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Effects of GABA, Diazepam and Baclofen on Reflex Bradycardia in Rats

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ABSTRACT

The effect of GABA, diazepam and baclofen on epinephrine-induced reflex bradycardia was studied in pentobarbital-Na anesthetized rats. Intravenous injection of epinephrine (1.5 μg/kg) resulted in marked hypertension and bradycardia. It was observed that in rats given epinephrine (1.5 μg/kg i.v.) 15-30 mins after a prior intracerebroventricular (i.c.v.) administration of either GABA (10-100 μg), diazepam (1-10 μg, a $GABA_A$ agonist) or baclofen (0.01-0.25 μg, a $GABA_B$ agonist) showed an enhanced epinephrine-induced reflex bradycardia as compared to the control group (saline i.c.v. injected). Pretreatment of animals with GABA, diazepam and baclofen, however, did not influence the effects of epinephrine on arterial blood pressure. The result thereby indicates that within the brain, GABA-sensitive cells and GABA receptor, both $GABA_A$ site and $GABA_B$ site, are involved in the regulation of cardiovascular function and may act through a central mechanism to enhance the vagal tone and/or attenuate the preganglionic sympathetic efferent activity which may lead to potentiation of the reflex bradycardia.

Alterations of γ-aminobutyric acid (GABA)-mediated transmission are probably involved in neurological disorders such as Parkinson's disease, Huntington's chorea, epilepsy, as well as some other behavior disorders[1,2]. Recently, enough experimental evidence has accumulated to suggest that the GABAergic system within the brain form essential links in the central regulation of cardiovascular function. For example, it has been shown that direct administration of GABA into brain ventricles or onto the ventral surface of the medulla or localized sites in the medial reticular formation resulted in reduced blood pressure and heart rate[3,4,5]. Clinically, there are many drugs of therapeutic importance which can directly influence GABA-mediated neurotransmission. These drugs include the barbiturates, benzodiazepines, baclofen and

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valproate, all of which may enhance GABA function. Although their pharmacologic properties have been well studied, there appears to be little information on the effect of these drugs on arterial baroreceptor functions. In our present study, rats were used to assess the effects of exogenous administration of GABA and its agonists such as diazepam and baclofen on epinephrine-induced bradycardia.

METHODS AND MATERIALS

Male Wistar rats weighing about 250 g were used. Prior to experiment, these animals had been housed in a controlled light and temperature environment. They were given free access to tap water and granular chicken feed supplied by Taiwan Sugar Corporation. On the day of experiment, prior to injection of the drug into the lateral cerebral ventricle, the rats were put under general anesthesia (sodium pentobarbital 30 mg/kg i.p.) and implanted with cannulae. The implantation of cerebroventricular cannulae was carried out according to the De Groot[6] coordinates: AP, 4.8; L, 2.5 and H, 3.0 mm. The cannulae were constructed by connecting a 10 μl Hamilton syringe via PE 10 tubing. During surgery, the correct positioning of each guide tube was verified by the rapid flow under gravity[7] of saline into the lateral cerebral ventricle. To monitor blood pressure and to administer intravenously, rats were implanted with polyethylene catheters (PE-50 tubing) through the left femoral vein and artery. The implanted arterial lines were attached to blood pressure transducers (Gould, type P23ID) for continuous recording of blood pressure. Heart rate was monitored with a Gould Biotach amplifier. All recordings were performed with a four-channel Gould 2400S polygraph[8]. Rectal temperature was maintained at 37.5±0.5°C throughout the course of the experiments by irradiation with infrared light.

All solutions were prepared in pyrogen-free glass-ware baked at 180°C for 5 hr before use. GABA (Sigma), diazepam (Hoffmann-La, Roche) and baclofen (Ciba-Geigy) was freshly prepared in 0.9% saline for intracerebroventricular (i.c.v.) administration. Epinephrine (USP) (Retired Servicemen's Pharmaceutical Plant of Taiwan) was administered intravenously (i.v.) by way of the femoral vein[9]. Appropriate saline-injected controls were always run simultaneously. Subsequent results were then statistically evaluated by Fisher's t-test.

RESULTS

After administration of GABA (10-100 μg), diazepam (1-10 μg) or baclofen (0.01-0.25 μg) into the lateral cerebral ventricle, the resting blood pressure (BP) and heart rate (HR) showed immediate change. The cardiovascular responses recovered 15-30 mins after treatment of drug and there was no significant difference compared with saline i.c.v. injected group (Fig. 1, 2, 3, between the columns). In saline-injected animals, intravenous administration of epinephrine (1.5 μg/kg) elicited marked hypertension and bradycardia (Fig. 1A, 2A, 3A). However, rats which were

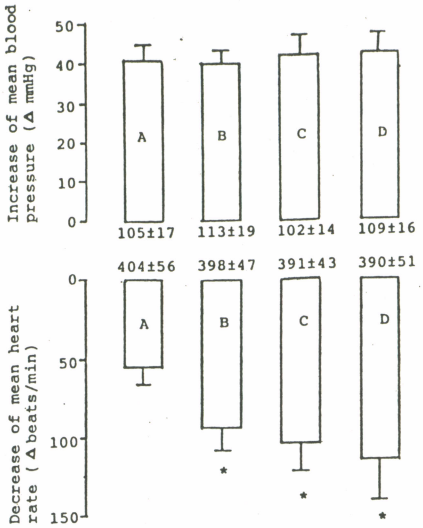

Fig. 1. Effect of i.c.v. injection of GABA on the bradycardia response to i.v. administration of epinephrine (1.5 ug/kg). Resting values for mean blood pressure (mmHg) and mean heart rate (beats/min) are given between the column (±S.E.). Maximal changes induced by i.v. injection of epinephrine are given by the columns as mean ± S.E. of 10 animals. A: saline-treated; B: GABA 10 ug, C: GABA 30 ug, D: GABA 100 ug treated. *Significantly different from saline-treated group, at $p < 0.05$.

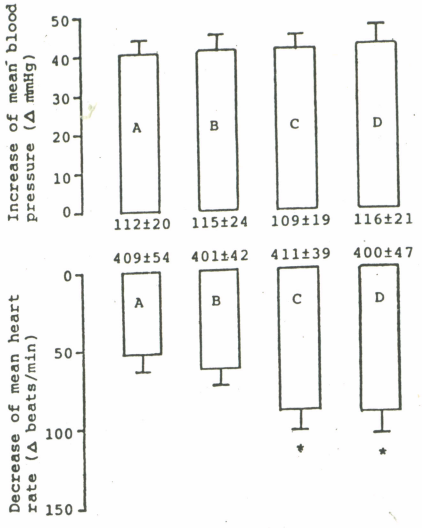

Fig. 2. Effect of i.c.v. injection of diazepam on the bradycardia response to i.v. administration of epinephrine (1.5 ug/kg). Resting values for mean blood pressure (mmHg) and mean heart rate (beats/min) are given between the column (±S.E.). Maximal changes induced by i.v. injection of epinephrine are given by the columns as mean ± S.E. of 10 animals. A: saline-treated; B: diazepam 1 ug, C: diazepam 3 ug, D: diazepam 10 ug treated. *Significantly different from saline-treated group, at $p < 0.05$.

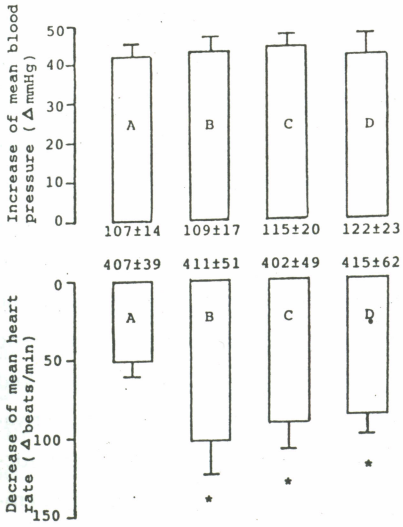

Fig. 3. Effect of i.c.v. injection of baclofen on the bradycardia response to i.v. administration of epinephrine (1.5 ug/kg). Resting values for mean blood pressure (mmHg) and mean heart rate (beats/min) are given between the column (±S.E.). Maximal changes induced by i.v. injection of epinephrine are given by the columns as mean ± S.E. of 10 animals. A: saline-treated, B: baclofen 0.01 ug, C: baclofen 0.05 ug, D: baclofen 0.25 ug treated. *Significantly different from saline-treated group, at $p < 0.05$.

given epinephrine (1.5 μg/kg i.v.) 15-30 mins after a prior i.c.v. injection with GABA (10-100 μg, Fig. 1), diazepam (3-10 μg, Fig. 2) or baclofen (0.01-0.25 μg, Fig. 3) (i.e. at the time when effects of these drugs on BP and HR had already recovered), exhibited bradycardia to a greater extent when compared to saline injected rats ($p < 0.05$, Fig. 1, 2, 3, lower columns). Pretreatment of animals with GABA, diazepam and baclofen, however, did not influence the effects of epinephrine on arterial blood pressure (Fig. 1, 2, 3, upper columns).

DISCUSSION

The arterial baroreflex system is regarded as one of the most powerful and rapidly acting homeostatic mechanisms for regulating blood pressure. An increase in arterial pressure stretches the baroreceptors located in the carotid sinus, aortic arch and other large central arteries. Signals from these are transmitted to the brain stem and hence back to the autonomic nervous system to reduce the sympathetic discharge and to increase the vagal discharge; both effects slow the heart rate and dilate the peripheral blood vessels thus restoring the blood pressure to normal range[10]. The central baroreceptor arc is polysynaptic, with the first synapse located in the nucleus tractus solitarii (NTS), and inhibiting neurons interposed between the NTS and the cardiovascular center[11,12]. It is generally accepted that the central baroreceptor arc contains serotonergic, dopaminergic and noradrenergic neurons. For example, activation of serotonergic receptors within the brain was found to depress adrenaline-induced bradycardia, whereas inhibition of these central serotonergic receptors facilitated adrenaline-induced bradycardia[13,14]. Blockade of dopaminergic receptors in the brain with either dopamine receptor antagonists or by destruction of dopamine neurones caused a significant reduction in reflex bradycardia[15,16]. In contrast, activation of dopaminergic receptor in the brain with either dopamine receptor agonists or by electrical stimulation on the rabbit, both noradrenergic and serotonergic neurons within the brain had been shown to participate in central baroreceptor-heart rate reflex pathways[17,18]. Recently, as mentioned during introduction, evidence suggests that GABAergic mechanisms are involved in the regulation of cardiovascular function. It was also implicated that GABA[19] acted directly or indirectly on central monoaminergic neuron to influence their brain contents and activities. According to the review of Bowery et al.[20], receptors for GABA in the mammalian brain are not homogenous, at least, two classes are known and have been designated $GABA_A$ and $GABA_B$ sites respectively. The $GABA_A$ sites represent all classical bicuculline-sensitive sites whereas $GABA_B$ sites are not affected by bicuculline and do not recognize many of the accepted GABA-mimetics such as isoguvacine and 3-aminopropanesulphonic acid. With regards to influence on membrane permeability, $GABA_A$ receptors are linked to chloride ion channels such that activation of the receptor increase the conductance of chloride across the neuronal mem-

brane, whereas $GABA_B$ site is unlikely to be associated with a chloride channel mechanism but possibly linked to a species of calcium ion channel. Sedative benzodiazepines are the group capable of enhancing the binding of GABA to the $GABA_A$ recognition site or increasing the lifetime of GABA activated chloride channels. On the contrary, baclofen (a muscle-relaxant) activates the $GABA_B$ site thereby producing its pharmacologic activities. The present results showed that direct administration of either GABA, $GABA_A$ agonist (diazepam) or $GABA_B$ agonist (baclofen) into the lateral cerebral ventricle caused an enhancement of epinephrine-induced reflex bradycardia, although the responses in arterial pressure were no different from those of the control. Thus, our result indicate that both the GABA-sensitive cells and GABA receptor, (including $GABA_A$ site and $GABA_B$ site) within several brain regions are involved in the regulation of cardiovascular function and act through a central mechanism to enhance the vagal tone and/or to attenuate the preganglionic sympathetic efferent activity that serve to potentiate reflex bradycardia. Our previous results[9] demonstrated that microinjection of GABA into the ventrolateral medullary area – where serotonin cells of the B_1 and B_3 groups were located[21] – produced a decrease in both mean arterial pressure and heart rate but enhanced epinephrine-induced reflex bradycardia. Needless to say, further investigations would be necessary to a better understanding of the intricate relationships between the serotonergic neuron, the catecholaminergic neuron, and the GABA-sensitive neurons in their regulation of cardiovascular function.

REFERENCES

1. OJA SS, KONTRO P, LÄHDESMÄKI P: Amino acids as inhibitory neurotransmitters. Progress in Pharmacology 1; 1-113, 1977.
2. COOPER JR, BLOOM FE, ROTH RH: The biochemical basis of neuropharmacology. 249-294 Oxford University Press, New York, 1982.
3. SWEET CS, WENGER HC, GROSS DM: Central antihypertensive properties of muscimol and related γ-aminobutyric acid agonists and the interaction of muscimol with baroreceptor reflexes. Can J Physiol Pharmacol 57; 600-605, 1979.
4. GUERTZENSTEIN PG: Vasodepressor and pressor responses to drugs topically applied to the ventral surface of the brain stem. J Physiol (Lond.) 224; 84-85, 1972.
5. ROBERT NW, PETER PB, ABBOTT JK, HREDAY NS: Endogenous GABAergic mechanisms in the medulla and the regulation of blood pressure. J Pharmacol Exp Ther 230; 34-39, 1984.
6. DE GROOT J: The rat hypothalamus in stereotaxic coordinates. J Comp Neurol 113; 389-400, 1959.
7. LIU HJ, LIN MT: Cholecystokinin-induced hypothermia: Possible involvement of serotoninergic mechanisms in the rat hypothalamus. Pharmacol 31; 108-114, 1985.

8. YU YC, YANG CP, HUANG CJ, LIU HJ: The diurectic action of Eucommiae Cortex and its effects on cardiovascular system. Bulletin of Taipei Medical College 15; 87-93, 1986.
9. YANG CP, LIN MT: Amino acids injected into the cerebroventricular system induce an enhancement of reflex bradycardia in the rat. Neuropharmacology 22; 919-922, 1983.
10. KIRCHHEIM HR: Systemic baroreceptor reflexes. Physiological reviews 56; 136-160, 1976.
11. KIRCHHEIM HR: Systemic arterial baroreceptor reflexes. Physiol Rev 56; 100-176, 1976.
12. KORNER PI: Integrative neural cardiovascular control. Physiol Rev 51; 312-367, 1971.
13. LIN MT, CHERN SI: Effects of 5-hydroxytryptamine alterations on reflex bradycardia in rats. Am J Physiol 236; R302-R306, 1979.
14. LIN MT, TSAY BL, FAN YC: Effects of 5-hydroxytryptamine, fluoxetine and chlorimipramine on reflex bradycardia in rats. J Pharmacol 32; 493-496, 1980.
15. CHEN FF, LIN MT: Effects of dopamine, apomorphine, gamma-hydroxybutyric acid, haloperidol and pimozide on reflex bradycardia in rata. J Pharmacol Exp Ther 214; 427-432, 1980.
16. LIN MT, TSAY BL, CHEN FF: Activation of dopaminergic receptors within the caudate-putamen complex facilitates reflex bradycardia in the rat. Jpn J Physiol 32; 431-442, 1982.
17. KORNER PI, HEAD GA: Effects of noradrenergic and serotonergic neurons on blood pressure, heart rate and baroreceptor-heart rate reflex of the conscious rabbit. J Auton Nerv Syst 3; 511-523, 1981.
18. WING LMH, CHALMERS JP: Effects of p-chlorophenyl-alanine on blood pressure and heart rate in normal rabbits and rabbits with neurogenic hypertension. Clin Exp Pharmacol Physiol 1; 219-229, 1974.
19. BARTHOLINI G: Present knowledge of GABA receptor agonists. In: Medicinal chemistry advances. F.G. De Las Heras., S. Vega. editor (Pergamon Press, New York 1980).
20. BOWERY NG, PRICE GW, HUDSON AL, HILL DR, WILKIN GP, TURNBULL MJ: GABA receptor multiplicity, Visualization of different receptor types in the mammalian CNS. Neuropharmacology 23; 219-231, 1984.
21. HOWE PRC, KUHN DM, MINSON JB, STEAD BH, CHALMERS JP: Evidence for a bulbospinal serotonergic pressor pathway in the rat brain. Brain Res 270. 29-35, 1983.

GABA, Diazepam和Baclofen對老鼠反射性徐脈之影響

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本實驗係探討GABA, Diazepam和Baclofen 對大白鼠，由靜脈投與Epinephrine（1.5 μg／kg）導致之反射性徐脈的影響。實驗組由側腦室投與GABA（10～100μg），Diazepam（1～10 μg）或Baclofen（0.01～0.25 μg），雖可使大白鼠的血壓及心跳有所改變，但此對心臟循環系的作用，約在15～30分鐘之內，可回復至與由側腦室投與 Saline 之對照組相同。於此時，再由靜脈投與Epinephrine，則Epinephrine所造成的反射性徐脈，在投與不同劑量之GABA, Diazepam或Baclofen組與投與Saline之對照組相比較，可見投與GABA組，Diazepam組或Baclofen組均有顯著的加強（$P < 0.05$），但對 Epinephrine 所產生的血壓上昇反應，兩組間沒有明顯的差異。依 Bowery 等的分類，中樞的GABA作用部位有二類，即$GABA_A$-Site及$GABA_B$-Site，而Diazepam 屬於$GABA_A$ agonist, Baclofen 則屬於 $GABA_B$ agonist。因此由以上GABA, Diazepam與Baclofen三者均可加強反射性徐脈的結果來看，似乎顯示腦中$GABA_A$-Site及$GABA_B$-Site二者均可能參與中樞性反射性徐脈的調節作用。

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