

Intracerebroventricularly injected *L*-arginine-induced vasopressin release is mediated by cGMP in rats¹

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KEY WORDS nitric oxide; argipressin; arginine; cyclic GMP; methylene blue; guanylate cyclase

AIM: To study the possible role of cGMP in the effect of *L*-arginine-induced arginine-vasopressin (AVP) release. **METHODS:** In anesthetized rats, saline, *L*-arginine, 8-Br-cGMP, *L*-arginine + methylene blue (an inhibitor of guanylyl cyclase) were injected intracerebroventricularly (icv) and the plasma vasopressin level was detected with radioimmunoassay. **RESULTS:** Both 8-Br-cGMP $2.53 \text{ g} \cdot \text{L}^{-1}$ and *L*-arginine $100 \text{ g} \cdot \text{L}^{-1}$ increased plasma AVP level [from (2.6 ± 0.3) to $(6.6 \pm 0.4) \text{ ng} \cdot \text{L}^{-1}$, and from (3.2 ± 0.5) to $(5.8 \pm 1.4) \text{ ng} \cdot \text{L}^{-1}$, respectively; $P < 0.01$] 5 min after the icv injection; methylene blue ($3.74 \text{ g} \cdot \text{L}^{-1}$) + *L*-arginine ($100 \text{ g} \cdot \text{L}^{-1}$) did not change plasma AVP level. **CONCLUSION:** cGMP was a mediator of the effect of *L*-arginine-induced AVP release.

Nitric oxide (NO) is a neuronal messenger widely distributed in the brain^[1,2]. The distribution is mainly revealed by the expression of nitric-oxide synthase (NOS) in various brain structures. NOS is highly expressed in the supraoptic and paraventricular nuclei of the hypothalamus where the neurohormones, arginine-vasopressin (AVP) and oxytocin, are produced^[3]. The role played by NO in the release of these hormones has been investigated^[4-6]. The observation that intracerebroventricular injection (icv) of *L*-arginine, the substrate of NOS, enhances AVP release in conscious rats^[6] has been confirmed in our laboratory in anesthetized rats^[4]. In the present

work, we intended to observe whether this *L*-arginine-induced AVP release is mediated by activation of soluble guanylyl cyclase with the formation of cyclic cGMP^[7], which is generally considered as the main mediator in the effect of NO.

MATERIALS AND METHODS

Materials *L*-Arginine and methylene blue were purchased from Sigma Chemical Co and 8-Br-cGMP was from RBI Chemical Co. Sep-Pak C₁₈ Cartridge for AVP extraction was from Millipore Chemical Co. Rabbit polyclonal antibody against AVP and the synthetic AVP used were from Peninsula Chemical Co. [¹²⁵I] NaI was from DuPont Chemical Co.

Protocol Sprague-Dawley rats ($\hat{\sigma}$, weighing $227 \pm 23 \text{ g}$, obtained from Shanghai Experimental Animal Center, certificate No 005) were under pentobarbital anesthesia ($60 \text{ mg} \cdot \text{kg}^{-1}$). A Narishige stereotaxic instrument was used to guide the tip of the needle of a microsyringe to 1.3 mm posterior to the bregma, 2.0 mm lateral to the midline and 4.7 mm below the skull surface. The left femoral artery was cannulated with a PE-50 polyethylene tubing filled with heparinized ($0.1 \text{ MU} \cdot \text{L}^{-1}$) saline for blood pressure recording or withdrawal of blood. The left femoral vein was cannulated for injection of an equal volume of 6 % dextran (molecular weight 40 000) in 5 % glucose solution to replace the blood loss. A 2-channel physiologic recorder (LMS-2B, Chengdu Instruments) was used to record the arterial blood pressure (ABP) and heart rate (HR). At least 15 min after stabilization of ABP and HR following the surgery the 1st blood sample was collected. The icv needle placement was verified by injection of neutral red just before sacrifice. Only rats with dye in the ventricular system were used.

The rats were divided into 4 groups: 1) icv of $10 \mu\text{L}$ saline for control ($n = 10$); 2) icv 8-Br-cGMP, a membrane permeable cGMP analog

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(25.3 μg in 10 μL saline, $n = 8$); 3) icv *L*-arginine 1.0 mg in 10 μL saline ($n = 8$); 4) icv methylene blue, an inhibitor of soluble guanylyl cyclase, 37.4 μg plus *L*-arginine 1.0 mg in 10 μL saline ($n = 7$). All icv were performed after the 1st collection of blood sample. The 2nd and 3rd collections of blood sample were taken 5 and 15 min respectively after the icv. Each blood sample was 2.0 mL.

Plasma AVP was extracted with Sep-Pak C₁₈ Cartridge and measured by radioimmunoassay⁽⁸⁾. All standards and blood samples were measured in duplicate. The lower detection limit of the assay was 0.3 $\text{ng} \cdot \text{L}^{-1}$. The intra- and interassay coefficient of variation were 5.5 % and 7.5 %, respectively.

Statistical analysis Data were expressed as $\bar{x} \pm s$ and were analyzed statistically by signed rank test for repeated measures for differences with time. The differences between groups were compared with the Wilcoxon rank sum test.

RESULTS

Before and after icv saline, the contents of plasma AVP were (2.9 ± 0.4) $\text{ng} \cdot \text{L}^{-1}$ (time 0), (3.3 ± 0.8) $\text{ng} \cdot \text{L}^{-1}$ (5 min), and (3.3 ± 0.7) $\text{ng} \cdot \text{L}^{-1}$ (15 min), respectively. No significant difference with time was detected. Neither mean ABP nor HR changed significantly: the mean ABP = (12.4 ± 0.7), (12.7 ± 1.1), and (12.5 ± 0.8) kPa respectively; HR = (410 ± 25), (408 ± 24), and (408 ± 26) beats per minute (bpm) respectively at 0, 5, and 15 min.

8-Br-cGMP by icv caused elevations of plasma AVP from an initial value of (2.6 ± 0.3) to (6.6 ± 0.4) $\text{ng} \cdot \text{L}^{-1}$ ($P < 0.01$) at 5 min and (4.6 ± 0.3) $\text{ng} \cdot \text{L}^{-1}$ ($P < 0.05$) at 15 min after application of this agent (Fig 1). The mean ABP was initially (13.0 ± 1.0) kPa, changed to (14.7 ± 1.5) kPa ($P < 0.05$) at 5 min and returned to (13.3 ± 1.2) kPa at 15 min after the injection. The HR decreased from (395 ± 13) to (377 ± 16) bpm ($P < 0.05$) at 5 min and returned to (387 ± 16) bpm at 15 min after the application of 8-Br-cGMP (Fig 2).

Injection of *L*-arginine in 10 μL saline into the lateral ventricle increased plasma AVP from a basal level of (3.2 ± 0.5) to (5.8 ± 1.4) $\text{ng} \cdot \text{L}^{-1}$ ($P < 0.01$) at 5 min and (4.7 ± 0.8) $\text{ng} \cdot \text{L}^{-1}$ ($P < 0.05$) at 15 min after icv (Fig 1).

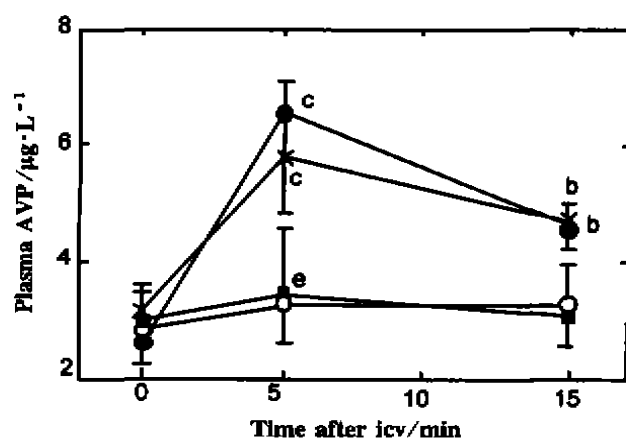


Fig 1. Stimulating effects of icv *L*-arginine (*L*-Arg) and 8-Br-cGMP on AVP release and inhibitory effect of methylene blue (MB) on AVP response to *L*-Arg. Saline (\circ , $n = 10$), 8-Br-cGMP (\bullet , 25.3 μg in 10 μL saline, $n = 8$), *L*-Arg (\times , 1.0 mg in 10 μL saline, $n = 8$), and MB 37.4 μg + *L*-Arg 1.0 mg in 10 μL saline (\square , $n = 7$). ^b $P < 0.05$, ^c $P < 0.01$ vs time-zero; ^e $P < 0.05$ vs *L*-arg.

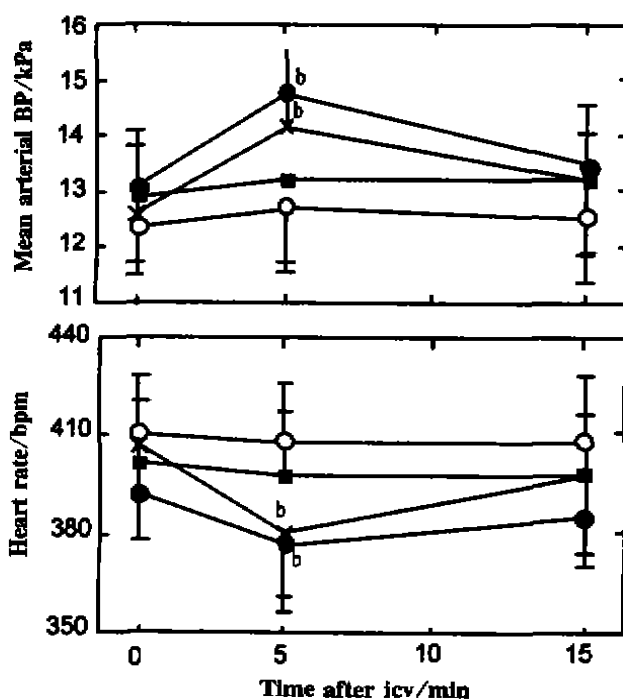


Fig 2. Blood pressure and heart rate after icv saline (\circ), 8-Br-cGMP (\bullet), *L*-Arg (\times), and methylene blue + *L*-Arg (\blacksquare). ^b $P < 0.05$ vs time-zero.

The mean ABP was elevated from a basal value of (12.6 ± 0.9) to (14.1 ± 1.5) kPa ($P < 0.05$) at 5 min and returned to (13.1 ± 1.4) kPa at 15 min after icv. The HR decreased from a start value of (407 ± 27) to (379 ± 23) bpm

($P < 0.05$) at 5 min and returned to (398 ± 23) bpm at 15 min (Fig 2).

Before and after icv of methylene blue together with *L*-arginine, the contents of plasma AVP at time 0, 5, and 15 min were (3.0 ± 0.5), (3.4 ± 0.4), and (3.1 ± 0.6) $\text{ng} \cdot \text{L}^{-1}$, respectively, the response of AVP to *L*-arginine being blocked by methylene blue ($P < 0.05$, vs *L*-arginine in saline, Fig 1). At the same time the mean ABP and HR did not change significantly. The mean ABP = (12.9 ± 0.9), (13.1 ± 1.1), and (13.1 ± 1.2) kPa, respectively; and the HR = (402 ± 24), (398 ± 25), and (398 ± 23) bpm, respectively, at 0, 5, and 15 min after icv (Fig 2).

DISCUSSION

A number of hormones, such as insulin, corticotrophin-releasing factor (CRF), luteinizing hormone-releasing hormone (LHRH), somatostatin, adrenocorticotropin, growth hormone, have been shown to be regulated by NO^[9]. Among them, growth hormone^[10] and insulin^[11] were suggested to be induced by NO via cGMP. In addition, the inhibitory effect of NO on prolactin release was also shown to be mimicked by cGMP^[12]. In the case of LHRH, however, it appears that NO acts on the cyclooxygenase enzyme rather than on soluble guanylyl cyclase^[13]. The cyclooxygenase enzyme converts the arachidonic acid to prostaglandin E₂ which activates adenylate cyclase to increase cAMP, thereby enhancing the exocytosis of LHRH. The mechanism of NO-mediated interleukin 2-induced CRF was also supposed to involve the activation of cyclooxygenase enzyme^[14]. Besides, the possibility of NO activation of other heme-containing enzyme, such as lipoxygenase and epoxygenase, also remains to be elucidated^[14]. Nevertheless, in the present study, it is demonstrated that the *L*-arginine-induced AVP release is mainly mediated by cGMP, since not only 8-Br-cGMP can stimulate plasma AVP release, but also the soluble guanylyl cyclase inhibitor can block the *L*-arginine-induced AVP release.

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cGMP 介导脑室注入 L-精氨酸诱导的大鼠血管加压素释放效应¹

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关键词 一氧化氮; 精氨酸加压素; 精氨酸; cGMP; 亚甲蓝; 鸟苷酸环化酶

目的: 探讨 cGMP 是否介导 L-精氨酸(一氧化氮合

酶底物)引起的血管加压素(AVP)释放增多效应。
方法: 用放射免疫法测定大鼠血浆中 AVP 水平。
结果: 侧脑室分别注射 L-精氨酸和 8-溴-cGMP (一种可透过膜的 cGMP 衍生物)能刺激血浆 AVP 水平增加[分别从(3.2 ± 0.5)升至(5.8 ± 1.4) ng·L⁻¹, 从(2.6 ± 0.3)升至(6.6 ± 0.4) ng·L⁻¹, P < 0.01], 同时注射 L-精氨酸和亚甲蓝(鸟苷酸环化酶抑制剂)对血浆 AVP 的水平没有影响。
结论: cGMP 介导 L-精氨酸引起的 AVP 释放增加效应。

Up-regulation of LPS-induced iNOS activity in dibutyryl cyclic AMP-differentiated rat astrocytes

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KEY WORDS lipopolysaccharides; nitric-oxide synthase; cyclic AMP; cell differentiation; up-regulation (Physiology); astrocytes; immunohistochemistry

AIM: To study the effect of dBcAMP on bacterial endotoxin LPS-induced NOS activity.
METHODS: Microscopic changes were observed. Nitrite levels were measured by fluorometric assay. NOS activity was measured by citrulline assay.
RESULTS: Within 3-4 h after the addition of dBcAMP 1 mmol·L⁻¹ to culture medium, a morphological transformation reminiscent of *in vivo* differentiation occurred. Coincubation with LPS and dBcAMP 1 mmol·L⁻¹ resulted in a marked increase in the nitrite production as compared with LPS alone. This increase was concentration- and time-dependent with a maximal effect after 24 h treatment. Nitrite production stimulated by LPS is parallel to the degree of cell differentiation. After a 24-h costimulation with LPS and dBcAMP, L-citrulline formation assay revealed a 3-fold increase in

NOS activity over LPS treatment alone. Simultaneous incubation with L-NAME, completely inhibited the stimulation effect of LPS/dBcAMP on nitrite production. Cycloheximide and dactinomycin also suppressed enhancement of NOS activity stimulated by LPS/dBcAMP, both in nitrite production and citrulline assay, indicating that the enhancement of NOS activity was due to the expression of inducible NOS (iNOS) gene and protein.
CONCLUSION: Inflammatory signals can trigger astrocytes to express substantially different levels of iNOS depending on their degree of differentiation.

NO, a potent and pleiotropic mediator of many biological functions, is synthesized from L-arginine by NOS. Three forms of NOS have been characterized^[1]. The constitutive endothelial and neuronal types are calcium-dependent. The third form of the enzyme is the calcium-independent inducible NOS (iNOS), which is only expressed in the presence of bacterial toxins or cytokines. This form of the enzyme produces high levels of NO which is used by the macrophage to kill tumor cells as well as invasive microorganisms including bacteria and viruses^[2]. The excessive production of NO by iNOS has also been implicated in the

Abbreviations used: cAMP = cyclic AMP; dBcAMP = N⁶,2'-O-dibutyryl cyclic AMP; GFAP = glial fibrillary acidic protein; LPS = lipopolysaccharide; L-NAME = N^G-nitro-L-arginine methyl ester; NO = nitric oxide; NOS = nitric-oxide synthase; PBS = phosphate-buffered saline.

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