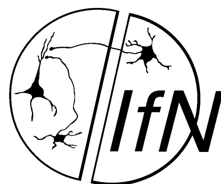
Research Report 1998/1999



IfN Project Group Neuropharmacology and FAN GmbH
Head : Prof. Dr. Klaus G. Reymann

The Project Group Neuropharmacology and FAN GmbH are concerned with the investigation of cellular mechanisms of synaptic plasticity and neurodegeneration using models of long-term potentiation (LTP) and stroke/ischemia. In 1998/1999 the focus was on the following topics:

1. Contribution of metabotropic glutamate receptors (mGluRs) to mechanisms of long-term potentiation (LTP)

Previous studies by this group have shown that activation of phospholipase C-coupled group I mGluRs and second messengers as protein kinase C (PKC) are crucial co-factors in the induction of LTP (for review: Manahan-Vaughan and Reymann, 1998 a,c,d). In collaboration with Dr. Balschun (Department Neurophysiology) we found that the efficacy of group I mGluR antagonists and Ca^{2+} channel blockers in blocking LTP maintenance is contingent upon the tetanization strength and the resulting $[Ca^{2+}]_i$ response (Wilsch et al., 1998). As our $[Ca^{2+}]_i$ measurements by high speed confocal laser scanning microscopy have shown, weak tetanization paradigms evoke a submaximal intradendritic $[Ca^{2+}]_i$ rise, whereas longer tetani evoke a high $[Ca^{2+}]_i$ plateau (Jäger et al., 1998). The elevation of $[Ca^{2+}]_i$ following weak tetanic stimulation could be impaired by an inhibition of group I mGluRs (Wilsch et al., 1998).

Therefore, by instigating Ca^{2+} release from intracellular stores, group I mGluRs enable a NMDA-dependent, input-specific LTP at moderate levels of synaptic activation.

The role of group II mGluRs, negatively coupled to adenylyl cyclase, in mechanisms of LTP was investigated with excitatory postsynaptic field potentials which were evoked from the CA1-region in rat hippocampal slices. Application of the group II specific agonists during tetanization resulted in a dose-dependent reduction of LTP. In contrast, group II mGluR antagonists caused the opposite effect, i.e. a dose-dependent enhancement of LTP. We concluded, that group II mGluRs are not essential for the induction of LTP but they may be involved in feedback mechanisms limiting the magnitude and duration of potentiation. In addition group II mGluR antagonists inhibit the early, but not the late depression following a depotentiating low frequency stimulation paradigm applied shortly after LTP-induction (Kulla et al., 1999).

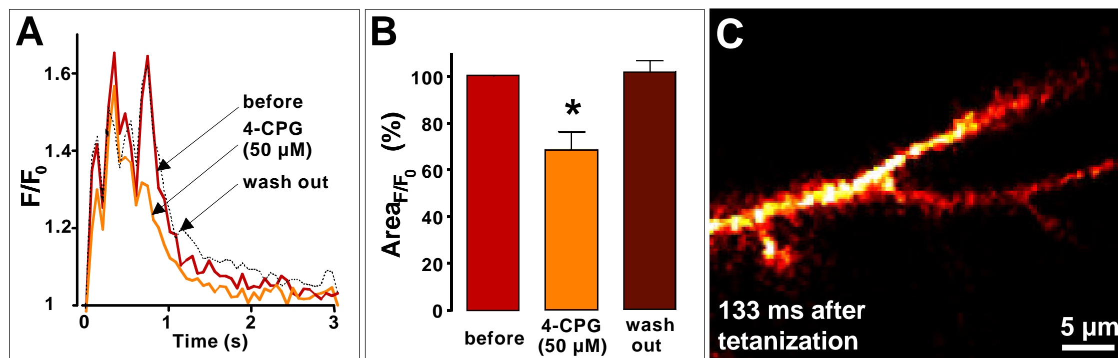


Fig. 1: Ca^{2+} imaging of the rise of $[Ca^{2+}]i$ in the dendritic tree of CA1 neurons filled with the Ca2+-sensitive dye Calcium Green-1 upon a tetanization (4*2 pulses at 100 Hz). (A, B) Bath application of 4-CPG (50 µM) led to a significant reduction of Area F/Fo, which was predominantly caused by a slower rise and earlier decay of $[Ca^{2+}]i$ (A). (C) Image of the Ca^{2+} response of a single neuron 133 msec following tetanization.

2. The role of calcium-induced calcium release and inositoltetrakisphosphate in LTP

In addition to inositoltrisphosphate (InsP3) sensitive intracellular calcium stores (ICSs) Ca^{2+} may be released from another group of ICSs, which are activated by receptors which bind to the plant alkaloid ryanodine. We investigated the role of ryanodine-sensitive ICSs in the expression of LTP using extracellular and intracellular recordings of excitatory potentials during the application of the RyanR-antagonists. The experiments proved that during tetanization the activation of post-synaptic ryanodine-sensitive ICSs is necessary for LTP expression (Szinyei et al., 1999). Increasing the number of tetani results in a reduction of the contribution of calcium-induced calcium release to the physiological response indicating that different post-synaptic Ca^{2+} sources complement each other during LTP induction.

We also tested the effect of inositol 1,3,4,5-tetrakisphosphate (InsP4) on (LTP). Intracellular application of InsP4 and EPSP recordings were carried out using the whole-cell

configuration. Induction of LTP in the presence of InsP4 (100 μM) resulted in a substantial enhancement of the LTP magnitude compared with control potentiation (Szinyei et al., 1999). Using an intrapipette perfusion system, it was established that application of InsP4 was required during induction of potentiation for this enhancement to occur. An enhancement of LTP was not observed if a non-metabolizable InsP3 analogue (2,3-dideoxy-1,4,5-trisphosphate) was applied intracellularly. Therefore InsP4 might function as a second messenger on its own and not only be a step of metabolism. ω-Conotoxin GVIA and different inhibitors of the endoplasmic reticulum-dependent Ca^{2+} release abolished the InsP4-induced LTP facilitation. These data indicate that InsP4 can modify the extracellular Ca^{2+} entry through upregulation of voltage-gated calcium channels (VGCCs), and/or activate postsynaptic InsP3-dependent Ca2+ release which may in turn contribute to the observed enhancement of LTP induced by InsP4 (Fig. 2).

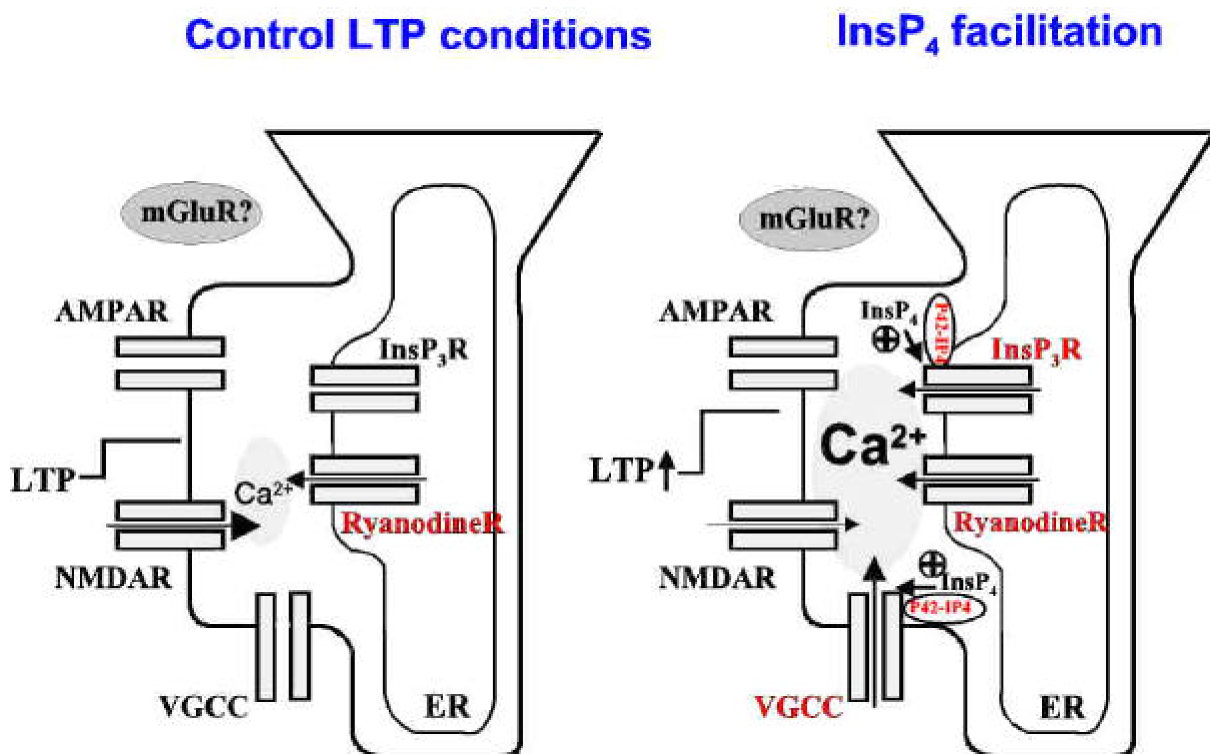


Fig. 2: Scheme illustrating the possible mechanisms of injected InsP4 on LTP facilitation in hippocampal CA1 pyramidal cells. A, under control LTP conditions NMDA and Ryanodine receptors (CICR) contribute to the potentiation. On the contrary, InsP3-dependent Ca2+ release probably does not. B, after the whole cell application of InsP4, InsP3-dependent Ca2+ release is activated as well as Ca2+ influx through VGCCs is enhanced which leads to a facilitated potentiation. Under physiological conditions, InsP4 is produced after activation of G-protein coupled receptors, such as mGluRs or muscarinic receptors. According to previous studies, InsP4 can interact with N-type VGCCs. The action of InsP4 on proteins in the plasma membrane or in the ER is possibly mediated by InsP4 binding proteins (InsP4BPs), such as p42IP4 (Aggensteiner et al. 1998). The metabolite InsP4 could therefore play the role of a second messenger.

3. Involvement of metabotropic glutamate receptor activation in hypoxic/hypoglycemic and ischemic injury

According to the excitotoxicity hypothesis changes in glutamate homeostasis is one of the key factors in the complex hypoxic/hypoglycemic events. Besides ionotropic glutamate receptors one could expect that also mGluRs are involved in mechanisms of necrosis and apoptosis. Previous experiments of this group have demonstrated that antagonists of mGluRs can protect neurons against failure of synaptic transmission in an electrophysiological *in vitro* model. Recently, protection was also obtained when the specific mGluR group I agonist 3,5-dihydroxyphenylglycine (DHPG) was applied from at least 10-20 minutes before the insult. Co-application of the PKC inhibitors staurosporine or chelerythrine attenuated the protective effect of DHPG. Our data suggest that group I mGluR agonists are only protective when present prior to the onset of the hypoxic/hypoglycemic insult and that activation of PKC is a critical, but not the only step in the protective mechanism (Schröder et al., 1999a). Recently we were able to demonstrate, that also agonists of group III mGuRs excert protection in hippocampal slices (Henrich-Noack et al., in press; Sabelhaus et al., submitted).

To focus on the influence of mGluRs on cerebral ischemia *in vivo* we established a 2-vessel occlusion-model of transient global ischemia in gerbils, in which the lesion occurs selectively in the CA1 layer of the hippocampus. Surprisingly, results obtained from this model differ markedly from our *in vitro* findings, for instance we did not see pronounced neuroprotection when we applied different mGluR antagonists. In contrast to reports of another group were our results obtained with agonists of group I and II mGluRs: (1S,3R)-1-aminocyclopentane-1,3-dicarboxylate ((1S,3R)-ACPD) led to a significant increase in neuronal damage (Henrich-Noack and Reymann, 1999). Interestingly, slow onset potentiation following ACPD-injection in rats was followed by late cell death (Manahan-Vaughan, et al., 1999a). A neuroprotective effect was observed only with (S)-4C3HPG, which acts as an antagonist at group I mGluRs and as an agonist at group II mGluRs. Animals treated with this compound showed almost the same level of healthy neurones as sham operated animals (Henrich-Noack et al., 1998). Moreover, we still detected significant neuroprotection when the compound was applied 65 min after ischemia. Recent controls, however, showed that in gerbils lowering the body and brain temperature could explain most of the effects of (S)-4C3HPG. In collaboration with Prof. Rauca (University Magdeburg) we found that in a model of transient focal ischemia in rats (middle cerebral artery occlusion, MCAO) this compound is highly protective independent of temperature changes. The receptor group(s) or subtype(s) responsible for the neuroprotective effect is/are not yet identified.

Ca^{2+} signalling in astrocytes which is induced by activation of mGluRs could also play an important role in protecting neurons by releasing survival factors upon activation. Therefore, the Ca^{2+} signalling elicited by several mGluRs agonists was investigated in hippocampal astrocytes using Ca^{2+}-sensitive fluorophores. Our results suggest the presence of at least two subpopulations of class I mGluRs recruiting from the truncated splice variants of mGluR1 and/or hitherto unknown glial-specific class I mGluRs (Bernstein et al., 1998).

In conclusion, the promising *in vitro* findings with mGluR compounds could be only partially confirmed *in vivo*. Further developments can be expected following the discovery of new receptor subtypes and more specific compounds.

4. Sodium entry pathways as new targets for neuroprotection

An interface-type organotypic hippocampal slice cultures prepared from 10 day old Wistar rats was established as an *in vitro* model to investigate also late mechanisms involved in ischemic brain injury and to monitor neuroprotective effects of drugs 24 h after the insult by propidium iodide (PI) staining of the nuclei of deceased cells. The NMDA receptor antagonist MK-801 protects organotypic slice cultures both in the short-term and the long-term range whereas the AMPA/kainate receptor antagonists NBQX and the mGluR group I and II agonist ACPD do not.

A prominent feature of cerebral ischemia is the excessive intracellular accumulation of both calcium $[Ca^{2+}]_i$ and sodium $[Na^{+}]_i$, which results in subsequent cell death. To date, most studies have focused on the ion channels involved whereas the Na^{+}/Ca^{2+} exchanger has received little attention, although it couples the Na^{+} to the Ca^{2+} gradient and may thus significantly influence neuronal injury. The selective Na^{+}/Ca^{2+} exchange inhibitor KB-R7943, significantly improves recovery of population spike amplitudes in rat hippocampal slices after hypoxia/hypoglycaemia. Our data suggest that the Na^{+}/Ca^{2+} exchanger, operating in reverse mode, contributes to hypoxia/hypoglycemia-induced injury in CA1 neurons (Schröder at al., 1999b, Breder et al., in press). MK-801 and tetrodotoxin, an inhibitor of voltage-gated Na^{+} channels, also reduce neuronal damage even when administered only after the insult.

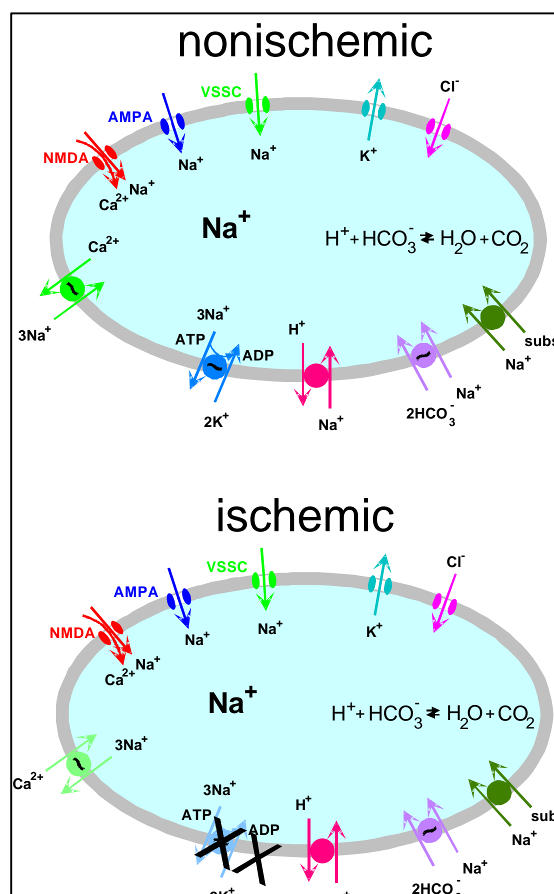


Fig. 3: Ion channels and transporters influencing Na^+ homeostasis under normal and ischemic conditions. Ischemic conditions result in the loss of function of the Na^+/K^+-ATPase and the reversal of the Na^+/Ca^{2+} exchanger.

5. Protease thrombin as an endogenous mediator of neuroprotection

The extracellular serine protease thrombin, a well known key player in blood coagulation and platelet activation, has been found to be expressed in different brain regions. In collaboration with Prof. Reiser and Dr. Striggow (University Magdeburg, Striggow et al., in press) we have considered thrombin and its receptor as endogenous mediators of neuronal protection against brain ischemia. Exposure of gerbils to prior mild ischemic insults two days before a severe occlusion caused a robust ischemic tolerance of hippocampal CA1 neurons. This resistance was impaired if the specific thrombin inhibitor hirudin was injected intracerebroventricularly before each short-lasting insult. Thus, efficient native neuroprotective mechanisms exist and endogenous thrombin seems to be involved therein.

In vitro experiments using organotypic slice cultures of rat hippocampus revealed that thrombin can have protective but also deleterious effects on hippocampal CA1 neurons. Low, picomolar concentrations of thrombin or of a synthetic thrombin receptor agonist induced significant neuroprotection against experimental ischemia. In contrast, higher concentration of thrombin decreased further the reduced neuronal survival that follows the deprivation of oxygen and glucose, or even caused neuronal cell death by itself. Degenerative thrombin actions might be also relevant in vivo, since hirudin increased the number of surviving neurons when applied before a 6 min occlusion. Low thrombin concentrations induced intracellular Ca^{2+} spikes in fura-2 loaded CA1 neurons whereas higher concentrations caused a sustained Ca^{2+} elevation.

Thus, distinct Ca^{2+} signals may define whether thrombin initiates protection or not. Taken together, in vivo and in vitro data suggest that thrombin can determine neuronal cell death or survival following brain ischemia.

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