

Research report

Neuroprotection of *S*(+) ketamine isomer in global forebrain ischemia

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Abstract

The non-competitive *N*-methyl-D-aspartate (NMDA) receptor antagonist ketamine can block the action of excitotoxic amino acids in the central nervous system. *S*(+) ketamine has a 2–3 times higher anesthetic potency compared with the ketamine-racemate and also shows a higher neuroprotective efficacy *in vitro*. To determine the neuroprotective activity of *S*(+) ketamine compared with its *R*(–) stereoisomer *in vivo*, we examined the functional and neurohistological outcome in rats treated 15 min after global forebrain ischemia with *S*(+) ketamine in different dosages compared with *R*(–) ketamine. Influence of the treatment on regional cerebral blood flow (rCBF) and cortical oxygen saturation (HbO₂) was monitored over 1 h after the ischemia using laser doppler flowmetry and microphotospectrometry respectively. Sixty and ninety mg/kg of *S*(+) ketamine but not *R*(–) ketamine significantly reduced neuronal cell loss in the cortex compared with the saline treated group. No significant neuroprotection was observed in the hippocampus. Although no significant change in rCBF was found, *S*(+) ketamine restored the cortical HbO₂ to preischemic values. These results indicate that *S*(+) ketamine in higher dosages can reduce neuronal damage in the cortex after cerebral ischemia, possibly by improving the ratio of oxygen supply to consumption in the postischemic tissue. © 2001 Elsevier Science B.V. All rights reserved.

Theme: Disorders of the nervous system

Topic: Ischemia

Keywords: Glutamate; Laser-doppler flowmetry; Oxygenation; Histology

1. Introduction

Focal and global cerebral ischemia are critical events in many clinical situations, such as stroke, cardiac arrest, and cerebral trauma [39]. There is ample evidence that glutamate and other excitatory amino acids are at least partly responsible for the selective neuronal death after cerebral ischemia/anoxia [12,20,25,30]. Glutamate is an excitatory neurotransmitter in the central nervous system (CNS) [20,30]. Three ionotropic subtypes of the glutamate receptors have been identified: *N*-methyl-D-aspartate (NMDA), α -amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA), and kainate [25]. Energy depletion after brain ischemia results in an increased synaptic release and

decreased cellular reuptake of glutamate [20,29]. The accumulation and interaction of glutamate with its receptors contributes to a variety of pathological processes such as intracellular sodium/calcium influx, cellular swelling, and activation of enzymes, that catalyze the breakdown of proteins, lipids and nucleic acids leading to neuronal death [12,41]. Therefore, antagonists of the NMDA receptor are thought to be an effective therapeutic approach in the treatment of secondary neuronal injury after cerebral ischemia. However, despite numerous experimental studies investigating different substances [19,22,41], ketamine and dextrorphan are currently the only clinically available NMDA antagonists. Ketamine hydrochloride is an established dissociative anesthetic used in selected clinical procedures for over 25 years [13]. Ketamine interacts with the phencyclidine (PCP) binding site which is located in the NMDA receptor associated ion-channel [1]. It inhibits the influx of Na⁺ and Ca²⁺ cations through this channel thus noncompetitively antagonizing the actions of NMDA agonists like glutamate [1,7,17]. There is evidence that ketamine-induced anes-

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nesia is in part mediated by the interaction with the NMDA receptor complex [17,44]. This pharmacological action could provide therapeutic benefit in the treatment of glutamate induced mediated neuronal injury after cerebral ischemia. Ketamine has been shown to protect neurons from NMDA induced damage in vivo after ischemia [1,7,16,27], seizures [8], cerebral trauma [38] and after intracerebroventricular injection of excitotoxins [22–24] as well as in vitro [1,15]. However, unlike other anesthetics, which are CNS depressants, ketamine is reported to increase cerebral metabolism, with subsequent increase of local glucose utilization, cerebral blood volume and cerebrospinal fluid (CSF) pressure [3]. Thus, the overall effects of ketamine might reflect an interplay between the parenchymal neuroprotective effects and its prometabolic action [5]. Because of its chiral center at the C₂ atom of the cyclohexanone ring, ketamine exists as two isomers, the *R*(–) and the *S*(+) form [28,36]. The *S*(+) isomer shows approximately 2–3 times higher analgetic and hypnotic potency than the *R*(–) isomer [15,26]. Furthermore it has been shown that certain locomotor and psychomimetic side effects are less intense in the *S*(+) form [36,42,43] and the therapeutic index is greater compared with the racemate [28,42]. As recently demonstrated, *S*(+) ketamine also has a higher neuroprotective potency in vitro against NMDA-induced injury compared with the racemate and the *S*(–) form and can induce neuronal regeneration after axonal transection [15,45]. However, no studies evaluating the potential neuroprotective effects of the *S*(+) isomer in vivo have been performed so far. The purpose of the current study is to determine the differences in the neuroprotective properties of the ketamine isomers in vivo in a rat model of global forebrain ischemia induced by hypobaric hypotension. To assess the potential prometabolic effects of ketamine and its influence on cerebral microperfusion and oxygenation the present study was carried out with special focus on cerebral blood flow, cortical oxygen saturation, functional and histopathological outcome.

2. Materials and methods

2.1. Animals

30 adult male Wistar rats weighing 401 ± 68.0 g (mean \pm S.D.) were used. The animals were housed at constant temperature and humidity with free access to food and water.

2.2. Ketamine stereoisomer

The substance was obtained as sterile dry powder (Parke Davis, Detroit, MI, USA). The solution was prepared freshly before injection in sterile saline and the pH was adjusted to 7.0. *S*(+) ketamine was given in three different

dosages 30, 60, 90 mg/kg body weight (groups A, B, C), group D received 90 mg/kg of the *R*(–) ketamine, the control group E received sterile 0.9% saline. A third person randomly assigned 6 animals to each group. The investigator was blinded to the treatment during the entire experiment. Drugs or saline were administered via an intraperitoneal catheter 15 min after cerebral ischemia.

2.3. Laser doppler flowmetry (LD)

Regional cortical blood flow (rCBF) was measured by two TSI laser flow blood perfusion monitors (model BPM 403a; TSI, St. Paul, MN, USA). The principles and calibration procedures are reviewed elsewhere [2]. The probe positioning was performed as previously described by Heimann et al. [14]. In brief, the needle probes were fixed to a micromanipulator and placed over the intact dura. The probe was translocated by the motor-driven micromanipulator (0.1 μ m resolution; Märzhäuser, Germany) to 25 positions in 300 μ m steps within the cranial window. Therefore, one scanning procedure results in values from 25 different locations within the cranial window. The median of these scanned flow values was used as a measure of regional blood flow. All flow values are expressed as arbitrary units (LDU).

2.4. Cortical hemoglobin oxygenation (HbO₂)

HbO₂ was determined using the Erlangen micro-lightguide spectrometer (EMPHO) (for review see [21]). In brief, white light from a xenon arc lamp is transmitted to the cortical tissue by a 250- μ m-lightguide. The backscattered light is led to a rotating bandpass filter disk by 6 micro-lightguides that surround the illuminating fibre. The monochromated light in the range of 502 to 632 nm is transmitted via a fluid lightguide to a photomultiplier. In this wavelength range, hemoglobin displays an oxygen saturation-dependent spectrum (oxygenated peak: 542 and 577 nm–deoxygenated peak: 556 nm). Thus the determination of oxygenated and deoxygenated hemoglobin can be calculated from the mixed spectrum of unknown HbO₂.

2.5. Experimental protocol

After recording stable baseline values of rCBF, and HbO₂ the lower part of the animal (excluding the thorax) was positioned in a negative pressure chamber which was connected to a standard vacuum cleaner. The right carotid artery was occluded and the barometric pressure within the chamber was reduced, causing a pooling of venous blood in the lower body of the animal. Via a phase triggered power supply, the power of the vacuum cleaner and hence the negative pressure in the chamber was controlled to an MAP of 40 mmHg. This level was maintained for 15 min, MAP and HbO₂ were continuously measured. The hypo-

baric hypotension was stopped and the right carotid artery reopened. In early reperfusion phase (15 min) MAP was recorded every min. rCBF and HbO₂ were measured sequentially beginning at 15, 30, 45 and 60 min after ischemia: HbO₂ data were obtained approx. 1.5 min after rCBF.

2.6. Neurofunctional test

Body weight and general impairment were investigated the day before and daily for 6 days after cerebral ischemia. To test motor skills and coordination, animals were placed on a horizontal rope and the time they were able to balance on the rope was measured (horizontal rope test). Animals were also placed on a plane which was inclined with increasing angle. The maximum angle, that the animals could maintain their position was recorded (inclined plane test).

2.7. Quantitative histology

Six days after the experiment the animals were killed in deep anesthesia using in vivo perfusion fixation with phosphate-buffered paraformaldehyde (4%) at pH 7.4 after brief flushing with 0.9% saline. The brain was removed and embedded in paraffin. The brain tissue blocks were cut serially into coronal slices of 3- μ m thickness and stained both with hematoxylin-eosin (HE) and cresyl violet. Quantitative histology was performed using standard sections of the parietal cortex and hippocampus. Images of respective structures were obtained by using a light microscope equipped with a $\times 10$ lens (Zeiss). The images were projected onto the screen of an Amiga 2000 computer using a color camera (Ikegami) and a genlock interface. Standardized grids were superimposed over the video image and viable nerve cells were counted according to identification criteria which are described elsewhere [40]. Counting was conducted by investigators blinded to the respective experimental group. Nerve cell counts obtained from both hemispheres of each animal were analyzed for side differences and — since those were absent — were averaged for final evaluation.

2.8. Statistical analysis

All data are reported as means \pm STD, together with medians \pm S.E.M. Statistical significance was tested using an ANOVA followed by pairwise multiple comparison procedures according to Student-Newman-Keuls or Dunn's test, two group comparison was performed using either Wilcoxon or Mann-Whitney Rank Sum test (Sig-mastat; Jandel Scientific, Erckrath, Germany). Differences were assumed statistically significant at $P < 0.05$.

3. Results

All experimental animals included in the study had physiologic baseline parameters (pO₂ = 93.4 \pm 4.8 mmHg; pCO₂ = 39.2 \pm 1.5 mmHg, SO₂ = 96.2 \pm 0.3%, pH_a = 7.35 \pm 0.01, MAP = 74.9 \pm 2.8 mmHg). The baseline rCBF value before induction of cerebral ischemia was 37.06 \pm 3.97 LDU. No significant difference between the baseline values of the different treatment groups was observed ($P > 0.05$, Fig. 1a). During cerebral ischemia the CBF values dropped significantly to 10.43 \pm 1.11 LDU ($P < 0.001$). The treatment groups showed no significant differences in rCBF values during or after hypobaric hypotension (Fig. 1a).

The preischemic HbO₂ value was 63.32 \pm 1.83%. No significant difference of the baseline values between the treatment groups has been observed ($P > 0.05$). During cerebral ischemia the values decreased significantly to 31.88 \pm 2.60% ($P < 0.001$, Fig. 1b). During reperfusion, the HbO₂ values were significantly higher in the 30 mg/kg S(+) ketamine treatment group compared to the saline treated controls at timepoints 15, 30, 45 and 60 min after cerebral ischemia ($P < 0.05$). 60 mg/kg S(+) ketamine and 90 mg/kg S(+) ketamine caused significantly higher HbO₂ values only 30 and 45 min after cerebral ischemia ($P < 0.05$). The R(−) isomer did not have any effects on the HbO₂ values (Fig. 1b).

Body weight dropped significantly 1 day after cerebral ischemia ($P < 0.05$, Table 1). Animals slowly regained body weight and by day 4 no significant difference to the preischemic values were found. Also, no significant differences between treatment groups could be detected (Table 1).

Scores on both the horizontal rope and the inclined plane test were significantly decreased on day 1 after the experiment and increased until day 6 ($P < 0.05$, Tables 2 and 3). The values improved in both tests, on day 4 post ischemia no significant differences to the preischemic values were found. In the horizontal rope test we found a slightly better scoring in all of the S(+) ketamine treated groups, however, these differences did not reach statistical significance.

Histological data of the different hippocampal regions are presented in Fig. 2. Cerebral ischemia produced significant neuronal cell necrosis in all hippocampal regions compared to the sham treated control ($P < 0.05$). Neuron loss was bilateral without hemispheric differences. The ketamine treated groups showed a tendency towards a dose-dependent reduction of the neuronal cell loss; however, no statistically significant difference between the different ketamine treatment groups compared with the saline treated group was found.

In the cortex, ischemia induced severe cell loss in the saline treated group compared to the sham treated control ($P < 0.001$, Fig. 3). Ketamine treatment ameliorated the neuronal injury in a dose dependent manner. However,

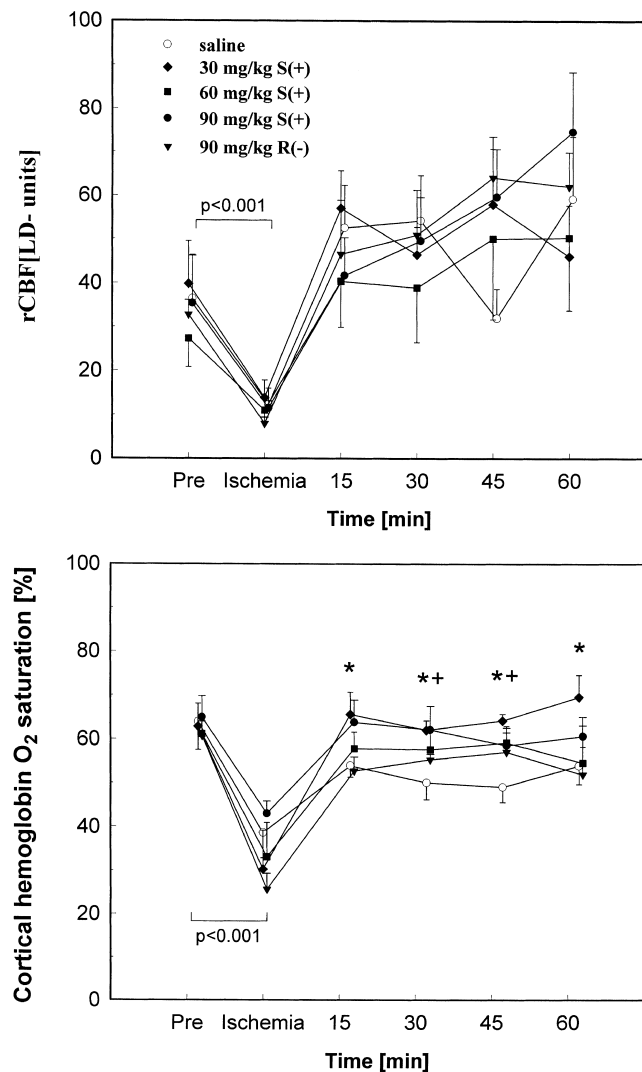


Fig. 1. (a) Laser Doppler flow values before, during, and 60 min after cerebral ischemia in the different treatment groups. The LD probe was moved by a motor-driven micromanipulator to 25 positions within a cranial window (see Materials and methods). The median of individual readings represents regional CBF (rCBF). All flow values are expressed as arbitrary units (LDU). Data are depicted as mean \pm S.E.M. During ischemia flow was significantly reduced in all groups without group differences ($P < 0.001$). (b) Cortical oxygen saturation (HbO₂) measured by microphotospectrometry, values expressed in percent saturation. During ischemia HbO₂ decreased significantly in all groups ($P < 0.001$) without group differences; * = $P < 0.05$, 30 mg/kg S(+) compared to saline treated animals, + = $P < 0.05$, 60 and 90 mg/kg S(+) compared to saline treated animals. Data are depicted as mean \pm S.E.M.

only the 60 and the 90 mg/kg S(+) isomer treated groups showed significant cell preservation ($P < 0.05$). The R(-) isomer did not show significant cell protection compared to the saline treated group.

4. Discussion

In the present study we compared the potential neuroprotective effects of different dosages of S(+) ketamine

and of the stereoisomer R(-) ketamine given after global forebrain ischemia. Although no significant neuronal protection could be observed in the hippocampus, S(+) ketamine significantly reduced neuronal cell loss in the cortex. The R(-) stereoisomer did not induce this effect.

Several studies demonstrated neuronal protection by ketamine after global or focal brain ischemia [7,16]. In contrast, other studies showed either no beneficial effects [18,35] or indicated even negative consequences of ketamine [6]. Ketamine has a number of undesirable effects, that could be responsible for those reported failures to protect neurons in vivo. In particular, ketamine has been reported to increase intracranial pressure [33]. This effect may be induced either by dilating cerebral vessels due to activation of the cholinergic vasodilator system [34] or by increase of cerebral metabolism with consecutive higher cerebral blood flow (CBF) [11,37]. Surprisingly, we did not observe significant changes of CBF in the ketamine treated groups. Several studies indicate that ketamine can cause both, inducing and depressing effects on cerebral metabolism. One study indicated that ketamine shows activating effects only at lower, subhypnotic dosages (5 mg/kg), whereas higher dosages as used in the present study seem to have more depressive actions on cerebral metabolism [10]. Certainly we cannot exclude that the cranial window changed the intracranial space architecture and thereby increased the cerebral compliance. It is noteworthy, that ketamine shows neuroprotective actions only in very high dosages [7,24], although the estimated dosage sufficient to block NMDA receptors is only 20 mg/kg [7]. Thus, ketamine administered in a moderate dose which is sufficient to block NMDA receptor interaction might not cause neuroprotective effects due its pro-metabolic side effects in this concentration range.

In a series of studies, ketamine has been shown to have distinct effects in different anatomic localizations of the brain [9,31,32]. Structures of the limbic system, particularly the hippocampus, showed a large increase of glucose metabolism; whereas, decreased metabolism was observed in the somatosensory and auditory system. The CBF monitoring in our study was limited to the cortex, potential CBF effects in deeper structures of the brain could not be detected.

The failure of significant neuroprotection in the hippocampus in our study may be in part caused by a ketamine induced metabolic activation in this area, which antagonized the beneficial NMDA-blocking effects of the ketamine treatment. Moreover, it is possible that the comparably long ischemic phase of 15 min used in this study induced too much primary damage in the very ischemia-sensitive region of the hippocampus to observe protective actions of postischemic treatment against secondary neuronal injury. In fact, most of the studies observing neuroprotection by ketamine in the hippocampus had an ischemic period of 10 min [7] or less.

The tissue hemoglobin oxygen saturation measurements after cerebral ischemia showed significantly higher HbO₂

Table 1

Body weight (g) of experimental animals at baseline and up to 6 days after 15 min global forebrain ischemia. All animals lost weight significantly at days 1 and 2, and thereafter recovered^a

	Saline	30 mg/kg S(+)	60 mg/kg S(+)	90 mg/kg S(+)	90 mg/kg R(-)
Baseline	423.0±19.1	386.5±26.6	421.0±25.5	406.8±18.7	368.0±30.1
(n)	(6)	(6)	(6)	(6)	(5)
Day 1	361.0±23.9	357.0±18.1	363.8±25.9	358.0±11.1	330.2±29.2
(n)	(6)	(6)	(6)	(6)	(5)
Day 2	360.2±28.5	355.3±18.3	358.0±29.9	354.3±14.1	330.2±31.6
(n)	(5)	(6)	(6)	(6)	(5)
Day 3	369.3±22.3	366.0±20.8	364.6±30.9	361.3±13.4	342.4±25.8
(n)	(6)	(6)	(6)	(6)	(5)
Day 4	367.0±25.9	373.6±22.2	370.1±29.7	367.5±13.2	347.0±33.5
(n)	(5)	(5)	(6)	(6)	(4)
Day 5	373.5±20.9	372.3±18.5	376.3±28.0	375.1±14.7	356.2±28.1
(n)	(6)	(6)	(6)	(6)	(5)
Day 6	378.8±21.1	379.5±21.5	402.6±28.7	386.5±18.9	366.0±26.4
(n)	(6)	(6)	(6)	(6)	(5)

^a There were no group differences.

Table 2

Horizontal Rope Test: Time rat could hold to horizontal rope (s) (at baseline and up to 6 days after 15 min global forebrain ischemia)^a

	Saline	30 mg/kg S(+)	60 mg/kg S(+)	90 mg/kg S(+)	90 mg/kg R(-)
Baseline	15.7±2.1	13.8±3.5	11.3±2.7	16.7±4.0	18.7±3.0
(n)	(6)	(6)	(6)	(6)	(5)
Day 1	5.7±1.6	7.8±3.0	5.2±1.8	8.3±1.8	9.0±5.3
(n)	(6)	(6)	(6)	(6)	(5)
Day 2	4.6±0.7	12.2±4.0	5.7±1.7	11.3±4.0	9.8±5.1
(n)	(5)	(6)	(6)	(6)	(5)
Day 3	6.4±1.3	10.0±0.4	8.2±1.5	11.4±3.9	13.1±4.4
(n)	(6)	(6)	(6)	(6)	(5)
Day 4	6.6±1.7	15.2±4.0	10.7±2.0	12.9±3.8	11.8±4.1
(n)	(5)	(5)	(6)	(6)	(4)
Day 5	8.0±1.8	14.8±3.5	14.7±3.3	13.6±3.6	13.0±3.8
(n)	(6)	(6)	(6)	(6)	(5)
Day 6	9.2±2.1	12.2±2.9	17.6±3.7	14.9±3.7	17.0±4.4
(n)	(6)	(6)	(5)	(6)	(5)

^a After ischemia results in all groups were significantly worse at day 1 and then recovered without group differences.

Table 3

Inclined Plane Test: Maximal inclination of rotating plane (degree) tolerated by rat without falling off (at baseline and up to 6 days after 15 min global forebrain ischemia)

	Saline	30 mg/kg S(+)	60 mg/kg S(+)	90 mg/kg S(+)	90 mg/kg R(-)
Baseline	53.5±1.8	53.7±1.7	52.8±1.7	53.2±2.0	50.6±2.0
(n)	(6)	(6)	(6)	(6)	(5)
Day 1	49.3±4.7	45.5±2.2	42.3±2.0	42.3±1.8	46.0±3.1
(n)	(6)	(6)	(6)	(6)	(5)
Day 2	48.6±3.6	49.2±2.5	43.2±2.7	48.7±2.4	46.2±1.5
(n)	(5)	(6)	(6)	(6)	(5)
Day 3	48.2±2.7	50.0±2.4	47.0±2.3	42.8±3.2	46.4±1.8
(n)	(6)	(6)	(6)	(6)	(5)
Day 4	45.8±1.6	52.0±0.9	50.5±2.1	50.0±2.5	51.5±1.6
(n)	(5)	(5)	(6)	(6)	(4)
Day 5	50.5±3.3	54.8±1.1	52.2±2.0	51.5±2.2	50.0±1.6
(n)	(6)	(6)	(6)	(6)	(5)
Day 6	52.3±3.7	54.5±1.6	55.0±1.9	56.7±2.3	55.6±3.8
(n)	(6)	(6)	(5)	(6)	(5)

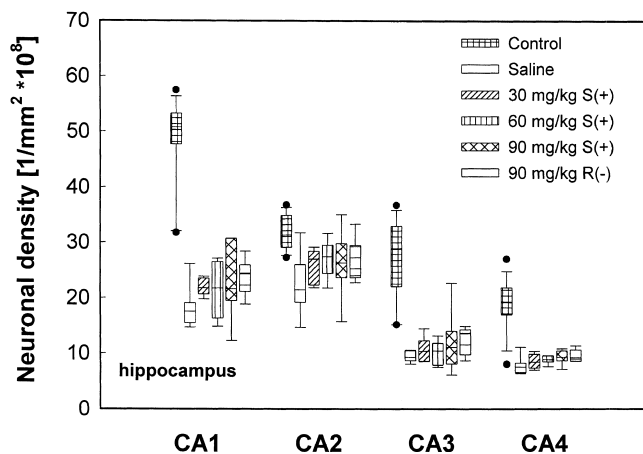


Fig. 2. Number of hippocampal neurons remaining after cerebral ischemia treated with saline or ketamine isomers in different doses (neurons/mm²·10⁸). Control shows sham treated animals. Cell counts were significantly reduced in all ischemic groups without displaying significant group differences. Data are depicted as mean±S.E.M., dots show 5th/95th percentiles.

values in the *S*(+) ketamine treated groups regardless of the dose. This is in accordance with a study using the NMDA antagonist Mk-801 after focal ischemia in the rat, which also found a higher tissue HbO₂ in the postischemic cortex [4]. Interestingly, in that study the authors also found no change in the postischemic CBF induced by Mk-801 and could show that the effects on HbO₂ are due to a reduced oxygen extraction rate rather than a change in the oxygen supply. The authors indicate that blocking the NMDA receptor may reduce the increased postischemic oxygen consumption. Indeed, our saline and *R*(-) ketamine-treated groups show a decreased postischemic

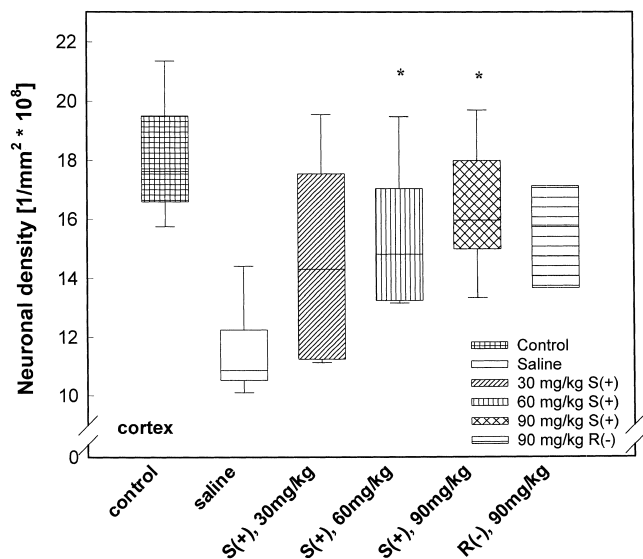


Fig. 3. Number of cortical neurons remaining after cerebral ischemia treated with saline or ketamine isomers in different doses (neurons/mm²·10⁸). Control shows sham treated animals. Data are depicted as mean±S.E.M., *=*P*<0.05 compared to saline treated animals.

HbO₂ throughout the observation period, whereas, the *S*(+) ketamine animals recovered completely. This, in turn, correlates with the significant neuronal cell protection in the cortex induced by *S*(+) ketamine.

The current data showed neuronal protection mostly in the cerebral cortex, whereas other structures such as hippocampus were less protected. This may explain why the horizontal rope test which among others tests motor coordination did not show more than a trend towards better performance. Other possibly non-protected brain areas (such as basal ganglia and the cerebellum) contribute to the overall performance in this test. Hence, this might be the reason for the failure to detect significant differences despite the protective properties of *S*(+) ketamine in the cortex.

The failure of 90 mg *R*(-) ketamine to show significant protective effects may in part be caused by a shorter half live and a lower availability of the drug compared to *S*(+) ketamine.

In conclusion, the present results demonstrate that systemic postischemic administration of *S*(+) ketamine has a positive effect on oxygen saturation and cortical neuronal cell death after global forebrain ischemia. The required high dosages did not show any toxicity. Moreover, *S*(+) ketamine seems to have a higher in vivo neuroprotective potency compared with its *R*(-) stereoisomer.

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