KETAMINE FAILS TO PROTECT AGAINST ISCHAEMIC NEURONAL NECROSIS IN THE RAT

M. L. JENSEN AND R. N. AUER

Ketamine has been in clinical use for more than two decades. In recent years it has become clear that, in addition to its effect as a dissociative anaesthetic, ketamine is an effective blocker of NMDA excitatory amino acid receptors in the brain [1-4]. The NMDA receptor has been implicated as the subtype of excitatory amino acid receptor responsible for neuronal necrosis in hypoxia–ischaemia [5, 6]. As ketamine protects hippocampal neurones from anoxia in vitro, and as this agent crosses the blood–brain barrier when administered systemically, we have investigated the potential usefulness of ketamine in preventing ischaemia-induced neuronal degeneration and death.

MATERIALS AND METHODS

Ischaemia was induced in male Wistar rats by a modification of the model developed by Smith and co-workers [7]. Briefly, forebrain ischaemia was induced by a combination of carotid clamping and hypotension. The rats were anaesthetized in 3 % halothane, the trachea intubated, and the lungs ventilated using a Starling type ventilator, with 66 % nitrous oxide and 0.7 % halothane in oxygen. A tail vein was cannulated and a continuous infusion of suxamethonium 2 mg h⁻¹ was administered to maintain continuous paralysis. A tail artery was cannulated and arterial pressure was recorded continuously using a Statham transducer (Gould P50, Oxnard, Calif.). Arterial pressure recordings were digitized every 6 s and recorded on a computer hard disk. These readings were used later to calculate the mean intra-ischaemic arterial pressures in the various groups (table I). A central venous catheter was inserted via the right jugular vein and attached to a pre-warmed 10-ml heparinized syringe.

After a period of stabilization of approximately 30 min, the rats were given trimetaphan 5 mg kg⁻¹ i.v. The rats were exsanguinated into the pre-warmed heparinized syringe. When arterial pressure reached 50 mm Hg, two clamps were placed around the carotid arteries. Arterial pressure recordings were digitized every 6 s and recorded on a computer hard disk. These readings were used later to calculate the mean intra-ischaemic arterial pressures in the various groups (table I). A central venous catheter was inserted via the right jugular vein and attached to a pre-warmed 10-ml heparinized syringe.

SUMMARY

Ketamine is a dissociative anaesthetic known to be an N-methyl-D-aspartate receptor blocker. Since the NMDA excitatory receptor on neurones is implicated in ischaemic neuronal necrosis, ketamine might be expected to have a beneficial effect in cerebral hypoxia–ischaemia. Ketamine was tested in a rat model of forebrain ischaemia allowing 7 days recovery. Ketamine 6 mg kg⁻¹ i.v. was administered 5–10 min before ischaemia in one group of rats, and ketamine 60 mg kg⁻¹ day⁻¹ i.m. for 3 days and 7 continuous days after ischaemia in two other groups. An additional group received ketamine 24 mg kg⁻¹ i.v. before ischaemia and 120 mg kg⁻¹ day⁻¹ i.m. after ischaemia for 7 days continuously. Control rats received ischaemia but no treatment. The results were compared with untreated controls by neuropathological examination of the entire brain, sectioned subserially. There was no significant difference in necrosis between treated and untreated groups after any of the ketamine regimens. The findings demonstrate that systemically administered ketamine fails to protect the brain against hypoxic–ischaemic injury in the rat.
KETAMINE AND ISCHAEMIC BRAIN DAMAGE

TABLE I. Dosage of ketamine and physiological variables for each group (mean ± SEM). Number of injections received per day - group 3: 2 (two rats) or 3 (four rats); group 4: 2 (four rats) or 4 (two rats); group 5: 2 (all in group). * Average of readings sampled every 6 s over entire ischaemic period. † Duration with both carotids clamped and AP ≤ 50 mm Hg

| n | Survival (days) | Ketamine (mg kg$^{-1}$) | Before ischaemia | After ischaemia | I.v. < 45 min post-ischaemia | I.m. dose per day | No. of days ketamine post-ischaemia | AP before ischaemia (mm Hg) | Mean intra-ischaemic AP* (mm Hg) | Mean ischaemic duration† (min:s) | AP after ischaemia (mm Hg) | Glucose (mmol litre$^{-1}$) | PCO$_2$ (kPa) | PO$_2$ (kPa) | pH |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Group 1 | 8 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 123 ± 2 | 49.5 ± 0.2 | 9:13 ± 0.02 | 128 ± 3 | 7.8 ± 0.9 | 4.51 ± 0.15 | 17.52 ± 1.28 | 7.415 ± 0.018 |
| Group 2 | 7 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 118 ± 3 | 47.5 ± 0.3 | 9:07 ± 0.02 | 125 ± 4 | 6.9 ± 0.7 | 4.6 ± 0.19 | 14.69 ± 1.16 | 7.425 ± 0.017 |
| Group 3 | 7 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 125 ± 4 | 50.3 ± 0.2 | 9:13 ± 0.02 | 133 ± 3 | 7.9 ± 0.5 | 4.76 ± 0.2 | 13.82 ± 1.09 | 7.389 ± 0.016 |
| Group 4 | 7 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 115 ± 3 | 48.5 ± 0.3 | 9:09 ± 0.02 | 118 ± 2 | 6.5 ± 0.4 | 4.9 ± 0.19 | 16.04 ± 1.2 | 7.396 ± 0.012 |
| Group 5 | 7 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 120 ± 3 | 47.3 ± 0.2 | 9:05 ± 0.02 | 121 ± 2 | 9.5 ± 0.4 | 4.71 ± 0.15 | 16.63 ± 0.91 | 7.389 ± 0.017 |

After 9 min of ischaemia, the carotid clamps were released and blood reinfused. The trachea was extubated and the animal allowed to awaken, to survive for 1 week.

The schedules, doses and routes of administration of ketamine are given for each group in Table I.

A subgroup of two of the six rats receiving ketamine for 7 days (group 4) received 60 mg kg$^{-1}$ day$^{-1}$ in four divided doses rather than two divided doses in order to test for possible lack of efficacy resulting from decreasing blood concentrations of ketamine between insufficiently frequent doses of drug.

All rats were sacrificed 1 week after ischaemia. Rats were re-anesthetized with 2–3% halothane and the trachea intubated. A thoracotomy was performed, and trans-cardiac perfusion was carried out using 4% formaldehyde, phosphate-buffered to pH 7.35.

Sections were double stained with 1% acid fuchsin and 0.1% cresyl violet, and the number of acidophilic neurones, previously shown to be necrotic [8], was counted under light microscopy.

Integers representing the degree of neuronal necrosis were assigned for the cerebral cortex and striatum, the main telencephalic brain regions vulnerable to ischaemic damage, as follows: in the hippocampus, where total cell counts were available from previous quantitative studies [9], necrotic neurones were visually counted directly and given a percentage (Table II). In the cerebral cortex, where total cell counts were unavailable, 10–100 necrotic cells per section were assigned the integer 1, 100–1000 necrotic neurones were given 2, and greater than 1000 necrotic neurones

<table>
<thead>
<tr>
<th>No. cells dead</th>
<th>Total No. cells</th>
<th>% Cells necrotic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>8326</td>
<td>12405</td>
</tr>
<tr>
<td>Group 2</td>
<td>5316</td>
<td>11762</td>
</tr>
<tr>
<td>Group 3</td>
<td>5583</td>
<td>8263</td>
</tr>
<tr>
<td>Group 4</td>
<td>8224</td>
<td>13979</td>
</tr>
<tr>
<td>Group 5</td>
<td>7597</td>
<td>14276</td>
</tr>
</tbody>
</table>

Brains were removed the following day, processed in graded ethanol and xylol, and embedded in paraffin. Subserial sectioning was performed in order to obtain sections from the cerebral cortex, hippocampus, caudate nucleus, diencephalon, brain stem and cerebellum in all animals.

TABLE II. Extent of neuronal necrosis in the hippocampus, CA1 region. Differences not significant (Student t test)

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TABLE III. Damage scores (mean ± SEM) in each group for cells of the neoconex. See text for details of scoring

<table>
<thead>
<tr>
<th>Group</th>
<th>Damage Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>2.5 ± 0.5</td>
</tr>
<tr>
<td>Group 2</td>
<td>2.5 ± 0.3</td>
</tr>
<tr>
<td>Group 3</td>
<td>2.3 ± 0.4</td>
</tr>
<tr>
<td>Group 4</td>
<td>2.2 ± 0.4</td>
</tr>
<tr>
<td>Group 5</td>
<td>2.0 ± 0.5</td>
</tr>
</tbody>
</table>

3. The scores for both sides of the brain were summed to give the regional damage index for cerebral cortex (table III).

RESULTS

Clinical observations

Control rats (group 1) subjected to 9 min of ischaemia showed normal grooming and eating behaviour 24 h after surgery.

Rats given pre-ischaemic ketamine (group 2) showed no delay in awakening after operation and were indistinguishable from control rats.

Rats given post-ischaemic ketamine (groups 3, 4 and 5) could be weaned from the ventilator in 2–2.5 h, in contrast to rats in groups 1 and 2, where tracheal extubation was possible after 30 min. For the duration of time during which they received ketamine injections, post-ischaemic rats in these three groups were in a clinically dissociated, sluggish state and reacted minimally to repeated i.m. injections of ketamine. This was taken to reflect adequate central effect of the drug.

Neuropathology

Ischaemia produced the well-described acidophilic neuronal change in cerebral cortex and hippocampus [10]. Acidophilic neurones showed blurring of the cytoplasmic organelles including the Nissl substance, and a loss of nuclear detail. Macrophages were occasionally present, and this was not used as a criterion for neuronal necrosis. The cardinal criterion used was the intense cytoplasmic acidophilia [8, 9] which was either present or absent.

No significant difference in neuronal necrosis was seen in the ketamine pretreated group, the pre- and post-treated group, or in the two groups receiving 3 and 7 days of post-ischaemic ketamine (tables II, III).

Damage was seen consistently in the GABA-ergic lateral reticular nucleus of the thalamus, as described previously [10]. The caudate nucleus was examined in all rats, but was not affected in any animals. One animal in group 1 and one in group 4 showed tectal infarcts in the inferior colliculi, but the characteristic audiogenic running seizures, seen after ischaemia in rats (Voll and Auer, in preparation) were not present after this relatively short duration of ischaemia. Cerebellar damage was uniformly absent.

DISCUSSION

The present findings demonstrate that ketamine, a centrally acting potent NMDA receptor blocker [4], is not effective in preventing ischaemic neuronal necrosis in the rat brain.

Ketamine acts at the NMDA receptor by non-competitive antagonism, at an allosteric site distinct from the ion channel [11]. Numerous in vitro studies based on cell culture or tissue slice preparations have demonstrated the effectiveness of ketamine in blocking NMDA mediated excitatory responses [1–3, 12–16].

The NMDA subtype of excitatory amino acid receptor has been implicated in neuronal necrosis produced by hypoxia–ischaemia [17]. Based on these findings, and on a protective effect of ketamine against hypoxia seen in cultured cortical neurones [18, 19] and in the hippocampal slice [19], a beneficial effect might be suspected in vivo.

Ketamine has been shown to be effective in preventing neuronal excitotoxicity in vivo produced by NMDA excitatory receptor agonists. Systemic injection of ketamine 30 mg kg⁻¹ i.p. and pentobarbitone 36 mg kg⁻¹ i.p., 30 min before intra-hippocampal injections of either quinolinic acid (120 μmol) or NMDA (50 μmol) resulted in partial protection of hippocampal neurones [20].

In view of these properties of ketamine as an NMDA receptor antagonist, and of its demonstrated in vivo protective action against toxicity from NMDA agonists [20], the failure of ketamine to protect against ischaemic neuronal necrosis may seem surprising in the light of mounting evidence that glutamate release and NMDA receptor activation are involved in hypoxic–ischaemic neuronal death [21]. The clinically dissociated state of the animals seen after ketamine administration would seem to indicate adequate penetration of the brain by the drug. Even in the CA1 zone of the hippocampus, where NMDA receptor sites are concentrated [22, 23], no
beneficial effect was seen in protecting selectively vulnerable neurones. Hence, it appears as though the in vivo testing for prevention of neuronal necrosis in a whole animal ischaemia model constitutes a more stringent criterion than in vitro testing for drugs reported to be effective against hypoxia–ischaemia in tissue culture and slice preparations [1–3, 12–15, 24]. It may be that only NMDA antagonists of even higher potency than ketamine, such as MK-801 [25] may be effective in preventing ischaemic neuronal death [26, 27].

Ketamine is known to activate cholinergically mediated cerebral vasodilatation, causing an increase in cerebral blood flow (CBF), and increased intracranial pressure [28, 29]. One might presume that any increase in post-ischaemic CBF would have been of benefit in the present study.

Few studies have examined the effect of ketamine in cerebral ischaemia. One research group found that ketamine anaesthesia increased stroke-mediators cerebral vasodilatation, causing an increase in cerebral blood flow (CBF), and increased intracranial pressure [28, 29]. One might presume that any increase in post-ischaemic CBF would have been of benefit in the present study.

Application of excitatory amino acid blockers in clinical situations should be based ideally on a demonstrated beneficial effect on both brain structure and function in animals. The present study demonstrated neither aggravation nor mitigation of the clinical or neuropathological sequelae of transient cerebral ischaemia. Thus, it seems likely that ketamine is neither harmful nor beneficial in clinical situations which may cause cerebral ischaemia.

ACKNOWLEDGEMENTS

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REFERENCES


