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Ketanserin reduces the postischemic EEG and behavioural changes following Endothelin-1-induced occlusion of the middle cerebral artery in conscious rats

Research Article

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Abstract: We modeled the common clinical conditions of human stroke in fully conscious rats through an occlusion of the middle cerebral artery (MCAO) by means of unilateral microinjection of Endothelin-1 (ET1) in the vicinity of the artery (EMCAO model). Since the role of serotonin (5-HT) system in the regulation of the cerebral blood flow has been known for long time and no data are available at present for the effects of 5-HT antagonists in focal ischemia models, we further tested whether a blockade of the serotonin-2A (5-HT2A) receptors by ketanserin (20 min post-ET1) would diminish the late EMCAO-induced functional and morphological changes. The long-term neurological (postural reflex) and electroencephalogram (EEG) changes in the somatosensory cortical region (S1FL) were used to assess the effects of ketanserin on the post-ischemic changes. The study was supplemented by a histopathological examination of S1FL area and striatum of both hemispheres. The EMCAO/ ketanserin-treated rats showed much smaller neurological deficits than the EMCAO rats treated with vehicle. This effect was observed on day 3 and lasted until the end of experiments - 14 days after EMCAO. The depression of alpha and beta EEG frequencies found after EMCAO was significantly and earlier restored following ketanserin. Notably, there was not augmentation of the pathological slow EEG waves at day 3 post-ET1 in the EMCAO ketanserin-treated rats compared with that observed in the EMCAO vehicle-treated rats. Although there were mild morphological changes in the penumbral S1FL cortical region after EMCAO, ketanserin reduced the histopathological difference between the ipsilateral and contralateral cortical S1FL regions, but did not change the difference between striatum of both sides. Ketanserin reduced the infarct size in ipsilateral hemisphere (mainly cortex). In conclusion, the results showed that treatment with ketanserin at the early stage of stroke may reduce the consequences of ischemia by improvement of functional and morphological recovery at later stages. Ketanserin appears to be a promising candidate for mitigating the consequences of stroke.

Keywords: *Cerebral ischemia • EEG • Endothelin-1 • Ketanserin • Neurological assessment • Conscious rat*

1. Introduction

Increased knowledge of the complex pathophysiology of the cerebral ischemic stroke has led to the development of a great number of candidates for neuroprotective drugs with different mechanisms of action. Most of these drugs are designed to decrease the central responses to excitatory amino acids (e.g., glutamate) by attenuating their excessive release or by blocking their receptors. The glutamate antagonists, however, have a number of adverse effects: agitation, altered sensory perception and hallucinations, disorientation, learning and memory impairments [1]. For this reason, new pharmacological approaches for neuroprotection of brain tissue after stroke are now demanded. Along with the increased release of excitatory amino acids during ischemia, the pathophysiology of stroke is also characterized by abnormal release of other neurotransmitters, e.g.,

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dopamine, noradrenaline, and serotonin (5-HT), with that of serotonin being the most considerable [2]. The role of the 5-HT system in the regulation of the cerebral blood flow has been known for long time [3,4]. It is well documented that 5-HT exerts a vasoconstriction mediated mainly through 5-HT_2 receptors and particularly, through the 5-HT_{2A} receptors [4]. The antagonists of the 5-HT_2 receptors are used now in the clinics for treatment of hypertonic and Burger diseases, some psychiatric disorders and sleep disturbances [5-6]. However, data for their use as neuroprotective drugs in human acute cerebrovascular disorders are lacking. 5-HT_2 receptor antagonists have been shown to have neuroprotective properties in models of global and forebrain transient ischemia [7-10]. However, in models of focal ischemia, the data about their effects are not sufficient (obtained only in models of permanent focal ischemia) and contradictory [11-13]. Importantly, animal models of focal transient ischemia are the most relevant to the pathophysiology of human stroke, since reperfusion frequently occurs after stroke as a result of recanalization. These models can thus provide information about the dynamics of the post-ischemic changes, and may have an important predictive value for the outcome and treatment of stroke. Such a pharmacological model on rats has been introduced as a reliable tool towards understanding the pathophysiological mechanisms of human stroke [14-16]. In this model, occlusion of the middle cerebral artery (MCAO) was induced in rats via intracerebral microinjection of Endothelin-1 (ET1) – the so-called EMCAO model. While some consequences of EMCAO on blood perfusion, behavior and histopathology have been explored in certain extent [14-21], the corresponding changes of bioelectrical activity are less investigated [22-25]. The brain electrical signals are in the list of mandatory outcome measures according to the recommendations from the first Stroke Academic Industry Roundtable meeting for the preclinical evaluation of neuroprotective and recovery enhancing drugs [26]. The rational of the present study was to examine whether a blockade of the 5-HT_2 receptors by means of ketanserin (mainly 5-HT_{2A} receptor antagonist [5,27]) administered in the acute phase of MCAO-induced ischemia would diminish the functional deficits and histopathological changes in a rat model of focal transient cerebral ischemia.

2. Material and Methods

2.1. Animals and surgery

The experiments were performed on 47 male Wistar rats (270-310 g) according to a protocol approved by the Local Research Ethical Committee. The details of surgery, MCAO, neurological assessment, EEG recording and analysis are published elsewhere [24]. Briefly, under anaesthesia, the rat was placed in a stereotaxic device (Narishige Sci. Instr. Lab., Tokyo) and secured in flat skull position. A 21-gauge stainless steel guide cannula was implanted in the left hemisphere 1mm dorsal to the piriform cortex (A+0.2 mm, L5.2 mm and D7.0 mm below the surface scalp, all referred to bregma [28]), and was used later for insertion of an injection needle for ET1 delivery in the vicinity of MCA. A 27-gauge dummy rod was inserted into the lumen of the guide cannula to maintain the guide passable. For EEG recordings, a small stainless steel screw electrode 1.2 x 2.0 mm was fixed epidurally above the S1FL (somatosensory cortex, forelimb area) at 1.2 mm anterior to bregma and at 4.0-4.5 mm lateral to midline [28]. Another two miniature stainless steel screws, one fixed on the skull above frontal bone and the other, posterior to lambda, were used for ground and common reference, respectively. Two wires were inserted into the dorsal neck muscles for recording muscle activity and rat movements. Then, a mound of self-curing resin Duracryl (Spofa Dental, Prague, Czech Republic) was built up around the cannula, screws, and wires forming a cap to secure them to the skull together with the female connector.

2.2. MCA occlusion

The occlusion of MCA was performed by microinjection of Endothelin-1 (ET1, human/porcine Endothelin, Sigma Chemical Company, St. Louis, MO, USA), via a 27-gauge needle inserted into the implanted guide cannula and protruded 1.0 mm from its tip. ET1 was prepared as a 60 pmol solution in 3 µl sterile saline and was intracerebrally delivered in unanaesthetized wake rat by means of a Hamilton syringe (Hamilton Co., Reno, Nevada) connected to the injection needle by a short polyethylene tubing.

2.3. Chronic EEG recording and EEG spectral analysis

Recording of EEG started 7-10 days after surgery and was performed on entirely conscious rat. Two baseline recordings were made for each animal during two consecutive days. Another, third baseline EEG recording was made immediately before ET1. EEG epochs of 4-s

Table 1. Neurological deficit scoring in posture reflex/hang test.

Deficit	Score	Description
No	3	Normal posture
Slight	2	Contralateral forelimb flexion
Moderate	1	Contralateral forelimb flexion + Reduced resistance to lateral pushing toward the paretic side
Severe	0	Contralateral forelimb flexion + Reduced resistance to lateral pushing toward the paretic side + Body twisting or circling toward the paretic side

duration were chosen off-line from 10-min EEG records in each session, and were afterwords stored in computer files. Calculation of the power spectra was performed by means of fast Fourier transformation (FFT) (program 1t of the BMDP Statistical Software, Los Angeles, 1990). Spectral densities were computed in each 0.25 Hz bin.

2.4. Assessment of neurological function

The neurobehavioral test, which we have previously described in detail [22,24] represented the modified postural reflex/hang test developed by Bederson et al. (1986) [29]. Briefly, the degree of abnormal upper body posture was assessed by suspending rat by the tail and observing twisting of the thorax and extension of forelimbs. Then, rat was placed on a table with the rat's tail held by hand and resistance to ipsilateral and contralateral lateral pressure applied behind the shoulder was assessed. The following scale of scoring was used: 3, no deficit; 2, slight deficit; 1, moderate deficit; and 0, severe deficit. A description of behavioural scoring is given in Table 1. All behaviors were scored in a blind fashion.

2.5. Experimental Protocol

The experimental groups are described in Table 2. Three animals out of 47 died during implantation of the electrodes and the cannula, mainly due to anaesthesia. During the infusion of ET1, the mortality rate was 2.2%, or one animal out of 44. Of the remaining 43 animals, 4 fulfilled the exclusion criteria and were considered non-ischemic because they did not demonstrate any of the above described neurological deficits during the initial period (0-20 min) after the ET1 infusion, and were therefore discarded. Of the remaining 39 ischemic rats, 15 were treated intraperitoneally (i.p.) with ketanserin tartrate (Tocris Cookson Ltd., UK), and 24 with the vehicle of ketanserin, distilled water. The drug was injected in a dose of 6 mg/kg i.p. in a volume of 2 ml/kg of body weight. We choose this dose, which is bigger than it is necessary for 5-HT_{2A} receptor blockade [5] with

Table 2. Experimental groups.

Animal number			Remained
Before surgery	47		
Died during surgery	3		44
Died during the ET infusion	1		43
Excluded from further analysis: had no neurological deficits 0-20 min post-ET1	4		39
Rats included in different analysis(total number 39)	EEG recording, neurological assessment and histology (methyleneblau)	Histology: TTC or Cresyl-violet staining(without EEG electrodes)	
Ketanserin-treated	8	7	15
Vehicle-treated	15	9	24
Total	23	16	39

the presumption that this dose is in the range of those shown to exert neuroprotective effects in other models of global and forebrain ischemia in rats (1 to 20 mg/kg) [8,9,13].

Twenty three rats (out of 39) were implanted with electrodes. Of these, 8 were treated with ketanserin and 15 were treated with its vehicle. The experiment was divided into two parts: First, 3 μl of saline (vehicle of ET1) was infused in the vicinity of MCA, followed by i.p. injection of ketanserin 20 min later. Second, after an interval of 15-16 days, the same rats were rendered ischemic by intracerebral infusion of ET1, followed again by an i.p. administration of ketanserin. Thus, we were able to compare the effects of EMCAO/Ketanserin on EEG and behavioral output measures to those of Saline/Ketanserin applied to the same animals and measured at the same time points. In the subgroup of animals treated with vehicle and equipped with electrodes (number15), an identical procedure was conducted with the differences that the animals were injected intraperitoneally with distilled water instead of ketanserin.

The EEG changes and the neurological deficits after EMCAO in each group of rats were assessed in 12 time sessions: H1 (1h), H4 (4h), D1 (day 1), D3 (day 3-4), D7 (day 7-8), D14 (day 14-15) after saline infusion, and the same 6 sessions were used to test the same rats after ET1 infusion.

2.6. Histology

Following the last experimental session (14-15 days after ET1), the rats with EEG electrodes (number 23) were deeply anesthetized with Thiopental (Biochemic GmbH) and then 3 µl of Methylenblau HCl (SERVA, Heidelberg) as a 5% solution in distilled water were delivered via the injection needle inserted into the guide cannula. The brains were removed, stored in formalin, and after about 5 days, cut into sections and examined for the location of the blue spot. It was found to be in the piriform cortex in the vicinity of the MCA [28,30].

In a subgroup of 16 rats (out of 39), morphological examination of the brain tissue was performed 3 days post-ET1 either by means of 2,3,5-triphenyltetrazolium chloride (TTC) staining (8 rats) or cresyl violet (CV) staining (8 rats). The procedure for the TTC staining and the quantification of brain volume has been previously described in details [24]. Total ischemic volume in the ipsilateral hemisphere was determined in percentage of the volume of the contralateral (control) hemisphere. For the CV staining, the rats were transcardially perfused with 150 ml saline, followed by 500 ml 4% paraformaldehide in 0.1M phosphate buffer, pH 7.2. The brains were placed in the same fixative, to which 20% sucrose was added for 2-20 days. Sections of 30-µm thickness were cut in the coronal plane with a freezing microtome. The sections were mounted and stained with 0.5% cresyl violet.

2.7. Statistical analysis

The EEG spectral densities for all epochs in a session were averaged for each rat, each time session, and each treatment condition (Saline/Vehicle, Saline/Ketanserin, EMCAO/Vehicle, and EMCAO/Ketanserin). The General Linear Model procedure was used for statistical analysis of the EEG data (Statistica 6.0, Stat Soft. Inc., Tulsa, U.S.A., 2001). Three-way Hotelling-Lawley MANOVAs were made with repeated measures on two factors for each 0.25 Hz bin of the EEG spectra (from 1 to 32 Hz). Factors were: (1) “Ketanserin” as a between-subject factor with two levels: ‘Vehicle’ and ‘Ketanserin’; (2) “EMCAO” as a first within-subject factor with two levels: ‘Saline’ and ‘ET1’; and, (3) “Time” as a second within-subject factor with 7 levels (‘T0’, ‘H1’, ‘H4’, ‘D1’, ‘D3’, ‘D7’, and ‘D14’). Multiple comparison tests (Hotelling F-contrasts for LS means at 95% significance level) were performed for each 0.25 Hz bin of the power spectra and for each pair of treatment conditions: EMCAO/Vehicle vs. Saline/Vehicle, Saline/Ketanserin vs. Saline/Vehicle, EMCAO/Vehicle vs. EMCAO/ketanserin, and EMCAO/Ketanserin vs. Saline/Ketanserin. *P*-values of less than 0.05 were considered statistically significant.

Neurological scores were analyzed with nonparametric statistical procedures on ranks (Statistica 6.0, Stat Soft. Inc., Tulsa, U.S.A., 2001): Friedman and Kruskal-Wallis ANOVA followed by post hoc analysis with Wilcoxon matched pairs test (to compare rats treated with Saline/Vehicle and EMCAO/Vehicle) and Mann-Whitney U-test (to compare EMCAO/Vehicle rats with EMCAO/Ketanserin rats).

The comparison between the volume of infarct in vehicle- and ketanserin-treated rats was performed using Mann-Whitney U test.

3. Results

3.1. EEG data

EMCAO produced a pronounced development of EEG changes in S1FL cortex, ipsilateral to the hemisphere to where ET1 was delivered (Figure 1). MANOVAs (for each of the 125 bins of the EEG spectra) (Figure 1A) showed that the effect of ET1 strongly depended on time: significant interactions “EMCAO” x “Time” for many frequencies except for the fastest ones. The “EMCAO” x “Ketanserin” x “Time” interactions were statistically significant for frequency bands in 5.75-10.5 and 12.5-16.0 Hz meaning thus that the effects of EMCAO for these EEG frequencies depended on both time and post-ET1 treatment (ketanserin or its vehicle). The post-hoc analysis showed clear evolution of EEG changes in time (Figure 1B-G). At H1 (1 hour post-ET1, Figure 1B) and H4 (4 hours post-ET1, Figure 1C) the cortical EEG power densities in EMCAO/Vehicle rats dropped with about 20% of their pre-ischemic power (for most of EEG frequencies). At day 1, the fast waves were still depressed (Figure 1D). At day 3, (Figure 1E) the slow waves began to recover and even to augment when compared with Saline/Vehicle rats. Thereafter, at day 7 (Figure 1F), some restoration of these EEG changes occurred, but no complete resolution of the averaged spectral EEG profile was seen until day 14-15 after ET1 (Figure 1G). No electrographic seizures were observed during the entire period of experiments.

Ketanserin did not influence the early changes (at H1 and H4) in EEG induced by EMCAO. On the next day (Figure 1D), however, ketanserin was able to eliminate the suppression by ET1 of the fast EEG waves (contrasts EMCAO/Vehicle – EMCAO/Ketanserin were above the critical F-values for frequencies 12.5-32.0 Hz). At day 3 (Figure 1E), the EEG theta and slow alpha waves in EMCAO/Ketanserin rats were significantly attenuated as compared to those in EMCAO/Vehicle rats: EMCAO/Vehicle – EMCAO/Ketanserin contrasts were above the

Figure 1. Effects of intracerebral injection of ET1 (60 pmol) in the vicinity of left MCA in conscious rats on the EEG power spectra in the left somatosensory cortex, forelimb region (SIFL) during quite waking and the effects of ketanserin (6 mg/kg i.p.) administered 20 min after ET1. At **A** – MANOVA results for each 0.25 Hz-bin of the power spectra from 1 to 32 Hz expressed as log Fisher (F)-values (vertical lines). Only frequency bins where the main effects of factor "EMCAO", factor "Ketanserin" and their interactions with factor "Time" were significant at $P < 0.05$ were depicted. At **B, C, D, E,** and **F** – EEG spectral profiles: EEG power density values for each bin of 0.25 Hz from 1 to 32 Hz expressed as percentage of the corresponding values before treatment (or combination of treatments) and plotted on a logarithmic scale. The horizontal line at 2.0 represents the corresponding pre-treatment values taken as 100%; H1, H4, D1, D3, D7, and D14 are time sessions after treatments as follows: 1h; 4h; day 1; day 3; day 7; day 14. Four horizontal lines with vertical bars below each group of EEG spectral profiles at B, C, D, E, and F indicate those frequency bins where statistical significance was achieved in comparisons between each pair of the four conditions (from top to bottom): EMCAO/Vehicle vs. Saline/Vehicle, Saline/Ketanserin vs. Saline/Vehicle; EMCAO/Vehicle vs. EMCAO/ketanserin, and EMCAO/Ketanserin vs. Saline/Ketanserin (The bars are log-transformed Fisher's PLSD comparisons, $p < 0.05$).

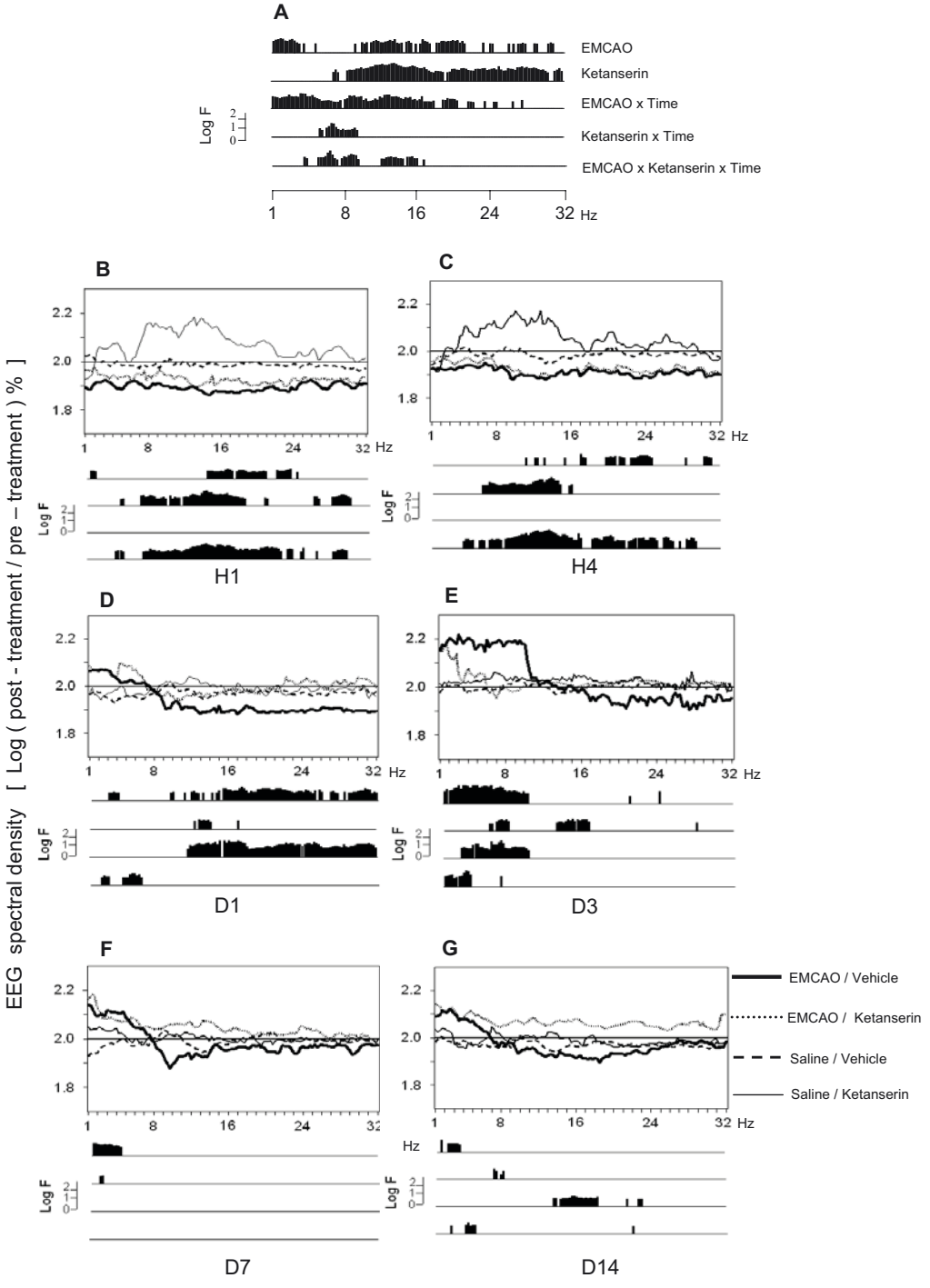
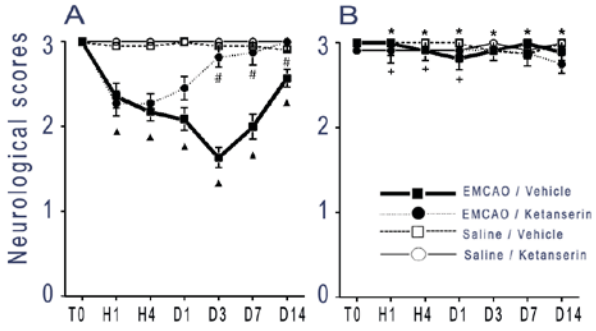


Figure 2. Effects of ketanserin in non-ischemic rats (Saline/Ketanserin) and ischemic rats (EMCAO/Ketanserin) compared to the effects of vehicle in non-ischemic rats (Saline/Vehicle) and ischemic rats (EMCAO/Vehicle) on postural reflex of the contralateral (**A**) and ipsilateral body side (**B**) before ET1 (T0), at the 1h (H1), 4h (H4), day 1 (D1), day 3 (D3), day 7 (D7), and day 14 (D14). Data are presented as mean ± S.E.M. and were analysed as follows ($p < 0.05$): ▲ EMCAO/Vehicle vs. Saline/Vehicle, * EMCAO/Vehicle$_{contra}$ vs. EMCAO/Vehicle$_{ipsi}$, and + EMCAO/Ketanserin$_{contra}$ vs. EMCAO/Ketanserin$_{ipsi}$.(Wilcoxon matched pairs test); # EMCAO/Vehicle vs. EMCAO/Ketanserin (Mann-Whitney U-test).



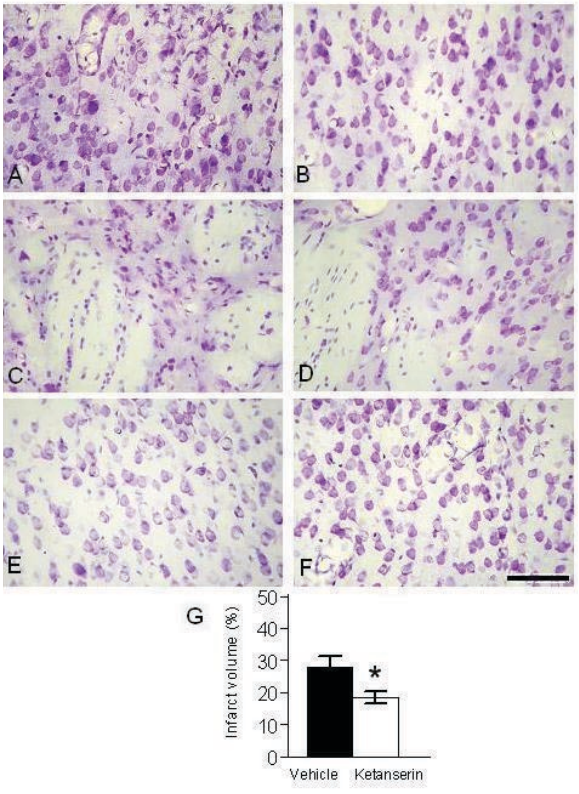
critical F-values for frequencies between 3 and 10 Hz. At day 7 (Figure 1F) and day 14 (Figure 1G), ketanserin was not able to reduce the small residual effect of ET1 on the slowest EEG waves but still augmented the reduced by ET1 power of beta waves.

Ketanserin injected i.p. alone (in non-ischemic rats) caused augmentation of the EEG power density mainly in the range of theta-alpha-slow beta waves measured at H1 and H4: Saline/Ketanserin – Saline/Vehicle comparisons were statistically significant for frequencies 6.25 - 18.25 Hz (Figures 1B and 1C).

3.2. Assessment of neurological functions

All Saline/Vehicle-treated rats exhibited postural reflex score of 3 for both sides of the body when examined from the first hours until day 14 post-ET1 (Figure 2). The neurological scores for the contralateral side of the body of rats with ischemia (Figure 2A) were significantly lower than those of the same rats infused with saline instead of ET1: EMCAO/Vehicle vs. Saline/Vehicle. The effect of ET1 was time-dependent since the nonparametric Friedman ANOVA showed significant Chi.Sqr (N=20, df=6) = 35.6 ($p < 0.0001$), and the Wilcoxon matched pairs test showed a maximum of the deficit in the EMCAO/Vehicle vs. Saline/Vehicle rats at day 3 after ET1. Then a partial recovery of the neurological score began at day 7, with a contralateral forelimb flexion being still evident in some animals at day 14. The neurological scores of the ipsilateral side of EMCAO/Vehicle rats (Figure 2B) did not change by ET1 and significantly differed from those in the contralateral side.

Figure 3. Microphotographs of layer II of the S1FL region of the cerebral cortex and dorsolateral striatum in rats treated with ketanserin or its vehicle (Cresyl violet staining). On the left (A,C,E) – ipsilateral hemisphere, on the right (B,D,F) –contralateral hemisphere. On A and B – S1FL in control (vehicle-treated) rat, on E and F – S1FL in ketanserin-treated rat. On C and D – Striatum in control (vehicle-treated) rat. Sections of 30-μm thickness stained with 0.5% cresyl violet. Scale bar – 50 μm. On G – infarct volume derived from seven TTC stained sections. Mean ± SEM are presented (Mann-Whitney test, * $p < 0.05$).



The EMCAO/Ketanserin rats showed much smaller contralateral neurological deficit than the EMCAO/Vehicle rats from the day 3 and until the last day of the experiments (Figure 2A), as indicated by Kruskal-Wallis ANOVA by ranks and by the significant differences between EMCAO/Vehicle and EMCAO/Ketanserin rats (Mann-Whitney U-test). Ketanserin treatment was able to disentangle the differences between contralateral (Figure 2A) and ipsilateral (Figure 2B) scores beginning from D3 and until the end of experiment.

3.3. Histology

The effect of EMCAO on the histopathology (Cresyl-violet staining) in the S1FL region ipsilateral to the ET1 delivery (where the electrode for EEG recording was situated) (Figure 3A) was mild: no obvious cell loss compared to the contralateral S1FL region (Figure 3B) was observed, although both the swollen pale neurons and dark cells were present in this ipsilateral cortical

region. In ischemic rats treated with ketanserin less histological alterations in the ipsilateral (to the ET1 delivery) S1FL cortex were noted (Figure 3E) relative to EMCAO/Vehicle rats (Figure 3A). Importantly, the comparison between the ipsilateral (Figure 3E) and the contralateral side (Figure 3F) of this cortical region after ketanserin revealed practically no difference. No obvious effect of ketanserin was seen in striatum (not shown) compared to the severe damage in the ipsilateral striatum in vehicle-treated rat, which justified the successful EMCAO (Figure 3C).

Ketanserin decreased the infarct volume derived from postmortem TTC-stained brain sections at 3 days following EMCAO by about 33% ($p < 0.05$) (Figure 3G). The decrease of infarct volume by ketanserin was mainly due to the decrease of volume of infarct in the ipsilateral cortex.

4. Discussion

In the present study, ET1 was used as a tool to produce severe and sustained, but ultimately reversible unilateral MCA occlusion in order to provide an animal model of focal cerebral ischemia with reperfusion [14-25]. The quantitative EEG and the neurological examination both showed a substantial effect of ET1. This effect exhibited a specific time dynamics. Importantly, the present study demonstrated that the 5-HT_2 receptor antagonist ketanserin reduced the EEG changes and the neurological deficits in this model of cerebral ischemia. In parallel, ketanserin reduced the infarct size in the ipsilateral hemisphere (mainly in cortex) and the histopathological difference between the ipsilateral and contralateral somatosensory S1FL cortical regions, but did not change the morphological abnormalities in striatum.

We demonstrated an impairment of EEG after ET1-induced ischemia, which followed a specific time course: (1) suppression of all EEG waves (statistically significant for most of the fast waves) at the early stage of EMCAO (1-4 hours after ET1); (2) some restoration of the amplitude of the slow waves, but still with concomitant depression of the amplitude of fast alpha and beta waves at 24 h; (3) an augmentation of the EEG slow waves power (in delta, theta and slow alpha ranges) 3-7 days after ET1; and, (4) no complete restoration of the averaged spectral EEG profile at day 14-15 with slow delta waves remaining in the EEG. The early acute EEG changes appear predictive in terms of brain function, given the severe reduction (up to 50%) in the ipsilateral cortical cerebral blood flow (CBF) measured after 60 pmol of ET1 1h and 4h after ET1 in the same model of EMCAO [17]. The observed reduction of EEG amplitude in the acute phase of ischemia was probably due also to depletion of energy stores and breakdown of energy metabolism [31]. The same suppression of the EEG waves in all frequency bands has been reported in rats 1-2 h after MCAO by filament technique [32], and the sensitivity of synaptic transmission to ischemia was proposed to be one of the factors accounting for the impairment of EEG in the penumbra [33].

The initial decrease of EEG power several hours after ET1 was followed 24 h later by a partial restoration of EEG power in delta, theta and alpha bands. The recovering of EEG power might be explained by restoring the cortical blood flow to its control levels 16-22 h after EMCAO [17]. Later (at day 3-4 post-ET1), the EEG spectral density of delta, theta and slow alpha waves increased significantly while that of the beta waves remained still slightly depressed. The increase in the amplitude of the delta waves in the ipsilateral cortex, which is indicative for a decrease in basal neuronal activity, was due to the appearance of polymorphic delta activity in the EEG. This activity is thought to be a hallmark involved in the pathological outcome in rat focal ischemia [34-35]. The high-amplitude delta waves are shown to be a common phenomenon of ischemic brain injury also in humans [36-37]. The injection of 60 pmol of ET adjacent to MCA did not trigger electrographic or behavioural seizures until the end of our experiment as opposite to higher dose of ET1 which may lead to non-convulsive seizure activity in EEG within 2 h after the onset of ischemia [25].

The period of full maturation of brain damage in this model of MCAO has been shown to take place between days 3 and 7 post-EMCAO [14-16]. We have previously shown that 3 days post-EMCAO of about 30% of the tissue of the ipsilateral hemisphere was without viable mitochondria identified by 2,3,5 - triphenyltetrazolium chloride (TTC) staining [24]. It may be therefore suggested that the suppression of the EEG activation in the ipsilateral S1FL cortex 3 days post-EMCAO (increase in the slow EEG waves and decrease in the fast EEG waves amplitude) was partly due to the ischemic morphological alterations of cells in this area. Our histological results showed that these alterations were not severe, probably because the EMCAO had been shown to spare the forelimb sensory-motor cortex, representing thus the peri-infarct region or penumbra in this model [38]. However, the success of the MCAO in rats was proven by the severe damage of cells in the ischemic core, namely, the dorsolateral neostriatum, demonstrated by TTC and cresyl-violet staining. In addition, besides S1FL cortex, other injured brain regions in the ipsilateral hemisphere, which send

afferents to S1FL [39] may be also responsible for the observed changes in EEG recorded from this cortical region and for the neurological deficits after EMCAO.

Laboratories worldwide have so far examined the neuroprotective properties of various substances. Given that serotonin plays a pathological role in a number of low blood flow conditions [2-4], the properties of some serotoninergic receptor agonists and antagonists as putative neuroprotectants have been explored. The antagonists of 5-HT_2 receptors such as ritanserin, ketanserin and mianserin were shown to reduce the volume of the brain infarct, to improve behavioral performance, or to have beneficial effects on the local cerebral blood flow in models of global and forebrain ischemia and in models of permanent focal ischemia in rats and gerbils [7-13]. However, data about the effects of the 5-HT_2 receptor antagonists in models of transient focal ischemia are lacking. These models mimic closer the clinical situation of human stroke than the permanent ones. We found that ketanserin (mainly 5-HT_{2A} receptor antagonist [5,27]), administered 20 min after the ET1 delivery near MCA, hastened the recovery of EEG: while in EMCAO/vehicle rats, the fast EEG waves restored not earlier than at day 3 post-ET1, in the EMCAO/ketanserin rats, the restoration of fast alpha and beta waves was evident already 24 h after ET1. In addition, ketanserin enhanced the recovery of EEG: (1) restored the early EMCAO-induced suppression of the fast beta waves and (2) decreased the ET1-induced augmentation of delta, theta and alpha waves at day 3. These findings reinforce the possibility for participation of 5-HT_{2A} receptors in the pathogenesis of ischemic neuronal damage, because both, the suppression of the fast waves by the ischemia and the augmentation of the slow waves, were supposed to be electrophysiological signs of MCAO-induced ischemic alterations [32,34,35]. The slowest delta waves, which were augmented after ET1 at this time period, however, did not disappear completely after ketanserin treatment. It seems that ketanserin was able only to reduce, but not to completely eliminate the ET1-induced functional EEG disturbances. Although there were mild alterations in the amount of cellular damage in the EMCAO/ketanserin rats compared to the EMCAO/vehicle rats, the drug eliminated the histopathological difference between the ipsilateral and contralateral cortical S1FL areas as assessed by means of cresyl-violet staining three days post-ET1 and reduced the infarct size in the ipsilateral hemisphere, mainly in cortex. In favor of the alleviating effects of ketanserin of the EMCAO-induced alterations were also the present findings that the EMCAO/ketanserin rats showed much smaller neurological deficit than the EMCAO/vehicle rats and that ketanserin eliminated the difference between the neurological scores measured for the contralateral and ipsilateral body sides beginning from day 3 until the last day of the experiments.

We showed that in non-ischemic conditions ketanserin seriously influenced the balance between the excitatory and inhibitory influences on cortical neuronal circuitry: it strongly augmented the cortical EEG power, especially in the alpha band, 1-4 hours after the injection. This finding is in agreement with the view that antagonism at 5-HT_2 receptors promotes inhibitory influences [40] and synchronization in cortex [41]. In conditions of ischemia, ketanserin, administered shortly after ET1 may attenuate the ischemic excitatory amino acid efflux as was shown recently for another 5-HT_2 receptor antagonist adatanserin [42]. Hence, it is very probable that ketanserin, administered 20 min after ET1, may indirectly decrease the excitation, exerted by glutamate during the early phase of the EMCAO-induced ischemia [43] and thereby to reduce its consequences at a later stage.

Another possible mechanism of ketanserin action on the observed ET1-induced alterations might be related to the synergistic effect between ET1 and 5-HT [44]. It is very probable that ketanserin disturbed this synergistic effect and thereby reduced the consequences of the ET1-induced ischemia. The vascular action of ketanserin [3] is another mechanism by which the effects of ketanserin may be explained. In MCAO models, the concentration of 5-HT in the ischemic regions elevates dramatically immediately after occlusion and during the early phase of the reperfusion [2]. The strong vasoconstrictory action of serotonin is well documented [3] and this effect of 5HT was shown to be exerted through activation of $5HT_2$ receptors [4]. In pathological conditions of reduced CBF, as after thrombotic infarction in rats, ketanserin prevents the reduction of CBF [13]. Thus, under conditions of ischemia following the ET1-induced MCAO, the vascular effects of ketanserin may be also an important determinant of its neuroprotective properties.

Ketanserin has the highest affinity for the serotonin 5-HT_{2A} receptor, but also binds less potently to other non-serotonergic receptors, for example, the alpha-adrenergic receptors [45]. Many studies have shown that ketanserin can block vascular alpha 1-adrenoceptors [46,47]. What is more, to exhibit its competitive 5-HT_2 receptor antagonism, the coexisting alpha 1-adrenoceptor antagonist property of ketanserin was shown to be very important [48]. Therefore, it is not excluded that the neuroprotective effects of ketanserin at the high dose used was mediated not only by 5-HT_{2A}, but also via alpha adrenergic receptors.

In conclusion, the results showed that treatment with ketanserin at the early stage of ET1-induced ischemia

was able to reduce the long-term ischemia-induced functional and histopathological changes. A compound with these properties might be an interesting candidate for alleviating the consequences of stroke.

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