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# Delayed Treatment with YM90K, an AMPA Receptor Antagonist, Protects against Ischaemic Damage after Middle Cerebral Artery Occlusion in Rats

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**Abstract**

The neuroprotective effect of an α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) receptor antagonist YM90K [6-(1*H*-imidazol-1-yl)-7-nitro-2,3(1*H*,4*H*)-quinoxalinedione monohydrochloride] has been examined in a rat middle cerebral artery occlusion model.

Intravenous infusion of YM90K (2·5–20 mg kg$^{-1}$ h$^{-1}$ for 4 h) starting immediately after occlusion of the middle cerebral artery significantly reduced the cortical infarct volume 24 h after occlusion compared with the control group. The protection at the highest dose was 39% ($P < 0·05$). Similar protective effects were observed when YM90K (20 mg kg$^{-1}$ h$^{-1}$ for 4 h) was delayed up to 2 h after middle cerebral artery occlusion (45% reduction, $P < 0·05$). CNS1102 [*N*-(1-naphthyl)-*N'*-(3-ethylphenyl)-*N'*-methylguanidine hydrochloride], a non-competitive *N*-methyl-D-aspartate (NMDA) receptor antagonist, also reduced the cortical infarct volume when 1·13 mg kg$^{-1}$ was administered by intravenous bolus injection immediately after middle cerebral artery occlusion, followed by intravenous infusion at 0·785 mg kg$^{-1}$ h$^{-1}$ for 4 h (35% reduction, $P < 0·05$). This neuroprotective effect was not observed when administration was delayed 1 h after middle cerebral artery occlusion.

These results suggest that AMPA receptors might play a more important role than NMDA receptors in the late development of neuronal cell damage after focal cerebral ischaemia and that AMPA receptor blockade would be one beneficial strategy in treating acute stroke.

Excessive increase of glutamate in the synaptic cleft is an important factor in post-ischaemic development of neuronal damage (Benveniste et al 1984). There is much evidence demonstrating the neuroprotective effects of *N*-methyl-D-aspartate (NMDA)-type glutamate receptor antagonists in experimental ischaemia models (Simon et al 1984; Boast et al 1988; Park et al 1988; Minematsu et al 1993a, b; Sauer et al 1995). Recently, the involvement of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA)-type glutamate receptors in the development of post-ischaemic neuronal damage has also been demonstrated (Gill 1994). The neuroprotective effects of AMPA receptor antagonists such as 2,3-dihydroxy-6-nitro-7-sulphamoylbenzo(*F*)quinoxaline (NBQX) or 1-(aminophenyl)-4-methyl-7,8-methylenedioxy-5*H*-2,3-benzodiazepine (GYKI52466) have been shown in many experimental models of cerebral ischaemia (Sheardown et al 1990; Gill et al 1992; Xue et al 1994; Graham et al 1996). These compounds are promising candidates as therapeutic agents, because they are effective when administered during the post-ischaemic period. They also lack the psychotomimetic action and neurotoxicity (Izumisawa et al 1995) of some NMDA receptor antagonists (Koek et al 1988; Olney et al 1991). Previous studies have also demonstrated that 6-(1*H*-imidazol-1-yl)-7-nitro-2,3(1*H*,4*H*)-quinoxalinedione monohydrochloride (YM90K), an AMPA receptor antagonist (Ohmori et al 1994), has neuroprotective effects in models of both transient global

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cerebral ischaemia (Shimizu-Sasamata et al 1996; Kawasaki-Yatsugi et al 1997) and focal cerebral ischaemia (Shimizu-Sasamata et al 1996; Yatsugi et al 1996; Yao et al 1997). The binding affinity of YM90K for AMPA receptors is similar to that of NBQX. Also, YM90K suppresses audiogenic seizures in DBA/2 mice almost as effectively as NBQX when administered intracerebroventricularly. However, the anti-convulsive effect of YM90K was three times more potent than that of NBQX when given by systemic administration (Shimizu-Sasamata et al 1996). The neuroprotective effect of YM90K on delayed neuronal death induced by transient global cerebral ischaemia in gerbils was about twice as potent than that of NBQX when administered systemically (Kawasaki-Yatsugi et al 1997). In this model, therapeutic time-windows for YM90K and NBQX were 6 and 1 h, respectively. Although about 83% of gerbils treated intraperitoneally with 60 mg kg$^{-1}$ NBQX died, only 17% of gerbils died when treated with the same dose of YM90K. The serious toxicity of NBQX was probably because of its nephrotoxicity (Xue et al 1994). These in-vivo profiles suggest that YM90K might be more suitable for clinical use than NBQX, although their in-vitro profiles resemble each other very closely.

In this study, to examine the usefulness of YM90K as a therapeutic agent for acute stroke in man, the dose-response and post-treatment efficacy of YM90K were investigated in a rat middle cerebral artery occlusion model. In addition, the therapeutic time-window of efficacy was compared with that of *N*-(1-naphthyl)-*N'*-(3-ethylphenyl)-*N'*-methylguanidine hydrochloride (CNS1102), a selective non-competitive NMDA receptor antagonist (Reddy et al 1994). Because this compound is neuroprotective in focal cerebral ischaemia models (Minematsu et al 1993a, b; Aronowski et al 1994; Cohen et al 1994; Meadows et al 1994), and is the only non-competitive NMDA receptor antagonist for which neuroprotective effect in stroke in man has been demonstrated (Knapp et al 1997), comparing the neuroprotective profiles, especially the therapeutic time-windows of efficacy, of YM90K and CNS1102 is important for estimation of the possible usefulness of YM90K as a therapeutic agent for acute stroke.

## Materials and Methods

*Induction of focal ischaemia*

Experiments were performed on male Fischer 344 rats, 280–350 g (Charles River Japan, Yokohama, Japan). Under sodium pentobarbital anaesthesia (50 mg kg$^{-1}$, i.p.) the left external jugular vein was cannulated to enable continuous infusion. Two days later the proximal portion of the left middle cerebral artery was permanently occluded as described by Tamura et al (1981). Briefly, under 2% halothane anaesthesia the temporal muscle was simply retracted via a *trans*-retro-orbital approach without removal of the temporal muscle and zygomatic arch, and a left subtemporal craniectomy was performed. The dura was incised with a fine needle and the stem of the middle cerebral artery was electrocauterized just medial to the olfactory tract and then cut to ensure the completeness of the occlusion. After surgery anaesthesia was turned off. During surgery and drug administration rectal temperature was maintained at 37 ± 1°C by use of a heating blanket and overhead lamp.

In a separate experiment, the influence of YM90K and CNS1102 on brain temperature and other physiological parameters was examined under similar experimental conditions (n = 4–5 in each group). A polyethylene tube (PE-50) was inserted into the tail artery before middle cerebral artery occlusion to monitor blood pressure and to obtain blood samples. Brain temperature was monitored with a needle-type thermoprobe (BAT-12, Physitemp Instrument, Clifton, NJ) inserted into the left striatum (anterior: 0·0 mm, lateral: 2·5 mm from bregma, depth: 3·5 mm from skull surface) through a guide cannula implanted stereotaxically 3–5 days before ischaemia.

All procedures were in accordance with the guidelines of the Animal Ethical Committee of Yamanouchi Pharmaceutical Co. Ltd.

*Drug administration*

YM90K and CNS1102 were synthesized in our laboratory. YM90K and CNS1102 were dissolved in saline made alkaline with a few drops of 1 N NaOH solution. These solutions were then adjusted to pH 8·5 and 7·4, respectively, with 1 N HCl. In each respective control group the same volume of saline at the same pH was administered using the same infusion speed.

In a dose–response study, YM90K was administered at doses of 1, 2·5, 5, 10 or 20 mg/60 mL kg$^{-1}$ h$^{-1}$ (n = 8–12 per group) over 4 h by intravenous infusion immediately after middle cerebral artery occlusion. In a further study to determine the time-window of efficacy YM90K was administered at a dose of 20 mg kg$^{-1}$ h$^{-1}$ over 4 h by intravenous infusion beginning 1, 2 or 4 h after middle cerebral artery occlusion (n = 8–10 per group). CNS1102 was administered by intravenous bolus injection at 1·13 mg mL$^{-1}$ kg$^{-1}$, followed by intravenous infusion at 0·785 mg/3 mL kg$^{-1}$ h$^{-1}$ for 4 h, starting either

immediately or 1 h after middle cerebral artery occlusion (n = 8–10 per group).

*Histological evaluation*

The rats were anaesthetized with sodium pentobarbital (50 mg kg$^{-1}$, i.p.) 24 h after middle cerebral artery occlusion and their brains were fixed by transcardiac perfusion with 10% formalin neutral buffered solution. The animals were decapitated and the heads left in 10% formalin neutral buffered solution for 24 h. After this the brains were removed. The fixed brain was embedded in paraffin and eight 5-$\mu$m coronal slices were cut and stained with haematoxylin and eosin. Regions of cerebral damage in each slice were delineated under light microscopy by an observer unaware of the drug treatment for each rat. The areas of striatal and cortical damage were quantitatively assessed using an image analyser system (Luzex, Nireco, Tokyo, Japan). The volume of ischaemic damage was calculated by integrating these areas using the distance between each stereotaxic level according to the co-ordinates of König & Klippel (1963). The endpoints for integration were anterior 10·5 mm to anterior 0·35 mm from the interaural line.

*Statistical analysis*

Data are means ± s.e.m. Infarct volumes were compared by one-way analysis of variance followed by Dunnett's multiple-range test or Student's *t*-test. Physiological parameters among experimental groups were compared by two-factor repeated-measure analysis of variance. $P < 0.05$ was regarded as indicative of significance.

## Results and Discussion

Animals receiving high doses (10 and 20 mg kg$^{-1}$ h$^{-1}$) of YM90K became mildly sedated during administration. Flat body posture and head waving were observed during administration of CNS1102. These behavioural changes disappeared 1 to 2 h after termination of the intravenous infusion. They were thought to be induced by blockade of AMPA receptors in the case of sedation (Gill 1994) or NMDA receptors in the case of stereotyped behaviour (Koek et al 1988). There were no significant differences between physiological parameters measured for saline- and YM90K (20 mg kg$^{-1}$ h$^{-1}$)-treated groups, or for saline- and CNS1102-treated groups (Table 1).

The AMPA receptor antagonist YM90K and the NMDA receptor antagonist CNS1102 had significant neuroprotective effect when administered immediately after induction of focal cerebral ischaemia. Brain temperatures and other physiological parameters were not influenced by effective doses of either compound, indicating that the neuroprotective effects of these compounds were not a result of non-specific effects such as hypothermia.

Table 1. Effects of YM90K and CNS1102 on physiological parameters.

| Parameter | Treatment | Pretreatment | 2 h | 4 h | 24 h |
|---|---|---|---|---|---|
| YM90K | | | | | |
| Mean arterial blood pressure (mmHg) | Saline | 99·0 ± 7·8 | 105·3 ± 3·4 | 112·8 ± 3·8 | – |
| | YM90K | 96·0 ± 8·5 | 119·8 ± 11·8 | 119·8 ± 11·2 | – |
| pH | Saline | 7·422 ± 0·009 | 7·364 ± 0·011 | 7·349 ± 0·018 | |
| | YM90K | 7·425 ± 0·006 | 7·335 ± 0·016 | 7·294 ± 0·024 | – |
| $PaCO_2$ (mmHg) | Saline | 40·7 ± 1·1 | 40·3 ± 1·2 | 39·2 ± 1·1 | – |
| | YM90K | 41·1 ± 1·9 | 42·7 ± 1·0 | 41·1 ± 2·0 | – |
| $PaO_2$ (mmHg) | Saline | 83·1 ± 3·4 | 90·8 ± 0·7 | 107·7 ± 10·9 | – |
| | YM90K | 108·4 ± 16·7 | 115·2 ± 13·3 | 95·1 ± 3·1 | – |
| Rectal temperature (°C) | Saline | 37·6 ± 0·1 | 36·8 ± 0·1 | 36·5 ± 0·2 | 37·4 ± 0·2 |
| | YM90K | 37·5 ± 0·1 | 36·4 ± 0·2 | 36·0 ± 0·3 | 37·4 ± 0·3 |
| Brain temperature (°C) | Saline | 37·8 ± 0·1 | 36·3 ± 0·1 | 36·0 ± 0·2 | 36·9 ± 0·2 |
| | YM90K | 37·6 ± 0·1 | 36·0 ± 0·2 | 35·6 ± 0·3 | 36·9 ± 0·3 |
| CNS1102 | | | | | |
| Mean arterial blood pressure (mmHg) | Saline | 107·8 ± 6·4 | 101·2 ± 7·2 | 108·0 ± 6·3 | – |
| | CNS1102 | 91·0 ± 8·5 | 117·3 ± 11·3 | 106·8 ± 6·9 | – |
| pH | Saline | 7·432 ± 0·006 | 7·428 ± 0·006 | 7·413 ± 0·012 | – |
| | CNS1102 | 7·426 ± 0·013 | 7·394 ± 0·024 | 7·388 ± 0·011 | – |
| $PaCO_2$ (mmHg) | Saline | 41·4 ± 0·5 | 39·4 ± 1·0 | 38·3 ± 1·5 | – |
| | CNS1102 | 42·1 ± 1·6 | 39·1 ± 2·0 | 38·3 ± 0·8 | – |
| $PaO_2$ (mmHg) | Saline | 108·1 ± 10·9 | 89·8 ± 0·4 | 92·1 ± 2·6 | – |
| | CNS1102 | 98·3 ± 11·9 | 117·5 ± 11·9 | 120·7 ± 10·4 | – |
| Rectal temperature (°C) | Saline | 37·2 ± 0·1 | 37·1 ± 0·3 | 36·7 ± 0·2 | 37·4 ± 0·2 |
| | CNS1102 | 37·1 ± 0·2 | 36·9 ± 0·4 | 36·7 ± 0·2 | 37·4 ± 0·1 |
| Brain temperature (°C) | Saline | 37·6 ± 0·1 | 36·9 ± 0·4 | 36·7 ± 0·2 | 37·4 ± 0·1 |
| | CNS1102 | 37·2 ± 0·2 | 36·3 ± 0·5 | 35·9 ± 0·1 | 36·5 ± 0·2 |

Immediate administration of YM90K at doses of 2·5–20 mg kg$^{-1}$ h$^{-1}$ resulted in significant reductions in cortical (penumbra region) infarction volume 24 h after middle cerebral artery occlusion compared with the control group (Table 2). At a dose of 20 mg kg$^{-1}$ h$^{-1}$ a 39% reduction in cortical infarct volume was observed. There were no differences between the control group and all doses of YM90K-treated groups in striatal (core region) infarct volumes (Table 2). The neuroprotective effect of YM90K administered immediately after middle cerebral artery occlusion in this study is consistent with that reported elsewhere (Shimizu-Sasamata et al 1996). In that study YM90K was administered as a single intravenous bolus of 30 mg kg$^{-1}$ followed by intravenous infusion at 10 mg kg$^{-1}$ h$^{-1}$ for 4 h. Because of its relatively low solubility under the conditions used YM90K precipitated in the renal tubules; in this study the regimen was changed to avoid this possibly serious side-effect and these tests closely modelled possible clinical regimens for man. Under these conditions YM90K did not induce serious side-effects even at the highest dose used in this study. YM90K had a marked neuroprotective effect when an infusion of 0·5 mg kg$^{-1}$ h$^{-1}$ was administered intravenously for 6 h in a previous cat middle cerebral artery occlusion study (Yatsugi et al 1996). At that dosage precipitation in renal tubules did not occur, suggesting its therapeutic potential in treating stroke in man.

The neuroprotective effect of CNS1102, a selective non-competitive NMDA receptor antagonist, was also examined. This compound has a high affinity for the NMDA ion-channel site (concentration resulting in 50% inhibition, IC50 = 36 nM) and low affinity for $\sigma$ receptors (IC50 = 2540 nM) (Reddy et al 1994). This compound has neuroprotective effects in focal cerebral ischaemia models (Minematsu et al 1993a, b; Aronowski et al 1994; Cohen et al 1994; Meadows et al 1994), and is the only non-competitive NMDA receptor antagonist with demonstrated neuroprotective effect against stroke in man (Knapp et al 1997). Intravenous CNS1102 (1·13 mg mL$^{-1}$ kg$^{-1}$) followed by intravenous infusion at 0·785 mg/3 mL kg$^{-1}$ h$^{-1}$ for 4 h immediately after middle cerebral artery occlusion also reduced the cortical infarct volume by 35% compared with the control group (Table 3). This effect is consistent with other results (Minematsu et al 1993a) which used nearly the same administration regimen. Although the dose–response of CNS1102 was not examined in the current study, the dosage of CNS1102 was thought to be sufficient, as evidenced by typical behavioural changes such as head weaving generally attributed as being caused by NMDA receptor blockade. Furthermore, the extent of protection by CNS1102 was similar to that of YM90K when administered immediately after middle cerebral artery occlusion, indicating that this dose regimen is suitable for comparing the effects of YM90K and CNS1102.

Both CNS1102 and YM90K resulted in significant neuroprotective effect when administered immediately after middle cerebral artery occlusion, suggesting that NMDA and AMPA receptors play similarly important roles in the time immediately after the development of neuronal damage after focal cerebral ischaemia because both CNS1102 (Minematsu et al 1993b) and YM90K (unpublished data) have short biological half-lives (approximately 60 min) in plasma after administration. The extent of protection by these two compounds were also similar to that seen previously with other

Table 2. The neuroprotective effect of YM90K on cortical and striatal infarcts 24 h after middle cerebral artery occlusion in rats.

| | Infarct volume (mm$^3$) | |
|---|---|---|
| | Cortex | Striatum |
| Saline | 90·2 ± 6·8 | 53·4 ± 3·1 |
| YM90K (mg kg$^{-1}$ h$^{-1}$) | | |
| 1·0 | 76·0 ± 6·7 | 44·7 ± 1·2 |
| 2·5 | 61·6 ± 7·6* | 45·0 ± 2·7 |
| 5 | 60·8 ± 7·6* | 50·4 ± 3·6 |
| 10 | 57·2 ± 11·2* | 52·6 ± 3·6 |
| 20 | 55·2 ± 7·5* | 49·9 ± 3·4 |

Saline or YM90K was intravenously infused over 4 h, starting immediately after middle cerebral artery occlusion. Each value is the mean ± s.e.m. *$P < 0·05$, significantly different from the result for the saline group (one-way analysis of variance followed by Dunnett's multiple range test).

Table 3. The neuroprotective effect of CNS1102 on cortical and striatal infarcts 24 h after middle cerebral artery occlusion in rats.

| | Infarct volume (mm$^3$) | | | |
|---|---|---|---|---|
| | 0 h | | 1 h | |
| | Cortex | Striatum | Cortex | Striaum |
| Saline | 100·5 ± 6·8 | 43·2 ± 1·7 | 107·9 ± 8·6 | 43·5 ± 0·5 |
| CNS1102 | 65·5 ± 11·1* | 41·0 ± 3·8 | 106·7 ± 7·2 | 44·8 ± 0·7 |

Saline or CNS1102 (1·13 mg kg$^{-1}$ intravenous bolus + 0·785 mg kg$^{-1}$ h$^{-1}$ for 4 h) was administered starting either immediately (0 h) or 1 h after middle cerebral artery occlusion. Each value is the mean ± s.e.m. *$P < 0·05$, significantly different from the result for the respective saline group (Student's $t$-test).

glutamate receptor antagonists such as NBQX (Graham et al 1996) and 5-methyl-10,11-dihydro-5*H*-dibenzo(*a*,*d*)cyclohepten-5,10-imine hydrochloride (MK-801) (Hatfield et al 1992) in models of permanent middle cerebral artery occlusion (Tamura's method). These previous data and the results of the current study indicate that the extent of protection of NMDA and AMPA receptor antagonists when given immediately after the induction of ischaemia in permanent middle cerebral artery occlusion models are very similar.

Simultaneous comparison of the therapeutic time-windows of efficacy for YM90K and CNS1102 under the same experimental conditions is important for estimating the possible usefulness of YM90K as a therapeutic agent for acute stroke. In the 1- or 2-h delayed YM90K-treated groups there were also significant reductions of infarct volume (Table 4). The protection in cortical infarct volume in the 1-h and 2-h delayed treatment groups was 36% and 45%, respectively. This result was similar to that of the group treated with the same dose ($20 mg kg^{-1} h^{-1}$) immediately after occlusion. When given 4h after middle cerebral artery occlusion, however, there was no significant difference between the infarct volumes of saline- and YM90K-treated groups (Table 4). This result is consistent with the data for NBQX, which reduces infarct volume when given up to 90min after middle cerebral artery occlusion (Graham et al 1996). GYKI52466, a non-competitive AMPA receptor antagonist, was also reported to be protective when given 2h after middle cerebral artery occlusion (Smith & Merdrum 1992). These previous observations and the results of this study suggest that AMPA receptors might play a crucial role in preventing neuronal damage up to 2h after the onset of ischaemia in this model.

There was no significant reduction in the infarct volume in the CNS1102-treated group treated 1h after middle cerebral artery occlusion (Table 3). The neuroprotective effect of CNS1102 was eliminated when given 1h after occlusion despite the significant protection of the same dose when given immediately after occlusion. It has been reported (Meadows et al 1994) that CNS1102 resulted in significant neuroprotection when administered 1h after ischaemic onset in the rat middle cerebral artery occlusion model employing an administration regimen similar to that used in the current study. Although the reason for this discrepancy is unclear, there are some possibilities. In the Meadows study brain ischaemia was induced by intraluminal suture middle cerebral artery occlusion and CNS1102 was administered to anaesthetized rats, whereas in the current study CNS1102 was administered to conscious rats. It is reported that anaesthesia modified ischaemic brain damage in rodents (Freund et al 1990). The different experimental procedures in the two studies might explain the lack of effect of CNS1102 in the current study.

Another non-competitive NMDA receptor antagonist, MK-801 has been reported (Hatfield et al 1992) to be effective even when administered 1h after ischaemia in the same permanent middle cerebral artery occlusion model as was used in the current study. It has been reported that there is interstrain variation in the volume of cerebral infarction after standardized middle cerebral artery occlusion in rats (Duverger & Mackenzie 1988). This includes the Fischer 344 rats used in this study and the Sprague-Dawley rats used in the Hatfield study. The infarct volumes of these two strains might be different, and this difference might contribute to the positive effect of MK-801 and the negative effect of CNS1102. It has also been reported that MK-801 induces reduction of brain or body temperature; this affects neuronal damage (Ginsberg & Busto 1989) in rodent cerebral ischaemia models, especially in global ischaemia models, in the dose range described by Hatfield et al (Buchan & Pulsinelli 1990; Buchan et al 1991;

Table 4. The therapeutic time-window for the efficacy of YM90K on cortical and striatal infarct volumes 24h after middle cerebral artery occlusion in rats.

| | Infarct volume ($mm^3$) | | | | | |
|---|---|---|---|---|---|---|
| | 1h | | 2h | | 4h | |
| | Cortex | Striatum | Cortex | Striatum | Cortex | Striatum |
| Saline | 77·1 ± 11·2 | 40·2 ± 2·8 | 69·6 ± 10·8 | 32·8 ± 2·6 | 108·8 ± 6·9 | 49·6 ± 0·4 |
| YM90K | 49·1 ± 7·1* | 31·2 ± 3·3 | 38·6 ± 5·8* | 24·5 ± 3·9 | 102·2 ± 5·6 | 48·3 ± 0·6 |

Saline or YM90K ($20 mg kg^{-1} h^{-1}$ for 4h) was intravenously infused starting 1, 2 or 4h after middle cerebral artery occlusion. Each value is the mean ± s.e.m. $*P < 0.05$, significantly different from the result for the respective saline group (Student's *t*-test).

Hayward et al 1993). It is unknown whether or not this effect contributes to the MK-801-induced neuroprotective effect, because physiological parameters such as body temperature were not measured by Hatfield et al (1992). It is strongly suggested that CNS1102 at the intravenous dose used in this study ($1{\cdot}13\,mg\,kg^{-1}$) does not induce reduction of brain temperature in the focal ischaemia model (Hasegawa et al 1994) and so these pharmacological differences between MK-801 and CNS1102 might contribute to the observed negative effect of CNS1102 in the current study.

Results from transient middle cerebral artery occlusion in spontaneous hypertensive rats showed that MK-801 was ineffective when given after middle cerebral artery occlusion, whereas NBQX was effective when administered even after a 90-min delay (Xue et al 1994). Margaill et al (1996) also reported that MK-801 was ineffective when given 30 min after middle cerebral artery occlusion at a dose found to be effective when given just before ischaemia in a transient middle-cerebral-artery occlusion model in Sprague-Dawley rats. Although NMDA receptor antagonists have neuro-protective effects in many experimental stroke models, taken together these reports and the current study show that the therapeutic time-window for efficacy of NMDA receptor antagonists in focal ischaemia might not be longer than those of AMPA receptor antagonists.

The elevation of intracellular $Ca^{2+}$ concentration, $[Ca^{2+}]_i$, after an excessive increase of glutamate in the synaptic cleft plays a critical role in ischaemia-induced cell damage (Siesjö 1981). This elevated $[Ca^{2+}]_i$ is thought to be mediated by several pathways: NMDA receptor-linked ion channels, which are highly permeable to $Ca^{2+}$, but are blocked in a voltage-dependent manner by $Mg^{2+}$ (Wong & Kemp 1991); voltage-sensitive calcium channels (Miller 1991); metabotropic glutamate receptors, which mediate $Ca^{2+}$ release from intracellular stores (Sugiyama et al 1987); AMPA receptor-linked ion channels lacking the GluR2 subunit, which are also permeable to $Ca^{2+}$ and $Na^+$ (Hollmann et al 1991; Verdoorn et al 1991); and the $Na^+$–$Ca^{2+}$ exchanger, which might reverse during ischaemia (Siesjö & Bengtsson 1989).

$Ca^{2+}$ entry through a combination of these pathways would lead to the accumulation of toxic levels of $[Ca^{2+}]_i$. That both YM90K and CNS1102 show significant neuroprotective effect in this study suggests that blockade of one of these pathways is enough to provide some neuroprotection in focal ischaemia. Blockade of AMPA receptors will reduce the depolarization of the postsynaptic cells, thus preventing, to some extent, the alleviation of the voltage-dependent block of NMDA receptors with $Mg^{2+}$, leading to reduction of $Ca^{2+}$ entry into the cell (first pathway). This reduction in depolarization of the cell will also reduce $Ca^{2+}$ influx through voltage-sensitive calcium channels (second pathway). In addition, blockade of AMPA receptors will reduce $Ca^{2+}$ (fourth pathway) and $Na^+$ entry through these receptors. This reduction of $Na^+$ entry will suppress the reversal of the $Na^+$–$Ca^{2+}$ exchanger (fifth pathway). Thus there is the possibility that blockade of AMPA receptors by YM90K could reduce $Ca^{2+}$ influx more efficiently than a blockade of NMDA receptors by CNS1102 via the general action of YM90K of attenuating glutamate-induced neuronal depolarization. This might contribute to its wider therapeutic time-window compared with CNS1102. Careful and precise experiments will be needed to prove this hypothesis.

In conclusion, this study has demonstrated that AMPA and NMDA receptors are important in the neuronal damage that follows focal ischaemia in rats. The most important result was that YM90K had a wider therapeutic time-window than CNS1102 under the same experimental conditions, suggesting that AMPA receptors play an important role in the late phase of neuronal cell damage in this model. Because several hours usually pass between onset of occlusion and the start pharmacological therapy in clinical situations, these data suggest the beneficial effects of an AMPA receptor blockade on acute stroke in man.

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