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The effect of eliprodil on the evolution of a focal cerebral ischaemia in vivo

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Abstract

The purpose of the present study was to evaluate in vivo the effect of a non competitive antagonist of the NMDA receptor, eliprodil, on the size of a focal ischaemic insult and on its temporal evolution in a rat model, using a spin-echo diffusion magnetic resonance imaging multislice technique. Rats were either injected with 1 mg/kg i.v. of eliprodil or with the vehicle only (placebo) 5 min after middle cerebral artery occlusion, or not injected (controls). Ten coronal slices were acquired every hour, up to 7 h after occlusion of the artery, and the volume of hyperintense signals was measured at each time point and for each animal. Diffusion magnetic resonance images revealed that the administration of eliprodil reduced significantly (by 50% or more) the volume of ischaemia, up to 7 h after occlusion, particularly in the cortex of the ipsilateral hemisphere. The results show the potential efficacy of eliprodil to reduce the cerebral ischaemic volume after arterial occlusion, thus confirming the interest of glutamate receptor antagonists in the treatment of ischaemia.

Keywords: Cerebral ischaemia; Middle cerebral artery occlusion; Diffusion magnetic resonance imaging; Eliprodil; NMDA receptor antagonist

1. Introduction

A cerebral ischaemic accident produces a cascade of interrelated events including a sudden decrease of intracellular pH, a drop of the energy stores followed by ionic imbalance, tissue depolarisation, release of excitotoxic amino acids and a massive cytoplasmic entry of Ca^{2+}, leading to the formation of cytotoxic oedema (for reviews see Miller et al., 1992; Siesjö, 1992a,b). Other mechanisms, among which the formation of free radicals (Carney et al., 1992), of polyamines (Dempsey et al., 1985, 1988) and the production of unsaturated free fatty acids, in particular arachidonate, responsible for the synthesis of prostaglandins and leukotrienes (Kirino, 1982; Dempsey et al., 1985), intervene during the ischaemic process and severely affect cell viability. In the absence of reperfusion, the ischaemic process leads, with time, to rupture of the blood–brain barrier and to cell death (for review see Hara et al., 1993).

The involvement of excitatory amino acids during ischaemia has led to the suggestion that blockade of ionotropic glutamate receptors with specific antagonists might have a valuable neuroprotective action (for review see Hossmann, 1996). In the case of the NMDA receptor, such neuroprotection has been sought for either by modulation of the receptor with non competitive antagonists such as channel blockers (e.g., 5-methyl-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclo-heptene-5,10-imine; MK 801) or its blockade with competitive antagonists such as the piperidine derivatives, 4-(3-phosphonopropyl)-2-piperazine-carboxylic acid (CCP; Boast et al., 1988) and *cis*-4-(phosphonomethyl)-2-piperidine-carboxylic acid (CGS 19755; Simon and Shiraishi, 1990). Among the non-competitive NMDA receptor antagonists, ifenprodil [(±)-(*R**,*S**)-α-(4-hydroxyphenyl)-β-methyl-4-(phenyl- methyl)-1-piperidineethanol-(*R*,*R*)-2,3-dihydroxybutenedioate (2:1) (salt), hemihydrate] and its derivative, eliprodil [(±)-α-(4-chlorophenyl)-[(4-fluorophenyl)methyl]-1- piperidineethanol], have been shown to occupy the polyamine site of the receptor (Carter et al., 1990) and to be effective to reduce the focal ischaemic cytotoxic oedema in the rat, mouse and

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cat (Gotti et al., 1988; Carter et al., 1988). The efficacy of these drugs, shown at late time after middle cerebral artery occlusion by infusion or using repetitive injections, relies upon their capacity to antagonize the neurotoxic effect of spermidine and spermine when these are present in the extracellular space. These polyamines should then act as possible modulators of the NMDA receptor (Paschen, 1992). Early blockade of the polyamine site of the receptor might then protect against the insult more efficiently.

To demonstrate the neuroprotective effect of a drug with standard techniques is cumbersome as individual animals have to be killed at different time points. In this respect, diffusion magnetic resonance imaging (D-MRI) is of special interest, first because it allows visualization of the insult and its follow-up in vivo (Moseley et al., 1990; Mintorovitch et al., 1991; Finelli et al., 1992; Benveniste et al., 1992; Sevick et al., 1992; Hoehn-Berlage, 1995) and, secondly, because it is accepted that the reduction of the apparent diffusion coefficient of water, due to the ischaemic insult corresponds to the development of the cytotoxic oedema (Hossmann et al., 1994; Perez-Trepichio et al., 1995). The non-invasiveness and the high spatio-temporal resolution of D-MRI, have allowed the use of this methodology to follow the action of pharmacological agents (Elger et al., 1994; Müller et al., 1995; Le Bars et al., 1996).

The present report deals with a study on the effect of eliprodil on the volume and the evolution of focal cerebral ischaemic infarcts in a rat model in vivo using D-MRI. The results will be discussed as tentative answers to the following questions: (a) whether eliprodil has any significant effect upon ischaemic volume after a single injection, (b) whether the drug effect persists during the whole experimental period (7 h) and (c) whether the drug has a potential efficacy of action upon the peripheral territory of the ischaemic insult.

2. Materials and methods

Twenty eight rats (8 treated, 10 placebos and 10 controls; Sprague–Dawley males, 260–380 g body weight) were used for the experiments in accordance with the directives of the French Ministry of Agriculture (decree No. 87-848, October 19, 1987, authorization No. 6549 to RM).

2.1. Surgery

Focal ischaemia was induced under anaesthesia (1% halothane) by middle cerebral artery occlusion according to the method of Koizumi et al. (1986) as modified by Roussel et al. (1994). The technique consists in the insertion of a nylon thread (0.17 mm thick, 20 mm long with an end bulb 0.3 mm thick and 3 mm long) into the external carotid artery and pushing it up to the origin of the middle cerebral artery via the internal carotid. Eliprodil (1 mg/kg, 0.1 ml/100 g body weight; Synthélabo, Bagneux) was injected into the femoral vein 5 min after the occlusion of the middle cerebral artery. Control animals were not injected and 'placebo' animals were injected with the vehicle solution only (1 ml/kg of 1 mmol/l citric acid in 5% glucose physiological saline solution).

2.2. Magnetic resonance imaging

The animals were positioned in the magnetic resonance magnet with a stereotaxic head holder, kept under anaesthesia (1% halothane) and maintained at a body temperature of 37°C with a heating blanket, throughout the MRI experiment. Imaging was performed in a horizontal magnet of 2.35 Tesla (40 cm bore diameter), interfaced with an MSL console (Bruker, France), and equipped with an unshielded 12 cm diameter gradient insert. A circular surface coil (2.5 cm diameter) was used for excitation as well as for detection. Images were acquired every hour up to 7 h post-occlusion of the middle cerebral artery ($i = 1–7$) with a spin-echo diffusion-weighted sequence (Echo time = 80 ms, Repetition time = 2000 ms, slice thickness = 1 mm, matrix 128×128, field-of-view = 30 mm, number of acquisition per phase encoding step = 8). The two diffusion sensitizing gradient pulses, 23 ms width, were applied in the read direction (along the X-axis, left–right). The time between the two rising edges was 33.5 ms and the gradient strength $b = 1200$ s/mm^2. In order to eliminate the potential influence of changes in T_2 of tissue water during development of the oedema, the diffusion-weighted sequence was alternated with a T_2-weighted spin-echo sequence (number of acquisitions = 4) using the same parameters without diffusion sensitizing gradient pulses. For each rat, two sets of 5 interleaved coronal slices, covering the whole ischaemic territory, were acquired. The total acquisition time was about 1 h.

2.3. Histochemical validation

At the end of the imaging procedure the animals were killed with an excess of halothane, the brains were removed and quickly frozen in isopentane cooled with liquid nitrogen for histochemical analysis. Brain slices (10 μm thick) were made with a cryotome (Leica, Germany) and stained according to Goldner's modification of Masson's trichrome reaction (Hould, 1984).

2.4. Post-processing and statistics

All magnetic resonance images were transferred to a SUN workstation. Image post-processing was performed using software from IDL (Research Systems, CO). Pure diffusion images were obtained on a pixel to pixel basis by dividing diffusion-weighted and T_2-weighted images. The effect of eliprodil treatment as a function of time was

assessed by determining, on each slice, the surface of the hyperintense area calculated from diffusion images and attributed to the ischaemic insult. The total ischaemic volume $V(t_i)$ was estimated as the sum of the hyperintense surfaces S_j ($j = 1–10$) times the slice thickness, delineated manually as regions of interest at each time point and for each image. The hyperintense surface area of the cortex was delineated manually on coronal slices and with comparison with the stereotaxic atlas of the rat (Paxinos and Watson, 1982). The subcortical volume was calculated as the difference between the total and the cortical volumes.

The statistical significance of differences was calculated by a multiple analysis of variance (MANOVA) for repeated measures, using SPSS/WIN + (SPSS, 6.1.3). The repeated measures were the volumes at each time point while the treatment regimens were used as groups. The analysis was performed twice, using either the control or the placebo animals as reference for the contrast comparison. The level of statistical significance was chosen at $P < 0.05$ and the test was one-sided.

3. Results

3.1. Validation of the MR images

Fig. 1 shows, 7 h after the occlusion of the middle cerebral artery, the hyperintense signals observed on the coronal magnetic resonance image of a control animal (Fig. 1a) and of an animal injected with eliprodil (Fig. 1c). The magnetic resonance images were similar to those obtained after histochemical staining from the same animals (Fig. 1b and d).

3.2. Volume of the ischaemic insult

Reduction of the total ischaemic volume was observed at each time point after injection of the drug ($n = 8$), whether compared to placebo ($n = 10$) or to control ($n = 10$) animals (Fig. 2). One hour after the occlusion of the artery, the total ischaemic volume in the eliprodil-treated animals was reduced by 56% and 45%, respectively, when compared to the placebo animals and to the controls (18.9 ± 4.2 mm^3 vs. 42.9 ± 14.0 mm^3 and vs. 34.4 ± 8.5 mm^3; mean $\pm$ S.E.M.). Seven hours after occlusion of the middle cerebral artery, the effect persisted at 60% and 49%, respectively (53.4 ± 15.6 mm^3 vs. 134.8 ± 38.4 mm^3 in the placebos and vs. 104.7 ± 25.2 mm^3 in the controls). When the statistical analysis was performed with MANOVA for repeated measurements, a significant difference for the evolution of the total ischaemic volume in treated and in placebo animals was found at all time points ($0.03 < P < 0.05$) with the exception of the 3rd hour post-occlusion ($P = 0.06$). A statistically significant difference was found between treated and control animals from 3 to 5 h post-occlusion ($P < 0.05$).

The ischaemic volume in the cortex of the eliprodil-treated animals, 1 h after artery occlusion, was reduced by 93% and 90%, respectively, when compared to placebo animals and to controls (0.8 ± 0.3 vs. 11.4 ± 4.8 mm^3 and

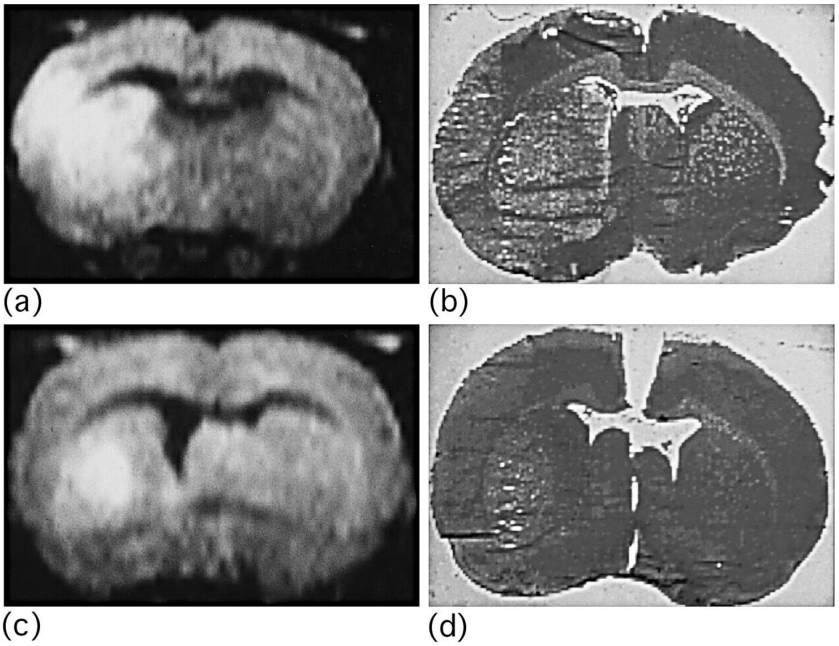

Fig. 1. Comparison between diffusion magnetic resonance images and histochemical staining. (a) Pure diffusion coronal image of a control animal showing a hyperintense signal corresponding to the occluded territory of the middle cerebral artery, 7 h after occlusion; (c) Similar coronal image in an eliprodil-injected animal; (b) Goldner's trichrome staining on a corresponding coronal section of the same animal shown in (a); (d) Goldner's trichrome staining on a corresponding coronal section of the same animal shown in (c). Magnification × 10.

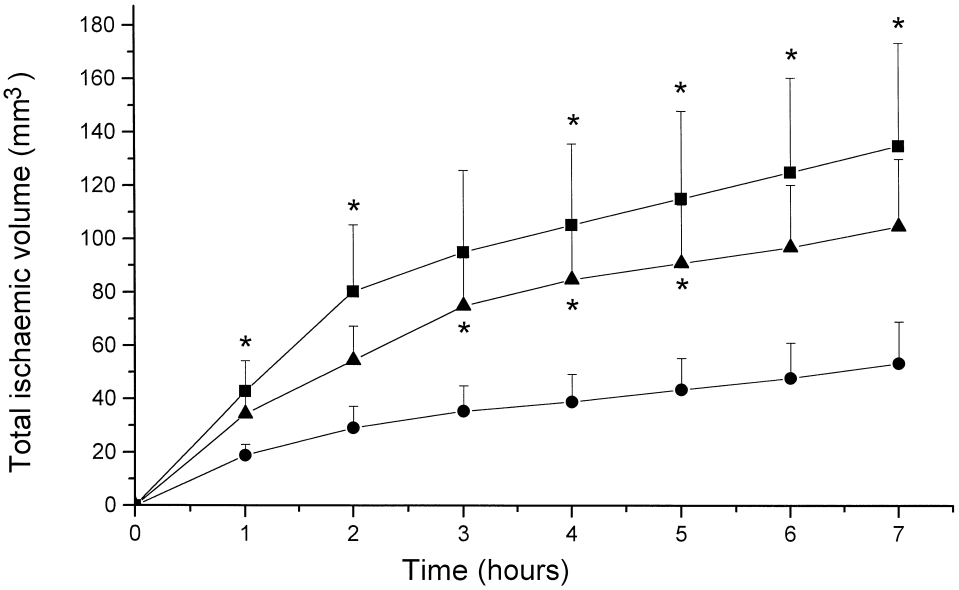

Fig. 2. Effect of eliprodil (1 mg/kg, i.v.) on the evolution of the magnetic resonance hyperintensities attributed to the global ischemic volumes. The results are presented as means ± S.E.M. The significance, determined by MANOVA, is shown by the asterisks ($P < 0.05$). Symbols: (■) placebo, (▲) control, (●) eliprodil-injected animals.

vs. 8.3 ± 4.7 mm^3; mean ± S.E.M.) (Fig. 3). Seven hours after the occlusion, the effect persisted at 85% and 78%, respectively (9.4 ± 4.1 mm^3 vs. 64.4 ± 26.0 mm^3 in the placebos and vs. 42.5 ± 14.9 mm^3 in the controls). Using MANOVA, a significant difference in the evolution of the ischaemic volume was found at all time points between the treated and the placebo groups, and from the 3rd to the 7th hour post-occlusion between treated and control animals.

The subcortical ischaemic volume in the eliprodil-treated group (Fig. 4) was reduced by 43% and 31%, respectively, when compared to the placebo and the control animals 1 h after middle cerebral artery occlusion (18.0 ± 4.0 vs. 31.6 ± 7.3 mm^3 and vs. 26.2 ± 7.1 mm^3; mean ± S.E.M.) and by 37% and 36%, respectively, 7 h after the artery occlusion (44.0 ± 11.6 vs. 70.5 ± 14.0 mm^3 and vs. 68.9 ± 14.3 mm^3). No statistically significant difference in size of the

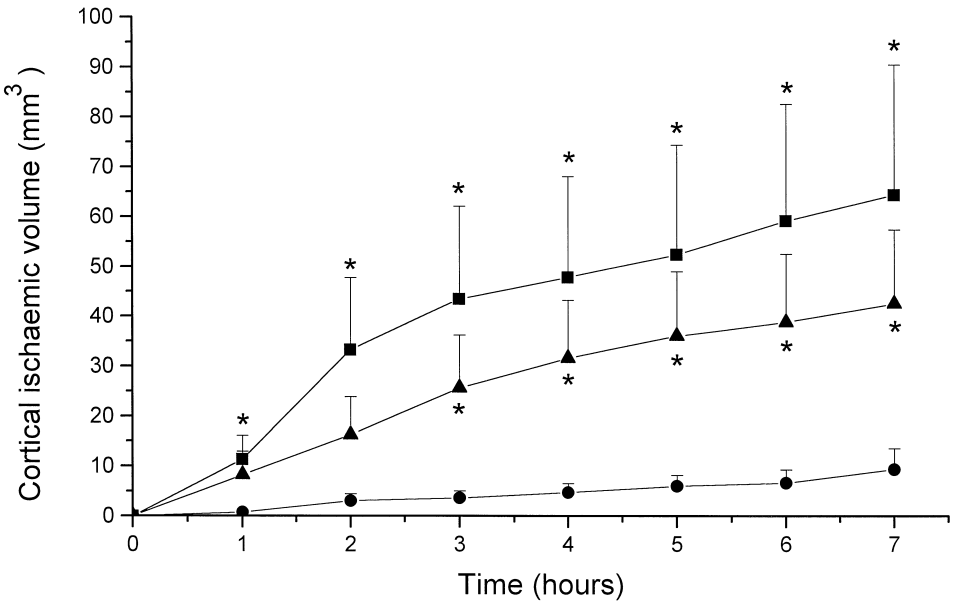

Fig. 3. Effect of eliprodil (1 mg/kg, i.v.) on the evolution of the magnetic resonance hyperintensities attributed to the cortical ischemic volumes. The results are presented as means ± S.E.M. The significance, determined by MANOVA, is shown by the asterisks ($P < 0.05$). Symbols: (■) placebo, (▲) control, (●) eliprodil injected animals.

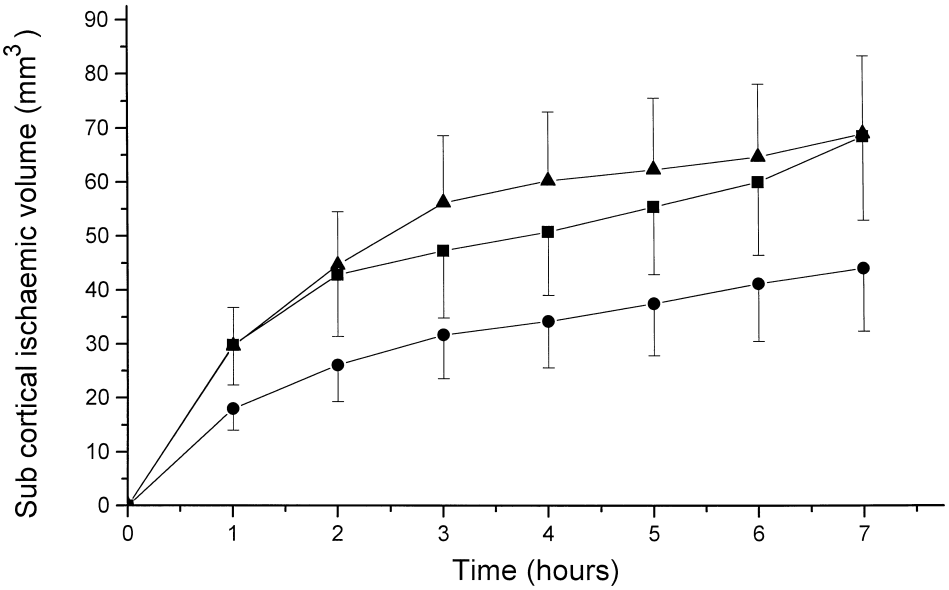

Fig. 4. Effect of eliprodil (1 mg/kg, i.v.) on the evolution of the magnetic resonance hyperintensities attributed to the subcortical ischemic volumes. The results are presented as means ± S.E.M. Symbols: (■) placebo, (▲) control, (●) eliprodil-injected animals.

subcortical ischaemic volumes between treated and either of the untreated groups was found on MANOVA.

4. Discussion

The present data indicate that, in a focal irreversible ischaemia model, eliprodil decreased the total volume of infarction, as determined by the reduction in the volume of diffusion abnormalities. One hour after the occlusion of the middle cerebral artery, comparison of the infarction volume between animals treated with the drug and either the placebo or the control groups indicated a possible neuroprotective effect of this NMDA receptor antagonist, as previously suggested (Gotti et al., 1988). Multiple Analysis Of Variance (MANOVA) revealed that the evolution with time of the ischaemic volume was significantly different in treated animals and in placebo or control animals as regards to total volume and, particularly, cortical ischaemic volume. It has to be stressed that for the time points for which the difference between the treated and untreated groups did not reach statistical significance, the P value was less than 0.1. However, it is likely that the small sample size and the important variability in the development of infarction were responsible for a reduction of the statistical power.

In spite of the variability, the sequential magnetic resonance images obtained in this study allowed us to follow the effect of the drug during the whole experimental period and to observe that the efficacy of a single injection of eliprodil appeared as soon as the 1st hour and persisted during the 7 h of the experiment.

The data correlate well with those from other reports showing that blockade of the glutamate receptor(s) of the NMDA or the α-amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA) type results in reduction of the ischaemic insult. Non-competitive NMDA receptor antagonists such as ifenprodil, its derivative, eliprodil, and MK-801 were shown in some reports to be effective to reduce the cytotoxic oedema (Gotti et al., 1988; Duval et al., 1992 and Lo et al., 1994, respectively). These compounds have different sites of action, the chloride channel in the case of MK-801 (Gill et al., 1996) and the polyamine site for eliprodil and ifenprodil (Carter et al., 1988, 1990). Other experimental compounds, competitive antagonists of the NMDA receptor, have been studied in various ischaemic models. These compounds, [*R*]-4-oxo-5-phosphonorvaline (MDL-100453; Hasegawa et al., 1994), CPP (Boast et al., 1988), D-*E*-2-amino-4-methyl-5-phosphono-3-pentenoic acid (CGP 40116; Sauer et al., 1993), CGS 19755 (Simon and Shiraishi, 1990), 2*R*,4*R*,5*S*-(2-amino)-4,5-(1,2-cyclohexyl)-7-phosphonoheptanoic acid (NPC 17742; Nishikawa et al., 1994), all have comparable pharmacological effects upon the size of the ischaemic oedema.

It appears, moreover, that the main effect of NMDA receptor antagonists, as shown for MK 801 in particular, may be directed to modulation of the still functioning vasculature in the ischaemic peripheral territory as suggested by an increased regional cerebral blood flow (Roussel et al., 1992; Torregrosa et al., 1994). Comparatively, antagonists of the AMPA/kainate receptor, such as 2,3-dihydroxy-6-nitro-7-sulfamoylbenzol(F)quinoxaline (NBQX) and the benzodiazepine derivative, 1-(4-aminophenyl)-4-methyl-7,8-methylene-dioxy-5*H*-2,3-benzodiazepine hydrochloride (GYKI 5246), have been shown to be effective

12–24 h after global (Buchan et al., 1991; Le Peillet et al., 1992) and focal (Gill et al., 1992; Smith and Meldrum, 1992; Xue et al., 1994) ischaemia without a negative influence upon blood flow.

Discussion of the present results should consider the possibility of a preferential topographical site of action for eliprodil. Previous results have shown that, in this type of ischaemic model, NMDA receptor antagonists may act more efficiently upon the cortical portion of the ischaemic lesion (Lo et al., 1994; Gill et al., 1996). Two patterns of ischaemic development were observed in the present experiments in the placebo and control groups: some animals developed global (subcortical and cortical) infarction and others had a limited subcortical infarction only. This outcome was unpredictable under the present experimental conditions, but certainly is sufficient explanation for the variability of the model. The difference in the significance level of eliprodil action on the ischaemic volumes measured in the cortical and in the subcortical areas indicates that the effect of the NMDA receptor antagonist was more important in the cortex. It also suggests that the persistence of a residual blood flow, probably due to arterial anastomosis and/or collateral recruitment, delayed the occurrence and lessened the severity of the ischaemic process, giving more opportunities for drug intervention. It should also be emphasized that, in this experimental model, the ischaemic insult first appears subcortically at the striatal level and eventually reaches the cortex in both antero-posterior directions. The action of a neuroprotective agent in the later reached cortex should thus be expected to be more effective.

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