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Non-NMDA Receptors Mediate Both Pressor and Depressor Actions of the Cardiovascular-Reactive Areas in the Brainstem of Cats

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Abstract

L-glutamate (Glu), an important excitatory transmitter in the central nervous system, is mainly mediated via two kinds of ionotropic Glu receptors: N-methyl-D-aspartate (NMDA) and α-amino-3-hydroxy-5-methyl-4-isoxazole proprionic acid (AMPA)/kainate (non-NMDA) receptors. Microinjection of Glu (0.1 M, 30 nL) into gigantocellular tegmental field (FTG), dorsomedial medulla (DM) and rostral ventrolateral medulla (RVLM) induced increases of the systemic arterial pressure (SAP) and the sympathetic vertebral nerve activities (VNA), while its microinjection into caudal ventrolateral medulla (CVLM) induced decreases of SAP and VNA. In this study, the 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), a non-NMDA antagonist, was used to examine the effects of non-NMDA receptors on Glu-induced cardiovascular responses. Cats were anesthetized intraperitoneally with a mixture of urethane (400 mg/kg) and α-chloralose (40 mg/kg) and paralyzed with gallamine triethiodide (4 mg/kg, i.v. per hour). CNQX blocked the Glu-induced pressor responses in FTG, DM and RVLM but potentiated the depressor responses in CVLM. These results suggest that non-NMDA receptors modulate the central pressor and depressor responses in an opposite direction. On the other hand, activation of DM and RVLM neurons by application of AMPA (5 mM, 30 nL) evoked pressor responses. These AMPA-induced responses were significantly blocked by CNQX. Interestingly, CNQX itself induced pressor responses in many stimulated points of the pressor areas (FTG: 6/9; DM: 13/24; RVLM: 6/13), indicating a tonic release of Glu mediating depressor effects. In conclusion, non-NMDA receptors within the pressor (FTG, DM and RVLM) and depressor (CVLM) areas may play different modulatory roles in cardiovascular integration. The depressor mechanism mediated by non-NMDA receptors is tonically activated by the release of endogenous Glu in these pressor and depressor areas.

Key Words: CNQX, glutamate, gigantocellular tegmental field, dorsomedial medulla, ventrolateral medulla, vertebral nerve activities

Introduction

The rostral ventrolateral medulla (RVLM) is generally accepted as the origin of descending sympathetic outflow from the medulla for maintaining basal vasomotor tone (11, 13, 26). RVLM neurons are tonically inhibited by projections from neurons within the caudal ventrolateral medulla (CVLM) (1, 13). CVLM may also exert sympathetic inhibition through its direct bulbospinal pathways (19, 22, 25). In addition to RVLM, neurons in the dorsomedial medulla (DM) share the function of vasomotor integration, and stimulation of this region produces pressor responses in cats (2, 4 -10, 31, 32, 36, 39), rats (38), and rabbits (14). Direct projection has been demonstrated from DM to sympathetic neurons of the intermediolateral column (IML) of the spinal cord (21, 34). Furthermore, both DM and RVLM are

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independent from rostral neural structures in the maintenance of vasomotor tone, since no reduction of the resting SAP and the Glu-induced increases of SAP and sympathetic nerve activities in DM and RVLM were observed after precollicular decerebration (7). In contrast, after precollicular decerebration, the pressor effects upon Glu stimulation of the gigantocellular tegmental field (FTG) are greatly diminished (7).

Glu is an excitatory transmitter in the mammalian central nervous system. Based on the mechanism of signal transduction, Glu activates two kinds of receptors: ionotropic and metabotropic glutamate receptors (11, 27). Metabotropic glutamate receptors act through coupling to cellular effectors via GTP-binding proteins (11). The role of mGluRs in physiological function is not entirely clear. The excitatory effects is mainly due to activate ionotropic glutamate receptors, including N-methyl-D-aspartate (NMDA) receptor, α-amino-3-hydroxy-5-methyl-4-isoxazole proprionic acid (AMPA) receptor, and the kainate receptor. In mammalian central nervous system (CNS), activation of the non-NMDA (AMPA/kainate) receptors is thought to be responsible for the excitatory transmission at glutamatergic synapses, which are characterized by their rapid onset and decay in action (3). Such actions are antagonized by the 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) which is a potent non-NMDA antagonist and is widely used to examine the effect of non-NMDA receptors (17, 27). In our previous study using glutamic acid diethylester (GDEE, a non-NMDA receptor antagonist) and D-2-amino-5-phosphonovaleric acid (D-AP5, a NMDA receptor antagonist), we have shown that non-NMDA receptors are more responsible for the production of pressor responses than NMDA receptors during Glu activation in the pressor FTG, DM, and RVLM (10). GDEE is comparatively weaker in potency on non-NMDA receptors. GDEE also decreases cholinergic action (23) and causes hyperpolarization in neurons (41). This suggests that GDEE may induce a non-specific inhibition. Thus, it is worthwhile to further examine whether the inhibition of Glu-induced response in the pressor areas is really caused by the blockade of non-NMDA receptors or just a side effect by GDEE? CNQX, a newer compound, is more potent than GDEE in antagonizing the action of non-NMDA receptors and widely used to examine the effect of non-NMDA receptors (17, 27) and thus is used in the present study. In addition, the present study also examined the effects elicited by activation of non-NMDA receptors via exogenous application of AMPA and blockade of endogenous non-NMDA receptor activities by CNQX.

In general, the regulation of SAP depends on the sympathetic nerve activity. We have compared the vertebral sympathetic nerve activities (VNA) and renal nerve activities (RNA) during Glu activation of the pressor areas in the medulla, and found that RNA was not consistent with the change of SAP and was more affected by the baroreflex (31). Therefore, the vertebral nerve activities (VNA) was chosen to represent sympathetic nerve activities in this study.

Methods

General Preparations

Twenty-three adult cats, weighing 2.4 - 3.2 kg, were intraperitoneally anesthetized with a mixture of urethane (400 mg/kg) and α-chloralose (40 mg/kg) and paralyzed with gallamine triethiodide (4 mg/kg per hour, i.v.). The general procedures were as previously described (9). These included: artificial ventilation to maintain the end-tidal CO_2 at approximately 4%, maintenance of the rectal temperature at 37.0 ± 0.5 °C through a controlled heating pad, cannulation of the left femoral vein for drug administration. Besides, we cannulated the polyethylene (PE) tube from the left femoral artery to a point close to abdominal aorta for monitoring systemic arterial pressure (SAP), mean SAP (MSAP), and heart rate (HR). All recordings were made on a Gould ES-1000 polygraph.

Nerve Recording

Activities of the left vertebral sympathetic nerve were recorded as previously described (6). In six animals, the renal nerve activities were also recorded at the same time. The nerve was desheathed, cut at the distal end, and hooked on a bipolar platinum electrode. The nerve activities were amplified by a differential amplifier (bandpass: 10 - 3k Hz), rectified and integrated by an integrator (Gould 13-4615-70) with a reset time of 5 seconds. Nerve signals were monitored with an oscilloscope (Tektronix 5113) and stored on a tape recorder (Neuro Data DR-886) for later analysis.

Brain Stimulation

The head of the animal was fixed in a David-Kopf stereotaxic apparatus. The floor of the 4th cerebroventricle was exposed by removal of the cerebellum for better stimulation of the pressor and depressor areas. These areas included FTG (9 - 12 mm rostral to obex), RVLM (4 - 6 mm rostral to the obex), DM (3 - 5 mm rostral to the obex), and one depressor area of the CVLM (from 1 mm caudal to 1 mm rostral to obex). The stereotaxic coordinates were modified by the cytoarchitectonic atlas of the brainstem: (i)

The obex was used as a reference stereotaxic zero. (ii) The glass pipette was inserted into the brainstem at an angle of 34° which was perpendicular to the floor of the 4th cerebroventricle.

Microinjection into cardiovascular-reactive sites in the brainstem was carried out through a multibarrel glass micropipette (outside tip diameter 35 μm). One barrel was filled with 3 M NaCl and inserted with a platinum wire as an electrode for electrical stimulation (rectangular train pulses of 0.5 msec duration, 80 Hz, 50 μA in 15 sec). The following drugs were used: Glu (0.1 M, containing 0.5 % pontamine sky blue, Sigma) or AMPA (5 mM, Sigma), and different concentrations of CNQX (0.1, 1 or 5 mM, RBI). All chemicals were dissolved in saline at pH 7.4. If Glu induced positive responses in the stimulated point, then the CNQX was given 30 minutes later. Three minutes after pretreatment with CNQX, Glu was administered again to compare the Glu effect before and after CNQX. Chemicals were microinjected into the brain loci over a 5 sec period with pressured nitrogen gas by a pneumatic pump (Pneumatic Pressure System, Model PPS-2, Medical Systems Greenvale, New York, USA).

Idntification of Stimulation Points

At the end of each experiment, the animal was sacrificed with an overdose of pentobarbital, i.v. Two sets of brain sections in series were cut by a cryostat (50 μm); one set of section was left unstained for gross identification of the chemical injection point which was marked with pontamine sky blue; the other set was stained with cresyl violet for detailed histology.

Data Analysis

Percentage changes in SAP and VNA that responded to microinjection of Glu were analyzed by comparing the baseline (before microinjection of Glu) and peak responses. All data were presented as mean ± standard error of mean (mean ± s.e.m.), and were analyzed by Student *t*-test. The *p* value less than 0.05 was considered to be statistically significant.

Results

Effects of CNQX on the Glu-Induced Pressor/Depressor Responses

As a routine practice same as all other previous studies in the brain, control microinjections of solvent for Glu, CNQX and AMPA produced no significant change in SAP and VNA. Microinjection of Glu (0.1M, 30 nL) into DM produced rises of SAP (48.9 ± 6.6% vs. resting SAP) and VNA (58.9 ± 14.6 % vs. resting VNA, n=15). CNQX in a low concentration (1 mM, 50 nL) did not affect the Glu-induced pressor responses (SAP: 52.3 ± 12.4 %, VNA: 42.5 ± 35.9 %, n=5), while at a higher concentration (5 mM in 50 nL) significantly attenuated the Glu-induced pressor responses on SAP (15.8 ± 4.1 %, $p<0.001$, n=15) and VNA (9.2 ± 4.4 %, $p<0.05$, n=15, Fig. 1B). Similar effects were observed in FTG (Fig. 1A) and RVLM (Fig. 1C) when CNQX was applied at a higher concentration (5 mM, 50 nL) in these areas. The effects of CNQX, in a higher concentration (5mM), to Glu-induced responses are summarized on Fig. 2. The Glu-induced pressor responses on SAP and VNA in FTG, DM and RVLM were significantly inhibited by CNQX. Such inhibition of Glu-induced responses were recovered 1.5 to 2 hours after CNQX injections.

Microinjection of Glu into CVLM produced depressor responses (SAP: -26.2 ± 3.1 %, VNA: -16.5 ± 6.5 % vs. resting level, n=13). A lower

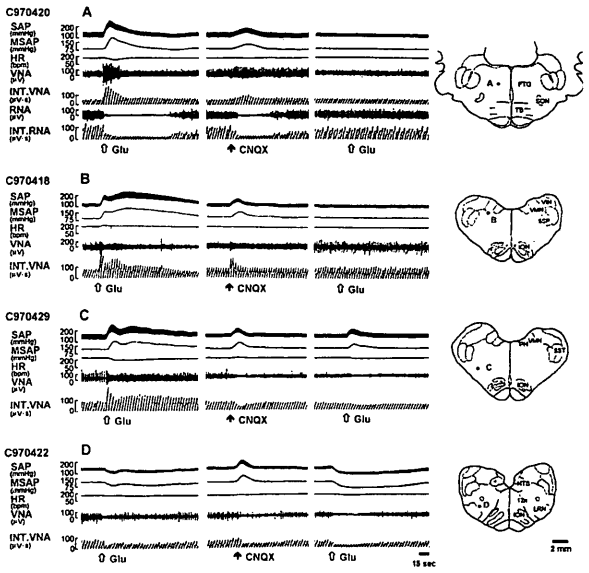

Fig. 1. Effects of CNQX on the Glu-induced responses.
Glu (0.1 M, 30 nL) and CNQX (5 mM, 50 nL) were used in all experiments. **A.** Microinjection of Glu in FTG (C940420, 11 mm rostral to obex as the dot shown in the brain drawing) produced an increase of SAP concomitant with an increase of VNA but a decrease of RNA. After pretreatment with CNQX, the Glu-induced responses were greatly reduced. Note that before blockade, CNQX itself produced an increase of SAP concomitant with VNA increase and RNA decrease. The responses were smaller than that of the control response induced by Glu. **B.** In DM (C970418, 4 mm rostral to obex), CNQX itself produced increases of SAP and VNA. After pretreatment with CNQX, the Glu-induced pressor responses were blocked by CNQX. **C.** In RVLM (C970429, 4 mm rostral to obex), CNQX also produced an increase in SAP but a decrease in VNA. The Glu-induced pressor responses were attenuated by CNQX. **D.** In CVLM (C970422, at the obex level). Glu produced decreases in SAP and VNA. CNQX alone induced a marked increase of SAP concomitant with a decrease in VNA. After pretreatment with CNQX, the Glu-induced depressor responses were much potentiated.

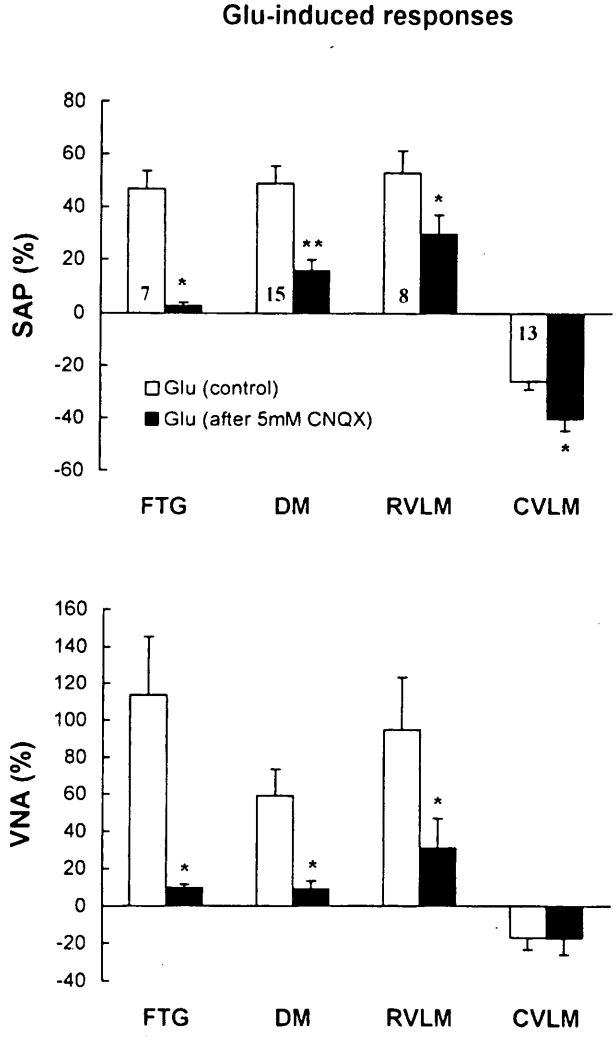

Fig. 2. Effects of CNQX on the responses of SAP and VNA induced by Glu in the pressor or depressor areas.
Microinjection of CNQX (5 mM, 50 nL) markedly antagonized the increases of SAP and VNA in FTG, DM and RVLM. In CVLM, the Glu-induced decrease of SAP was enhanced after CNQX injection. Single asterisk (*) indicates p less than 0.05 and double asterisk (**) indicates p less than 0.001. The number shown on the X-axis indicates the number of stimulated points.

concentration of CNQX (1 mM) did not affect the depressor responses induced by Glu in CVLM (SAP: -27.0 ± 10.1 %, VNA: -12.3 ± 9.6 %, n=5). A higher concentration of CNQX (5 mM, 50 nL) significantly potentiated the Glu-induced depressor responses only on SAP (-40.7 ± 4.4 %, $p<0.05$) but not affected the change of VNA (-17.3 ± 8.1 %, n=13) (Fig. 1D, Fig. 2). In two cases, the Glu-induced depressor responses were reduced by CNQX.

Effects of CNQX on the AMPA-Induced Pressor Responses in DM and RVLM

Specifically, the effects of non-NMDA receptors in DM and RVLM neurons were further

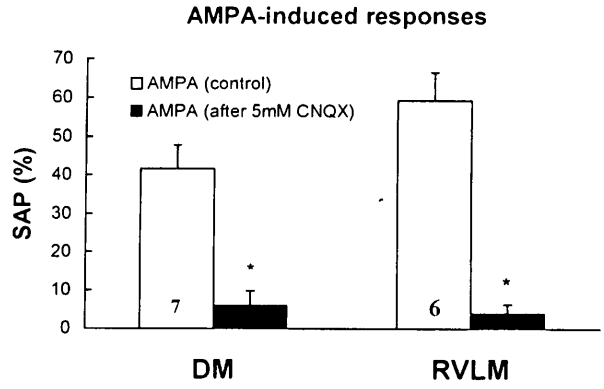

Fig. 3. Effects of CNQX on the pressor response induced by AMPA in the DM and RVLM.
The AMPA (5 mM, 30 nL) produced pressor responses in DM and RVLM. These AMPA-induced pressor response were significantly attenuated by CNQX (5 mM, 50 nL). Single asterisk (*) indicates p less than 0.05. The number shown on the X-axis indicates the number of stimulated points.

examined. Microinjection of AMPA (5 mM, 30 nL) into DM and RVLM produced pressor responses (Fig. 3), which were blocked by pretreatment with CNQX (DM: from 41.6 ± 6.1 % to 6.1 ± 3.8 %, n=7; RVLM: from 59.3 ± 7.1 % to 4.0 ± 2.4 %, n=6).

CNQX Itself Produced Pressor Responses

To our surprise, microinjection of CNQX (5 mM, 50 nL) into FTG, DM and RVLM produced an increase of SAP similar to that elicited by Glu (Fig. 1A, 1B and 1C). In CVLM, CNQX also increased the SAP but decreased the VNA (Fig. 1D). Table 1 summarizes the effects of microinjection of CNQX into these pressor areas or the depressor area. CNQX produced predominantly pressor responses, when it was applied into either the pressor or depressor areas (Table 1). To rule out that the CNQX-induced pressor effect was a result of Glu leakage from the multibarrel glass pipette, another group of experimentes was carried out by using four-barrel micropipetts, filling only with CNQX in different concentrations (0.1, 1, 5 mM) and with 3 M NaCl for electrical stimulation. Microinjection of CNQX in ascending concentrations (0.1,1 and 5 mM, 50 nL) into the DM produced dose-dependent increases of SAP and VNA (Fig. 4).

Discussion

Excitatory amino acid (EAA) receptors play an important role in central cardiovascular integration (5-7, 9, 26, 27). The present results show that administration of CNQX (5 mM) into the pressor areas of FTG, DM and RVLM reduces the Glu-induced increases in SAP and sympathetic VNA, while CNQX

Table 1. SAP and VNA Responses by CNQX (5mM, 50 nL Microinjected into FTG, DM, RVLM and CVLM

	CNQX (5 mM, 50 nL)		
	pressor	depressor	no response
FTG			
SAP (%)	23.0 ± 10.9		
VNA (%)	33.7 ± 13.9		
n/Total	6 / 9		3 / 9
DM			
SAP (%)	30.7 ± 5.9	-15.0 ± 5.0	
VNA (%)	25.9 ± 8.4	0.0 ± 0.0	
n/Total	13 / 24	4 / 24	7 / 24
RVLM			
SAP (%)	31.8 ± 7.7	-12.0 ± 2.5	
VNA (%)	12.2 ± 14.8	-10.5 ± 1.5	
n/Total	6 / 13	4 / 13	3 / 13
CVLM			
SAP (%)	23.3 ± 7.0	-15.3 ± 5.3	
VNA (%)	-10.3 ± 6.0	-10.7 ± 7.8	
n/Total	8 / 14	3 / 14	3 / 14

Note that microinjection of CNQX itself produced pressor responses in many stimulated points of the pressor areas, FTG, DM and RVLM, and the depressor CVLM. Except FTG, CNQX could produce depressor responses in some points of the above areas. Values are mean ± s.e.m., the percentage change (%) was analyzed by comparing to the basal level. n/Total indicates the number (n) among total stimulated points that produced such response.

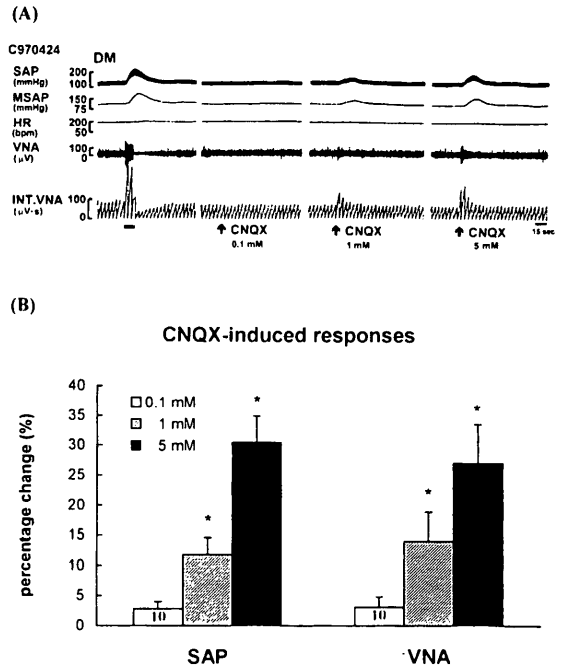

Fig. 4. Microinjection of CNQX alone into the DM produces a dose-dependent increase of SAP and VNA. (A) Stimulation at DM was accomplished by a multibarrel micropipette that contained different concentrations of CNQX only (no Glu). From left to right: electrical stimulation (50 μA, 15 sec), CNQX at a lower dose (0.1 mM, 50 nL), medial dose (1 mM, 50 nL) and higher dose (5 mM, 50 nL). Note that CNQX at a lower dose (0.1 mM, 50 nL) did not change the resting SAP. CNQX produced an increase of SAP in a dose-dependent manner after the concentration reached 1 mM. (B) Histograph shows cardiovascular effects of different CNQX concentrations (0.1, 1 and 5 mM, 50 nL) microinjected into the DM. The number shown on the X-axis indicates the number of stimulated points. * indicates $p<0.05$ (paired t test) compared with basal value.

administration into the depressor area of CVLM augments the Glu-induced decreases of SAP and VNA. Therefore, the phenomenon of an opposite effect of pressor and depressor non-NMDA receptor antagonism on cardiovascular regulation is clear. These opposite antagonistic actions of CNQX in the pressor and depressor areas are consistent with the finding in rats by Kobayashi *et al.* (20). Besides, the antagonized effect of CNQX is consistent with those of other non-NMDA antagonists GDEE (10) and 6,7-dinitro-quinoxaline-2,3-dione (DNQX) (13).

The CNQX, in a high concentration dose of 5 mM, significantly inhibited the Glu- induced pressor responses on FTG, DM and RVLM and potentiated the depressor responses on CVLM. Besides, CNQX itself could produce pressor responses on these Glu-induced pressor areas. In the present study, we used a concentration of CNQX in 5 mM, a midway to the concentration (1 and 10 mM) using on decerebrate cats by Nattie *et al.* (28). In such a dose range, they did not observe any toxic effect. Furthermore, in our study, these inhibition of Glu-induced responses were recovered 1.5 to 2 hours after CNQX injections.

Gamma-aminobutyric acid (GABA)-containing neurons in CVLM send inhibitory outflow to RVLM and tonically inhibit the neuron pacemaker characteristics (33, 34). Neurons of CVLM contain non-NMDA receptors (26). It is possible that blockade of non-NMDA receptors in CVLM alters the level of Glu, which in turn modulates the release of other neurotransmitters, like GABA, and thereby resulting in tonic inhibition. Blockade of non-NMDA receptors in the depressor CVLM leads to an increase of SAP and an decrease of VNA (shown in the middle panel of Fig. 1D). This observation indicates an endogenously excitatory inputs to the inhibitory CVLM neurons. The CNQX-induced pressor responses could be due to a disinhibition of RVLM neurons (24, 26). However, in this study, CNQX did not antagonize the Glu-induced depressor responses in CVLM. This finding implies that some CVLM sympathoinhibitory neurons are not tonically active and can only be

excited by exogenous Glu stimulation.

Unexpectedly, microinjection of CNQX into many pressor areas produced an immediate increase of SAP similar to that of Glu (FTG: 6/9, DM: 13/24; RVLM: 6/13). We also considered the possibility that CNQX is a partial agonist similar to Glu, but no information so far is available in this regard including those from the manufacturer RBI company. It is noteworthy that changes in VNA during the CNQX-induced pressor response were variable; VNA might increase, decrease or not change. Furthermore, during the pressor response induced by either Glu or CNQX, VNA and RNA reacted in an opposite direction (see Fig. 1A). This observation is consistent with our past study that different sympathetic nerves may be integrated by separate mechanisms (6, 31). The CNQX-induced immediate pressor responses may involve a complicated process of neurotransmitter interaction. One possibility is that CNQX may block the adjacent inhibitory interneurons which are tonically driven by endogenous Glu release. Unfortunately, no direct evidence has been reported in this regard. Our previous studies have demonstrated that microinjection of glycine, an inhibitory transmitter, to the pressor DM and RVLM increased the SAP. Thus, glycine may work through an inhibition of the inhibitory-interneurons located nearby the pressor neurons (37). It is worthwhile to mention, however, it does not exclude the possibility that neurons receiving synapses with the inhibitory pathway are originating far from the injection site. GABA is also an inhibitory neurotransmitter. In the rat spinal cord, CNQX can mediate through the non-NMDA receptors to block the inhibitory postsynaptic potential (IPSP) that is activated by glycinergic and GABAergic interneurons (40). However, whether CNQX producing pressor responses through a similar inhibition of inhibitory interneurons remains to be explored.

The pressor FTG, DM and RVLM, and the depressor CVLM are all sensitive to Glu to evoke changes of SAP. In these areas, neurons showing positive Glu-immunoreactivity may also show immunoreactivity to other chemical transmitters as well, i.e., norepinephrine (7), enkephalin, substance P, vasoactive intestinal peptide, somatostantin (18), neuropeptide Y (16), GABA (33) and serotonin (29), etc. These transmitters may interact with each other and also affect the non-NMDA receptors. As a result, besides Glu, CNQX may also interact with other neurotransmitters to affect cardiovascular responses. In fact, microinjection of CNQX in these pressor and depressor areas may increase, decrease or not alter the SAP and VNA. This suggests that complicated mechanisms mediating Glu receptors activation are colocalized in the same area responsible for cardiovascular integration. Thus, stimulation inputs in a brain area may involve many complicated connections and networks.

Taken together, CNQX attenuates the Glu-induced pressor responses of FTG, DM and RVLM but potentiates the depressor response of CVLM. This implies that CNQX antagonizes a pressor action within the pressor areas of FTG, DM and RVLM or within the depressor CVLM. From our results, AMPA did induce pressor responses in both RVLM and DM. Similar to Glu, both AMPA-induced and Glu-induced pressor responses were blocked by CNQX. Thus, non-NMDA receptors are the major type of receptors underlying the Glu-induced pressor responses. These findings also suggest that non-NMDA receptors may play an opposite modulation role within the pressor and depressor areas responsible for cardiovascular integration. Besides, based on the pressor effect induced by CNQX, it seems very likely that a depressor mechanism, probably inhibitory interneurons existing in the vicinity of pressor areas, is tonically activated by the endogenous Glu. Such depressor mechanism, which is mediated by non-NMDA receptors, could be blocked by CNQX.

Acknowledgments

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