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# The Effect of Glycine on the CNS Regulation of Cardiovascular Response

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## ABSTRACT

*Three groups of conscious adult male Wistar rats, i.e. intact, catecholamine (CA)-depleted, and serotonin (5-HT)- depleted, were all administrated with 30, 100 and 300 μg of glycine via the lateral cerebral ventricular cannulation (i.c.v.) to evaluate the effects of glycine on cardiovascular response. At these doses, glycine resulted in a dose-dependent decrease in heart rate and mean arterial pressure, but it increased the sensitivity of the baroreflex to change the heart rate. In intact rats, pretreatment with glycine did not change the adrenaline-induced pressor response, yet it enhanced adrenaline-induced reflex bradycardia. However, in CA-depleted and 5-HT depleted groups, the abilities of glycine to induce hypotension, bradycardia as well as to enhance adrenaline-induced reflex bradycardia were significantly attenuated. It indicated that the central monoaminergic systems involve the effect of glycine on cardiovascular functions.*

Recently, glycine has been strongly suggested to be one of the major inhibitory neurotransmitters in the central nervous system[1,2,3]. Animal studies have demonstrated that glycine caused hypothermia in rats through intraperitoneal (i.p.) or intracerebroventricular (i.c.v.) administration [4]. However, there were few investigations concerning the influence of glycine on cardiovascular function in conscious rat. In the present study, we present the effects of glycine on heart rate, arterial pressure, and adrenaline-induced reflex bradycardia in intact conscious rats. Furthermore, the brain catecholamine-depleted or serotonin-depleted rats were also used to evaluate whether the regulation of cardiovascular function by glycine may be mediated by central monoaminergic systems.

## MATERIALS AND METHODS

### 1. Animals & their preparation

Adult male Wistar rats about 250 g were

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used in all the following experiments. Rats were implanted with cerebroventricular cannulae under general anesthesia (sodium pentobarbital 60 mg/kg i.p.) one week before used in experiments and housed individually in wire-mesh cages with free access to tap water, granular chicken feed at room temperature (25±1.0°C) under natural light-dark cycles. The implantation method was followed the De Groot[5] coordinates: AP. 7.0; Lat. 1.0 and Hor. 0.1 mm. The correct positioning of each guide tube of the cannulae was verified by the rapid flow of saline into the lateral cerebral ventricle under gravity[6]. Cathethrization via left femoral vein and artery with polyethylene catheters (PE-50 tubing) was performed 24 hours before experiments under the reanesthetized condition for blood pressure monitoring and intravenous adrenaline administration[7].

### 2. Experimental design

The rats were divided into 3 groups to study the individual effects of central administration of glycine on cardiovascular function. They are (1) intact control rats, (2) CA-depleted rats, which were intraventricularly administrated with 120 $\mu$g $\alpha$-methyl-p-tyrosine methylester 4 hours before tested, (3) 5-HT-depleted rats, which were intraperitoneally administrated with 250 mg/kg p-chlorophenylalanine ethylester 72 hours before used. Then we assessed the vasopressor and bradycardiac responses of each group of animals under different i.v. doses of adrenaline administration.

### 3. Drugs

Drugs (glycine, adrenaline, $\alpha$-MT and PCPA) used in this research were obtained from Sigma Chemical Co. (St. Louis, MO). Drug solutions were prepared in pyrogen-free glassware which was baked at 180°C for 5 hr. before use. All solutions were freshly prepared in 0.9% saline on the day of testing.

### 4. Measurements of cardiovascular function

On the day of experiment, animals were placed on holders and the previously implanted arterial lines were connected to the blood pressure transducers (Gould, type P23XL) for continuous recording of blood pressure. The heart rate was monitored with a Gould Biotach amplifier. Then the rats were left undisturbed until the steady state conditions were reestablished. Glycine and $\alpha$-MT were administrated to rats through the i.c.v. cannulae whereas p-chlorophenylalanine was via i.p. injection. Adrenaline, used as a vasopressor substance, was administrated via the femoral vein catheter. On the other hand, the appropriate vehicle-injected controls were also simultaneously performed with the drug-treated animals.

## RESULTS

### 1. Effects of glycine on heart rate, arterial blood pressure and adrenaline-induced reflex bradycardia in intact conscious rats.

A dose-related decrease in both heart rate and arterial pressure was noted, as shown in Table 1, after direct administration of glycine into the lateral cerebral ventricle of intact conscious rats with the dose ranging from 30 to 300 $\mu$g. In control groups, administration of saline vehicle only resulted in a insignificant transient drop in mean arterial pressure. Our data also demonstrated a dose-dependent enhancement of reflex bradycardia after administration with varying doses of glycine to the animals which had previously received intravenous injection of either 1.25 or 2.5 $\mu$g/kg body weight of

Table 1. Effect of intracerebroventricular administration of glycine on cardiovascular responses in intact conscious rats

| Treatment | Control values | Minimum values | Difference | Time to maximun fall (min) | Time of recovery (min) |
|---|---|---|---|---|---|
| Mean arterial pressure (mm Hg) | | | | | |
| 0.9% saline | 115±17 | 112±16 | -3±0.5 | 0.2±0.1 | 0.5±0.3 |
| Glycine 30$\mu$g | 117±15 | 107±11 | -10±2.1 * | 2.6±0.4 * | 4.1±0.9 * |
| Glycine 100$\mu$g | 114±11 | 89±16 | -25±4.4 * | 5.7±0.9 * | 8.5±1.7 * |
| Glycine 300$\mu$g | 108±14 | 71±14 | -37±5.6 * | 7.3±1.5 * | 11.2±2.0 * |
| Heart rate, beats/min | | | | | |
| 0.9% saline | 405±51 | 400±49 | 5±1.2 | 0.5±0.3 | 0.9±0.4 |
| Glycine 30$\mu$g | 411±59 | 403±50 | -8±1.4 * | 3.0±0.7 * | 5.6±0.9 * |
| Glycine 100$\mu$g | 408±62 | 391±59 | -17±2.5 * | 6.6±1.3 * | 10.4±2.7 * |
| Glycine 300$\mu$g | 410±55 | 364±69 | -46±8.6 * | 9.1±1.7 * | 15.0±2.9 * |

* Significantly different from control value (0.9% saline), p value less than 0.05 (Student's t-test). The values are experessed as the mean ± SEM. of 6 animals.

adrenaline. (shown in Fig. 1). Although it exhibited a greater bradycardia when administrated with adrenaline 10 minutes after a prior treatment with glycine, however, the pretreatment of glycine did not influence the effects of adrenaline on arterial blood pressure.

2. Effects of glycine on cardiovascular function and adrenaline-induced reflex bradycardia in CA or 5-HT-depleted conscious rats.

The dose-related hypotensive and bradycardiac effects of glycine were significantly attenuated after CA-depletion with $\alpha$-MT or 5-HT-depletion with PCPA as show in the Fig. 2. The glycine potentiation of adrenaline-induced reflex bradycardia was also diminished, as shown in the Fig. 3, when pretreated with $\alpha$-MT or PCPA.

## DISCUSSION

It has demonstrated that direct administration of glycine into the cerebral ventricular resulted in acute decreases in basal heart rate as well as arterial pressure in both our previous research using anesthetized rats[8] and the present study using conscious rats. Pretreatment of animals with glycine greatly enhanced the adrenaline-induced reflex bradycardia. However, pretreatment with $\alpha$-MT or PCPA significantly reduced the effects of glycine on cardiovascular function. $\alpha$-MT, a potent inhibitor of tyrosine hydroxylase [9], reduced the brain content of catecholamines by means of inhibiting the rate-limiting enzyme of the catecholamine biosynthesis processes[10]. Similarly, the brain content of serotonin was reduced in rats treated with PCPA[11]. The arterial baroreflex system is regarded as one of the most powerful and rapidly action homeostatic mechanisms for blood pressure regulation. It is generally accepted that the central baroreceptor arc is polysynaptic: Its primary synapse is in the nucleus of the tractus solitarius (NTS) and its inhibitory neurons interpose between the NTS and the cardiovascular

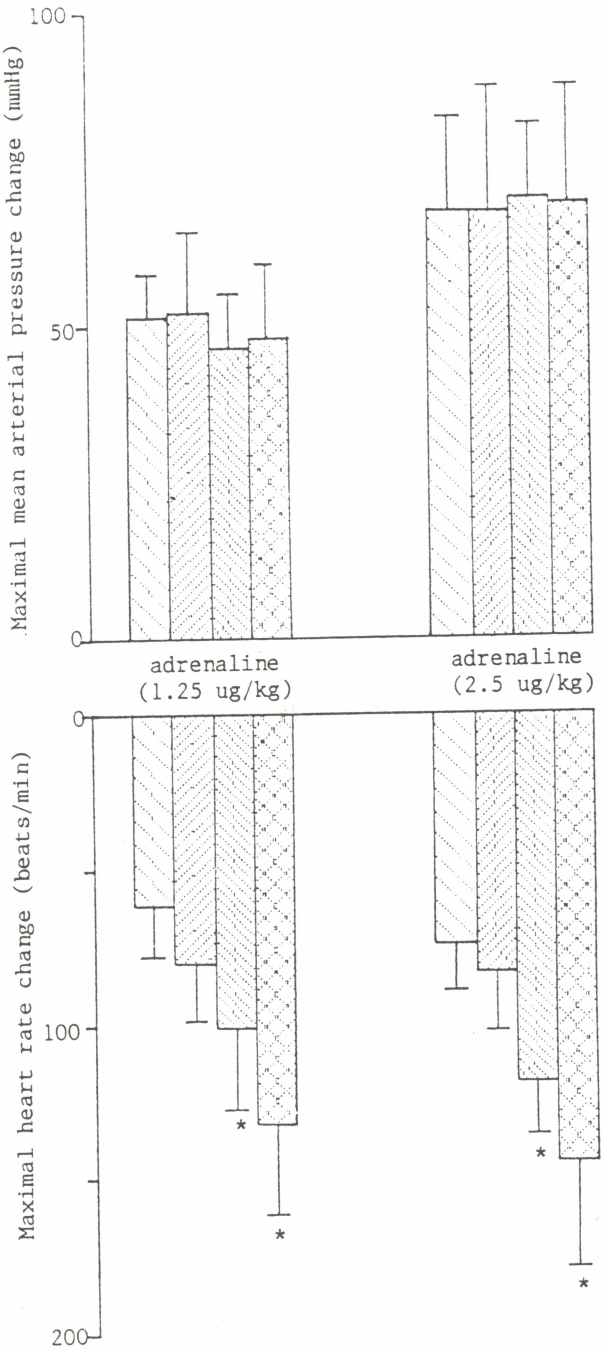


Fig. I Effects of intracerebroventricular administration of inhibitory amino acid on cardiovascular responses induced by intravenous injection of adrenaline in intact conscious rats.
*Significantly different from control values (0.9% saline group), at $p < 0.05$ (Student's t-test). The values are expressed as the mean ± SEM of 6 animals.

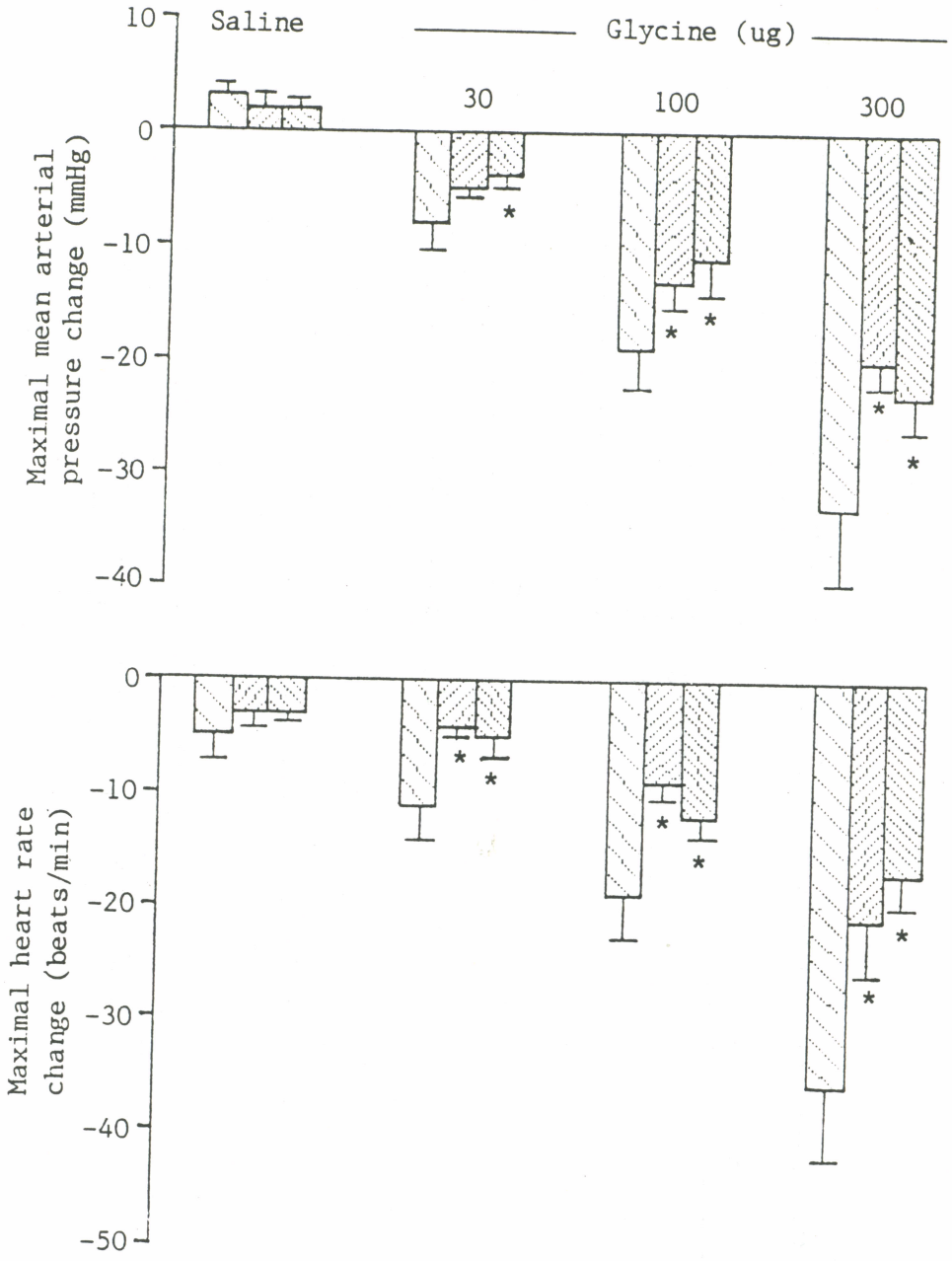


Fig. 2 Effects of intracerebroventricular administration of glycine on cardiovascular responses in catecholamine or serotonin depleted conscious rats. *Significantly different from corresponding control values (0.9% saline group), at $p < 0.05$ (Student's t-test). The values are expressed as the mean ± SEM of 6 animals.

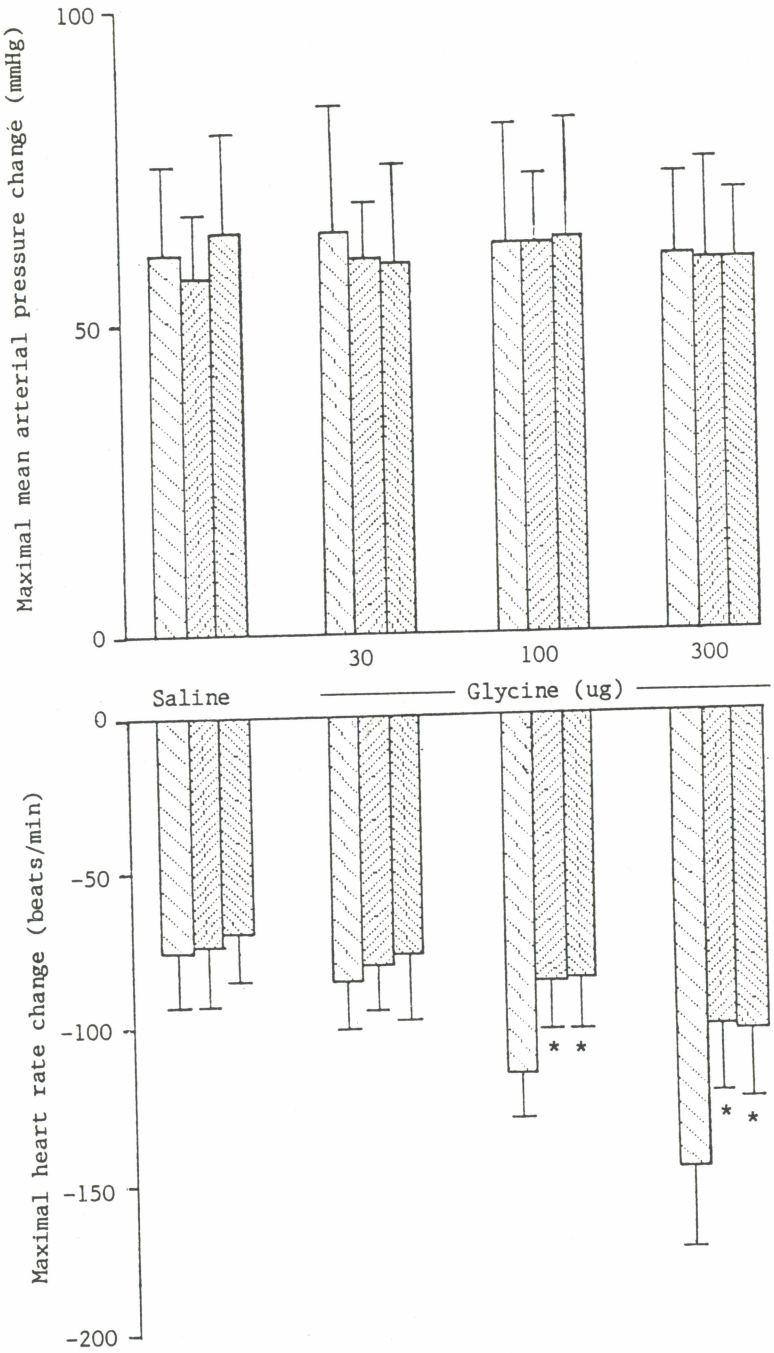


Fig. 3 Effects of intracerebroventricular administration of glycine on cardiovascular responses induced by intravenous injection of adrenaline in catecholamine or serotonin depleted conscious rats. *Significantly different from corresponding control values (0.9% saline group), at $p < 0.05$ (Student's t-test). The values are expressed as the mean ± SEM of 6 animals.

center[12,13]. However, recent accumulated evidences imply that the central baroreceptor arc in the rat contains monoaminergic synapses. For instance, it was found that activation of the serotoninergic receptors within the brain could depress the adrenalline-induced bradycardia, whereas it facilitated a adrenaline-induced bradycardia while these receptors were inhibited[14,15]. It also resulted in a significant decrease in reflex bradycardia when either brain dopamine receptors were blocked by dopamine receptor antagonists or the dopaminergic neurons were destructed[16,17]. Activation of brain dopaminergic receptors by dopamine receptor agonists or by electrical stimulation of dopamine neurons led to an enhancement of the adrenaline-induced bradycardia in rats. It has been shown in the rabbits that both noradrenergic and serotoninergic neurons in the brain participate in the central baroreceptor-heart rate reflex pathway[18,19]. On the other way, it was also known that glycine influenced the brain neurotransmitter content and activity by means of direct or indirect effects on the central monoaminergic neurons. So far as we know, the key mechanism to regulate the cardiovascular function lies in the reciprocal relationship between the vagal tone and the sympathetic efferent activity. According to our present results, glycine seemed to act through a central mechanism to facilitate the vagal efferent activity and/or inhibit preganglionic sympathetic efferent activity, which leads to hypotension, bradycardia in rats. However, the effects of glycine on normal cardiovascular function and adrenaline-induced reflex bradycardia were significantly reduced after pretreatment of rats with $\alpha$-MT to deplete catecholamines or with PCPA to deplete serotonin. We suggest that glycine is involved in the central regulation mechanisms of cardiovascular function and its effects appear to be mediated by central serotoninergic and catecholaminergic systems. The more precise action site and mechanisms of glycine on cardiovascular function will be assessed by further experiments.

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# 甘氨酸對心血管功能之中樞神經調節作用

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## 摘　要

本實驗係探討中樞神經性單胺系統參與甘氨酸對清醒大白鼠心血管之影響。實驗中清醒之大白鼠分爲三組:(一)對照組，(二)以 α-MT 耗竭腦中兒茶酚胺含量之實驗組，(三)以 PCPA 耗竭腦中血清張力素含量之實驗組。由大白鼠之側腦室投予甘氨酸，劑量分別爲 30，100 或 300μg，來探討甘氨酸對心血管之作用。結果顯示甘氨酸可導致大白鼠心跳減慢，平均血壓下降和加強由腎上腺素所造成的反射性徐脈現象，此作用隨甘氨酸使用之劑量增加而增加。大白鼠投予甘氨酸後再由靜脈投予腎上腺素，並不會改變因腎上腺素所引發的壓力反應，但甘氨酸的預先投予會加強腎上腺素所造成的反射性徐脈。若投予 α-MT 來耗竭大白鼠腦中兒茶酚胺之含量，或投予 PCPA 來耗竭大白鼠腦中血清張力素之含量，則可顯著地抑制甘氨酸所引起的心跳減慢，血壓下降及反射性徐脈加強等作用。由實驗結果得知，甘氨酸與中樞性單胺系統調節心臟血管系統之功能有關。

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