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Nitric oxide and L-type calcium channel influences the changes in arterial blood pressure induced by central angiotesin II

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ABSTRACT

This study has determined whether voltage dependent calcium channels are involved in ANG II-induced pressor effect by means of nitrergic mechanism. We have previously shown the central involvement of angiotensin II (ANGII) in the arterial blood pressure. The antipressor action of L-Type calcium channel antagonist, nifedipine, has been studied when it was injected into the trird ventricle (3rdV) prior to ANG II. The influence of nitric oxide (NO) on nifedipine antipressor action has also been studied by utilizing N^W-nitro-L-arginine methyl ester (L-NAME) (40μg.0.2μl^{-1}) a nitric oxide synthase inhibitor (NOSI) and L-arginine (20μg.0.2μl^{-1}), a nitric oxide donor agent). Rats Holtzman 200-250g, with cannulae implanted into the 3rd V were injected with ANG II into the 3rd V. MAP increased after ANG II injection. Such increase was potentiated by the prior injection of L-NAME. Losartan injected into the 3rdV, prior to ANG II, blocked the pressor effect of ANGII. PD 123319 decreased the pressor effect of ANGII. Rats pretreated with either 50μg.0.2μl^{-1} or 100μg.0.2μl^{-1} of nifedipine, followed by 25 pmol.0.2μl^{-1} of ANGII, decreased ANGII-pressor effect. However, L-NAME (40μg.0.2μl^{-1}) potentiated the pressor effect of ANGII, which was blocked by the prior injection of nifedipine and L-arginine (20μg.0.2μl^{-1}). These data have shown the correlation between L-Type calcium channel and a free radical gas nitric oxide (NO) in the central control of ANG II-induced pressor effect. This suggests that L-Type calcium channel participates in both short and long term neuronal actions of ANG II with the influence of nitrergic ways.

KEYWORDS

Blood pressure;
Angiotensin;
Calcium channel;
Nitric oxide;
CNS.

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INTRODUCTION

NO plays an important role in the hydromineral and cardiovascular regulation and influence several central angiotensin physiological parameters[1-4]. L-NAME increases blood pressure which is at least in part salt sensitive[5]. A major factor determining a neuronal Ca^{2+}-dependent signal is the opening of permeability pathways for Ca^{2+} in the cell membrane[6]. However, the interaction between nifedipine/NO of the CNS on the cardiovascular regulation has not been demonstrated yet. The objective of this study was to determine the role of voltage-sensitive calcium channels in ANGII-induced pressor response when ANG II was injected into the lateral 3rdV. We tested the action of nifedipine, L-Type calcium channel antagonist, and L-arginine a NO donor, which were injected into the 3rd V on ANGII-induced pressor effect. We also studied the influence of a nitric oxide synthase inhibitor, L-NAME, injected into the 3rdV on the nifedipine effect.

MATERIAL AND METHODS

The Medical Ethics Committee of the Universidade Estadual Paulista UNESP approved all protocols in this study.

Male Holtzman rats weighing 250-300g were anesthetized with zoletil 50mg/Kg (tiletamine chloridrate 125mg and zolazepan chloridrate 125mg) into quadriceps muscle. A stainless steel cannula with 10 and 12-mm long and 0.7-mm OD was implanted into the LV according to the coordinates of Paxinos and Watson atlas rat brain[7].

After the animals recovery from brain surgery (5 days) PE-10 polyethylene tubing connected to PE-50 tubing was inserted into the abdominal aorta through the femoral artery.

The study of arterial blood pressure, means arterial pressure (MAP), was started 5 days after the brain surgery. Each animal was used to 3 times.

The drugs were injected into the 3rdV by using a Hamilton micro syringe (5µl) connected by a PE-10 polyethylene tubing (25cm) to a needle (0.3mm o. d.), which was introduced into the brain through the cannula previously fixed to the animals' head.

Direct mean arterial blood pressure (MAP) was record in conscious rats in a test cage, without access to food or water.

The results are reported as mean ±S.E.M. The ANOVA and Newman-Keuls post-hoc test were used to determine the significance. The values were considered statistically significant with 5% level ($P<0.05$).

RESULTS AND DISCUSSION

At the end of the experiments, the rats were anesthetized with ether perfused with saline and buffered formalin. The brains were removed, fixed in 10% formalin, frozen to -25^{0}C and cut into 20-30µm coronal sections. Only animals in which the injection was placed in the LV were use in this study.

Effects of nifedipine and L-arginine on the increase of MAP induced by the injection of ANGII into the 3rd V figure 1

Microinjections of ANG II into the 3rd V cause an increase in MAP compared to control (15±2mmHg vs. control 4±1mmHg $P<0.05$). The microinjection of saline+nifedipine into the 3rd V caused no change in the MAP(3,5±1mmHg). Nifedipine 50µg injected into the 3rd V followed by ANG II decreased the ANGII-induced increase in MAP (11±1mmHg $P<0.01$). Nifedipine 100µg injected into the 3rdV followed by ANG II blocked the ANGII-induced increase in MAP (5±1 mmHg $P<0.01$). The injection of L-arginine also

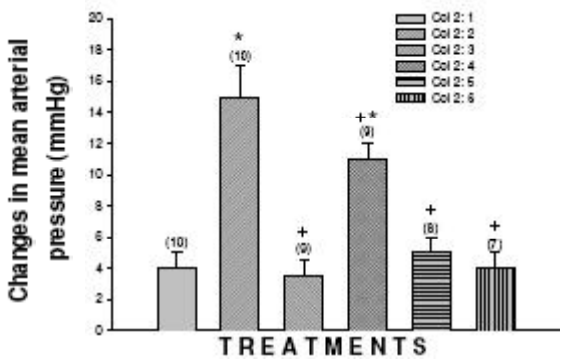

Figure 1: Mean arterial pressure following 3rdV injection of saline 0.15M NaCl (SAL), SAL+ANGII, SAL+Nifedipine 100µg (NIF), NIF 50µg followed by 25 pmol ANGII, NIF 100µg followed by 25 pmol ANGII. Number of animals at the top of each column. Data are means ± S.E.M. ANOVAs as follows: $F_{(5,47)}=79.6$, $P<0.01$.*$P<0.05$ (Neuman-Keuls post-hoc test) vs. SAL+SAL, $^+P<0.05$ vs. SAL+ANGII

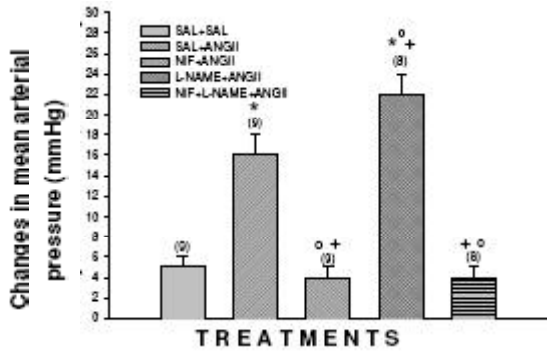

Figure 2: Mean arterial pressure following 3rd V injection of saline 0.15 M NaCl (SAL), SAL+ANGII, NIF 100µg followed by 25 pmol ANGII, L-NAME (LNA) followed by 25 pmol ANGII, NIF+LNA followed by 25 pmol ANGII. Number of animals at the top of each column. Data are means ± S.E.M. ANOVAs as follows: $F_{(4,38)}$ =81.3 P< 0.01.*P<0.05 (Neuman-Keuls post-hoc test) vs. SAL+SAL, +P<0.05 vs. SAL+ANGII and OP<0.05 vs.LNA+ANGII

blocked the pressor effect of ANG II (4±1 mmHg P<0.01). L-arginine injected alone into the 3rdV produced no change in the MAP.

Actions of nifedipine and L-NAME injected into the LV on ANGII pressor effects figure 2

ANGII produced an increase in the MAP. Nifedipine injected prior to ANGII produced a decrease in the MAP. The increased of MAP after ANGII was potentiated after injection of L-NAME. Rats injected with nifedipine 100µg prior to L-NAME followed by ANGII presented a decrease in the MAP.

In these experiments microinjections of ANG II into the 3rd V caused increase in the MAP, which was blocked by nifedipine and potentiated by L-NAME. The microinjection of L-arginine into the LV decreased the pressor effect of ANG II. The injection of nifedipine combined with L-NAME, which were injected prior to ANG II into the 3rd V, blocked the potentiation effect of L-NAME. The injection of L-NAME into the MnPO increased the MAP[8]. The treatment with L-NAME induced an increase in arterial blood pressure, which is at least in part salt sensitive. The action of L-NAME may be due to a local vasoconstriction. Furthermore, the salt-sensitive component appears to be ANG II-dependent, as it was associated with increase of plasma ANGII levels and could be reversed by the treatment with ANG II receptor antagonist[5].

These data, suggest that structures surrounding cerebral ventricles may release ANG II, which acts as a neurotransmitter resulting in postsynaptic effects, which in turn influence blood pressure control. Angiotensinergic neural pathways and calcium channels are important in neural function and may have important homeostatic roles, particularly related to cardiovascular function by involving NO. It has been demonstrated that NO antagonizes the vasoconstrictive and pro-atherosclerotic effects of ANGII, whereas ANGII decreases NO bioavailability by promoting oxidative stress. In the results of the present study we can suggest that nifedipine may have interfered with Ca^{2+} influx in the presynaptic terminals, where L-type calcium channels play important roles in the modulating presynaptic neurotransmitter release. It may also have altered Ca^{2+}-dependent signal events in postsynaptic neurons since previous studies demonstrated the permissive effects of voltage sensitive calcium channels (VSCCs) on NMDA receptor-mediated Ca^{2+} influx. In most neurons of the central nervous system, there are at least two major classes of calcium channel: voltage sensitive (VSCCs) and receptor-operated calcium channels (ROC). Ours results are strongly supported by the results of Li et al., and Saad et al., that demonstrated the hypertensive effect of ANGII was significantly enhanced by prior microinjection of L-NMMA into paraventricular nucleus (PVN) showing that the effect of ANGII in arterial pressure interact with nitric oxide[9-12].

CONCLUSION

The main find of these experiments is that nifedipine with or without L-NAME blocked ANGII-induced MAP increase when injected into 3rdV. By this result can explain that the pressor effect of ANGII into the circunventricular structures utilizes L-Type calcium channel to exert this effect. The nitric oxide also participates in ANGII effect. The influence of L-Type calcium channel and NO utilizing cGMP pathways on ANG II pressor effect can explain the results of this experiment. Finally we demonstrated the important role of ANGII in central regulation of arterial blood utilizing the calcium channel and nitric oxide.

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