GABAergic effects on the depressor responses elicited by stimulation of central nucleus of the amygdala

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Ciriello, John, and Stefanie Roder. GABAergic effects on the depressor responses elicited by stimulation of central nucleus of the amygdala. Am. J. Physiol. 276 (Heart Circ. Physiol. 45): H242–H247, 1999.—GABAergic inputs have been demonstrated in the central nucleus of the amygdala (ACe). However, the contribution of these inhibitory inputs to the cardiovascular responses elicited from the ACe is not known. Experiments were done in chloralose-anesthetized, paralyzed, and artificially ventilated male Wistar rats to investigate the effects of microinjections of GABA, the selective GABA_A-receptor antagonist bicuculline, or the GABA_A-receptor antagonist phaclofen, in the ACe on the mean arterial pressure (MAP) and heart rate (HR) responses elicited by L-glutamate (Glu) stimulation of the ACe. Microinjections of Glu in the ACe elicited decreases in MAP (−13.7 ± 1.6 mmHg) and HR (−5.3 ± 1.9 beats/min). The MAP and HR responses elicited by Glu stimulation of the ACe were significantly reduced (89%) by the prior microinjection of GABA in the same ACe site. In addition, at some sites in the ACe at which microinjection of Glu did not elicit depressor responses, Glu injections in the presence of phaclofen elicited decreases in MAP (−9.5 ± 1.0 mmHg) and variable changes in HR. On the other hand, the magnitude of the depressor responses elicited during stimulation of the ACe site in the presence of bicuculline was significantly attenuated (60%), whereas phaclofen had no effect on the magnitude of the depressor responses elicited by Glu stimulation of the ACe. These data suggest that GABAergic mechanisms in the ACe alter the excitability of ACe neurons involved in mediating changes in systemic arterial pressure and HR.

METHODS

General procedures. Experiments were done in 38 male Wistar rats (230–508 g) anesthetized with α-chloralose (60 mg/kg iv, initially, supplemented by additional doses of 30 mg/kg every 1–2 h) after induction with equithesin (0.3 ml/100 g ip). All experimental procedures were done in accordance with the guidelines on the use and care of laboratory animals as set out by the Canadian Council on Animal Care and approved by the Animal Care Committee at the University of Western Ontario. Polyethylene catheters (PE-50) were inserted in the femoral artery and vein for the recording of AP and the administration of drugs, respectively. AP was recorded through a Statham P23XL transducer, and HR was monitored with a 7P4DEF Grass tachograph triggered by the AP pulse, both of which were continuously recorded on a Grass 79D polygraph. The trachea was cannulated, and the animals were artificially ventilated (model 683; Harvard Apparatus, Natick, MA) with room air and 95% oxygen. The animals were paralyzed with pancuronium bromide (initial dose 1 mg/kg iv, followed by supplementary doses of 0.5 mg/kg every 30 min; Pavulon) to eliminate the possibility that the cardiovascular responses elicited during stimulation of the brain were secondary to muscular activity or related to respiratory changes (3). All surgical procedures were done before the administration of the paralyzing agent. During the nonsurgical portions of the experiment, the animals were allowed to recover periodically from the paralyzing agent to determine the depth of anesthesia by examining withdrawal reflexes. Body temperature was maintained at 37 ± 0.2°C by a heating pad controlled by a Yellow Springs model 73 temperature controller.

Microinjections in the ACe. The head of the animal was fixed in a Kopf stereotaxic instrument, and access to the ACe was obtained by a partial bilateral parietal craniotomy. All exposed nervous tissue was covered with cotton pellets soaked in warm Dow Corning 360 medical fluid (Dow Corning, Midland, MI) to prevent drying. The region of the ACe (19) was systematically explored for sites that elicited cardiovascular responses. A cardiovascular responsive site was defined as a site at which the center of the injection site was histologi-
cally verified in the ACe and at which a change of $\approx 5 \text{ mmHg}$ and/or 5 beats/min in mean arterial pressure (MAP) and/or HR, respectively, was elicited by the microinjection (20–40 nl) of Glu (1.0 M in 0.9% saline; Sigma Chemical, St. Louis, MO; see Refs. 3, 8, and 10). Glu was delivered using triple-barreled glass micropipettes (total tip diameter, 35–45 µm) by the application of pressurized nitrogen pulses controlled by a picospritzer (General Valve, Fairfield, NJ; see Ref. 3). The injected volumes were measured by direct observation of the fluid meniscus in the micropipette by using a microscope fitted with an ocular micrometer (3).

At a cardiovascular responsive site in the region of the ACe, 100 nl of 0.9% physiological saline ($n = 5$ rats) or GABA (0.1 M; $n = 9$ rats; Sigma; see Refs. 20 and 26) were microinjected from the second barrel of the multiple-barreled pipette. The ACe was restimulated with Glu at 2–5 min and 30–60 min after the microinjection of either the saline or GABA. Cardiovascular responsive sites in the ACe were also tested in the presence of the GABAA-receptor antagonist bicuculline methiodide (0.4 mM; $n = 12$ rats; Sigma; see Ref. 25) or the GABAB-receptor antagonist phaclofen (5 mM; $n = 21$ rats; Research Biochemical International; see Ref. 25) as described above for the saline and GABA experiments. Bicuculline or phaclofen was microinjected (40–100 nl) in cardiovascular responsive sites in the ACe, and the same sites were restimulated with Glu at 2–5 min and 30–60 min after the microinjections.

**RESULTS**

The baseline MAP and HR were 121.8 ± 1.5 mmHg and 386.5 ± 5.8 beats/min, respectively, in the chloralose-anesthetized rat. Stimulation of histologically verified sites ($n = 29$ rats) in the region of the ACe (Fig. 1) with the excitatory amino acid Glu elicited decreases in MAP (−13.7 ± 1.6 mmHg) and HR (−5.3 ± 1.9 beats/min).

Microinjections of GABA in cardiovascular responsive sites in the ACe elicited no changes in AP or HR. However, the depressor (Fig. 2A) and bradycardic (Fig. 2B) responses elicited by Glu stimulation of the ACe were significantly (89%) reduced at 2–5 min after the microinjection of GABA in the ACe ($n = 6$ rats; Fig. 2). The cardiovascular responses to Glu stimulation returned to approximately control levels at 30–60 min after the microinjection of GABA (Fig. 2).

**Fig. 1.** A: bright-field photomicrograph of neutral red stained transverse section through the region of the amygdala (at ~6.7 mm rostral to the interaural line) showing the location of a Pontamine sky blue deposit in the ACe corresponding to an injection site that elicited cardiovascular responses. Arrow points to approximately the center of the 100-nl injection. Scale bar, 0.5 mm. ABL, basolateral amygdaloid nucleus; ACe, central nucleus of the amygdala; AIM, intercalated amygdaloid area; ic, internal capsule; st, striatal terminalis. B: series of representative drawings through the region of the amygdala extending from 6.2 to 7.2 mm rostral to the interaural line showing the location of Glu microinjection sites that elicited depressor and bradycardic responses and at which the effect of GABA (○), bicuculline (▲), or phaclofen (■) on the depressor and bradycardia responses elicited by Glu stimulation was tested. Asterisks in B represent sites at which no cardiovascular responses were elicited by Glu stimulation alone; however, in the presence of phaclofen, Glu elicited depressor responses. Scale bar in B, 1 mm. ACo, anterior cortical amygdaloid nucleus; AL, lateral amygdaloid nucleus; AMe, anterior medial amygdaloid nucleus; En, endopiriform nucleus; Pir, piriform cortex.
Similarly, microinjections of the GABA_A-receptor antagonist bicuculline in cardiovascular responsive sites in the ACe did not elicit cardiovascular responses. However, the bicuculline microinjection in a cardiovascular responsive site in the ACe (n = 12 rats) significantly attenuated (60%) the magnitude of the cardiovascular responses elicited by Glu stimulation at 2–5 min after the microinjection of the antagonist (Fig. 2). The cardiovascular responses to Glu stimulation were also observed to return to approximately control values by 30–60 min after the bicuculline microinjection (Fig. 2). Unlike the effects of GABA and bicuculline on the depressor responses, the microinjection of the GABA_B-receptor antagonist phaclofen in a cardiovascular responsive site in the ACe (n = 12 rats) did not alter the magnitude of the depressor and bradycardic responses to Glu stimulation of ACe (Figs. 3 and 4A).

To test the possibility that a tonic inhibition by GABAergic neurons on ACe neurons prevented the activation of these neurons to elicit cardiovascular responses, at nine sites in the ACe at which Glu

Fig. 2. Bar graphs showing the changes in mean arterial pressure (MAP; A) and heart rate (HR; B) elicited by Glu stimulation of the ACe before (control) and at 2–5 min and 30 min after GABA or bicuculline (hatched bars) microinjections in the same site in the ACe. Note that the magnitude of the depressor and bradycardic responses to Glu stimulation (open bars) of the ACe were not different from those elicited with Glu at 5 and 30 min after the microinjection of phaclofen. However, at ACe sites at which Glu stimulation failed to initially elicit cardiovascular responses (hatched bars), depressor responses were elicited by Glu stimulation at 5 and 30 min after microinjection of phaclofen in the same site in the ACe. n, No. of rats.

microinjection did not elicit cardiovascular responses (Fig. 1), phaclofen was microinjected in these sites, and the sites were restimulated with Glu. Glu stimulation 5–30 min after a microinjection of phaclofen elicited decreases in MAP (−9.5 ± 1.0 mmHg) that were accompanied with variable changes in HR (Figs. 3 and 4). Depressor responses could not be elicited from these sites by Glu at 60 min after the injection of phaclofen (Fig. 4B). Phaclofen alone did not elicit significant changes in MAP and HR (Fig. 4) at these ACe sites.

Control microinjections of physiological saline in the same sites in the ACe (n = 5 rats) from which depressor (−19.3 ± 2.5 mmHg) and bradycardic responses (−5.0 ± 1.6 beats/min) were elicited by Glu had no effect on the magnitude of the cardiovascular responses (MAP, −17.0 ± 1.3 mmHg; HR, −5.0 ± 1.6 beats/min) elicited by Glu at 2–5 min after the saline microinjection.
DISCUSSION

This study has demonstrated that GABAergic mechanisms in the ACe alter the cardiovascular responses elicited by activation of ACe neurons. This is based on the finding that microinjections of GABA or bicuculline, a selective GABAA-receptor antagonist, significantly attenuated the magnitude of the depressor and bradycardic responses elicited by selective activation of ACe neurons by Glu. In addition, it was found that Glu stimulation of ACe neurons at some sites alone was unable to elicit cardiovascular responses; however, in the presence of the GABAB-receptor antagonist phaclofen, Glu stimulation elicited decreases in AP. The observation in this study that Glu stimulation of ACe neurons in the anesthetized rat elicited depressor and bradycardic responses is consistent with previous studies using both electrical and chemical stimulation of ACe (4, 6, 7, 15). Neurons in the ACe that are thought to be involved in mediating these cardiovascular responses have been shown to have a low or no spontaneous discharge rate (18, 24). This low or lack of spontaneous activity in ACe neurons has been suggested to be due to a tonic inhibition by the GABAergic interneurons found within the ACe (1, 13, 16, 27, 28). ACe neurons with low spontaneous activity have been shown to increase their discharge rate during iontophoretic applications of GABA antagonists (14, 16). It is therefore possible that some of the cardiovascular output neurons in the ACe stimulated by the Glu in this study were under tonic inhibition by GABAergic interneurons. If this suggestion is accepted, then stimulation of ACe neurons after withdrawal of this tonic inhibition would be expected to elicit the cardiovascular response, and stimulation of ACe neurons in the presence of GABA would be expected to elicit responses smaller in magnitude. Consistent with this suggestion, it was found that injection of the GABAB antagonist phaclofen had no effect on the depressor responses elicited by stimulation of the ACe. However, at some ACe sites where Glu alone did not elicit cardiovascular responses, Glu microinjections made after the microinjection of phaclofen at the same site elicited decreases in sys-

Fig. 4. Representative experiments (A and B) showing arterial pressure (AP) and HR responses elicited by Glu stimulation of the ACe before (control) and after microinjection of phaclofen in the ACe and Glu stimulation at 5 and 30 min after phaclofen was microinjected in the same sites in the ACe. Note in A that Glu stimulation of the ACe (control) elicited decreases in AP and HR that were not different in magnitude from the depressor and bradycardia responses elicited by Glu at 5 and 30 min after phaclofen microinjection. In addition, note in B that Glu stimulation of the ACe elicited no cardiovascular changes (control). However, microinjections of Glu at 5 min after phaclofen microinjection in the same site in the ACe elicited a decrease in AP. Microinjections of Glu (control, 5 min, 60 min) or phaclofen were made at the times indicated by arrows. Scale bar, 1 min.

Fig. 5. Schematic diagram showing a proposed neuronal circuit by which GABAergic neurons (GABA) acting through the activation of either GABAA or GABAB receptors in the ACe may alter the control of arterial pressure by the ACe, BST, bed nucleus of the stria terminalis; Glu, glutamate neuron; NE, noradrenergic neuron; VLM, ventrolateral medulla; +, excitatory input; −, inhibitory input; ?, putative neurotransmitter in this pathway not known.
temic AP. In addition, microinjection of GABA in cardiovascular responsive sites in the ACe reduced the magnitude of the depressor responses elicited by Glu stimulation.

It was also found that microinjection of GABA or the GABA_A- and GABA_B-receptor antagonists alone did not elicit changes in AP or HR when microinjected directly in ACe sites at which microinjection of Glu elicited depressor responses. Similar results with microinjections of GABA agonists and antagonist in the ACe have been previously obtained in the awake rat (25). These observations suggest the possibility that GABA interneurons within the ACe may not be directly involved in mediating the cardiovascular responses elicited by stimulation of the ACe. These data also suggest that the ACe cardiovascular neurons are not activated by inhibition of GABA inputs alone but may require the activation of an excitatory input along with the selective blockage of GABA_B receptors to elicit cardiovascular responses.

An unexpected finding in this study was that bicuculline injections attenuated the depressor responses elicited by Glu stimulation of the ACe. This suggests that Glu may have also activated GABA interneurons that mediate the depressor responses via the GABA_A receptor. The mechanism by which GABA may mediate the depressor responses is not clear. However, it has been demonstrated that, in the ACe, GABAergic neurons themselves are innervated by GABA terminals and Glu terminals (27, 28). Therefore, it is possible that the Glu-mediated depressor responses were due to the activation of GABA neurons that inhibited ACe output neurons. It has previously been shown that the lateral ACe contains a large number of GABA neurons that terminate on medial ACe output neurons (27, 28). Medial ACe neurons have been shown to innervate brain stem autonomic nuclei (13, 28). It is therefore possible that bicuculline injections may have affected GABA_A receptors on medial ACe output neurons, and this resulted in an inhibition of the Glu-mediated depressor responses. This suggestion is consistent with the recent demonstration that, in the chronic rat, a slight increase in AP is observed after relatively large injections of bicuculline in the amygdala (10).

An alternate possibility is that GABA may also act on presynaptic terminals to alter the release of norepinephrine (2, 29, 30). GABA, acting via GABA_A receptors, may function to inhibit the release of norepinephrine from the presynaptic terminals. The ACe has been shown to receive a noradrenergic input from brain stem catecholaminergic neurons (23), and activation of noradrenergic systems in the ACe have been shown to elicit increases in AP (17). The finding that microinjections of GABA reduced the depressor responses is consistent with this suggestion. Finally, the possibility must be entertained that the prior microinjections of GABA directly hyperpolarized the cell membrane of the ACe output neurons, and this resulted in the attenuation of the effects of Glu on these neurons.

Perspectives. A neuronal circuit by which GABAergic neurons in the ACe may influence the control of AP by the ACe is schematically shown in Fig. 5. On the basis of the data obtained in this study and that available in the literature, it is proposed that glutamnergic inputs exert an excitatory effect on ACe GABAergic neurons. Stimulation of these GABAergic neurons leads to the activation of an output pathway, likely through the bed nucleus of the stria terminalis, that may be involved in mediating the AP response to stimulation of the ACe (22). The bed nucleus of the stria terminalis in turn may activate neurons within the ventrolateral medulla that mediate the decrease in AP (8) by either exerting an effect on either inhibitory interneurons that are antecedent to sympathetic premotor neurons or directly on the sympathetic premotor neurons themselves (8). The final output neuron from the ACe to the bed nucleus of the stria terminalis may be GABAergic, as direct GABAergic connections from the ACe to the bed nucleus of the stria terminalis have been demonstrated (27). However, these output neurons may also contain other putative neurotransmitters (20). Within the ACe, GABAergic interneurons may also be involved in mediating the depressor response via GABA_A receptor mechanisms, as it was found that bicuculline attenuated the decrease in AP to activation of the ACe. Therefore, it is possible that GABAergic neurons activated by the glutamnergic input inhibit, through GABA_A receptors, ACe output neurons that are also GABA containing. In addition, these GABAergic interneurons may themselves be under inhibitory control through GABA_B mechanisms (27), as it was found in this study that blocking these receptors allowed for the expression of a depressor response to Glu stimulation. Noradrenergic inputs may also be involved in modulating the inhibitory effects of the GABA interneurons.

In summary, these data have shown that GABAergic mechanisms, through the activation of GABA_A and GABA_B receptors in the amygdala, modulate the cardiovascular responses to stimulation of ACe neurons. These data suggest that GABA acting via the GABA_A receptors was involved in mediating the depressor responses, whereas GABA acting via the GABA_B receptor was involved in inhibiting the depressor responses.

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