Experimental Physiology

Comparative study of NMDA and AMPA/kainate receptors involved in cardiovascular inhibition produced by imidazoline-like drugs in anaesthetized rats

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The depressor mechanism of imidazoline-like drugs is believed to result from activation of I_1-imidazoline receptors (I_1R) and/or α_2-adrenoceptors within the central nervous system, which are associated with the glutamatergic system. The rostral ventrolateral medulla (RVLM) has been recognized as a specific target area that mediates the depressor action of imidazoline-like drugs. The objective of this study was to determine the comparative effects of blockade of the central glutamate receptor subtypes *N*-methyl-D-aspartate (NMDA) or α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA)/kainate on the cardiovascular actions of imidazoline-like drugs (clonidine and moxonidine) in anaesthetized rats. Intracerebroventricular (I.C.V.) injection of the NMDA receptor antagonist MK801 or the AMPA/kainate receptor antagonist 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) produced similar reductions in blood pressure (BP) and heart rate (HR) to those induced by I.C.V. injection of clonidine. Intracerebroventricular injection of the glutamate receptor antagonist kynurenic acid not only abolished clonidine-induced hypotension and bradycardia but converted the responses to a pressor action and tachycardia. Unilateral injection of MK801 or CNQX into RVLM significantly attenuated intra-RVLM clonidine-induced decreases in BP and HR. We also found that unilateral injection of a selective I_1R agonist, moxonidine, significantly decreased BP and HR, which were also attenuated to a similar extent by prior injection of MK801 or CNQX. In conclusion, these data show that blockade of central (RVLM) NMDA and AMPA/kainate receptors produces similar attenuation of the decrease in BP and HR induced by clonidine or moxonidine. It is suggested that both NMDA and AMPA/kainate receptors are involved in the cardiovascular inhibition produced by imidazoline-like drugs, which is probably at least partly dependent on an I_1R mechanism in the RVLM.

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Activation of I_1-imidazoline receptors (I_1R), α_2-adrenoceptors (α_2AR), or both in the brain is suggested to be a major mechanism responsible for the hypotension associated with imidazoline-like drugs (centrally acting drugs) such as clonidine and moxonidine (Ernsberger *et al.* 1990; Haxhiu *et al.* 1994; for review see Guyenet, 1997). It is well known that the rostral ventrolateral medulla (RVLM) is a key region regulating cardiovascular function and is involved in the depressor action of imidazoline-like drugs (Punnen *et al.* 1987; Drolet *et al.* 1990; Haxhiu *et al.* 1994). Interestingly, it has been demonstrated that the biological effects induced by activation of I_1R and α_2AR are closely related to glutamate release and the functional states of glutamate receptors (Tingley & Arneric, 1990; Milhaud *et al.* 2000). Furthermore, the hypotension resulting from systemic administration of clonidine is abolished by glutamate receptor blockade in spontaneously hypertensive rats (Jastrzebski *et al.* 1995). Therefore, there is a close relationship between the glutamatergic system and the centrally hypotensive mechanism within the brain.

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It is well known that there are two subtypes of ionotropic glutamate receptors, *N*-methyl-D-aspartate (NMDA) and α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA)/kainate receptors, which play an important role in regulating cardiovascular activity (for review see Dampney, 1994*a*,*b*; Chen *et al.* 1997; Sved *et al.* 2002). Previous studies, however, have focused on the interaction between NMDA receptors and imidazoline-like drugs. The evidence from our and other laboratories has demonstrated that central NMDA receptors contribute to the cardiovascular inhibition produced by imidazoline-like drugs (Tingley & Arneric, 1990; Jastrzebski *et al.* 1995; Zhang & Abdel-Rahman, 2002; Wang *et al.* 2003*b*, 2004). However, it is not clear whether another glutamate receptor subtype, the AMPA/kainate receptor, mediates the actions of imidazoline-like drugs and, if so, whether, compared with the NMDA receptor, the AMPA/kainate receptor is equally important in mediating the central depressor mechanism. It is reported that, compared with the NMDA receptor, the AMPA/kainate receptor in the RVLM may play a major role in control of cardiovascular function (Abrahams *et al.* 1994). Therefore, the main objective of this study was to observe the comparative mediation of the central NMDA and AMPA/kainate receptors, especially in the RVLM, in the cardiovascular effects produced by clonidine (a mixed agonist of I_1R and α_2AR). In order to further clarify the acting receptor mechanism responsible for this interaction, a selective I_1R agonist, moxonidine (Ernsberger *et al.* 1993; Haxhiu *et al.* 1994), was used in some experiments.

Methods

Animal preparation

Male Sprague–Dawley rats (weighing 260–350 g) were used, supplied by Sino-British SIPPR/BK Laboratory Animal Ltd. All animals received humane care in compliance with institutional animal care guidelines. Methods for general procedures, RVLM microinjection and histological procedures were similar to those previously described (Wang *et al.* 2003*b*, 2004).

Briefly, after induction of anaesthesia with pentobarbitone sodium (60 mg kg^{-1}, I.P., Sigma chemicals, St. Louis, MO, USA), catheters were inserted into the left femoral artery and the left femoral vein for recording blood pressure (BP) and for drug administration, respectively. Blood pressure was sequentially measured and displayed on one channel of a recording system (XW2004, SMMU, Shanghai China) by a computer, and heart rate (HR) was computed from the BP waveforms and displayed on another channel of the recording system. Blood pressure and HR were recorded continuously. Following tracheotomy, each rat was paralysed with gallamine triethiodide (10 mg kg^{-1} initially, I.V. supplemented with 4 mg kg^{-1} as needed, Sigma chemicals) and was mechanically ventilated with oxygen-enriched room air. Ventilation parameters were adjusted to maintain the arterial partial pressure of O_2 at approximately 100 mmHg and the arterial partial pressure of CO_2 below 40 mmHg. Urethane was injected intravenously to maintain surgical anaesthesia (1.1–1.3 g kg^{-1}, I.V., Sigma chemicals), and anaesthetics were supplemented when necessary. Depth of anaesthesia was gauged by the stability of BP and HR and the absence of a pressor response to paw pinch. Body temperature was maintained at about 37°C with a heating pad and an infrared lamp.

Intracerebroventricular (I.C.V.) and RVLM injections

The anaesthetized rat was fixed on a stereotaxic frame (Narishige, Scientific Instruments, Japan). The rats which received I.C.V. injections were subjected to a limited craniotomy. Intracerebroventricular injection was performed using a 5 μl syringe (Hamilton, Reno, NV, USA). The co-ordinates for lateral cerebral ventricles were determined from the rat atlas of Paxinos & Watson (1998) and were 1.0 mm lateral to the mid-line, 1.5 mm caudal to Bregma and 4.5 mm deep from the bone surface. The volume of drug injection was 5 μl, delivered over a period of approximately 30 s. At the end of each experiment, 5 μl of 2% Pontamine Sky Blue solution was injected into the lateral ventricle to verify the correct position of I.C.V. injection.

In the rats which received RVLM microinjection, the dorsal surface of the medulla was surgically exposed by occipital craniotomy and partial cerebellectomy. The dura was opened and retracted to expose the obex, the vertex of which was taken as a landmark for the stereotaxic co-ordinates. A multibarrel micropipette with tip diameter 20–50 μm for microinjection was inserted unilaterally into the RVLM using a micromanipulator (Narishige). The co-ordinates for RVLM were 2.3–2.8 mm rostral to the obex, 1.8–2.0 mm lateral to the mid-line and 2.8–3.2 mm below the dorsal surface of the medulla. Drugs were pressure injected through glass pipettes as previously described (Wang *et al.* 2003*b*). The injection was made over a period of 5–10 s, and the injection volume (100 nl) was carefully measured by observing the movement of the fluid meniscus along a reticule in a microscope. Accurate placement of the pipette and functional identification of the RVLM pressor area were accomplished by microinjection of L-glutamate (2 nmol). Microinjection of 100 nl of artificial cerebrospinal fluid (ACSF) served as the vehicle control. Finally, 100 nl of 2% Pontamine Sky Blue solution was injected into the RVLM to mark the site for subsequent histological identification.

Intracerebroventricular and RVLM injected drugs

All chemicals, including clonidine, moxonidine, L-glutamate, non-selective glutamate receptor antagonist

Table 1. Baseline values of MAP and HR in experimental groups

Groups	*n*	MAP (mmHg)	HR (beats min^{-1})
I.C.V. injections			
ACSF + ACSF	5	106 ± 6	394 ± 18
ACSF + clonidine	5	108 ± 4	414 ± 17
KYN + clonidine	9	110 ± 4	389 ± 18
MK801 + clonidine	10	113 ± 5	403 ± 19
CNQX + clonidine	8	112 ± 5	415 ± 16
RVLM microinjections			
ACSF + ACSF	5	102 ± 5	397 ± 16
ACSF + clonidine	5	109 ± 4	404 ± 15
MK801 + clonidine	10	104 ± 3	393 ± 17
CNQX + clonidine	8	108 ± 4	381 ± 13
ACSF + moxonidine	4	103 ± 5	369 ± 17
MK801 + moxonidine	6	98 ± 5	377 ± 16
CNQX + moxonidine	7	101 ± 4	410 ± 18

n is the number of the rats in each group. I.C.V., intracerebroventicular; RVLM, rostral ventrolateral medulla; ACSF, artificial cerebrospinal fluid; KYN, kynurenic acid.

kynurenic acid (KYN), NMDA receptor antagonist dizocilpine (MK801) and AMPA/kainate receptor antagonist 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), were obtained from Sigma. Clonidine, moxonidine, MK801 and L-glutamate were directly dissolved in ACSF (mM: 133.3 NaCl, 3.4 KCl, 1.3 $CaCl_2$, 1.2 $MgCl_2$, 0.6 NaH_2PO_4, 32.0 $NaHCO_3$ and 3.4 glucose, pH was adjusted to 7.4 with HCl or NaOH). Kynurenic acid and CNQX were initially dissolved in 0.1 M phosphate buffer solution and then diluted in ACSF to the final concentration. MK801 and CNQX were chosen as the antagonists for NMDA or AMPA/kainate receptors based on their specificity to block the respective receptor agonist (Wong *et al.* 1986; Honore *et al.* 1988).

Experimental protocols

Rats were divided into five groups ($n = 5$–10 rats each; Table 1) for I.C.V. injections and seven groups ($n = 5$–10 rats each; Table 1) for RVLM microinjections to investigate the effects of blockade of glutamate receptors, including the NMDA and AMPA/kainate receptor subtypes, on the haemodynamic responses to clonidine or moxonidine injected into the lateral ventricle or RVLM. In I.C.V. injection experiments, BP and HR responses to I.C.V.-injected clonidine (20 nmol) 10 min after pretreatment with ACSF (100nl), KYN (100 nmol), MK801 (50 nmol) or CNQX (10 nmol) were continuously monitored for at least 60 min. In the RVLM microinjection experiments, a transient pressor action in response to microinjection of glutamate (2 nmol) was used to chemically identify the RVLM pressor area. At least 30 min after L-glutamate microinjection, the values of BP and HR responses to intra-RVLM clonidine (5 nmol) or moxonidine (4 nmol) were continuously recorded (for at least 60 min) 10 min after intra-RVLM pretreatments with ACSF (100 nl), MK801 (500 pmol) or CNQX (200 pmol). The values of BP and HR at 10 min intervals after I.C.V. and intra-RVLM injection of clonidine or moxonidine were collected for further statistical analysis.

Histological analysis for I.C.V. and RVLM injections

At the end of the experiment, the animal was perfused transcardially with 0.9% NaCl and 10% formalin. The brainstem was removed, stored overnight in 10% phosphate-buffered formalin, and then transferred to fixative containing 30% sucrose. Frozen brain tissue was sectioned in the coronal plane (50 μm) and stained with Neutral Red. The correct position of I.C.V. injection was confirmed by the lateral ventricle being filled with blue dye. The centres for drug injections within the RVLM were reconstructed from the dye spots according to the atlas of Paxinos & Watson (1998).

Statistical analysis

Blood pressure was expressed as mean arterial pressure (MAP). All values are expressed as means ± S.E.M. The averaged value of MAP or HR was calculated every 10 min after injection of a test agent or vehicle. Statistical comparisons between different groups at corresponding time points were made by the repeated measures one- or two-way ANOVA followed by Student–Newman–Keuls *post hoc* test. These analyses were performed by software (SigmaStat 3.5). Differences were considered to be significant at $P < 0.05$.

Results

Effects of pretreatment with KYN, MK801 or CNQX on BP and HR responses to I.C.V. injection of clonidine

A total of 37 rats were used to determine whether I.C.V. injections of ACSF, KYN, MK801 or CNQX influence clonidine-induced cardiovascular effects. The baseline values for MAP and HR were similar for the different experimental and control groups (ANOVA; Table 1). Figure 1 shows original traces of the BP and HR responses to I.C.V.-injected clonidine 10 min after pretreatment with ACSF, KYN, MK801 or CNQX. Intracerebroventricular injection of clonidine (20 nmol) produced marked decreases in BP (-32 ± 4 mmHg, $P < 0.05$) and HR (-96 ± 19 beats min^{-1}, $P < 0.05$) compared with I.C.V. injection of ACSF (5 μl). The maximal hypotensive and bradycardic effects evoked by I.C.V. clonidine were reached within 30 min. The decreased

BP and HR induced by I.C.V. clonidine took more than 60 min to return completely to control levels. The reductions in BP evoked by I.C.V. injection of clonidine were significantly attenuated after I.C.V. pretreatment with MK801 (50 nmol) or CNQX (10 nmol) compared with pretreatment with I.C.V. vehicle (ACSF, 5 μl). Pretreatment with CNQX but not MK801 attenuated the clonidine-induced decrease in HR. Furthermore, the recovery time from the actions of clonidine was significantly shorter in groups pretreated with MK801 or CNQX than in the ACSF pretreatment group. It is worth noting that the magnitude of attenuation of the clonidine-induced depressor action between MK801 and CNQX groups was not significantly different. Interestingly, after pretreatment with KYN (100 nmol), the depressor and bradycardic responses to I.C.V. clonidine were not only abolished, but were reversed to pressor (22 ± 4 mmHg, $P < 0.05$) and tachycardic actions (55 ± 11 beats min^{-1}, $P < 0.05$). The time courses of changes in MAP and HR evoked by clonidine after pretreatment with ACSF, KYN, MK801 and CNQX are shown in Fig. 2. Notably, I.C.V. injections of KYN, MK801 or CNQX had no significant effect on baseline MAP and HR (Table 2).

Effects of pretreatment with MK801 and CNQX on BP and HR responses to RVLM microinjection of clonidine or moxonidine

Twenty-eight rats were used to determine the role of NMDA and AMPA/kainate receptors in the RVLM in mediating the effects of clonidine. The baseline values for MAP and HR for each group are shown in Table 1. Figure 3 shows original traces of BP and HR responses to clonidine microinjected unilaterally into the RVLM 10 min after pretreatment with ACSF, MK801 or CNQX. Unilateral injection of clonidine (5 nmol) into the RVLM following an equal volume (100 nl) of ACSF caused gradual reductions of BP and HR. Notably, the 10 min interval may not have permitted the maximal depressor effect to be reached because it required more than 60 min for the maximal response to

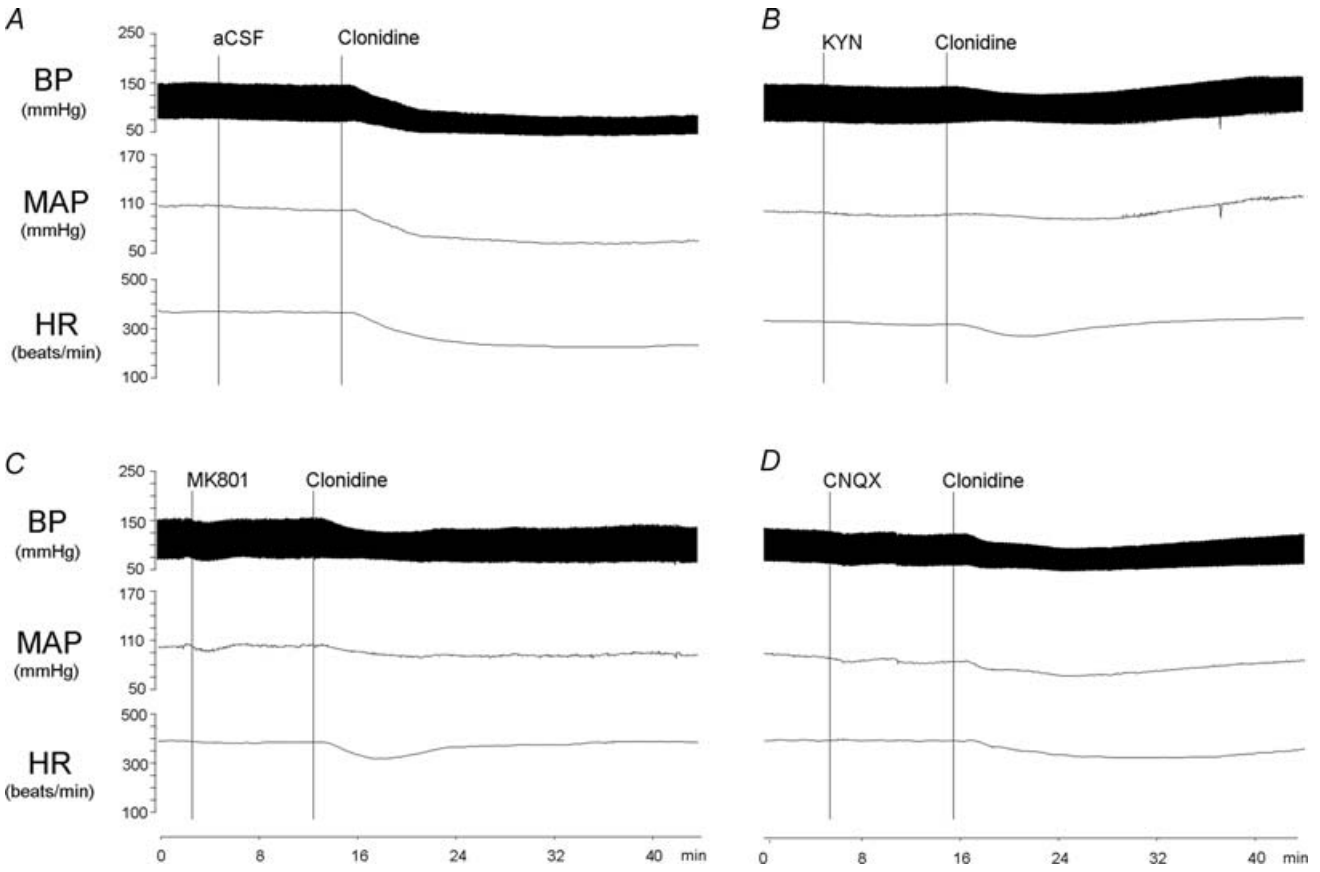

Figure 1 Effects of Intracerebroventricular injection of glutamate receptor antagonists on clonidine actions
Representative traces showing the responses of blood pressure (BP), mean arterial pressure (MAP) and heart rate (HR) to intracerebroventricular injections of clonidine (20 nmol) 10 min after pretreatment with ACSF (5 μl, *A*), KYN (100 nmol, *B*), MK801 (50 nmol, *C*) or CNQX (10 nmol, *D*).

Table 2. Effects of injections of kynurenic acid (KYN), MK801 and CNQX on MAP and HR

		MAP (mmHg)		HR (beats min^{-1})	
Groups	*n*	Pre	Post	Pre	Post
I.C.V. injections					
ACSF	5	106 ± 6	105 ± 4	394 ± 18	396 ± 19
KYN	9	110 ± 4	107 ± 5	389 ± 18	382 ± 17
MK801	10	113 ± 5	110 ± 5	403 ± 19	391 ± 16
CNQX	8	112 ± 5	109 ± 6	415 ± 16	401 ± 15
RVLM microinjections					
ACSF	5	102 ± 5	103 ± 5	397 ± 16	406 ± 17
MK801	16	101 ± 3	97 ± 4	385 ± 18	378 ± 16
CNQX	15	105 ± 4	101 ± 4	396 ± 13	372 ± 15

n is the number of the rats in each group. I.C.V., intracerebroventicular; RVLM, rostral ventrolateral medulla; ACSF, artificial cerebrospinal fluid.

occur after RVLM microinjection of clonidine. Compared with ACSF pretreatment, intra-RVLM injection of MK801 (500 pmol) or CNQX (200 pmol) resulted in a significant attenuation of the decreases in BP and HR elicited by intra-RVLM clonidine. The attenuation of the actions of clonidine in the presence of MK801 and CNQX was similar. The time course of effects of blockade of NMDA or AMPA/kainate receptors in the RVLM on MAP and HR responses elicited by intra-RVLM clonidine are shown in Fig. 4. As indicated in Fig. 4, in the CNQX or MK801 treatment group, the decreases in BP and HR returned almost to control levels about 30–40 min after intra-RVLM clonidine. Similar to I.C.V. injections, intra-RVLM MK801 or CNQX did not modify the resting values of BP and HR (Table 2).

In a further 17 rats (basal MAP and HR shown in Table 1), we studied the interaction between NMDA or AMPA/kainate receptors in the RVLM and moxonidine. Similar to intra-RVLM clonidine, unilateral injection of 4 nmol of moxonidine into the RVLM considerably reduced BP and HR compared with the vehicle group. The peak responses of MAP (−20 ± 3 mmHg) and HR (−45 ± 9 beats min^{-1}) occurred about 10–20 min after moxonidine injection, and the recovery time for BP and HR was about 50–60 min. Decreases in BP and HR induced by moxonidine were significantly attenuated by prior injection of MK801 or CNQX. We also observed that the

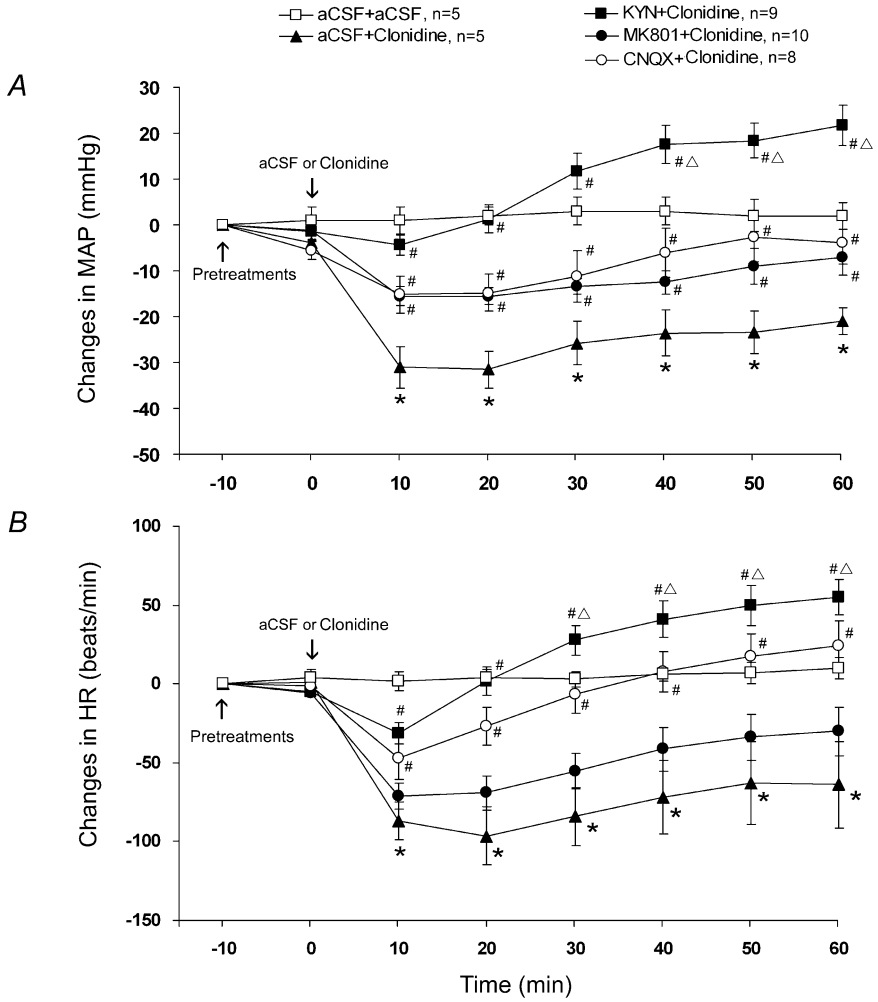

Figure 2. Changes in MAP (*A*) and HR (*B*) induced by intracerebroventricular injections of clonidine (20 nmol) following pretreatment with ACSF (5 μl), KYN (100 nmol), MK801 (50 nmol) or CNQX (10 nmol)
n is the number of rats in each group, * or $^{\Delta}P < 0.05$ *versus* ACSF + ACSF; #$P < 0.05$ *versus* ACSF + Clonidine at corresponding time points.

degree of attenuation of the decrease in BP and HR induced by intra-RVLM moxonidine was not significantly different between MK801 and CNQX pretreatment groups. Figure 5 presents the time course of effects of prior injection MK801 or CNQX on MAP and HR in response to intra-RVLM moxonidine.

Distribution of drug injections in oblongata medulla

The histological study performed in the I.C.V. injection experiments showed that the lateral ventricle was filled with blue dye, and cannula tunnel placement for I.C.V. injections was confirmed to be correct, suggesting correct placement for I.C.V. injections (data not shown). Figure 6 illustrates the distributions of the drug microinjection sites in medulla oblongata. All drug microinjection sites were located in the rostral medulla, just ventromedial to the compact portion of nucleus ambiguus, similar to the sites that we have published previously (Wang *et al.* 2003*b*).

Discussion

The present study used glutamate receptors and their subtype antagonists to provide evidence that the hypotension and bradycardia produced by central clonidine or moxonidine are mediated by glutamate receptors in the central nervous system. A new important finding from this work was that NMDA and AMPA/kainate receptors were of similar importance in mediating the central depressor mechanism of clonidine and moxonidine. Therefore, these data suggest that both NMDA receptors and AMPA/kainate receptors in the central nervous system, presumably in the RVLM, are involved in the centrally hypotensive action of imidazoline-like drugs, probably via an I_1R mechanism.

In order to detect the interaction between glutamate receptors and imidazoline-like drugs, KYN, MK801 and CNQX were chosen for their ability specifically to block glutamate, NMDA and AMPA/kainite receptors, respectively. In agreement with previous studies (Kiely &

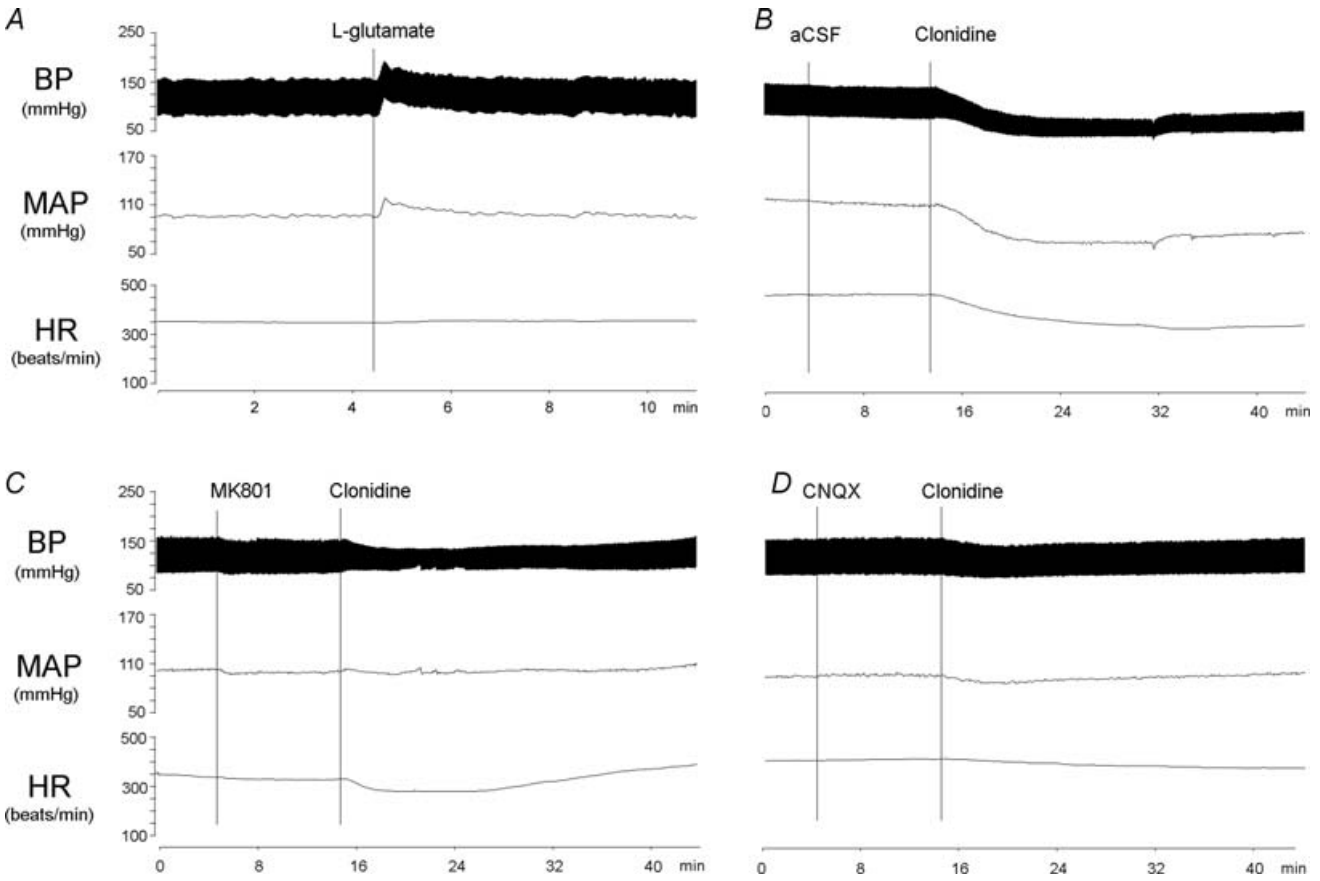

Figure 3. Representative traces showing the responses of BP, MAP and HR to microinjection of L-glutamate (*A*) and microinjection of clonidine (5 nmol) into the rostral ventrolateral medulla 10 min after pretreatment with ACSF (100 nl, *B*), MK801 (500 pmol, *C*) or CNQX (200 pmol, *D*)
The microinjection site was verified functionally by the pressor response to L-glutamate (2 nmol).

Gordon, 1994; for review see Sved *et al.* 2002), the data from this study also showed that centrally applied glutamate receptor antagonists failed to significantly affect the baseline BP and HR, suggesting that glutamate receptors, including NMDA and AMPA/kainate receptors, are not critically involved in tonic control of cardiovascular tone in normotensive rats. MK801 is a non-specific NMDA receptor antagonist, and at high doses it may affect the AMPA/kainate receptor. CNQX is a potent AMPA/kainate receptor antagonist, which also blocks the glycine modulatory site on the NMDA receptor complex. There might be a limitation that we did not further examine this cross-interaction. However, according to a previous study in rats (Kubo *et al.* 1993), injection of a similar dose of MK801 into the RVLM abolishes the pressor response evoked by NMDA (20 pmol) but does not affect that evoked by AMPA (3.7 pmol) or kainate (4.7 pmol). A similar dose of CNQX injected into the RVLM abolishes the pressor response evoked by AMPA and kainate but does not affected that of NMDA. Therefore, we believe that the doses of MK801 and CNQX used in this study can selectively and adequately block their corresponding receptors without affecting AMPA/kainate and NMDA receptors, respectively.

In this study, the first important finding was that NMDA or AMPA/kainate receptor blockade produced a very similar effect on the clonidine-induced decrease in BP and HR, suggesting that these two glutamate receptor subtypes may be equally important in mediation of the central depressor mechanism of imidazoline-like drugs. The interaction between the NMDA receptor and imidazoline-like drugs has been extensively demonstrated (Tingley & Arneric, 1990; Jastrzebski *et al.* 1995; Zhang & Abdel-Rahman, 2002). However, this is the first time that evidence has been obtained to suggest that, compared with the NMDA receptor, the AMPA/kainate receptor also plays an important role in mediating the central depressor action. We noted that the depressor action of intra-RVLM clonidine was slow to reach a maximum. Although the centres of injection sites were verified by histological analysis to locations within the RVLM, we excluded data if the centre of the injection site was outside the RVLM. Since the injection volume (100 nl) was somewhat larger than usual microinjection volume (50 nl), we did not confirm

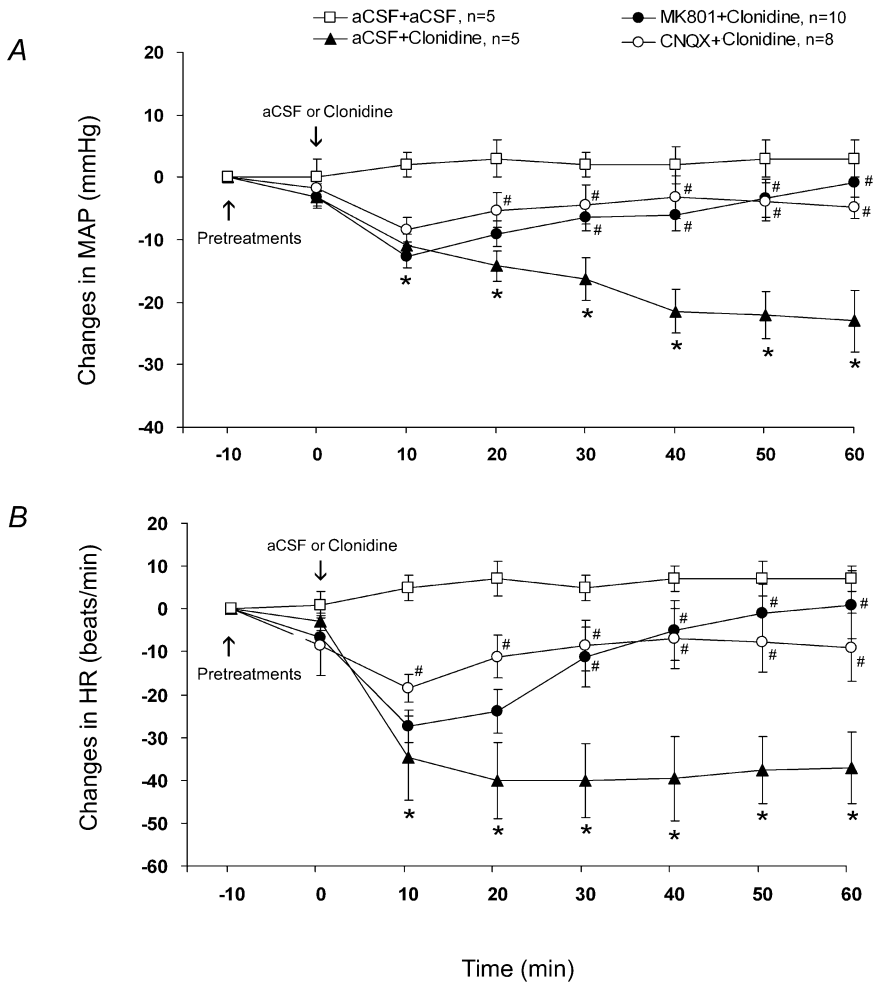

Figure 4. Changes in MAP (*A*) and HR (*B*) induced by microinjections of clonidine (5 nmol) following pretreatment with ACSF (100 nl), MK801 (500 pmol) or CNQX (200 pmol)
n is the number of rats in each group. **P* < 0.05 *versus* ACSF + ACSF; #*P* < 0.05 *versus* ACSF + clonidine at corresponding time points.

whether the area of spread of injected clonidine reaches the caudal ventrolateral medulla (CVLM). It is reported that injection of clonidine into the CVLM produces an excitatory effect on blood pressure and renal sympathetic nerve activity (Sesoko *et al.* 1998; Wang *et al.* 2003*a*). It is possible that the depressor action of intra-RVLM clonidine is buffered or slowed by the opposing effect in CVLM clonidine.

In I.C.V. or RVLM injection experiments, we found that the degree of attenuation of clonidine-induced decreases in BP was very similar after blockade of NMDA or AMPA/kainate receptors. It is notable that the glutamate receptor antagonist KYN not only had a greater effect on reducing I.C.V. clonidine-induced actions, but converted them to pressor and tachycardiac effects. Kynurenic acid is a non-selective glutamate receptor antagonist, which can block all ionotropic glutamate receptors, including NMDA and AMPA/kainate receptors, and this may explain why KYN produces a greater antagonism of the actions of clonidine than MK801 or CNQX alone. A single dose of antagonists was used in this work and, clearly, this may be considered a limitation. It is of importance that the dose-dependent effects of NMDA or AMPA/kainate receptor blockade on clonidine action are determined. The present data indicate that NMDA as well as AMPA/kainate receptors in the brain, especially in the RVLM, present a similar importance in modulation of the actions of clonidine. The exact mechanism(s) responsible for interaction between glutamate receptors and imidazoline-like drugs remain to be defined. It is reported that clonidine effectively stimulates the spontaneous release of the sympathoinhibitory transmitter γ-aminobutyric acid (GABA) in the RVLM, which is associated with enhancement of glutamate release (Perouansky & Grantyn, 1990). Stimulation of glutamate receptors increases GABA release and facilitates GABAergic synaptic activity (Jastrzebski *et al.* 1995). Increased GABA within the RVLM produces a significant sympathoinhibition

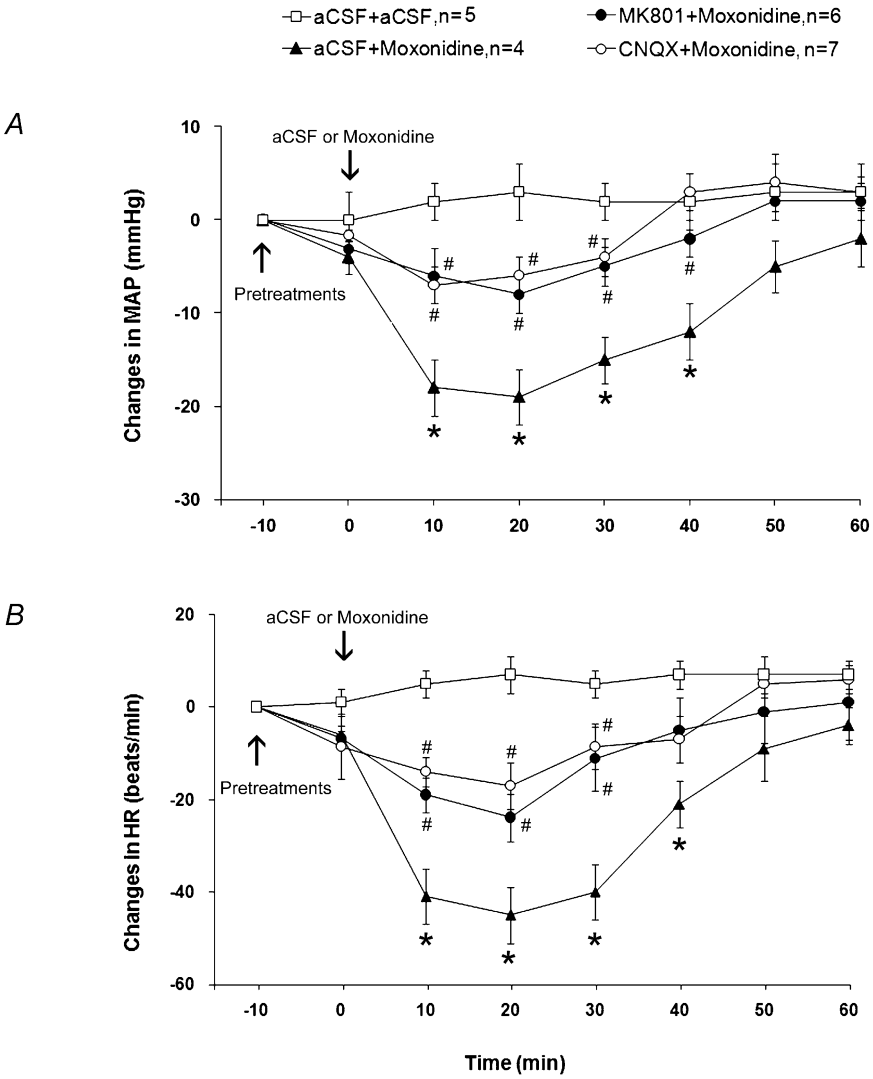

Figure 5. Changes in MAP (*A*) and HR (*B*) induced by microinjections of moxonidine (4 nmol) following pretreatment with ACSF (100 nl), MK801 (500 pmol) or CNQX (200 pmol)
n is the number of rats in each group, **P* < 0.05 *versus* ACSF + ACSF; #*P* < 0.05 *versus* ACSF + moxonidine at corresponding time points.

and hypotension (for reviews see Kubo & Kihara, 1987; Dampney, 1994*a*,*b*). However, the exact mechanism of clonidine-induced GABA release associated with glutamate release that underlies the interaction between clonidine and glutamate receptors requires further study.

Another important finding of the present study was that the effects of injection of moxonidine into the RVLM were significantly attenuated to the same extent by prior pretreatment with MK801 or CNQX. Since moxonidine is a selective I_1R agonist (Ernsberger *et al.* 1993; Haxhiu *et al.* 1994), these data strongly suggest that I_1R plays an important role in mediating the interaction between NMDA or AMPA/kainate receptors and central hypotensive mechanisms, which is consistent with the previous study (Zhang & Abdel-Rahman, 2002), although some studies suggest that, compared with α_2AR, the RVLM I_1R may play a predominant role in mediating the effects of imidazoline-like drugs (Ernsberger *et al.* 1990; Reis, 1996). In fact, contribution of I_1R, α_2AR, or both to centrally hypotensive mechanisms is a continuing and yet unresolved debate. In addition to I_1R mechanisms, however, it is unclear whether α_2AR is involved in the interaction between clonidine and glutamate receptors (for review see Guyenet, 1997). It has been reported that the effect of clonidine on the release of glutamate and GABA is prevented by the selective α_2AR antagonist yohimbine (Jastrzebski *et al.* 1995). Therefore, we believe that further experiments using specific antagonists for I_1R or α_2AR are warranted to characterize which receptor is responsible for mediating the interaction.

In summary, the present results showed that blockade of the different glutamate receptor subtypes produced a similar attenuation of clonidine-induced cardiovascular effects. We also demonstrated that the effects of moxonidine injected into the RVLM were reduced to the same degree by blockade of NMDA or AMPA/kainate receptors. Based on the findings of the present study, we conclude that NMDA receptors as well as AMPA/kainate receptors in the RVLM are of similar importance in mediating the effects of imidazoline-like drugs, which probably is, at least partly, dependent on an I_1R mechanism.

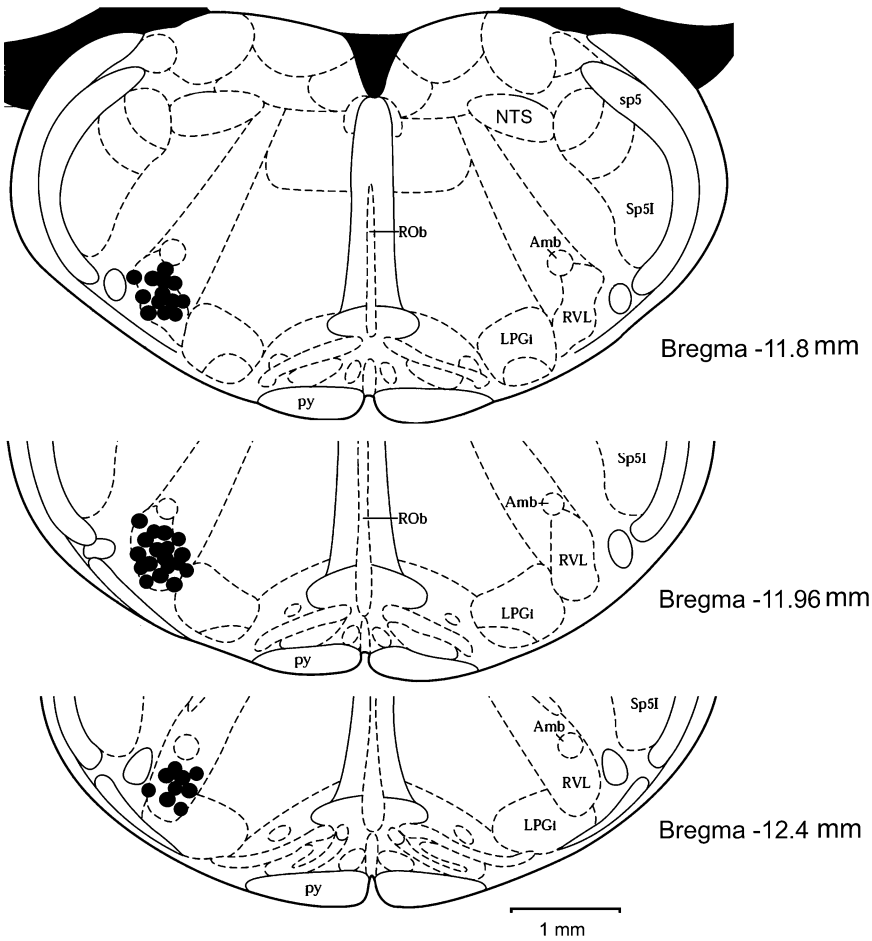

Figure 6. Distribution of microinjection sites (●) plotted on coronal sections
Amb, nucleus ambiguus; LPGi, lateral paragigantocelluar nucleus; ROb, raphe obscurus nucleus; RVL, rostral ventrolateral medulla; py, pyramidal tract; NTS, nucleus tractus solitarius; Sp5, spinal trigeminal tract; Sp5I, spinal trigeminal nucleus, interpolar part.

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