BIBLID: ISSN 0253-9756 Acta Pharmacologica Sinica 中国药理学报 1996 Jan; 17 (1): 49—52

Cardiovascular effects of injection of argipressin into lateral septal nuclei in rats

GAO Lie, JIANG Nai-Chang, LUO Qing-Hua[1], ZHU Zhu-Xia
(*Department of Physiology, Guiyang Medical College, Guiyang 550004, China*)

KEY WORDS argipressin; septal nuclei; blood pressure; heart rate

AIM: To determine whether argipressin (Arg) plays a role in central neural control of cardiovascular function by acting on the lateral septal nuclei (LSN). **METHODS**: Measuring mean arterial blood pressure (MAP) and heart rate (HR) responses followed microinjection of Arg into the LSN of rats anesthetized with urethane. **RESULTS**: Arg (100, 200, and 400 ng) injected into the LSN produced a dose-dependent hypertension and tachycardia. Maximal changes of MAP were 0.9±0.6, 2.3±1.3, 4.0±1.4 kPa, respectively; maximal changes of HR were 12±27, 50±33, and 89±27 bpm, respectively. Pretreatment of the LSN with a vasopressin 1 type antagonist d(CH_2)$_5$Tyr(Me)Arg abolished the MAP and HR responses produced by injection of Arg. Peripheral α-adrenergic blockade with phentolamine blocked the hypertension responses to injection of Arg into the LSN. **CONCLUSION**: Arg acts in the region of the LSN to exert a central action on the cardiovascular system that is mediates by stimulation of sympathetic outflow.

The lateral septal nuclei (LSN) region in rat receives vasopressinergic fibers from hypothalamic vasopressin synthetic nuclei[1,2] and is implicated in blood pressure regulation[3-6]. Arg facilitates excitatory transmission in slices of rat dorso-lateral septum[7]. Thus, Arg might act at the level of the LSN to influence cardiovascular function. The purpose of this study was to determine the effects of Arg injected into the LSN on cardiovascular system in anesthetized rats.

MATERIALS AND METHODS

Wistar rats of either sex weighing 293±*s* 23 g (n=53) were anesthetized with urethane (1 g·kg^{-1}, ip). Single-barrel metallic casing pipe was stereotaxically implanted into the right LSN, used to inject Arg (Sigma) as well as vasopressin 1 type antagonist [β-mercapto-β, β-cyclopentamethylenepropionyl[1], *o*-MeTyr[2], Arg[8]]-vasopressin (d(CH_2)$_5$Tyr(Me)Arg) (Sigma). Then, the rats received a left carotid arterial catheter for measurement of mean arterial blood pressure (MAP) and heart rate (HR), a jugular venous catheter for phentolamine (Endo Laboratories) injection.

Both Arg and [d(CH_2)$_5$Tyr(Me)Arg] were dissolved in artificial cerebrospinal fluid (CSF) (Arg: 0.2, 0.4, and 0.8 g·L^{-1} CSF; d(CH_2)$_5$Tyr(Me)AVP: 1 μg/0.5 μL CSF). Phentolamine was dissolved in 0.9 % saline (10 g·kg^{-1}·L^{-1}, iv). Pure CSF (0.5 μL) and 0.9 % saline (0.2 mL) were used as control groups to avoid the effects of nonspecific stimulation, such as volume, pH, temperature, velocity of injection.

The Arg injection sites were marked at the end of experiment by injection 0.5 μL of pontamine sky blue into the LSN. Brain was fixed in 10 % formaline and 50 μm sections were cut for histological localization of the injection sites.

All reported values are $\bar{x}\pm s$. Comparisons between groups were made using t-test.

RESULTS

Cardiovascular effects of Arg in LSN Injection of Arg (100, 200, and 400 ng) into the LSN produced a dose-dependent hypertension and tachycardia. Injection of the artificial CSF vehicle into the LSN had no detectable effects on MAP and negligible effects on HR (Fig 1).

Basal MAP and HR were not significantly different in the 4 groups, $P>0.05$ (Tab 1).

The hypertension and tachycardia apperaed at 2 min and 2.5 min, recovered at 15—40 min and 30—55 min, reached peak effects at 10 min and 20—25 min, respectively after administration of 100, 200, and 400 ng into LSN.

d(CH_2)$_5$Tyr(Me)Arg pretreatment on Arg effects The antagonist itself had no effects on blood pressure or heart rate when injected into the LSN. The average MAP and HR prior to injection of the blocker were 11.5±1.0 kPa and 358±66 bpm, respectively; after blockade values

[1] *Department of Biology, Guizhou Normal University, Guiyang 550001, China.*
Received 1994-08-29 Accepted 1995-01-16

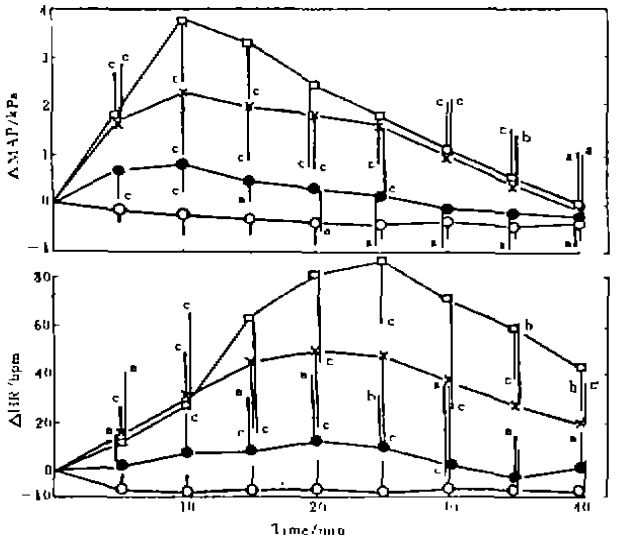

Fig 1. Changes in mean arterial pressure (△MAP) and heart rate (△HR) after injection of argipressin (Arg) into lateral septal nuclei (LSN) in anesthetized rats. Artificial CSF (○, $n=9$), Arg 100 (●, $n=9$), 200 (×, $n=10$), and 400 ng (□, $n=9$). $\bar{x}\pm s$. $^aP>0.05$, $^bP<0.05$, $^cP<0.01$ *vs* CSF.

Tab 1. Maximal changes of mean arterial blood pressure and heart rate after argipressin injection into lateral septal nuclei. $n=9-10$, $\bar{x}\pm s$. $^aP>0.05$, $^bP<0.05$, $^cP<0.01$ *vs* 0 ng.

Dose/ng	n	Basal	Maximal change
		Mean arterial blood pressure/kPa	
0	9	10.7±2.7	−0.5±0.1
100	9	11.2±4.4[a]	0.9±0.6[c]
200	10	11.0±4.4[a]	2.3±1.3[c]
400	9	10.4±3.6[a]	4.0±1.4[c]
		Heart rate/bpm	
0	9	356±24	−11±6
100	9	374±61[a]	12±27[a]
200	10	365±51[a]	50±33[c]
400	9	354±49[a]	89±27[c]

were 11.4±0.7 kPa and 352±68 bpm, respectively. Pretreatment of the LSN with the antagonist completely eliminated the MAP and HR responses to injection of 400 ng Arg into the LSN (Fig 2).

Phentolamine pretreatment on Arg effects

Treatment of rats with phentolamine (0.6 mg, iv) led to a decrease in MAP (10.5±2.5 kPa to 7.2±1.3 kPa) and an increase in HR (354±69 bpm to 397±82 bpm). Peripheral α-adrenergic blockade with phentolamine blocked completely the increase in MAP response to injection of 400

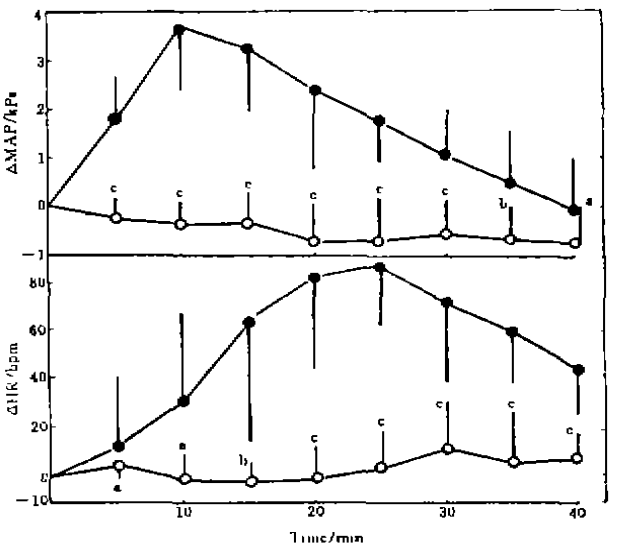

Fig 2. Antagonism of the responses induced by injection of Arg into LSN. Pretreatment of the LSN with d(CH$_2$)$_5$Tyr(Me)Arg (1 μg) eliminated hemodynamic responses to injection of Arg (400 ng) into LSN. Arg (●, $n=9$), d(CH$_2$)$_5$Tyr(Me)Arg + Arg (○, $n=5$). $\bar{x}\pm s$. $^aP>0.05$, $^bP<0.05$, $^cP<0.01$ *vs* Arg 400 ng.

ng Arg into the LSN. However, the peripheral administration of the α-adrenergic blocker had no effect on the HR responses to injection of 400 ng Arg into the LSN (Fig 3).

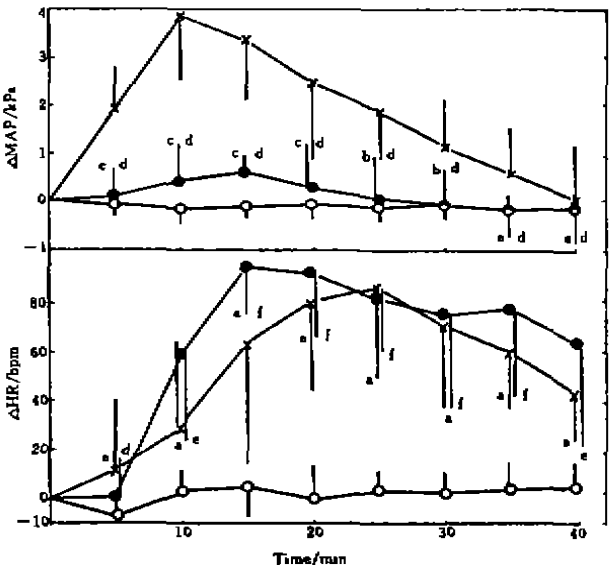

Fig 3. Antagonism of the responses induced by injection of Arg into LSN. Phentolamine (10 g·kg^{-1}·L^{-1}, iv) abolished the hypertension responses to injection of Arg (400 ng) into LSN. Arg 400 ng (×, $n=9$), Phentolamine + Arg (●, $n=7$), Phentolamine + CSF (○, $n=4$). $\bar{x}\pm s$. $^aP>0.05$, $^bP<0.05$, $^cP<0.01$ *vs* Arg 400 ng. $^dP>0.05$, $^eP<0.05$, $^fP<0.01$ *vs* Phentolamine + CSF.

DISCUSSION

In the present study, Arg microinjection into LSN caused a dose-dependent hypertension and tachycardia. This finding further demonstrated that the Arg plays a central role in cardiovascular regulation not only by affecting brain stem[4-9] and spinal cord[10,11] but also by affecting forebrain[12]. The responses induced by injection of Arg into the LSN were specific and not due to non-selective stimulation of this area, since injection of the vehicle (CSF) had no detectable effects on peripheral hemodynamics. In addition, Arg appeared to exert its effects within the LSN through a specific interaction with V1 type Arg receptors. Pretreatment with the V1 antagonist d(CH_2)$_5$Tyr(Me)Arg completely prevented the expected increase in arterial pressure and heart rate following subsequent injection of Arg into the LSN. However, the fact that the injection of the antagonist itself into the LSN did not alter blood pressure and heart rate suggests that the Arg in LSN had no tonic effects on cardiovascular activity in the anesthetized state. Peripheral α-adrenergic blockade with phentolamine completely blocked the increase in MAP response to injection of 400 ng Arg into the LSN. This indicated that the hypertension mainly mediated by sympathetic vasoconstrictor. The increases in blood presure and heart rate produced by injection of Arg into the LSN were neurally mediated since these responses were short-latent, almost abolished by peripheral α-adrenergic receptor blockade, and completely prevented by pretreatment of this central area with Arg antagonists. This indicated that the cardiovascular effects of Arg injected into the LSN resulted from stimulation of central neural mechanisms rather than leakage of the hormone into the peripheral circulation. Furthermore, our obsevations indicate that the elevations in blood pressure are accompanied by increase in heart rate, an unlikely possibility if the hypertension was peripheral in origin.

In conclusion, the present study indicates that Arg produces within the LSN a marked increase in blood pressure and heart rate that are mediated by the autonomic nervous system. The effect appears to be due to an action of Arg on V1 receptors.

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大鼠外侧隔核注射精氨加压素的心血管效应

高 列, 蒋乃昌, 罗庆华, 朱筑霞

(贵阳医学院生理教研室, 贵阳550004, 中国)

关键词 精氨加压素; 隔核; 血压; 心率

目的: 研究精氨加压素(Arg)在外侧隔核对心血管活动的影响. **方法**: 用乌拉坦麻醉大鼠, 在外侧隔核注入Arg, 记录平均动脉血压和心率变化. **结果**: Arg (100, 200和400 ng)使平均动脉血压分别升高0.9±0.6, 2.3±1.3和4.0±1.4 kPa; 心率分别加快12±27, 50±33, 和89±27 bpm. 用

d(CH$_2$)$_5$Tyr(Me)Arg 预先阻断外侧隔核的 V1型受体，可完全消除 Arg 在该核团的心血管效应. 用酚妥拉明预先阻断外周 α 受体，可完全消除 Arg 在外侧隔核的升压效应，但不影响其加快心率效应. **结论**：Arg 可作用于外侧隔核的 V1型受体，发挥对心血管活动的中枢控制作用.

BIBLID: ISSN 0253-9756 Acta Pharmacologica Sinica 中国药理学报 1996 Jan; 17 (1): 52—54

Toxicity to transferred rat embryos after aspirin treatment during preimplantation stage *in vivo*[1]

LOU Yi-Jia[2], DING Guang-Sheng, TU Zeng-Hong
(Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai 200031, China)

KEY WORDS aspirin; teratogens; blastocyst; embryo transfer; fetal development; drug-induced abnormalities

AIM: To explore the relationship between drug-induced blastopathies and post-implantation embryotoxicity or developmental defects. **METHODS**: Pregnant rats on d 3 were given intragastrically aspirin (0.25, 0.5, and 1 g·kg^{-1}). On d 4, the blastocysts were transferred into the uterine horns of pseudopregnant rats (made by mating with ♂ rats which had been given intragastrically 3-chloro-1,2-propanediol 5 mg·kg^{-1} for 5 d). Uterine contents were examined at term. **RESULTS**: The frequency of blastocysts with morphological alterations (FBMA) was increased on d 4 of gestation. The implantation rate was lower than that of the controls. A dose-related increase in resorption (55.2 %, 69.5 %, and 85.2 %) and malformation rate (3.8 %, 44.4 %, and 25 %), and decrease in viability rate of fetuses (44.8 %, 30.5 %, and 14.8 %) were observed in test groups with correlations to FBMA. **CONCLUTION**: Embryotoxicity and fetal malformations were induced by treatment of aspirin before implantation in a dose-dependent manner.

Preimplantation embryos were believed fairly resistant to teratogenic actions of chemicals to be obeying the "all-or-nothing" law[1,2], which, however, did not conform to all cases[2,3]. Previous works[4,5] showed that the preimplantation embryos were highly sensitive to the treatment of rats with aspirin causing both abnormal blastocysts and malformations in the surviving fetuses. The present study was carried out to analyze whether the toxic effects observed at term after preimplantation treatment with aspirin were induced by maternal effects or by direct effects of aspirin on the embryos.

MATERIALS AND METHODS

Aspirin (Shandong Xinghua Pharmaceutical Corp, China). Sprague-Dawley rats, 8 wk, virgins, weighing 186 ± *s* 13 g, ♂, adults, weighing 213 ± *s* 22 g, from Shanghai Institute of Planned Parenthood Research & Bantin and Kingman Universal Ltd were housed under 12-h light/12-h dark, 21 ± 1 ℃, 55 ± 5 % relative humidity for 2 wk before rats ♀ : ♂ (4 : 1) were mated during the night. The next morning when sperms were found in the vaginal smear was defined as d 0 of gestation. The mated females were divided randomly into experimental groups (n = 11) given on d 3 at 9:00 AM by ig aspirin 0.25, 0.5, and 1 g·kg^{-1} dissolved in 0.5 % CMC (almost equal to 1/2 LD_{50} of rats). Control group was treated with 0.5 % CMC 5 mL·kg^{-1}.

Pseudopregnancy in recipient ♀ rats Four ♀ rats were placed overnight with one ♂ rat which had been given ig 3-chloro-1,2-propanediol 5 mg·kg^{-1} × 5 d to be deprived of fertility ability[6]. The day when spermatozoa were found in the vaginal smear was taken as d 0 of pseudopregnancy.

Blastocysts evaluation and embryo transfer Pregnant and pseudopregnant females served as donors and recipients of embryos, respectively. On d 4 at 1 — 3 PM, the blastocysts were flushed from the uteri of donors using the HEPES-buffered medium M_2. The collected embryos were examined under a phase-contrast microscope (× 40)

[1] Project in part supported by National Natural Science Foundation of China, № 39170875.
[2] Now in *Department of Pharmacology, School of Pharmacy, Zhejiang Medical University, Hangzhou 310006, China.*
Received 1995-03-13 Accepted 1995-05-19