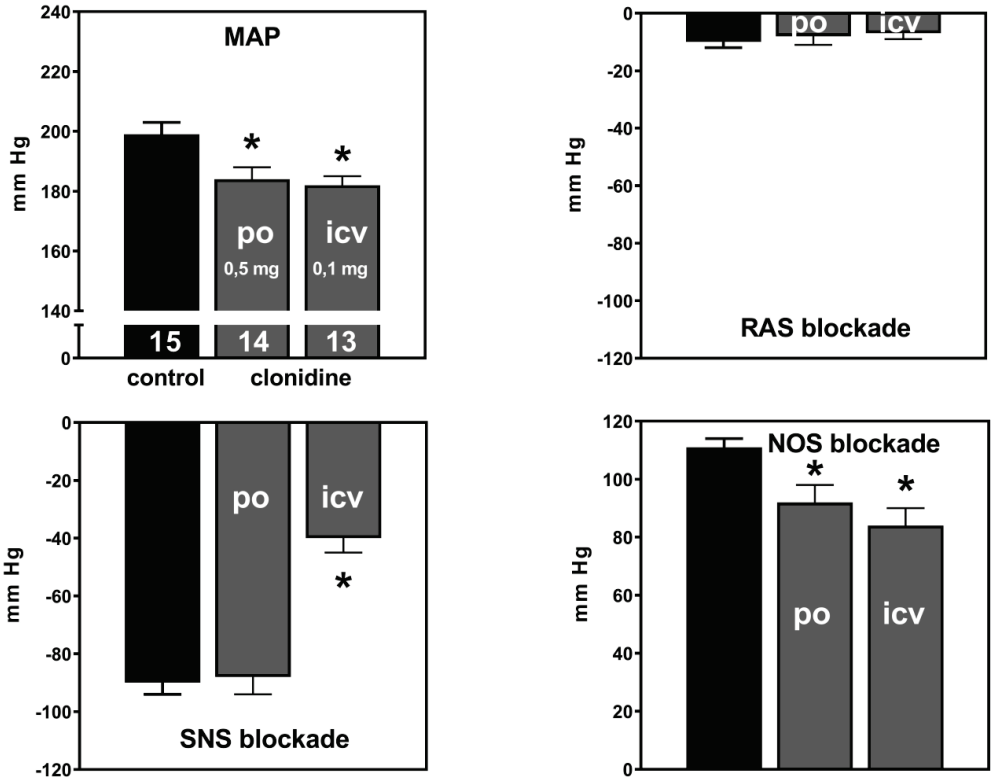

Fig. 1. Blood pressure effects of chronic peroral (po, 0.5 mg/kg/day) or intracerebroventricular (icv, 0.1 mg/kg/day) administration of clonidine to male salt-sensitive Dahl rats fed high-salt diet (4 % NaCl). MAP – basal mean arterial pressure, RAS blockade – MAP change after acute captopril injection (10 mg/kg iv), SNS blockade – MAP change after acute pentolinium injection (5 mg/kg iv), NOS blockade – MAP change after acute L-NAME injection (30 mg/kg iv). Data are means ± SEM, number of animals are given at the bottom of the columns. * significantly different ($p<0.05$) from the controls.

Table 1. Body weight, relative heart and kidney weights and residual blood pressure in male salt-sensitive Dahl rats fed high-salt diet (4 % NaCl) which were subjected to chronic peroral (po, 0.5 mg/kg/day) or intracerebroventricular (icv, 0.1 mg/kg/day) administration of clonidine for 4 weeks.

	Controls	**po Clonidine**	**icv Clonidine**
Number of rats	15	14	13
Body weight (g)	328±6	270±18*	316±8$^{\#}$
Heart weight (mg/ 100 g)	362±8	400±19	350±6$^{\#}$
Kidney weight (mg/ 100 g)	1001±14	840±22*	956±17$^{\#}$
Residual MAP (mm Hg)	71.7±1.4	65.5±2.0*	83.3±3.1*$^{\#}$

Data are means ± SEM, significantly different ($p<0.05$): * from the controls, $^{\#}$ from peroral Clonidine

BP reduction (-48±4 vs. -69±5 mm Hg in salt hypertensive animals not pretreated with icv clonidine administration).

Ren-2 transgenic rats

Chronic peroral or icv administration of clonidine also lowered BP of TGR with established angiotensin II-dependent hypertension, both treatments being similarly effective (Fig. 2). Intracerebroventricular clonidine administration reduced sympathetic tone as evidenced by the diminished BP response to acute pentolinium injection. This was not the case in TGR drinking clonidine solution in which sympathetic tone was not significantly altered but angiotensin II-dependent vasoconstriction was considerably reduced. There were no significant clonidine-induced changes in NO-dependent vasodilation (Fig. 2) or residual MAP (data not shown).

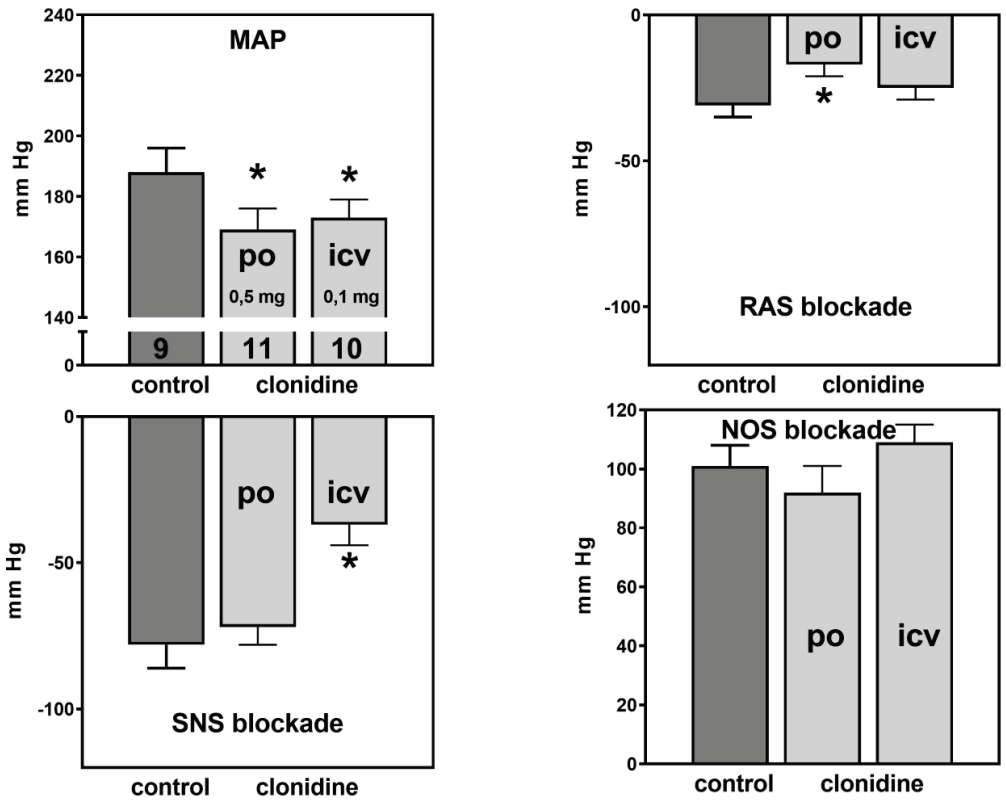

Fig. 2. Blood pressure effects of chronic peroral (po, 0.5 mg/kg/day) or intracerebroventricular (icv, 0.1 mg/kg/day) administration of clonidine to male heterozygous Ren-2 transgenic rats fed Altromin diet (0.45 % NaCl). MAP – basal mean arterial pressure, RAS blockade – MAP change after acute captopril injection (10 mg/kg iv), SNS blockade – MAP change after acute pentolinium injection (5 mg/kg iv), NOS blockade – MAP change after acute L-NAME injection (30 mg/kg iv). Data are means ± SEM, number of animals are given at the bottom of the columns. * significantly different ($p<0.05$) from the controls.

Discussion

Our study clearly demonstrated that chronic intracerebroventricular administration of α_2-adrenoceptor agonist clonidine lowered blood pressure in two different forms of experimental hypertension, i.e. Dahl rats withsalt hypertension and Ren-2 transgenic rats with angiotensin II-dependent hypertension. In both models the BP reduction was achieved by clonidine-induced attenuation of sympathetic tone which was elevated not only in salt hypertensive Dahl rats [23, 24] but also in heterozygous TGR [17, 25]. To our knowledge, this is the first report of the antihypertensive effects of chronic central α_2-adrenoceptor stimulation in either low-renin (Dahl rats) or high-renin (TGR) forms of experimental hypertension. The described sympatholytic mechanism of chronic icv clonidine treatment is in line with the earlier reports on antihypertensive effects of α_2/I_1 receptor agonists in SHR with genetic hypertension [9].

It is rather difficult to explain cardiovascular effects of chronic peroral clonidine treatment observed in the present study. In both rat models there was a similar degree of BP reduction induced by intracerebroventricular or peroral clonidine administration. However, peroral clonidine treatment did not affect sympathetic BP component in either Dahl rats or TGR. It should be noted that peroral clonidine treatment attenuated substantially angiotensin II-dependent BP component in TGR but this was not the case in Dahl rats On the other hand, peroral clonidine treatment of Dahl rats reduced their BP which seems to indicate enhanced vasodilation.

We must consider that clonidine stimulates α_2-adrenoceptors not only in the brain but also in the peripheral circulation. The activation of these receptors on vascular smooth muscle causes vasoconstriction [26] as it was demonstrated in the pithed rats subjected to ganglionic blockade [27]. In addition, clonidine also stimulates α_2-adrenoceptors located in the endothelium [28] causing the enhanced release of various vasodilator factors such as nitric oxide or prostacyclin [26, 29]. This vasodilator role of endothelial α_2-adrenoceptors becomes evident after the blockade of NO synthase or cyclooxygenase [30, 31]. Thus, we can speculate that the

long-term peroral administration of high clonidine doses (0.5 mg/kg/day) can induce substantial peripheral vasodilation, which might enhance sympathetic tone through baroreflex mechanisms.

Limitations of the study

Further experiments are necessary to evaluate other mechanisms, which might be responsible for the BP lowering elicited by peroral clonidine administration. One possibility is the attenuation of endothelin system, which was reported to be enhanced in these two forms of experimental hypertension – Dahl rats [32, 33] and TGR [34, 35]. It is also necessary to consider the enhancement of vasodilation mediated by prostacyclin, calcium-activated K^+ channels or endothelium-derived hyperpolarization factor that were reported to be important in salt hypertensive Dahl rats [36, 37] as well as in TGR [38, 39].

Conflict of Interest

There is no conflict of interest.

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