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Effects of MK-801 and ganglioside GM1 on postischemic prostanoid release and hippocampal lesion in gerbil brain

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Abstract. In this study Mongolian gerbils were submitted to a normothermic bilateral carotid ligation lasting 5 min. A noncompetitive antagonist of NMDA receptors, MK-801, 0.8 mg/kg, was injected i.p. 30 min before ischemia, or the ganglioside GM1, 30 mg/kg, was given i.p. for 3 days, twice a day. The morphology of the hippocampal CA1 neurones and the brain content of cyclooxygenase metabolites of arachidonic acid: prostaglandin 6-keto $PGF_{1\alpha}$ and thromboxane Tx B_2 were studied. Untreated ischemia induced the accumulation in brain of the 6-keto $PGF_{1\alpha}$ and Tx B_2 immunoreactive materials, and resulted in a lesion of 70% of CA1 neurones. In the MK-801- and GM1-pretreated groups the postischemic levels of Tx B_2 were significantly decreased. However MK-801 and GM1 did not prevent damage to the CA1 neurones in gerbils normothermic after ischemia, whereas a partial neuroprotection was observed in hypothermic, MK-801 treated gerbils. The results of this study indicate that NMDA receptors may participate in the mechanism of postischemic release of eicosanoids in brain. They also confirm a potential modulatory role of gangliosides. These results are discussed in terms of the involvement of cyclooxygenase metabolites of arachidonic acid in the mechanism of a selective delayed neuronal damage to the hippocampus CA1 after ischemia.

Key words: forebrain ischemia, GM1, hypothermia, MK-801, Mongolian gerbils, neurodegeneration, neuroprotection, NMDA receptors, prostanoids

INTRODUCTION

The release of arachidonic acid is one of the earliest biochemical responses to ischemia of the mammalian brain (Bazan 1970, 1989, Łazarewicz et al. 1972, Rehncrona et al. 1982, Yoshida et al. 1986, Abe et al. 1987, 1989). This effect may result from receptor and calcium-induced phospholipid degradation mediated by brain phospholipase A_2 and/or C. In turn a rapid accumulation in the brain of eicosanoids occurs in early stages of recirculation after ischemia (Gaudet et al. 1980, Shohami et al. 1982, Black et al. 1984, Dempsey at al. 1986, Dorman 1988, Patel et al. 1992). Prostaglandins and thromboxanes are the cyclooxygenase products of arachidonic acid metabolism, and therefore their accumulation during early stages of recirculation after ischemia indicates an extent of ischemia--evoked arachidonic acid release. The roles of thromboxane A_2 in pathogenesis of an ischemic neuronal injury, and neuroprotective effects of prostacyclin has been proposed (Wolfe and Coceani 1979, Awawd et al. 1983, Pluta et al. 1991). Brain prostanoids have been almost exclusively discussed in relation to microvessels and platelets (Moncada and Vane 1979), as the importance of vascular sites in the eicosanoid synthesis and functions has been well established (Gryglewski et al. 1988). A possible participation of the brain parenchymal sites in this process is not clear.

Studies *in vitro* on cultured neurones (Dumuis et al. 1988, Łazarewicz et al. 1988, 1990b, Sanfeliu et al. 1990, Tapia-Arancibia et al. 1990) have shown that a stimulation of NMDA receptors leads to a massive, calcium-dependent, phospholipase A_2-mediated release of arachidonic acid. A similar effect has been demonstrated in cultured cerebellar granule cells incubated in glucose- and oxygen-deprived medium, thus resembling ischemic conditions (Łazarewicz et al. 1990a). The neurotoxic action of excitatory amino acid neurotransmitters has been postulated to participate in the pathomechanism of neuronal damage in a variety of neurological disorders including brain ischemia, hypoglycemia and epilepsy (Simon et al. 1984, Wieloch 1985, Rothman and Olney 1986, Siesjö and Bengtsson 1989).

Thus, arachidonic acid release in ischemia may be partially attributed to a stimulation by endogenous agonists of the NMDA receptors in brain neurones, and this may result in eicosanoid production during recirculation. However, a direct evidence implicating the involvement of excitatory amino acids in the mechanism of *in vivo* eicosanoid release in ischemic brain is lacking. Also a participation of the pool of eicosanoids synthesised in neurones in the mechanism of selective neuronal injury should be evaluated.

The present work has been aimed at exploring these subjects using a well characterised *in vivo* model of transient forebrain ischemia in Mongolian gerbils. In these animals, due to incompleteness of the Willis' circle, there is a lack of connection between the carotid and vertebral arteries (Levine and Payan 1966). An accumulation of eicosanoids in gerbil brain after ischemia has been demonstrated (Gaudet and Levine 1979, Gaudet et al. 1980, Kempski et al. 1987). MK-801, a selective noncompetitive NMDA receptor antagonist (Wong et al. 1986) is known to interfere with brain neuronal signalling during ischemia (Koenig et al. 1990, Zabłocka et al. 1994), and to protect neurones in focal ischemia, although its neuroprotective effect in global brain ischemia has been disputed (McCulloch 1992). The other group of putative neuroprotective agents are gangliosides, that reduce *in vitro* the death of neurones induced by glutamate receptor agonists, hypoxia, and aglycemia (Favaron et al. 1988, Skaper et al. 1989, Facci et al. 1990, Laev et al. 1993). *In vitro* gangliosides modulate calcium fluxes in neurones (Guerold et al. 1992), stabilising calcium homeostasis after overexcitation (Manev et al. 1990a,b). *In vivo* gangliosides partially prevent a neuronal damage caused by excitotoxins and brain hypoxia/ischemia (Lombardi et al. 1989, Contestabile et al. 1990, Hadjiconstantinou et al. 1990, Karpiak et al. 1990, 1991a,b, Seren et al. 1990), and promote neuronal regeneration (Wójcik et al. 1982, Toffano et al. 1983).

Therefore, to assess the role of NMDA receptors in brain ischemia-induced production of eicosanoids in neurones, and in their involvement in the pathogenesis of ischemic neuronal damage, the effects of MK-801 and GM1 ganglioside pretreatment on postischemic accumulation of eicosanoids were confronted with their influence on ischemia-evoked selective neuronal injury in the hippocampus CA1.

METHODS

Animals

Adult Mongolian gerbils of both sexes, weighing 60 - 80 g, were fed on a standard diet with water *ad libitum*. The gerbils were randomly distributed to different groups (see the Results section). Each group consisted of 10 animals, in the exception of one GM1-treated group, which consisted of 5 gerbils. Due to a postischemic mortality of the gerbils, their final number in some groups decreased. The animals used for the analysis of brain eicosanoid content comprised untreated or drug-treated animals: naive, sham operated and submitted to 5-min bilateral carotid artery ligation (see below). Forebrain ischemia was usually followed by 5-min recirculation, but in some experiments also 0, 10, 15, or 20 min of recirculation was allowed. The animals were injected with MK-801, 0.8 mg/kg i.p. 30 min before surgery, or with GM1 (Sygen, Fidia S.p.A., Comune Fidia-Sinax, Abano Terme, Italy), 30 mg/kg i.p., twice a day for 3 days, the last injection 30 min prior to operation. Corresponding groups injected with 0.9% saline according to drug application schedules served as controls. Five groups, two control, two treated with MK-801, and one treated with GM1 were used for morphological studies after 14 days of recirculation. The protocols of these experiments were accepted by the local Ethical Committee.

Forebrain ischemia

The gerbils were anaesthetised with 4% halothane in a gas mixture containing 30% O_2 and 70 % N_2O. Two min before operation halothane concentration was reduced to 2%, and was kept on this level during ischemia. Common carotid arteries were isolated through an anterior midline cervical incision. Cerebral ischemia was induced by occlusion of both common carotid arteries with miniature aneurysmal clips for 5 min. In some groups of animals an anterior midline cervical incision was made and arteries were isolated without their occlusion (sham operation). During the whole surgery the animals were kept on a heating bed set at 38°C. After sewing up the wounds, the animals were kept for 5-20 min (measurements of brain eicosanoids), or for 3 h (morphological studies), in cages heated with an IR lamp to maintain a rectal temperature in the range of 37±1°C. The rectal temperature of control and drug-treated animals selected for a morphological study was monitored prior to drug application, anaesthesia, and ischemia, during carotid occlusion, and at the following times post ischemia: 5, 10, 15, 30, 60, 120, 180 min and after 24 h. Animals from one MK-801 treated group were not heated to allow the development of a spontaneous hypothermia.

Histology

Fourteen days after ischemia the animals were deeply anaesthetised with ether and subjected to an intracardial perfusion fixation with 4% neutralised formalin solution. The brains were removed, immersed in a 4% formalin solution for 1 week, transferred to abs. ethanol and embedded in paraffin. The 10 μm-thick cross sections from the dorsal part of the hippocampus were stained with cresyl violet.

Pathology

The neuronal damage to the CA1 regions of hippocampi was assessed by histopathology scoring. For this reason a five point scale was established: 0=no damage, 1=up to 30% of necrotic cells, 2=31% to 50% of necrotic cells, 3=51% to 70% of necrotic cells, 4=71% to 100% of necrotic cells. A histological examination was performed by a patho-

logist blind to the treatment groups. For each animal 5 sections of the central part of both hippocampi were analysed using a light microscope at x400 magnification. The density of viable CA1 pyramidal neurones was quantified on both sides, in randomly selected ten 0.1 mm portions per section, and an average number of the viable neurones was expressed in per cent of mean neuronal density in the CA1 region of the sham-operated animals, that was 33.1±2.5 per 0.1 mm. Then grades for each animal were determined using a described above scale. For all the experimental groups a number of animals exhibiting a specific grading was found. To calculate mean grades of histological damage for each group, gradings were multiplied by their incidence and the sum of the resulting products for a given group was divided by the total number of animals in each group.

Assay of brain eicosanoid contents

The gerbils were killed by decapitation and brain hemispheres were quickly removed, dissected at 0°C, and weighed samples were frozen in liquid nitrogen. The average time of brain dissection was about 30-45 s. The procedure of eicosanoid extraction was according to Powell (1980), in modification as described by Minamisawa et al. (1988). The samples of the brain cortex or of the hippocampus in which 6-keto prostaglandin $F_{1\alpha}$ (6-keto $PGF_{1\alpha}$) and thromboxane B_2, (Tx B_2) were assayed, were homogenised at 0°C in 4 ml of 0.05 M Tris-HCl buffer (pH 7.4) and centrifuged at 10,000g for 10 min. The supernatants were collected, diluted to 40 ml with 7.5 mM acetate buffer (pH 5.9) and adjusted to pH 3.0 with 1 N HCl. Aliquots of this extract were applied to octadecylsilyl silica microcolumns (Sep-Pak C_{18} cartridges, Waters Assoc., Milford, Massachusetts, USA), prewashed with 20 ml of methanol followed by 20 ml of water. The cartridges were washed with 20 ml aliquots of water, ethanol/water (15:85) and petroleum ether. Prostaglandins and thromboxane were eluted with 4 ml of methanol. Separate portions of this fraction were prepared for the Tx B_2 and 6-keto $PGF_{1\alpha}$ radioimmunoassay. Each portion was concentrated under nitrogen and suspended for the assay in 125 µl of the assay buffer. Commercial thromboxane B_2 and 6-keto $PGF_{1\alpha}$ [^{3}H] assay kits (Amersham, UK) were used for measurements of eicosanoids brain content. Radioactivity was counted in duplicate samples in a Beckman LS-9000 liquid scintillation counter with 10 ml of Bray's scintillation mixture. According to the manufacturers' declaration a cross reactivity of antisera against Tx B2 and 6-keto $PGF_{1\alpha}$ with other eicosanoids was negligible, therefore for a simplification the obtained values indicating the content of immunoreactive material will be quoted in this paper as the concentration of corresponding eicosanoids.

Statistical analysis

Mean values of eicosanoid content in brain ±SEM in each group were determined by a one-way analysis of variance (ANOVA), followed by Student's t test for grouped data. A statistical significance of histological damage was analysed using Mann-Whitney U test.

RESULTS

General observations

In the present study Mongolian gerbils from the untreated groups show no postischemic mortality (100% of animals survived). In the MK-801 pretreated group that had been heated for 3 h after ischemia to assure a relative normothermia, a 10% mortality was found. The mortality increased to 30% in an unheated group pretreated with MK-801. In the GM1-pretreated ischemic group all animals survived 14 days.

The rectal temperature of each group of animals submitted to 5 min carotid occlusion is shown in Figs. 1 and 2. The temperature of control (untreated) animals kept on the heated bed before and during surgery and then heated for 3 h after ischemia was stable in a range of 37±1°C. In a group of MK-801 pretreated gerbils heated in a similar way

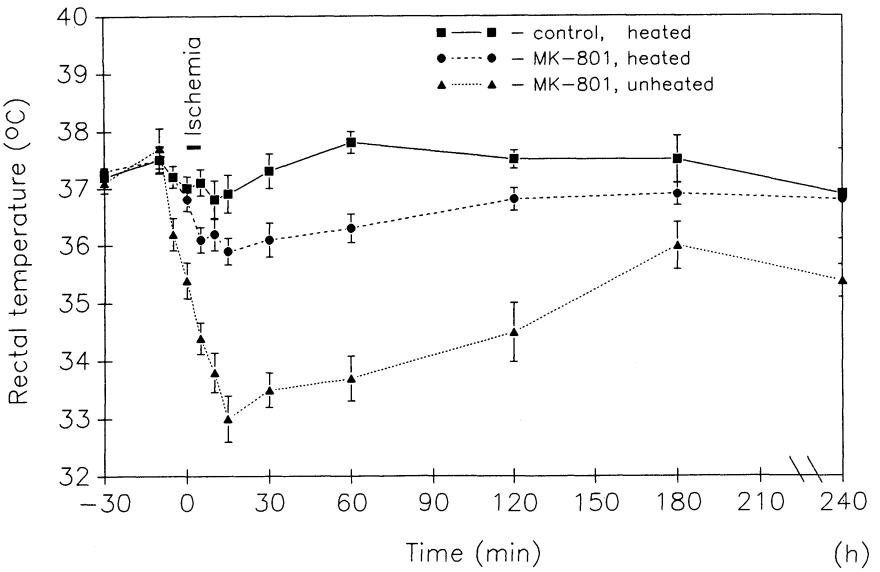

Fig. 1. Changes in rectal body temperature in gerbils submitted to 5 min forebrain ischemia: effect of MK-801 at different ambient temperature. MK-801 0.8 mg/kg b.w. was injected i.p. 30 min before bilateral carotid occlusion for 5 min (time O min - beginning of ischemia). Animals were either heated for 3 h (ambient temperature 25°C) or kept at room temperature 18°C. Values are means ±SEM from 10 animals in each group.

there was a 1°C decrease in rectal body temperature immediately after ischemia, with a tendency for increase to the basal value during the next two hours (Fig. 1). In a group of animals kept in a room temperature during and after ischemia there was a rapid fall of rectal temperature to the level of 33°C 5 min after ischemia, with a tendency to normalisation during the postischemic period. However even 3 h and 24 h after ischemia the mean value of rectal body temperature in this group was lower than in the control (Fig. 1). The rectal temperature in GM1 pretreated gerbils did not differ from the untreated control (Fig. 2).

Histology

Individual results and mean grades of histological damage to the hippocampal CA1 neurones are presented in Table I. An almost complete histological damage to CA1 neurones was observed in the saline treated gerbils from both control groups. The heated gerbils pretreated with MK-801 exhibited

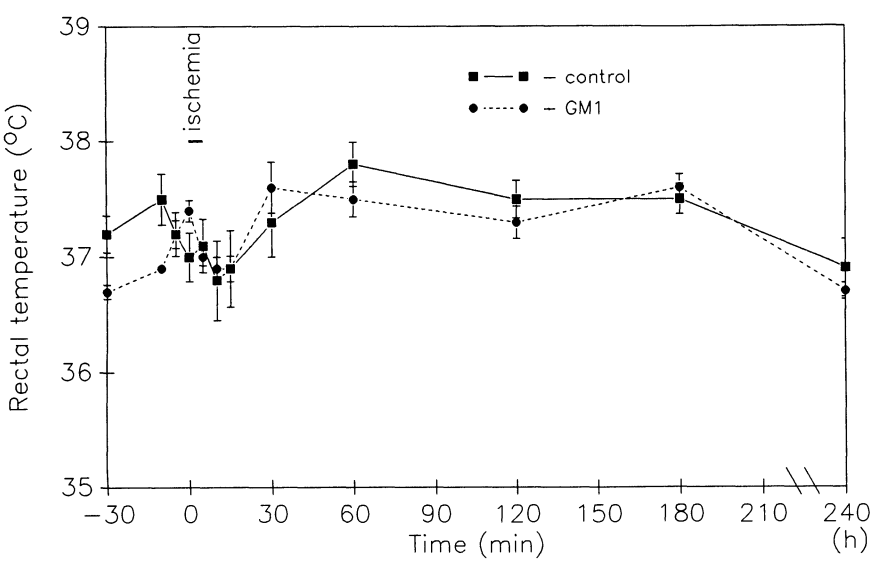

Fig. 2. Rectal temperature in GM1-pretreated gerbils submitted to 5-min ischemia (time O min - beginning of ischemia). GM1, 30 mg/kg b.w. was injected i.p. for 3 days twice a day. Animals were heated for 3 h after ischemia. Means ±SEM (n = 10 for the control and 5 for the GM1-treated groups).

TABLE I

Histological damage in CA1 area of gerbil hippocampus evoked by 5 min ischemia. Effect of pretreatment with MK-801 or GM1

Experimental group	n	CA1 damage						
		Incidence of each histological grading					Mean grade	Significance
		0	1	2	3	4		
Control to MK-801 (heated)	10	0	0	4	0	6	3.2	
MK-801 (heated)	9	0	0	1	1	7	3.7	ns
MK-801 (unheated)	7	1	1	3	2	0	1.9	$P<0.05$
Control to GM1 (heated)	10	0	0	0	1	9	3.9	
GM1 (heated)	5	0	0	0	1	4	3.8	ns

Experimental conditions described in Figs. 1 and 2. The animals were sacrificed 14 days after ischemia. Histological grading of the CA1 region was as follows: grade 0 - no cell necrosis, grade 1- damage to up to 30% of neurones, grade 2 - damage of 31-50% of neurones, grade 3 - damage of 51-70% of neurones, grade 4 - damage of 71 - 100% of neurones. Statistical significance of differences from the corresponding control groups was tested using two-tailed Mann-Whitney U test. The MK-801 - treated, unheated group differs significantly from the corresponding heated control and from the MK-801 - treated, heated groups. NS- nonsignificant.

extent of damage similar to the control group. A significant ($P<0.05$) protection of CA1 neurones was observed in a group of MK-801 pretreated gerbils allowed to develop hypothermia (compare Fig. 1). In the animals pretreated with GM1 the damage to CA1 neurones did not differ significantly from a control group.

Changes in brain content of 6-keto $PGF_{1\alpha}$ and Tx B_2

The basal levels of thromboxane B_2 and 6-keto $PGF_{1\alpha}$ in the brain cortex of naive, untreated gerbils were 6.0±0.12 ng/g w.w. and 10.8±1.33 ng/g w.w., respectively. These values were taken as 100% in Figs. 3-7. Occlusion of both common carotid arteries in gerbils for 5 min resulted in a significant increase of the content of Tx B_2 and 6-keto $PGF_{1\alpha}$ in the brain cortex after 5-min recirculation, as compared to naive and to the shame operated animals (Figs. 3-7). As shown in Fig. 5, a maximal accumulation of Tx B_2 appears 5 min after ischemia. Sham operation itself did not alter significantly the levels of eicosanoids studied. Pretreatment of gerbils with MK-801 had no effect on basal levels of 6-keto $PGF_{1\alpha}$ and Tx B_2 in the cortex of naive animals, although a slight decrease in the Tx B_2 level in MK-801 pretreated group of sham operated animals was noted (Fig. 3). An increase of Tx B_2 content in the cortex 5 min after ischemia was significantly in-

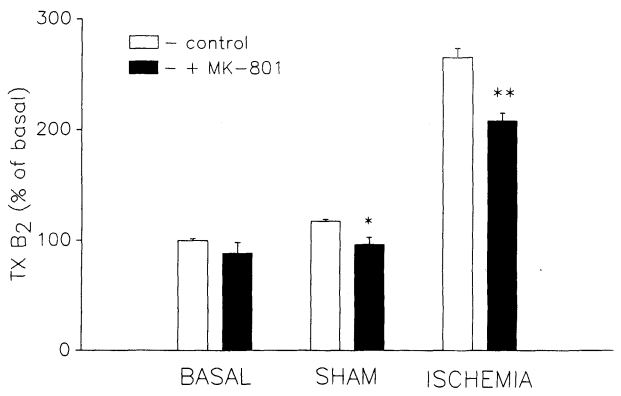

Fig. 3. Effect of MK-801 pretreatment on thromboxane B_2 content in gerbil brain cortex after 5-min forebrain ischemia. MK-801 was applied as described in Fig. 1. The animals were sacrificed for eicosanoid measurements after 5 min recirculation. Values are means ±SEM (n = 10). *$P<0.05$, **$P<0.01$ (ANOVA followed by Student's t-test), as compared to control.

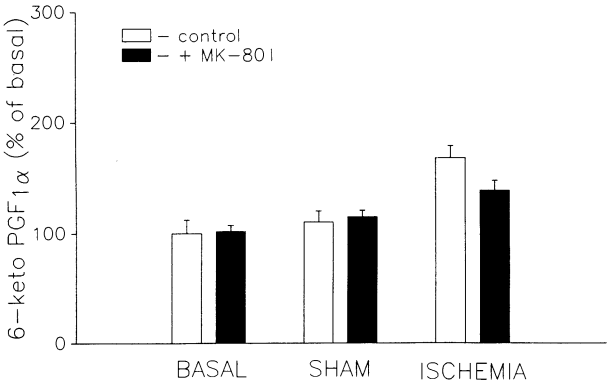

Fig. 4. Effect of MK-801 on changes in 6-keto $PGF_{1\alpha}$ content in gerbil brain cortex 5 min after 5-min global ischemia. MK-801 was given as described in Fig. 1. Values are means ±SEM (n=10). Differences statistically nonsignificant (P>0.05).

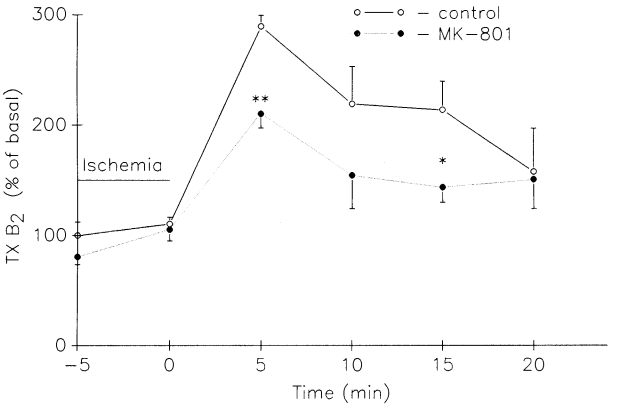

Fig. 5. Time-dependent accumulation of thromboxane B_2 in brain cortex of gerbils after 5 min forebrain ischemia: effect of MK-801 pretreatment. Dosage of MK-801 as described in Fig. 1. Values are means ±SEM (n=10).*P<0.05; **P<0.01 (ANOVA followed by Student's t-test), as compared to control.

hibited in the gerbils pretreated with MK-801 and with GM1 (Figs. 3 and 6), whereas an inhibition of postischemic increase in 6-keto $PGF_{1\alpha}$ contents by MK-801 and GM1 in these experimental conditions was nonsignificant (Figs. 4 and 7).

Changes in Tx B_2 content in the cortex of untreated and MK-801 pretreated animals during postischemic reperfusion (Fig. 5) indicate, that MK-801 uniformly attenuates the accumulation of Tx B_2 in the brain cortex within a 20 min period of recirculation.

Thromboxane B_2 accumulation during 5 min recirculation in the hippocampus of gerbils submitted to 5 min forebrain ischemia and the effect of pretreatment with MK-801 and GM1 did not differ from the effects seen in the brain cortex, presented in Figs. 3 and 6 (results not shown).

DISCUSSION

This study demonstrates that the pretreatment of Mongolian gerbils with a non-competitive antagon-

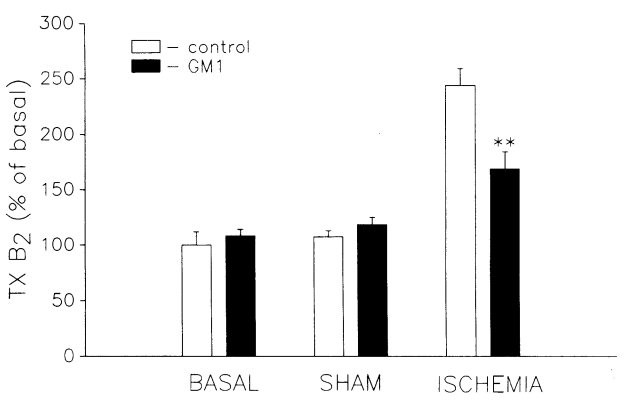

Fig. 6. Effect of GM1 on thromboxane B_2 content in gerbil brain cortex after 5-min forebrain ischemia. GM1 was applied as described in Fig. 2. The animals were sacrificed for the eicosanoid measurements after 5 min recirculation. Values are means ±SEM (n=10).**P<0.01 (ANOVA followed by Student's t-test), as compared to control.

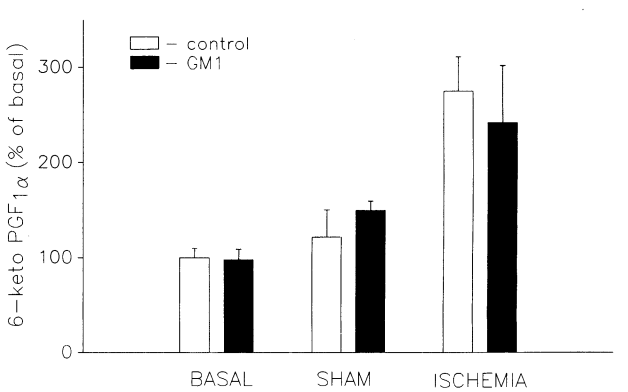

Fig. 7. Effect of GM1 on changes in 6-keto $PGF_{1\alpha}$ content in gerbil brain cortex 5 min after 5-min global ischemia. GM1 was given as described in Fig. 2. Values are means ±SEM (n=10). Differences statistically nonsignificant (P>0.05).

ist of NMDA receptors, MK-801 or with monosialoganglioside GM1 significantly reduces a postischemic accumulation of thromboxane B_2 in the brain cortex and hippocampus, without any protection to CA1 neurones from their delayed, selective damage.

An important role of arachidonic acid and its various metabolites for a normal function of the nervous system and in the pathogenesis of ischemic brain insult was suggested (Shimizu et al. 1979, Pappius and Wolfe 1983, Ueno et al. 1983, Piomelli et al. 1987, Minamisawa et al. 1988, Abe et al. 1989, Lynch and Voss 1990). An early accumulation of oxygenated metabolites of arachidonic acid in the brain, with a peak immediately after forebrain ischemia, was observed in several studies (Moskowitz et al. 1984, Kiwak et al. 1985, Dempsey et al. 1986, Minamisawa et al. 1988).

Postischemic formation of arachidonate metabolites in the brain reflects the release of arachidonic acid in the ischemic brain. This well known phenomenon (Bazan 1970, 1989, Łazarewicz et al. 1972) may be ascribed to the cholinergic and/or adrenergic agonist-dependent activation of phospholipase C and liberation of arachidonate from diacylglycerol (Ikeda et al. 1986, Yoshida et al. 1986, Abe et al. 1987, Strosznajder et al. 1987), and/or may depend on the NMDA receptor-mediated, Ca^{2+}-triggered activation of phospholipase A_2 (Łazarewicz et al. 1988, 1990a, Dumuis et al. 1988, Pellerin and Wolfe 1988, Tapia-Arancibia et al. 1990). The role of glutamate receptors in the mechanism of ischemic brain damage, and their possible involvement in the release of arachidonic acid in the nervous system during hypoxia, ischemia and hypoglycemia has been suggested (Simon et al. 1984, Wieloch 1985, Westerberg and Wieloch 1986, Abe et al. 1988, Siesjö and Bengtsson 1989, Łazarewicz et al. 1990a).

Reduction by MK-801 of ischemia-evoked accumulation of eicosanoids in the brain, which was shown in the present study, may indicate that in the brain cortex and hippocampus during and after ischemia, a significant portion of arachidonic acid and its metabolites - eicosanoids may be liberated in the process dependent on the activation of NMDA receptor-gated ionic channels permeable to Ca^{2+}. MK-801 is a powerful noncompetitive antagonist of NMDA subtype of glutamate receptors, acting at the phencyclidine binding site in the channel (Wong et al. 1986). Its high selectivity, demonstrated in early papers (Clineschmidt et al. 1982), has been recently disputed. Acute injections of MK-801 in doses similar to these used in our present study is known to induce bidirectional changes in glucose consumption in different brain structures (Nehls et al. 1990, Kurumaji et al. 1991). A specific dependence of this phenomenon on NMDA channel blockade, although not proven, is still highly probable. Recent studies of Charriaut-Marlangue et al. (1994) demonstrated an NMDA receptor-independent inhibition of protein biosynthesis in rat hippocampal slices by 10 μM MK-801. This is however a concentration 2 orders of magnitude higher than necessary to inhibit the NMDA channel. The dose of MK-801 used in our present study (0.8 mg/kg) is not excessive as compared to other studies on neuroprotection or behavioural pharmacology, thus the effects of MK-801 seems to be attributable to inhibition of NMDA channels. The effect of MK-801 can not be evoked by the MK-801-induced postischemic hypothermia, since normothermic conditions of the experiments were assured, and ischemia-evoked arachidonic acid release was shown to be insensitive to hypothermia (Busto et al. 1989).

Although other data indicate that MK-801 suppresses post-ischemic metabolic alterations resulting from pathological signal transduction in brain (Koenig et al. 1990, Zabłocka et al. 1994), there are also negative reports (Dempsey et al. 1991). In studies of Olsen and Kofod (1991), MK-801 exhibited only a non-significant inhibition of the anoxia-induced arachidonic acid release in the mouse brain.

The neurones that release arachidonic acid probably may directly participate in the synthesis of prostanoids in brain (Bishai and Coceani 1992). Moreover a concept of a transcellular eicosanoid synthesis (Maclouf et al. 1989) may be implicated

in the central nervous system. Neurones could play a role of donor cells liberating arachidonic acid, other intermediates, and transcellular messengers such as the platelet activating factor - PAF (Bazan 1989), and nitric oxide (Garthwaite et al. 1988), that can affect further steps of eicosanoid metabolism. Astroglia and endothelial cells may continue processing eicosanoids (Bruner et al. 1993). A major role of cerebral vessels in the synthesis of 6-keto $PGF_{1\alpha}$, a stable product of prostacyclin has been demonstrated (Abdel-Hakim et al. 1980a,b, Keller et al. 1985, Seregi et al. 1987, Herting and Seregi 1989), whereas thromboxane B_2, a stabile metabolite of Tx A_2, may also be produced in brain parenchyma (Wolfe et al. 1979).

The site of GM1-evoked inhibition of Tx B_2 synthesis is not clear. Previous studies demonstrated that GM1-lactone strongly inhibits eicosanoid formation in the ischemic rat brain without inhibition of arachidonic acid release (Petroni et al. 1989), which may suggest that gangliosides can interfere with further steps of arachidonate metabolism downstream the ischemic lipolysis. However GM1 was found to inhibit the release of arachidonic acid in endothelial cells incubated *in vitro* with different stimulators (Bressler et al. 1994). Therefore a complex mechanism of the GM1-induced inhibition of eicosanoid formation in the brain after ischemia may be proposed, including stabilisation of Ca^{2+} homeostasis in neurones that results in suppression of lipolysis, and interference with regulation of eicosanoid formation. Cyclooxygenase appeared to be susceptible to NO stimulation (Salvemini et al. 1993), whereas nitric oxide synthase, that is dependent on Ca-calmodulin stimulation, is present in the hippocampal CA1 neurones (Wendland et al. 1994). Thus gangliosides that bind calmodulin (Higashi and Yamagata 1992, Higashi et al. 1992) may prevent cyclooxygenase activation by interfering with calcium-calmodulin dependent NO formation.

A potent protective effect of MK-801 against the neurotoxicity of NMDA and in focal ischemia has been demonstrated, whereas its neuroprotection in forebrain ischemia has been disputed, since neuroprotection in global ischemia entirely depends on postischemic hypothermia evoked by MK-801 (Olney et al. 1987, Ozyurt et al. 1988, Ford et al. 1989, Schoepp et al. 1989, Buchan and Pulsinelli 1990). Thus our data demonstrating MK-801 protection of CA1 neurones only in hypothermic gerbils confirm these findings.

Neuroprotective effects of GM1 in different models of brain ischemia has been reported (Mahadik et al. 1989, Karpiak et al. 1990, Seren et al. 1990), whereas a failure of GM1 to improve outcome in neocortical focal ischemia in rats was observed by Mayer and Pulsinelli (1992). Gangliosides normalise intracellular calcium homeostasis acting downstream the NMDA receptors (Manev et al. 1990a,b, Guidotti et al. 1991), that is accompanied by inhibition of protein kinase C translocation and activation (Vaccarino et al. 1987). Gangliosides were shown to stabilise neuronal membranes and to prevent the ischemic Na^+/K^+-ATPase inhibition (Mahadik et al. 1989). Binding of calmodulin by GM1 (Higashi and Yamagata 1992, Higashi et al. 1992) may lead to a subsequent protection against the NMDA-induced, calcium and calmodulin-dependent activation of NO synthase and NO generation (Garthwaite et al. 1988, Dawson et al. 1993). Our negative results in gerbil experiments may indicate that in this model of 5-min forebrain ischemia more selective and powerful treatment is needed to damp the pathological signals mediating neuronal injury.

Dissociation of the effects of MK-801 and GM1 pretreatment on the postischemic release of cyclooxygenase metabolite Tx B_2 and neuronal damage in the hippocampus may indicate that the MK-801 and GM1-sensitive portions of the total pool of thromboxane is not substantial for the mechanism of selective delayed neuronal damage in the hippocampus CA1. The role of thromboxane A_2 in the mechanism of postischemic hypoperfusion leading to neuronal death has been suggested (Nakagomi et al. 1989). However the brain microcirculation depends on many mechanisms including the equilibrium between prostacyclin and thromboxane A_2 synthesis (Dirnagl 1993). In this study a tendency for attenuation of 6-keto $PGF_{1\alpha}$

formation, a stabile metabolite of prostacyclin was also observed. Petroni et al. (1989) reported equal inhibition by GM1-lactone of Tx B_2 and 6-keto $PGF_{1\alpha}$ release in the rat brain during and after ischemia. Thus, it is difficult to assess a possible influence of MK-801- and GM1-evoked inhibition of the prostanoid release on postischemic brain microcirculation.

We conclude that MK-801, a noncompetitive antagonist of NMDA receptors, and GM1, a less selective putative neuroprotecting agent, inhibit ischemia-evoked release of thromboxane in the gerbil brain, without a significant effect on injury to CA1 neurones. The results of MK-801 pretreatment may suggest a role of NMDA receptors in postischemic accumulation of cyclooxygenase metabolites in brain.

ABBREVIATIONS

NMDA N-methyl-D-aspartic acid
MK-801 (+)-5-methyl-10,11-dihydro-5H-dibenzo [a,d] cyclohepten-5,10-imine

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