Available online at www.sciencedirect.com
ScienceDirect
Neurochemistry International 49 (2006) 442–447
NEUROCHEMISTRY International
www.elsevier.com/locate/neuint

Therapeutic window of bradykinin B_2 receptor inhibition after focal cerebral ischemia in rats

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Received 7 November 2005; received in revised form 16 February 2006; accepted 20 February 2006
Available online 19 April 2006

Abstract

Following cerebral ischemia bradykinin/kinin B_2 receptors mediate inflammatory responses resulting in edema formation and secondary brain damage. However, the therapeutic window for B_2 receptor inhibition determining its potential clinical use has not been investigated so far. The aim of the current study was therefore to investigate the effect of delayed B_2 receptor inhibition on morphological and functional outcome following experimental stroke.

Rats were subjected to 90 min of middle cerebral artery occlusion (MCAo) by an intraluminal filament. Animals received 0.9% NaCl or 1.0 mg/kg/day Anatibant (LF 16-0687 Ms), a selective bradykinin B_2 receptor antagonist, for 3 days beginning at different time points after MCAo: 1, 2.5, 4.5, or 6.5 h ($n = 10$ per group). Neurological recovery was examined daily, infarct volume on day 7 after MCAo.

Animal physiology was not influenced by B_2 receptor inhibition. Significant improvement of functional outcome was observed when treatment was delayed up to 4.5 h after ischemia ($p < 0.05$ versus vehicle). Inhibition of B_2 receptors during ischemia, i.e. when the inhibitor was given 1 h after MCAo, reduced infarct volume in the basal ganglia and in the cortex by 49% ($p < 0.05$) and 26% ($p < 0.05$), respectively. Inhibition of B_2 receptors at later time points (2.5, 4.5, or 6.5 after MCAo) reduced penumbral damage, i.e. cortical infarction, by 19–26% ($p < 0.05$).

In conclusion, the current study shows that the therapeutic window of B_2 receptor inhibition extends for up to 6.5 h after MCAo. Our data therefore suggest that inhibition of kinin B_2 receptors represents a treatment strategy for ischemic stroke which may warrant clinical validation.

Keywords: Focal cerebral ischemia; Stroke; Kallikrein–kinin system; Bradykinin; Kinin receptors; Bradykinin B_2 receptor; Inhibition; Rats

1. Introductory statement

Ischemic stroke is the third leading cause of death and the main reason for severe disabilities in the industrialized world (Chalela et al., 2004). To date thrombolysis is the only approved treatment for ischemic stroke, however, over 95% of patients do not benefit from this therapeutic option, most frequently due to late hospital admission (The National Institute of Neurological Disorders and Stroke, 1995). Accordingly, novel therapeutic options preventing ischemic brain damage or delaying neuronal cell death until thrombolysis can be performed are at urgent clinical need.

Bradykinin is a nona peptide found in blood and tissue, which is cleaved from its pro-form kininogen by the protease kallikrein. Bradykinin has a half-time of less than 30 s and binds predominantly to kinin B_2 receptors, which are constitutively and ubiquitously expressed on mammalian cells, including neurons (Raidoo et al., 1996; Chen et al., 2000; Groeger et al., 2005). Activation of kinin B_2 receptors results, among others, in an increased vessel permeability and vasodilatation (Couture et al., 2001). Particularly due to these properties bradykinin and kinin B_2 receptors have been investigated for their ability to promote brain edema formation in various models of CNS injury, e.g. bacterial meningitis (Lorenzl et al., 1996), traumatic brain and spinal cord injury (Maier-Hauff et al., 1984; Unterberg et al., 1984; Unterberg

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0197-0186/$ – see front matter
doi:10.1016/j.neuint.2006.02.010

et al., 1986; Marmarou et al., 1999; Pruneau et al., 1999a; Stover et al., 2000; Plesnila et al., 2001; Gorlach et al., 2001; Pan et al., 2001; Kaplanski et al., 2002; Kaplanski et al., 2003; Hellal et al., 2003), and global cerebral ischemia (Kamiya et al., 1990, 1993; Kamiya, 1990; Lehmberg et al., 2003). In experimental stroke bradykinin brain levels are significantly elevated after reperfusion and kinin B_2 receptors are upregulated on dying neurons (Groeger et al., 2005). Kinin B_2 receptor knock-out mice show an almost 90% reduction of post-ischemic brain edema formation, less post-ischemic brain damage, and survive significantly longer than wild-type control mice (Groeger et al., 2005). Several studies using pharmacological bradykinin B_2 receptor antagonists demonstrated reduced brain edema and infarct formation following transient and permanent occlusion of the middle cerebral artery in mice and rats (Relton et al., 1997; Zausinger et al., 2002; Ding-Zhou et al., 2003). Although these studies provided important information on the role of kinin B_2 receptors for the pathophysiology of cerebral ischemia, inhibition of bradykinin B_2 receptors was not initiated later than 30 min after induction of cerebral ischemia, leaving the question for the potential clinical relevance of bradykinin B_2 receptor inhibition open. The aim of the current study was therefore to investigate the therapeutic window of bradykinin B_2 receptor inhibition following experimental stroke.

2. Experimental procedures

Male Sprague–Dawley rats (250–300 g body weight, Charles River Laboratory, Sulzfeld, Germany) were used for the current study. Before and during experiments animals were cared for in compliance with institutional guidelines of the University of Munich. All experiments were approved by the Animal Ethics Committee of the Government of Upper Bavaria, Germany.

2.1. Preparation, monitoring and surgery

Animals were fasted with free access to water the night before surgery. Rats were shortly anaesthetized with ether, intubated and mechanically ventilated as previously described (Zausinger et al., 2002). Anesthesia was maintained with a mixture of 1% halothane, 66% nitrous oxide, and 33% oxygen. Rectal and temporal muscle temperatures were recorded and used to maintain body and brain temperatures at 37.0 °C using thermostatically regulated feedback-controlled devices. The tail artery was cannulated for blood sampling, blood pressure monitoring, and administration of fluids and drugs. Blood gases, serum glucose, lactate, pH, and electrolytes were measured before, during, and after ischemia. Haemodynamic variables were monitored for 20 min after ischemia. Local cerebral blood flow (LCBF) of the middle cerebral artery territory was measured by laser-Doppler fluxmetry (LDF) (MBF3D, Moor Instruments, UK). For placement of the two laser-Doppler flow probes burr holes were made on each side of the skull over the somatosensoric cortex (5 mm lateral and 1 mm posterior of bregma) leaving the dura mater intact. Thereafter rats were placed in a supine position and immobilized using a stereotactic frame. LCBF was measured continuously during the whole duration of the experiment. The middle cerebral artery (MCA) was occluded using a 4-0 silicone-coated nylon monofilament inserted via the external carotid artery. Sufficient occlusion of the MCA was monitored by LDF (decrease of ipsilateral LCBF to 20–30% of baseline). Reperfusion was initiated by withdrawing the filament after 90 min.

2.2. Drug administration and treatment arms

Anatibant (LF 16-0687 Ms), the mesylate salt of (1-[[2,4-dichloro-3-[[(2,4-di-methylquinolin-8-yl)oxy] methyl] phenyl] sulfonyl]-*N*-[3-[[4-(aminoiminomethyl)phenyl] carbonylamino] propyl]-2(*S*)-pyrrolidinecarboxamide, MW 989) was stored as a powder at 4 °C and freshly dissolved in 0.9% NaCl daily. Anatibant (1 mg/kg body weight) was applied for 3 days by two daily s.c. injections ensuring constant blood levels of the drug.

Animals were randomly assigned to one of the five following treatment arms ($n = 10$ each): (1) vehicle treated controls (0.9% NaCl, handled as group 2), (2) $t = 1$ h (first dose of Anatibant Ms administered 1 h after MCAo, i.e. 30 min before reperfusion), (3) $t = 2.5$ h (first dose of Anatibant Ms administered 1 h after reperfusion), (4) $t = 4.5$ h (first dose of Anatibant Ms administered 3 h after reperfusion), and (5) $t = 6.5$ h (first dose of Anatibant Ms administered 5 h after reperfusion). The second dose was applied on the day of surgery six hours after the first dose. The next doses were given in the morning and in the afternoon of the following 2 days.

2.3. Quantification of functional outcome

A modified, six-point Bederson-test was performed from the first to the seventh day after surgery by an investigator blinded towards the treatment of the animals. All tests were performed in the morning and each rat was tested at the same time of the day. Zero points were given if the animal did not show any movement, one point for spontaneous circling, two points if circling appeared only after lifting the rat by the tail, three points for an asymmetry in fore paw strength, four points for an asymmetry in fore paw movement, and five points for normal behavior (Zausinger et al., 2002). Additionally, the body weight was measured daily.

2.4. Quantification of ischemic tissue damage

Seven days after MCAo animals were re-anaesthetized and brains were fixed by transcardial perfusion with 2% paraformaldehyde. Twenty sections with a thickness of 5 μm were prepared at 400 μm intervals throughout the MCA territory and stained with hematoxylin and eosin. The area of each hemisphere and the area of the infarcted cortex and basal ganglia were determined by digital planimetry (OPTIMAS 5.1, BioScan Inc., Edmonds, WA, USA). Absolute infarct volumes (V) were calculated using the following formula (A_n = area of infarcted tissue on section n; d = distance between sections = 0.4 mm):

$$V = d[A_1 + \ldots + A_{20}]$$

In order to correct for differences in brain size and hemispheric swelling, ischemic brain damage was expressed as percentage of the contralateral hemisphere.

2.5. Statistical analysis

Data are presented as mean ± standard deviation (S.D.) if not otherwise indicated. All data were analyzed using Kruskal–Wallis ANOVA on Ranks followed by Dunnet's procedure for post hoc analysis using Sigma Stat 2.0 Statistical Software (Jandel Scientific, San Rafael, California, USA). Statistical significance was assumed at $p < 0.05$.

3. Results

3.1. Mortality and physiological data

Six animals equally distributed among all experimental groups died within the first 24 h after MCAo due to subarachnoidal hemorrhage.

Physiological parameters (blood pressure, blood gases, serum glucose, lactate, pH and electrolytes) were within the normal range and not different between groups (representative data for the control and the 1 h treatment group are given in Table 1). Post-ischemic hyperthermia, a potential shortcoming of the currently used ischemia model, was not observed.