γ-AMINOBUTYRIC ACID RECEPTOR ON VASCULAR SMOOTH MUSCLE OF DOG CEREBRAL ARTERIES

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γ-Aminobutyric acid (GABA) had a relaxant effect on dog cerebral arteries which was blocked only by picrotoxin, a known antagonist of GABA receptors. Other agents, adrenoceptor and ganglion blocking agents, atropine, reserpine, tetrodotoxin and ouabain, had no effect on the inhibitory action suggesting the existence of GABA receptors in the vascular smooth muscle of dog cerebral arteries.

Introduction In spite of overwhelming interest in the mechanisms influencing the control of cerebral blood circulation, comparatively few studies have been concerned with the basic properties of the smooth muscle in cerebral blood vessels. Even the mechanical response of these vessels to neurogenic transmitters and vasoactive substances *in vitro* is still not fully understood. Recently we found that γ-aminobutyric acid (GABA), a putative postsynaptic inhibitory neurotransmitter in the central nervous system, causes relaxation of dog cerebral arteries. Thus, the present experiments were undertaken to explore this effect and to investigate the possible mechanism by which GABA inhibits the vascular smooth muscle of dog cerebral arteries.

Methods Dogs of either sex weighing on the average 11 kg were anaesthetized with sodium pentobarbitone (45 mg/kg i.p.), bled through the common carotid arteries and their brains removed. The basilar artery, middle and posterior arteries were dissected from the brain and used in these experiments. The external diameter of the basilar arteries was approximately 0.9 mm (0.7-1.2 mm, $n = 30$) and of the middle and posterior cerebral arteries about 0.8 mm (0.65-1.0 mm, $n = 38$).

For measurement of their mechanical responses the arteries were prepared in helical strips approximately 1-1.5 mm in width and 15 mm in length and mounted vertically in an organ bath containing 20 ml of Krebs-Ringer solution of the following composition (mM): NaCl 154, KCl 5.4, $CaCl_2$ 2.2, $NaHCO_3$ 6, dextrose 11, in distilled, deionized water. The bath medium was maintained at 37°C, pH 7.4 and equilibrated before and during the experiment with a gas mixture of 95% O_2 and 5% CO_2. A resting tension of 0.5 g was initially applied and maintained throughout the experiment and the preparations were allowed to equilibrate for 90 min in the bathing medium before the experiments were commenced. During the equilibration period, the bath solution was changed every 20 minutes. The muscle tension changes were recorded isometrically through a force-displacement transducer (Nihon Koden) and displayed on a polygraph.

In some experiments helical strips of aorta from rabbits (2-3 kg), rings of thoracic aorta of dog, and strips of mesenteric artery and portal vein of both rabbit and dog were used as model peripheral blood vessels for comparison.

Results GABA at 10^{-7} to 10^{-5} M caused relaxation in 9 of 14 strips of basilar and 8 of 11 strips of middle cerebral arteries. The inhibitory effect of GABA was much greater in presence of active tension (5-HT, 10^{-8} M) than at resting tension (Figure 1). However, in most of the basilar and middle cerebral arteries, the response was observed only at high GABA concentration (Figure 1a, 1b). In 5 of 14 basilar strips and 4 of 11 middle cerebral arterial strips, a low concentration was sufficient to cause relaxation (Figure 1c). Even after application of a high concentration of GABA (10^{-5} M), the strips completely recovered their original tension after the relaxation. This inhibitory effect of GABA was only observed in cerebral arteries, but not in dog and rabbit aorta, mesenteric artery and portal vein. Treatment with glycine and glutamate (10^{-8}-10^{-5} M) had no effect on either middle cerebral or basilar arteries (Figure 1a, 1b). After pre-treatment with picotoxin (10^{-5} M) for several min, the relaxing effect of GABA was almost completely abolished (Figure 1d); however, pretreatment with phentolamine (10^{-6} M), propranolol (10^{-6} M), atropine (10^{-6} M), pentolinium (10^{-5} M), tetrodotoxin (10^{-7} M), reserpine (4 mg/kg i.m., 24 h earlier) and ouabain

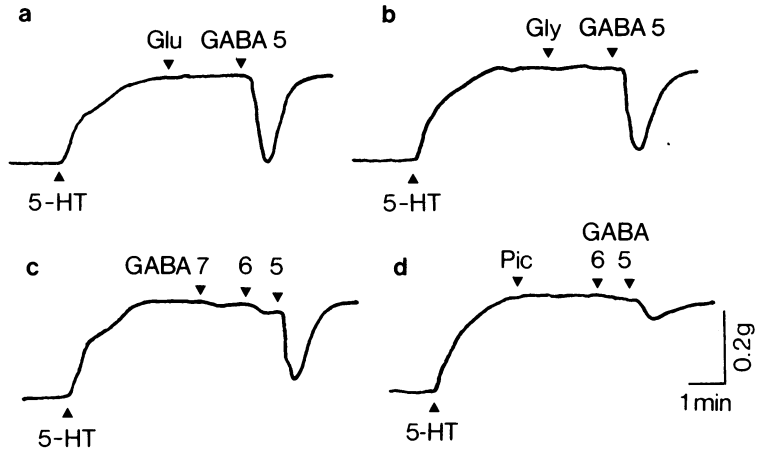

Figure 1 Relaxant effect of γ-aminobutyric acid (GABA), glutamate and glycine on the dog cerebral arteries. Relaxant effect was tested on the strips contracted by 5-hydroxytryptamine (5-HT) at 10^{-8} M. GABA 7 6 5 = 10^{-7} M, 10^{-6} M, and 10^{-5} M; glutamate (Glu) = 10^{-5} M; glycine (Gly) = 10^{-5} M; picrotoxin (Pic) = 10^{-7} M. (a) and (b) were from the same tissues; (c) and (d) were from the same tissues.

(5×10^{-8}-10^{-7} M) did not affect the response to GABA. Adenosine 5-triphosphate sodium (ATP) (10^{-5} M) and adenosine 5-monophosphate (10^{-5} M) caused similar relaxation of dog cerebral arteries but picrotoxin (10^{-5} M) failed to antagonize the effect.

Discussion GABA has a selective inhibitory effect on the dog cerebral arteries but not on the peripheral blood vessels of dog or rabbit. Other amino acids such as glycine and glutamate have no effect on the cerebral blood vessels. Since this inhibitory action of GABA was not blocked by adrenoceptor and ganglion blocking agents and by reserpine, atropine, and tetrodotoxin, the inhibitory effect of GABA is due to a direct action on the effector site rather than indirectly through the release of a chemical transmitter from the nerve endings. The antagonism of picrotoxin towards the response to GABA but not that induced by ATP or adenosine, plus the presence of GABA transaminase in cerebral arteries (Van Gelder, 1965), strongly suggest the existence of a specific GABA receptor in the vascular smooth muscle of the dog cerebral arteries.

This work was supported in part by the Nippon Tobacco and Salt Public Corporation and the Hawaii Heart Association. S.S. was visiting Professor at Kyoto University, School of Medicine during a sabbatical year of leave from the University of Hawaii. Reprint requests should be sent to Dr S. Shibata, University of Hawaii.

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(Received September 18, 1975)