Vasodilatation of the Hindquarters Induced by Antagonism of GABA_A Receptors in the Freely Moving Rat

Yumi TAKEMOTO

Department of Neurophysiology, Division of Integrated Medical Science, Programs for Biomedical Research, Graduate School of Biomedical Sciences, Hiroshima University, Kasumi 1-2-3, Minami-ku, Hiroshima, 734-8551, Japan

Abstract: An intracisternal injection of the GABA_A receptor antagonist bicuculline into conscious rats increased arterial pressure and decreased hindquarter resistance. Propranolol attenuated only the resistance response, but ganglionic block abolished both responses. These findings suggest that central GABA_A receptor blockade induces an autonomic pressor action with hindquarter vasodilatation. [The Japanese Journal of Physiology 55: 75–78, 2005]

Key words: GABA_A receptor antagonist, hindquarter resistance, adrenaline.

The depressor response to the intracisternal (i.c.) injection of gamma-aminobutyric acid (GABA) in the freely moving rat is accompanied by a preferential vasodilatation in the hindquarter vascular bed without significant changes in the renal and superior mesenteric vascular beds [1]. The present study investigated whether a blockade of GABA_A receptors with the selective antagonist bicuculline produced the opposite response in the hindquarter vascular bed, namely, vasoconstriction. An injection of bicuculline into the cisterna magna of conscious rats resulted in vasodilatation and a hypertensive response. The mechanisms of the responses were investigated with ganglionic and beta-adrenoceptor blocking agents. These experiments were carried out in accordance with the Guiding Principles for the Care and Use of Animals in the Fields of Physiological Sciences approved by the Physiological Society of Japan.

For a later injection of bicuculline (Sigma) solution, 17 male Wistar rats (335 ± 35 g) were anesthetized with sodium thiamylal (50 mg/kg, i. p.), and polyethylene cannulae were inserted into the cisterna magna via a hole drilled in the interparietal bone [2, 3]. After at least one week, each rat was reanesthetized and equipped with polyethylene cannulae into the external jugular vein for i.v. injections and into the aortic arch via the common carotid artery for a measurement of

arterial blood pressure. An electromagnetic flow probe (chronic implanted-type FC with an internal diameter of 2.0 mm, Nihon Kohden, Tokyo) was placed around the abdominal aorta just rostral to the bifurcation of the common iliac arteries. The lead wire of the flow probe and the distal end of each cannula were exited at the back of the neck [3, 4].

Data collection (08:00–19:00) began 2–3 days after probe implantation with animals freely moving in transparent home cages. By that time the rats were eating and drinking normally. Bicuculline methiodide (0.5, 1.0, and 2.0 nmol/10 μ l) or vehicle (0.9% saline) was injected i.c. in random order. The cardiovascular variables were allowed to return to baseline levels between injections. Hindquarter blood flow was determined with a flowmeter (MFV 1100 Nihon Kohden, Tokyo), and arterial pressure was measured with an amplifier (AP 621G Nihon Kohden, Tokyo). Flow and pressure signals were smoothed with RC circuits (time constant 1 s) to facilitate the determination of mean values. At the end of each recording session, each rat's aorta was carefully occluded by manual compression, and zero blood flow was confirmed with an oscillograph recording chart (RJG 4023 Nihon Kohden, Tokyo). For a detailed description of the methodology, see Takemoto [2–4].

An index of resistance (mean arterial pressure/

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Correspondence: Correspondence should be addressed to: Yumi Takemoto, Department of Neurophysiology, Division of Integrated Medical Science, Programs for Biomedical Research, Graduate School of Biomedical Sciences, Hiroshima University, Kasumi 1-2-3, Minami-ku, Hiroshima, 734-8551 Japan. Phone/Fax: +81-82-257-5128, E-mail: yumitake@hiroshima-u.ac.jp

hindquarter flow) was used to quantify changes in the vasomotor state. The data are expressed as means $\pm$ SD. Significant comparisons were detected by analyses of variance (ANOVAs) and paired t-tests. The hemodynamic data at each time point and each dose were compared with baseline control values by Dunnett's test. A $P < 0.05$ was considered significant in all cases.

The i. c. injections of bicuculline produced dose-dependent increases in systemic arterial pressure and hindquarter blood flow, but decreases in hindquarter resistance (Fig. 1). Figure 2 shows an example of the recordings of cardiovascular changes induced by bicuculline injection before and after systemic injection of dl-propranolol. In 5 conscious rats, DL-propranolol treatment (2.0 mg/kg, i.v.) given 10 min before bicuculline injection (2.0 nmol, i.c.) attenuated the bicuculline-induced hindquarter vasodilator changes (from $74 \pm 50\%$ to $29 \pm 30\%$, $p = 0.02$ in flow), but did not affect the pressor response (from $32 \pm 10\%$ to $33 \pm 16\%$, $p = 0.38$) measured 5 min after the bicuculline treatment. These results suggest that hindquarter vasodilatation induced by central stimulation with bicuculline is mediated by peripheral adrenaline release. Propranolol alone increased arterial pressure (109 ± 9.8 mmHg to 123 ± 15 , $p < 0.01$) and hindquarter resistance (24 ± 5.9 mmHg min 100 g/ml to 39 ± 8.6 , $p < 0.001$), consistent with previous observations [5].

Sympathetic nervous system activation and vasopressin release may contribute to the persistent pressor action of bicuculline even with propranolol beta-adrenoceptor antagonism. To evaluate this possibility, the effects of ganglionic block followed by systemic application of a vasopressin receptor antagonist on bicuculline-induced hemodynamic changes were examined. Control measurements were taken in conscious rats 5 and 10 min after bicuculline injection (2.0 nmol, i.c.), and chlorisondamine chloride (0.5 mg/kg/min, i.v.) was then infused for 10 min. Chlorisondamine treatment decreased arterial pressure from 116 ± 10 mmHg to 81 ± 11 mmHg ($p < 0.01$, $n = 6$), and this depression was maintained for more than 20 min. Bicuculline (2.0 nmol) was injected (i.c.) immediately after termination of the chlorisondamine infusion, and the vasopressin v1 receptor antagonist ($d[\text{CH}_2]_5^1$, O-Me-Thr², Arg⁸)-vasopressin (10 $\mu\text{g/kg}$, i.v.) was injected 5 min later. The vasodilator and pressor actions of bicuculline were completely blocked by the chlorisondamine ganglionic block (Fig. 3). There was no detectable effect of vasopressin antagonist even during the low arterial pressure produced by

Fig. 1. Time courses of the hemodynamic responses produced by bicuculline injection. Data collected before and after injections were treated with two-way ANOVAs (time: before and 1 to 30 min; dose: vehicle control and 0.5 to 2.0 nmol). Significant differences were then detected with Dunnett's test for comparisons at each time point v. the baseline and of each dose (0.5 to 2.0 nmol) v. no drug vehicle control; ** $p < 0.01$ and * $p < 0.05$ for time; $N = 6$. HQ: hindquarter.

ganglionic block.

Adrenaline release induced by i.c. injection of D-arginine [5] also produces hindquarter vasodilatation and pressor changes. However, D-arginine effects on both vasodilator and pressor actions are attenuated by propranolol treatment. Thus the pressor response to D-arginine appears to be due to a cardiovascular response to the release of adrenaline. In contrast, the pressor action produced by bicuculline injection persisted after propranolol treatment, but it was completely abolished by ganglionic block. This suggests that the pressor action of bicuculline was produced in part by vasoconstriction in other vascular beds by means of activation of the sympathetic nervous system. Preliminary examinations of resistance changes

Fig. 2. An example of recordings of mean arterial pressure (AP) and hindquarter flow (HQF) in bicuculline (bic.) injections before and after propranolol treatment. m: marks when the rat moved; i.c.: intracisternal injection.

Fig. 3. The effects of ganglionic block with chlorisondamine on bicuculline-induced hemodynamic actions; the block was followed by an injection of a vasopressin antagonist that produced no further significant changes. AP: arterial blood pressure; HQR: hindquarter resistance. The data were treated with paired *t*-tests.

in other vascular beds 5 min after bicuculline injection support this interpretation. Three subgroups of rats were monitored for bicuculline effects on resistance within the carotid, the superior mesenteric or the renal

vascular bed. Bicuculline injection (2.0 nmol, i.c.) produced the following hemodynamic changes in these subgroups: Carotid resistance increased by $141 \pm 43\%$ with a coincident $32 \pm 9.5\%$ pressor change ($n = 3$); superior mesenteric resistance increased by $124 \pm 57\%$ with a coincident $28 \pm 5.1\%$ pressor change ($n = 3$); and renal resistance increased by 65% with a coincident 24% pressor change ($n = 2$). These findings indicate that the effects of bicuculline on the central neural network may include adrenaline release and sympathetic nervous system activation. A recent study by Natarajan and Morrison [6] demonstrated that plasma adrenaline concentrations were elevated after a disinhibition of neurons in the rostral ventrolateral medulla of the rat with bicuculline. Intracisternal injections of dye reach the brainstem surface, including the rostral ventrolateral medulla [7]. These findings support the present hypothesis that bicuculline induces vasodilatation via adrenaline release. Consistent with this model, some GABA_A receptors regulating the sympatho-adrenal system appear to be tonically activated by endogenous GABA in freely moving rats.

The present results indicate that cardiovascular responses to GABA_A receptor blockade are not simply opposite to those of exogenous GABA. Rather a more complicated physiology was revealed because GABA_A receptor antagonism resulted in hindquarter vasodilatation instead of vasoconstriction. This result may be explained in this way: A previous study showed that exogenously applied GABA preferentially decreased vascular resistance in the hindquarter and carotid arteries, but not in the celiac, superior mesenteric, and renal arteries, in a manner dependent on the existing vascular tone [3]. Interestingly, exogenous GABA application produced larger decreases in resistance of the hindquarter and carotid vascular beds when the basal resistance was larger [3]. Thus the GABA treatment more robustly affected the two vascular beds that have higher resting tone. This preferential response to exogenously applied GABA in the brain may reflect varying levels of unbound GABA receptors not occupied by endogenous GABA. GABA receptors regulating the adrenal medulla and the non-responsive vascular beds appeared to be fully occupied by endogenous GABA in the other three vascular beds. If so, endogenous GABA in the brainstem would tonically inhibit adrenal activity and sympathetic nervous activity and thereby control other vascular beds, such as the superior mesenteric vascular bed. This hypothesis is supported by the present results, by the presence of adrenaline release [6], and by the generation of pos-

sible sympathetic vascular tone after central disinhibition with bicuculline.

In summary, the present study demonstrates that central disinhibition by a blockade of GABA_A receptors induces an autonomic nervous-mediated pressor action and hindquarter vasodilatation. A model of the possible central mechanisms that is consistent with these results and previous findings is proposed.

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