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Respiratory effects of halothane and AMPA receptor antagonist synergy in rats

Yoshihiro Shimazu, Kazuo Umemura *, Ken-ichi Kawano, Kazuya Hokamura, Hiroshi Kawazura, Mitsuyoshi Nakashima

Department of Pharmacology, School of Medicine, Hamamatsu University, 3600 Handa-cho, Hamamatsu 431-31, Japan

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Abstract

The influence of *N*-methyl-D-aspartate (NMDA) or α-amino-3-hydroxy-5-methylisoxazole-4-propionate (AMPA) receptor antagonists in combination with halothane anaesthesia on the respiratory system was investigated. Under 1.5% halothane anaesthesia, respiratory parameters including respiratory rate, minute volume, tidal volume, inspiratory and expiratory duration were measured before and after drug administration in rats. The AMPA receptor antagonists, 6-(1H-imidazol-1-yl)-7-nitro-2,3-(1H,4H)-quinoxalinedione hydrochloride, YM90K (5 and 10 mg/kg) and 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo(F)quinoxaline (NBQX, 15 mg/kg), which were administered intravenously for 30 min, significantly reduced the respiratory rate ($P < 0.01$) and minute volume ($P < 0.01$) and increased the tidal volume ($P < 0.05$) compared with values obtained before drug administration. None of these drugs affected respiratory parameters in the absence of anaesthesia. A NMDA receptor antagonist, MK-801 (0.5 mg/kg), which was administered intravenously for 30 min, also significantly reduced respiratory rate ($P < 0.01$), minute volume ($P < 0.01$) and tidal volume ($P < 0.01$) and prolonged inspiratory duration ($P < 0.05$). These results suggest that both AMPA and NMDA receptor antagonists cause respiratory depression under halothane anaesthesia in rats, although the mechanisms may be different for the two types of antagonists. © 1998 Elsevier Science B.V.

Keywords: Respiration; NMDA receptor antagonist; AMPA receptor antagonist; Halothane; Respiratory rate

1. Introduction

Recent studies indicate that ischaemia-induced neuronal damage may be linked to the toxicity exhibited by excitatory extracellular amino acids, particularly glutamate (Choi, 1988; Meldrum and Garthwaite, 1990). Consequently, glutamate receptor antagonists are good candidates for clinical study as stroke treatment compounds (Sheardown et al., 1990; Gill et al., 1991, 1992). Two functional subtypes of ionotrophic glutamate receptors in the central nervous system have been classified according to their preferred ligands, namely *N*-methyl-D-aspartate (NMDA) and α-amino-3-hydroxy-5-methylisoxazole-4-propionate (AMPA). Both NMDA and AMPA receptor antagonists have been reported to be neuroprotective in various models of experimental ischaemia. YM90K (6-(1H-imidazol-1-yl)-7-nitro-2,3-(1H,4H)-quinoxalinedione hydrochloride), a selective and potent AMPA receptor antagonist, has also been reported to be effective in rat and cat focal ischaemia models (Shimizu-Sasamata et al., 1996; Yatsugi et al., 1996). However, adverse effects of these antagonists may involve the respiratory system, since the generation of respiratory rhythm and its transmission to respiratory motoneurons involve a glutamate-like neurotransmitter acting on AMPA and NMDA receptors (Foutz et al., 1988, 1994; Liu et al., 1990; Greer et al., 1991; Pierrefiche et al., 1991, 1994; Abrahams et al., 1993; McManigle et al., 1994).

The most commonly used clinically and experimentally general anaesthetics, halothane, isoflurane, ether and ketamine, act on the NMDA and AMPA receptor complex in vitro (Carlá and Moroni, 1992). As a consequence, interactions with NMDA receptor antagonists and anaesthetics on the respiratory system may occur (Cassus-Soulanis et al., 1995). However, few investigations of the interaction between AMPA receptor antagonists and anaesthetics, especially inhalation anaesthetics, have been carried out. Therefore, the interactions between the AMPA receptor

* Corresponding author. Tel.: +81-53-4352271; fax: +81-53-4352270; e-mail: umemura@hama-med.ac.jp

Table 1
Respiratory and physiological parameters before administration of YM90K, NBQX or MK-801 to rats under halothane anaesthesia

Treatment (mg/kg)	n	RR	V_E	V_t	T_i	T_e	MABP	HR	Arterial blood gas		
									pH	PaO_2 (mmHg)	$PaCO_2$ (mmHg)
Saline	6	93 ± 9	167 ± 6	1.9 ± 0.2	234 ± 7	442 ± 58	92 ± 6	410 ± 18	7.35 ± 0.01	146 ± 1	40 ± 2
YM90K (5)	7	94 ± 6	167 ± 11	1.8 ± 0.1	242 ± 12	432 ± 32	89 ± 3	425 ± 12	7.37 ± 0.01	150 ± 3	40 ± 2
YM90K (10)	7	95 ± 7	155 ± 6	1.7 ± 0.1	264 ± 10	398 ± 46	94 ± 3	410 ± 14	7.40 ± 0.01*	150 ± 1	40 ± 2
NBQX (15)	6	98 ± 3	161 ± 4	1.6 ± 0.1	262 ± 14	361 ± 21	89 ± 3	385 ± 9	7.37 ± 0.01	147 ± 1	38 ± 1
MK801 (0.5)	6	94 ± 8	169 ± 9	1.8 ± 0.1	254 ± 10	413 ± 45	91 ± 4	408 ± 17	7.35 ± 0.01	145 ± 2	42 ± 2

Data represent means ± S.E.M. Values were obtained from 6–7 animals.
RR: respiratory rate (strokes/min), V_t: tidal volume (ml), V_E: respiratory minute volume (ml/min), T_i: inspiratory duration (ms), T_e: expiratory duration (ms), MABP: mean arterial blood pressure (mmHg), HR: heart rate (strokes/min).
* $P < 0.05$ versus saline control group.

antagonists, YM90K and NBQX (2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo(F)quinoxaline), the NMDA receptor antagonist MK-801 and halothane anaesthesia on the rat respiratory system were investigated.

2. Materials and methods

2.1. Animal preparation

Male Wistar rats (SLC, Shizuoka, Japan) weighing 180–230 g were used. The body temperature of the animals was maintained at 37.5°C with a heating-pad (K-module Model K-20, American Pharmaseal Company, Valencia, CA). Rats were anaesthetized with 1.5% halothane, 30% oxygen and 70% room air. The rats were not machine ventilated. The trachea was cannulated and fitted to the respiratory flow head (MLTF10L, ADInstruments Pty, NSW, Australia) connected to a spirometer (ML140, ADInstruments Pty, NSW, Australia). Respiratory rate, tidal volume, minute volume, inspiratory duration and expiratory duration were measured, using the MacLab/8s (ML780, ADInstruments Pty, NSW, Australia), before and 30 min after drug administration. A catheter for the administration of agents was placed in the femoral vein.

YM90K (5 and 10 mg/kg), NBQX (15 mg/kg) and MK-801 (0.5 mg/kg), which were dissolved in 0.9% NaCl (pH 8.5), were each administered by continuous infusion

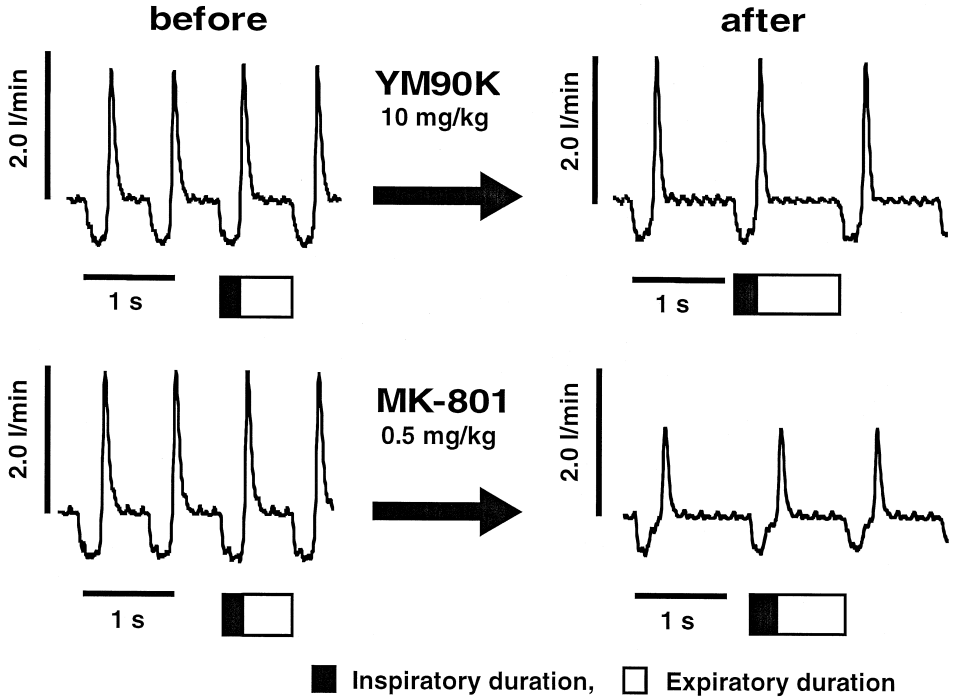

Fig. 1. Effects of YM90K (10 mg/kg) and MK-801 (0.5 mg/kg), which were administered intravenously for 30 min, on respiration under 1.5% halothane anaesthesia.

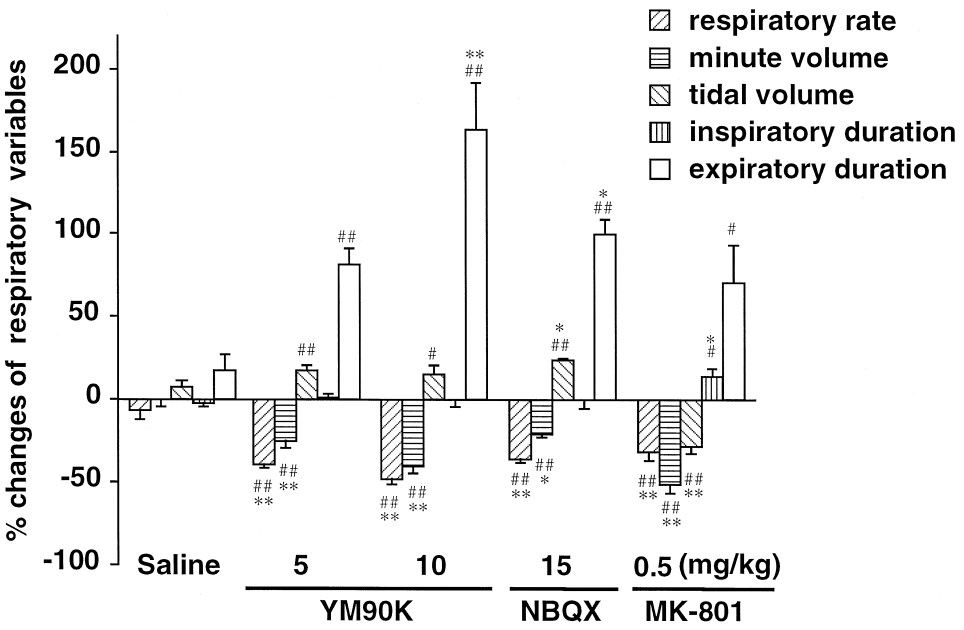

Fig. 2. The percent changes in respiratory parameters elicited by administration of YM90K, NBQX and MK-801 in rats under halothane anaesthesia. Data represent mean ± S.E.M. values for 6–7 animals. $^{*}P < 0.05$, $^{**}P < 0.01$ versus control group. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ versus before administration.

to 6–7 animals for 30 min. For the control group, saline was administered in the same manner to 6 animals.

In a separate experiment, the respiratory parameters of conscious rats were measured with a modified method described by Amis and Kurpershoek (1986). A catheter for drug administration was placed in the femoral vein under halothane anaesthesia. Wounds were infiltrated with 1% lidocaine and sutured. Rats were attached to a face-mask with the flow head connected to the spirometer and breathed room air. After conscious rats were acclimated to the face-mask, respiratory parameters were measured during the quiet waking state. YM90K (10 mg/kg), NBQX (15 mg/kg), MK-801 (0.5 mg/kg) or saline was administered intravenously to 5 animals for 30 min.

2.2. Drugs

YM90K was a gift from Yamanouchi Pharmaceutical Co. (Ibaraki, Japan). Halothane was purchased from Hoechst Japan (Tokyo, Japan), and NBQX and MK-801 were from Research Biochemicals (Natick, MA).

2.3. Statistical analysis

Data are presented as mean ± S.E.M. values. Data were analysed by a paired Student's *t*-test for comparisons between groups and by a one-way analysis of variance (ANOVA) followed by Dunnett's test for comparisons of more than three groups. A $P < 0.05$ value was considered significant.

Table 2
Respiratory parameters following administration of YM90K, NBQX or MK-801 to conscious rats

Treatment (mg/kg)	Before administration					After administration				
	RR	V_E	V_t	T_i	T_e	RR	V_E	V_t	T_i	T_e
Saline	110 ± 2	197 ± 13	1.8 ± 0.1	250 ± 11	303 ± 11	117 ± 5	205 ± 10	1.9 ± 0.1	259 ± 11	288 ± 13
YM90K (10)	108 ± 4	196 ± 5	1.8 ± 0.1	264 ± 10	298 ± 14	107 ± 5	198 ± 14	1.9 ± 0.1	275 ± 13	303 ± 18
NBQX (15)	110 ± 3	200 ± 14	1.8 ± 0.1	268 ± 9	284 ± 21	113 ± 3	201 ± 13	1.8 ± 0.1	270 ± 4	284 ± 9
MK801 (0.5)	105 ± 3	194 ± 14	1.9 ± 0.1	278 ± 8	304 ± 45	204 ± 26[a,d]	298 ± 19[b,d]	1.6 ± 0.1	174 ± 22[a,d]	148 ± 20[b,d]

Data represent means ± S.E.M. Values were obtained from 5 animals.
RR: respiratory rate (strokes/min), V_t: tidal volume (ml), V_E: respiratory minute volume (ml/min), T_i: inspiratory duration (ms), T_e: expiratory duration (ms).
[a] $P < 0.05$.
[b] $P < 0.01$ versus before administration.
[c] $P < 0.05$.
[d] $P < 0.01$ versus saline control group.

3. Results

Physiological parameters before YM90K, NBQX, MK-801 or saline administration were within the normal range (Table 1). A spirometer tracing is shown in Fig. 1. The percent changes in respiratory parameters due to YM90K, NBQX and MK-801 are shown in Fig. 2. Halothane anaesthesia alone slightly decreased respiratory rate and minute volume compared with those of conscious rats (Table 2). These values were not changed during 30 min halothane anaesthesia (Fig. 2).

YM90K, NBQX and MK-801 significantly ($P < 0.01$) reduced the respiratory rate due to prolongation of expiratory duration, compared with the respiratory rate before drug administration. MK-801 significantly ($P < 0.01$) reduced tidal volume and YM90K and NBQX significantly ($P < 0.05$) increased it. MK-801 significantly ($P < 0.05$) prolonged inspiratory duration, but YM90K and NBQX did not prolong it. All three drugs significantly ($P < 0.01$) reduced minute volume.

In conscious rats, arterial blood gas (pH, $PaCO_2$,PaO_2), heart rate and mean arterial blood pressure were not changed during the 1 h experimental period (data not shown). In conscious rats, YM90K and NBQX did not affect respiratory parameters. MK-801 affected respiratory parameters because of its distinct behavioural effects, such as sniffing and swaying, but did not depress them (Table 2).

4. Discussion

In this study, we set out to find if a synergy between NMDA or AMPA receptor antagonists and halothane anaesthesia existed. The doses of YM90K, NBQX and MK-801 used in this study were chosen because of their neuroprotective action in rats (Swan and Meldrum, 1990; Gill et al., 1992; Shimizu-Sasamata et al., 1996). YM90K and NBQX did not affect respiratory parameters during consciousness, nor were respiratory parameters suppressed by 1.5% halothane anaesthesia alone. However, a combination of halothane anaesthesia and AMPA receptor antagonists, which were used at the same doses as in conscious rats, suppressed respiratory rate and decreased respiratory minute volume. It has been reported that halothane inhibits AMPA-induced depolarization in mice cortical wedges (Carlá and Moroni, 1992). This suggests that halothane may act by modulating AMPA receptors. Based on our observations and others reports, halothane and AMPA receptor antagonists have a synergistic effect on respiratory rate.

Precise data concerning the respiratory effects of MK-801 in conscious rats could not be obtained in our study. This is because MK-801 has a convulsant action, causing phencyclidine-like behaviour in rats (Koek et al., 1988). However, MK-801 did not depress respiratory parameters in conscious rats. It has been reported that NMDA receptor antagonists impair respiratory activity (Foutz et al., 1988, 1994; Greer et al., 1991; Gill et al., 1992; Abrahams et al., 1993) and that combined administration of NMDA and AMPA receptor antagonists induces a lethal respiratory dysfunction (Foutz et al., 1994; McManigle et al., 1994). These findings suggest that a combination of a NMDA receptor antagonist and halothane anaesthesia suppresses the respiratory system.

Under halothane anaesthesia, the effects of AMPA and NMDA receptor antagonists on inspiratory duration and tidal volume were different. MK-801 prolonged inspiratory duration and reduced tidal volume, while YM90K and NBQX increased tidal volume but did not prolong inspiratory duration. These findings support evidence that activation of NMDA receptors, but not AMPA receptors, is a necessary step in the neural process in the brainstem that promotes the switch from inhalation to exhalation in the cat (Foutz et al., 1988, 1994; Monteau et al., 1990). The increase in tidal volume elicited by AMPA receptor antagonists may compensate for the reduction of respiratory rate and respiratory minute volume, while the NMDA receptor antagonist may inhibit this compensation.

In conclusion, the respiratory effects of halothane and AMPA or NMDA receptor antagonists are synergistic in rats, although the mechanisms of respiratory suppression appear to be different for NMDA and AMPA receptor antagonists. Further, AMPA receptors may play a key role in the generation of respiratory rhythm. Most importantly, however, are the clinical implications. Our findings suggest that a combination of AMPA or NMDA receptor antagonists and halothane may lead to serious and possibly fatal effects in humans. Therefore, until more is known about the normal function of these receptors, caution should be exercised in the clinical use of their antagonists.

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