BRAIN RESEARCH 1136 (2007) 13–19

available at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/brainres

BRAIN RESEARCH

Research Report

Evidence for a role for the group I metabotropic glutamate receptor in the inhibitory effect of tumor necrosis factor-α on long-term potentiation

*D. Cumiskey, M.P. Butler, P.N. Moynagh, J.J. O'Connor**

UCD School of Biomolecular and Biomedical Science, UCD Conway Institute of Biomolecular and Biomedical Research, Belfield, Dublin 4, Ireland

ARTICLE INFO

Article history:
Accepted 8 December 2006
Available online 2 January 2007

Keywords:
Tumor necrosis factor-α
Long-term potentiation
MCPG, MPEP
Group I mGluR
Ryanodine
Intracellular calcium store

ABSTRACT

Pro-inflammatory cytokines are known to be elevated in several neuropathological states that are associated with learning and memory. We have previously demonstrated in our laboratory that the inhibition of long-term potentiation (LTP) in the dentate gyrus region of the rat hippocampus, by tumor necrosis factor (TNF)-α, represents a biphasic response, an early phase dependent on p38 mitogen activated protein kinase (MAPK) activation and a later phase, possible dependent on protein synthesis. Many of the factors involved in the early modulation of LTP by TNF-α have yet to be elucidated. This study investigated if metabotropic glutamate receptors (mGluRs) are functionally linked to the inhibitory effect of TNF-α on LTP in the rat dentate gyrus in vitro. We report that the impairment of early-LTP by TNF-α is significantly attenuated by prior application of the group I/II mGluR antagonist MCPG and more specifically the mGluR5 antagonist MPEP. Since TNF-α is now known to cause transient increases in intracellular Ca^{2+} levels from ryanodine-sensitive stores, we explored the possibility that disruption of intracellular Ca^{2+} homeostasis could be involved. Ryanodine was found to significantly reverse the inhibition of LTP by TNF-α. From these studies we propose that the TNF-α inhibition of LTP is dependent upon the activation of TNFR1 and mGlu5-receptors. Importantly this study provides the first proof of the involvement of ryanodine-sensitive intracellular Ca^{2+} stores in TNF-α mediated inhibition of LTP.

1. Introduction

The pro-inflammatory cytokine tumor-necrosis factor (TNF)-α is elevated in the brain in a number of neuropathological states including trauma, ischemia, Parkinson's disease, multiple sclerosis and HIV-associated dementia (Iida et al., 2000; Kassiotis and Kollias, 2001; Sriram et al., 2002). The signaling mechanism underlying the ability of TNF-α to cause the cognitive dysfunction associated with many of these conditions has been probed, but to date no clear mechanistic understanding has been reached.

TNF-α and IL-1β at pathophysiological levels have been shown by many authors to inhibit long-term potentiation (LTP) in the CA1 and the dentate gyrus regions of the rat hippocampus (Cunningham et al., 1996; Murray and Lynch, 1998; Tancredi et al., 1992) but not by others (Stellwagen and Malenka, 2006). IL-1β has been shown to depress NMDA-receptor mediated field potentials in the dentate gyrus (Coogan

* *Corresponding author*. Fax: +353 1 716 7417.
E-mail address: john.oconnor@ucd.ie (J.J. O'Connor).

0006-8993/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.brainres.2006.12.019

and O'Connor, 1997) which may contribute to an impaired calcium ion influx during high frequency stimulation (HFS) (Cunningham et al., 1996). IL-1β has additionally been reported to inhibit voltage-dependent calcium channel (VDCC) function in the CA1 region (Plata-Salaman and Ffrench-Mullen, 1992; Plata-Salaman and ffrench-Mullen, 1994).

We have previously reported that TNF-α inhibition of early-LTP (1 h post-tetanus) could be blocked with a specific p38 MAPK inhibitor which had no effect on early or late-LTP (Butler et al., 2004). TNF-α has been shown to stimulate the release of Ca^{2+} from ryanodine-sensitive intracellular stores in dorsal root ganglion neurons with concomitant increases in the active phosphorylated forms of the stress kinases, c-Jun N-terminal kinase and p38 MAPK (Pollock et al., 2002). Both of these MAPKs have been shown to be involved in mGluR-dependent LTD.

The involvement of the mGluRs in hippocampal LTD has been well established (Bolshakov and Siegelbaum, 1994; Fitzjohn et al., 1999; O'Mara et al., 1995; Snyder et al., 2001), however their contribution to LTP has been a subject of unresolved debate (Bashir et al., 1993; Bortolotto et al., 1995; Chinestra et al., 1993; Manzoni et al., 1994; Richter-Levin et al., 1994; Riedel et al., 1995; Selig et al., 1995). To date, eight mGluR subtypes, designated mGluR1 to mGluR8, have been cloned from the mammalian brain. These are classified into three main groups (I, II, III) based on differences in sequence homology, coupling to second messenger systems and selectivity for various agonists (Anwyl, 1999; Conn and Pin, 1997). The group I mGluRs consists of subtypes mGluR1 and mGluR5, which couple preferentially to the Gq family of heterotrimeric G-proteins, Gq and G_{11}. Activation of the G-protein coupled receptor (GPCR) via agonist binding leads to a GDP/GTP exchange in the Gαq subunit of the Gαβγq-protein, which leads to the activation of phospholipase C (PLC)β, and the generation of important second messengers inositol-1-,4,5-trisphosphate (IP3) and diacylglycerol (DAG) from phosphatidylinositol 4,5 bisphosphate. IP3 causes the release of Ca^{2+} from intracellular stores and DAG the activation of protein kinase C (PKC). Group I antagonists have been shown to inhibit mGluR LTD in the dentate gyrus (Camodeca et al., 1999; O'Mara et al., 1995) and the CA1 region of the hippocampus.

In this study, we explored the role of mGluRs in mediating the inhibitory effect of TNF-α on LTP. We provide evidence that the TNF-α inhibition of LTP may be linked to mGluR5 receptor activation, as well as deregulated release of Ca^{2+} from ryanodine-sensitive intracellular Ca^{2+} stores.

2. Results

2.1. *The mGluR antagonists (RS)-MCPG and MPEP reduced the magnitude of LTP in the dentate gyrus*

LTP was induced by applying high frequency stimulation (HFS; eight trains of eight pulses at 200 Hz) to the medial perforant path of the dentate gyrus, which led to a robust LTP that was monitored for 1 h post-tetanus. We tested the effect of the group I/II metabotropic glutamate receptor (mGluR) antagonist (RS)-MCPG on the induction of LTP. MCPG (500 μM) was bath applied to the hippocampal slice 1 h pre-HFS and the fEPSPs responses recorded for 1 h post-tetanus. MCPG had no effect on baseline synaptic transmission but reduced the magnitude of LTP measured at 1 h post-tetanus (MCPG/LTP 151±4%, $n=5$ compared to 177±8% in control slices, $n=5$, $P<0.05$). A comparison of 100 to 120 min post tetanus between groups using ANOVA also revealed a significant difference ($F(1,8)=5.82$, $P<0.01$) (Fig. 1A). The specific group I mGluR5 antagonist MPEP (5 μM) was perfused through the hippocampal slice for 1 h prior to HFS. No effect on baseline synaptic transmission was observed, however, there was a small reduction in the magnitude of LTP which was significantly different from that observed in control LTP at 1 h post HFS (MPEP/LTP 140±5%, $n=4$ versus control LTP 167±7%, $n=5$, $P<0.05$). A comparison of 100 to 120 min post tetanus between groups using ANOVA also revealed a significant difference ($F(1, 7)=128.1$, $P<0.001$) (Fig. 1B).

2.2. *The group I/II mGluR antagonist MCPG attenuates TNF-α inhibition of LTP*

MCPG (500 μM) was added to the hippocampal slice 40 min prior to the addition of TNF-α (5 ng/ml), which was then perfused for 20 min before applying HFS. Evoked fEPSPs were monitored for 1 h post-tetanus. MCPG was found to significantly attenuate TNF-α mediated inhibition of early-LTP (MCPG/TNF-α LTP 152±12%, $n=5$ versus TNF-α/LTP 116±7%, $n=5$, 1 h post-tetanus, ANOVA, 100 to 120 min post tetanus ($F(1,8)=136.2$, $P<0.001$; Fig. 1A). Indeed, the MCPG/TNF-α LTP was not significantly different from MCPG/LTP at this same time-point (MCPG/TNF-α LTP measured 152±12%, $n=5$ versus MCPG/LTP of 151±4%, $n=5$, 1 h post-tetanus; Fig. 1A).

2.3. *The specific group I mGluR antagonist MPEP attenuates TNF-α inhibition of LTP*

The finding that the non-specific group I/II antagonist MCPG significantly attenuated TNF-α inhibition of LTP prompted us to extend this work and determine if TNF-α inhibition of LTP was mediated specifically through mGluR5 (Camodeca et al., 1999; Sriram et al., 2002; Wang et al., 2004). Slices were perfused with MPEP (5 μM) a selective mGluR5 antagonist (Malherbe et al., 2003) for 40 min, prior to the addition of TNF-α (5 ng/ml). HFS was then applied to the slice 20 min later and the electrophysiological response monitored for 1 h post-tetanus. MPEP markedly attenuated the TNF-α impairment of LTP; in slices pre-treated with MPEP, LTP measured 140±5%, $n=4$ compared to LTP of 102±9% in slices pre-treated with TNF-α alone, $n=6$ (ANOVA, 100 to 120 min post tetanus ($F(1,8)=58.6$, $P<0.01$; Fig. 1B). The magnitude of LTP measured at 1 h post-tetanus in MPEP/TNF-α slices was not significantly different from MPEP/LTP (MPEP/TNF-α LTP 137±4% versus MPEP/LTP 140±5% Fig. 1B).

2.4. *TNF-α had no effect on paired pulse depression in the dentate gyrus*

To investigate if TNF-α inhibition of LTP may have had a pre-synaptic component involving increased glutamate release, paired pulse stimuli were carried out at an inter stimulus interval (ISI) of 50 ms. We have previously reported on the role

of mGluRs in paired pulse depression in the dentate gyrus. TNF-α (5 ng/ml) had no effect on paired pulse depression (PPD) in the dentate gyrus when perfused for 2 h. Control PPD at 50 ms ISI was 87±3% (S1–S2)/S1 percent), $n=4$. At 2 h post TNF-α application, PPD was 85±4% ($n=4$, Fig. 2).

2.5. *Ryanodine attenuates the inhibitory effect of TNF-α on LTP*

In order to determine if TNF-α effects on LTP are in part affected by a rise in intracellular calcium levels ryanodine (which blocks the release of intracellular calcium from ryanodine-sensitive stores) was bath applied to the slice 40 min pre-TNF-α addition. Ryanodine was used at a concentration of 1 μM in these studies as this concentration has previously been demonstrated to activate the channel and stabilize it in a sub-conductance state, whilst higher concentrations lock the channel in a closed state (> 100 μM) (Fleischer and Inui, 1989; Meissner, 1994). Ryanodine had no significant effect on the induction of LTP at this concentration (ryanodine/LTP 155±9%, $n=6$, versus control LTP 164±6%, $n=6$; Fig. 3). Interestingly ryanodine was found to significantly attenuate the effects of TNF-α on LTP (ryanodine/TNF-α 172±12%, $n=4$ versus TNF-α alone 103±17% at 1 h, $n=6$ (ANOVA, 100 to 120 min post tetanus ($F(1,8)=127.9$, $P<0.001$; Fig. 3).

Fig. 4 is a summary graph of the effects of all the agents on LTP. For clarity each bar represents data analyzed at 1 h post-induction of LTP. Significant differences between groups are indicated by the asterisks (*$P<0.05$, **$P<0.01$).

3. Discussion

We have presented evidence in this study that the inhibition of LTP in the rat dentate gyrus caused by TNF-α is mediated in part by mGluR5 activation. The findings of this paper show that MCPG a non-specific group I/II antagonist and MPEP the specific mGluR5 antagonist have a small but significant effect on LTP on their own. Furthermore both antagonists significantly attenuate the TNF-α inhibition of LTP. Finally the inhibition of LTP was markedly attenuated by ryanodine, implicating the deregulated release of Ca^{2+} from intracellular stores in TNF-α inhibition of LTP. Future work can now target these newly identified areas in the TNF-α negative modulation of LTP.

Evidence to connect TNF-α to GPCR signaling pathways has come mainly to date from molecular studies on cell lines showing that TNF-α leads to the activation of downstream G-protein linked signaling molecules including PI3-K, PLC, PKC and PKA (Amrani et al., 1997; Chen et al., 2000; Hotta et al., 1999; Marchetti et al., 2004; Pascual et al., 2001). Interestingly TNF-α has been shown to have a direct effect on the state of activation of the GPCR by influencing the protein levels of a negative regulator of G-protein signaling RGS-7, which is found to be highly expressed in the mammalian

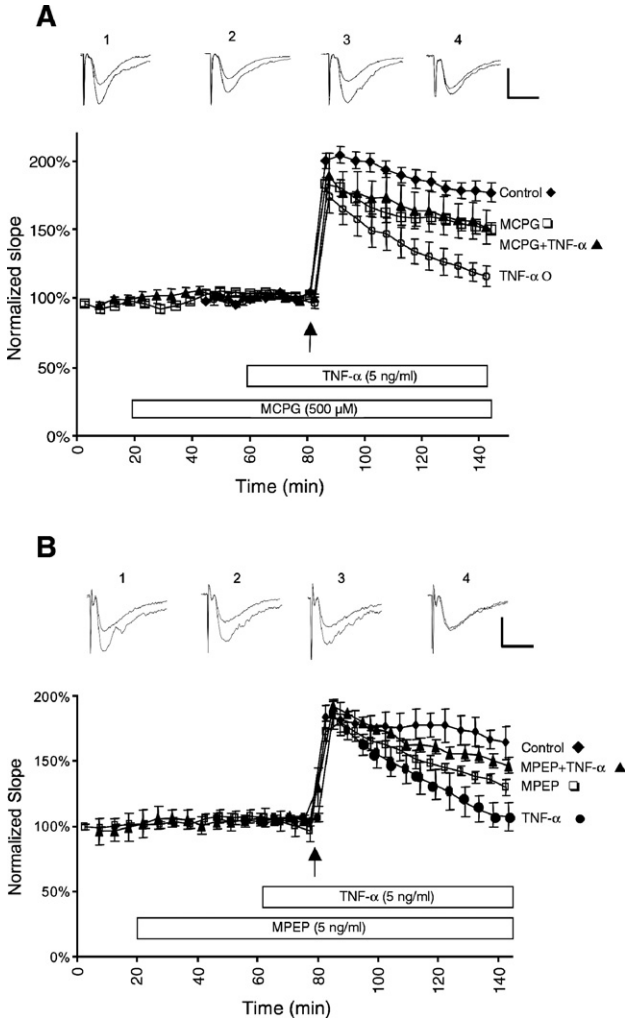

Fig. 1 – The mGluR antagonists MCPG and MPEP reverse the inhibitory effect of TNF-α on LTP. (A) Effects of the group I/II mGluR antagonist MCPG on LTP. Insets show single traces of representative fEPSP (1, control 10 min pre-tetanus and 1 h post-tetanus; 2, 10 min pre-tetanus MCPG treated slice and 1 h post-tetanus; 3, 10 min pre-tetanus MCPG+TNF-α treated slice and 1 h post-tetanus; 4, 10 min pre-tetanus TNF-α treated slice and 1 h post-tetanus. MCPG (500 μM) was added at time 20 min to the hippocampal slice and 1 h later HFS was applied. The magnitude of early-LTP was significantly lower in MCPG pre-treated slices at 1 h post-tetanus when compared to control LTP. TNF-α (5 ng/ml) was added at time 60 min and HFS applied 20 min later. TNF-α significantly inhibited LTP at 1 hr post tetanus. MCPG was found to significantly reverse the inhibitory effect of TNF-α on early-LTP. (B) Effects of the specific mGluR antagonist MPEP on LTP. Insets show single traces of representative fEPSP (1), control 10 min pre-tetanus and 1 h post-tetanus; (2) 10 min pre-tetanus MPEP treated slice and 1 h post-tetanus; (3) 10 min pre-tetanus MPEP+TNF-α treated slice and 1 h post-tetanus; (4) 10 min pre-tetanus TNF-α treated slice and 1 h post-tetanus. MPEP (5 μM) was allowed to perfuse through the slice for 60 min before applying HFS. MPEP significantly reduced the magnitude of LTP when compared to control LTP. TNF-α (5 ng/ml) was added at time 60 min and HFS applied 20 min later. TNF-α significantly inhibited LTP at 1 hr post tetanus. MPEP was found to significantly reverse the inhibitory effect of TNF-α on early-LTP. Scale bars: horizontal, 10 ms: vertical, 1 mV for both A and B.

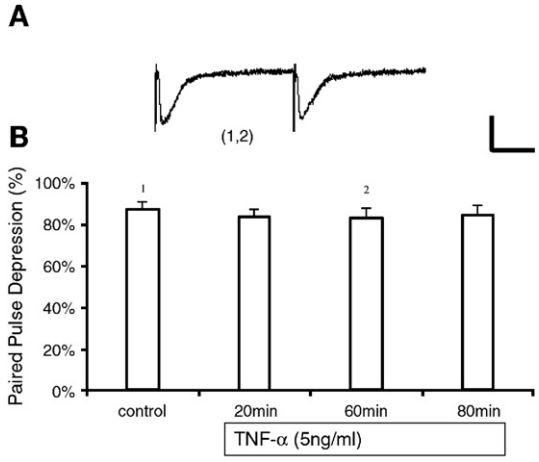

Fig. 2 – TNF-α has no effect on paired pulse depression (PPD) in the dentate gyrus. (A) Typical examples of traces showing paired pulse depression applied at inter stimulus intervals of 50 ms (1, control; 2, TNF-α 2 h following application). (B) Averaged data for the effect of TNF-α on paired pulse depression at the inter-stimulus interval of 50 ms. TNF-α had no significant effect on paired pulse depression at 1 h and 2 h post-TNF-α application. Scale bars: horizontal, 25 ms: vertical, 1 mV.

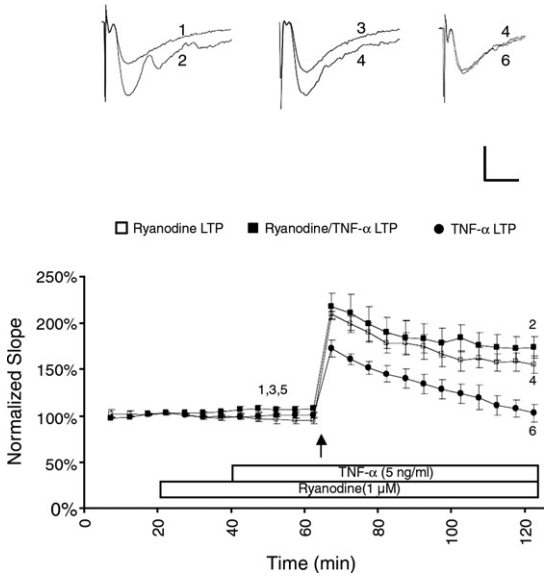

Fig. 3 – Ryanodine attenuates the inhibitory effect of TNF-α on LTP. Insets show single traces of representative fEPSP (1) control 10 min pre-tetanus; (2) control 1 h post-tetanus; (3) ryanodine (1 μM) treated slices 10 min before; (4) ryanodine treated slices 1 h post-tetanus; (5) Ryanodine/TNF-α treated slices 10 min before; (6) Ryanodine/TNF-α treated slices 1 h post-tetanus. Scale bars: horizontal, 10 ms: vertical, 1 mV. Slices were perfused with ryanodine (1 μM) for 40 min before HFS or with ryanodine 40 min prior to TNF-α addition which was perfused for 20 min before applying HFS. Evoked fEPSPs were monitored for 1 h post-tetanus. LTP was not significantly different in controls versus ryanodine treated slices. However ryanodine significantly attenuated the inhibitory effect of TNF-α on LTP ($n=6$ for controls and TNF-α treated slices, $n=4$ for ryanodine + TNF-α treated slices).

brain including the hippocampus. TNF-α leads to the rapid increase (15–20 min) of RGS-7 by a mechanism that involves p38 MAPK activation (Benzing et al., 1999; Benzing et al., 2002). RGS-7 increases the hydrolysis rate of active GTP-bound Gα subunits thereby causing their dissociation from downstream G-protein linked effector molecules and re-association with the Gβγ subunits returning to the inactive state. Interestingly a study by Butler et al. (2004) demonstrated a role for p38 MAPK in the TNF-α mediated inhibition of LTP. It would be interesting to examine the RGS-7-GPCR-signaling in relation to TNF-α inhibition of LTP in future studies.

The LTP seen in the presence of the mGluR antagonists alone (MCPG and MPEP) was found to be reduced when compared to control LTP in our experiments. This has previously been reported by some groups (Bashir et al., 1993; Bortolotto et al., 1995; Richter-Levin et al., 1994; Riedel et al., 1995), and not by others (Chinestra et al., 1993; Manzoni et al., 1994; Selig et al., 1995). Also work from mGluR5 knockout mice have shown that NMDA-receptor mediated LTP is impaired in these animals (Jia et al., 1998; Lu et al., 1997). However the concentrations of these compound that was chosen allowed for a significantly robust LTP to remain. The group I/II mGluR antagonist MCPG and the selective mGluR5 antagonist MPEP both reversed the TNF-α inhibition of LTP to levels comparable to the corresponding mGluR antagonist LTP. Since blockade of the group I mGlu5 receptor antagonizes TNF-α inhibition of LTP we suggest that TNF-α inhibition of LTP may depend upon the activation of the group I mGlu5 receptor. Activation of mGluR receptors by ACPD, a group I/II agonist can lead to p38 phosphorylation (Bolshakov et al., 2000). Our data suggest that mGluR receptor activation, involving mGlu5, causes p38 activation, which leads to the inhibition of LTP (Butler et al., 2004).

We examined how the group I mGluR is activated in this present study by considering the possibility of a pre-synaptic mechanism of action, one in which TNF-α could activate the group I mGluR through stimulating L-glutamate release from

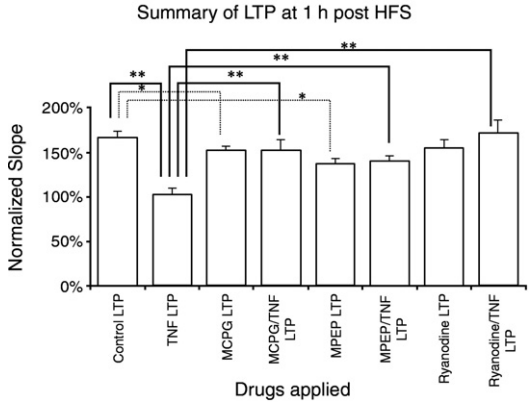

Fig. 4 – Summary graph of the effects of all the agents on LTP. For clarity each bar represents data analyzed at 1 h post-induction of LTP. Significant differences between groups are indicated by the asterisks. *$P<0.05$; **$P<0.01$.

glial cells in the immediate microenvironment. Paired pulse depression studies were performed. However no change in paired pulse depression was observed between TNF-α and control slices at 20 min post-TNF-α addition, the time-point at which HFS was applied making a pre-synaptic locus less likely. It is possible that the HFS protocol used to induce LTP in the present study leads to glutamate release from pre-synaptic terminals in turn activating the mGluR5 receptor. However studies utilizing paired pulse depression in the dentate gyrus have their limitations and further work would be necessary to indicate a presynaptic locus of affect.

Because TNF-α inhibition of LTP is attenuated by group I mGluR antagonists and the fact that mGluR5 activation is coupled to Ca^{2+} mobilization from intracellular stores prompted us to examine if disturbances in intracellular Ca^{2+} homeostasis could be a pivotal factor in the inhibition of LTP. Work by Pollock et al. (2002) on dorsal root ganglion (DRG) neurons provided the first proof that TNF-α application by itself could cause Ca^{2+} mobilization directly from ryanodine-sensitive intracellular stores with transient Ca^{2+} dependent inward currents being recorded from DRG neurons within an acute time frame. We decided to examine if the release of Ca^{2+} from such stores could underlie TNF-α mediated inhibition of LTP. This paper presents the first evidence that ryanodine, an inhibitor of ryanodine receptor Ca^{2+}-release channels significantly reversed the inhibition of LTP by TNF-α, while having no effect on the induction of LTP at the same concentration. Amyloid-β, which has recently being proposed to inhibit LTP through the activation of the TNF-R1 (p55) receptor (Wang et al., 2005), has previously been shown to reduce NMDA EPSCs (Chen et al., 2002; Snyder et al., 2005) and lead to transient intracellular Ca^{2+} rises in cultured hippocampal neurons (Smith et al., 2001). It would be intriguing to speculate if amyloid-β mediated inhibition of LTP could be similarly blocked with ryanodine. Interestingly, the release of Ca^{2+} from both the RyR and IP_3R have previously been demonstrated to be involved in amyloid-β mediated neurotoxicity (Ferreiro et al., 2004) and to involve the activation of calcineurin (Agostinho and Oliveira, 2003). Calcineurin is a key phosphatase that limits synaptic responses by causing the dephosphorylation of target proteins including inhibitor 1 protein, the regulator of protein phosphatase 1 (PP1) (Mulkey et al., 1994). Active PP1 can then lead to the dephosphorylation of Ca^{2+}/calmodulin dependent protein kinase II (CaMK II) and suppress LTP induction. We hypothesize that calcineurin could become activated following TNF-α application by a rise in Ca^{2+} from intracellular calcium stores and negatively affect the induction of LTP. Interestingly inhibition of calcineurin has been found to attenuate amyloid-beta- mediated inhibition of late-LTP in the dentate gyrus (Chen et al., 2002).

It is not fully clear how TNFR1 (p55) might play a role in the TNF-α mediated inhibition of LTP. Our data would seem to uncover a new cross talk role for the G protein coupled mGluRs and the p38 MAPK. There is a possibility that the cross talk between TNFR1 (p55) and the G-protein coupled mGluRs gives rise to an increase in Ca^{2+} levels leading to inhibition of LTP via a saturation or necrotic mechanism. Sufficient activation of mGluRs and reduced activation of TNFR1 is required for LTP. We propose that the inhibition of LTP by TNF-α involves activation of mGluRs and the p38 MAP kinase. Through high frequency stimulation at the induction of LTP, mGluRs are activated by glutamate while TNF-α activates TNFR1 (p55). The combined activation of these receptors may stimulate sufficient p38 MAP kinase activity to give rise to the TNF-α inhibition of LTP (see Fig. 5 for a schematic diagram).

In conclusion we have shown in this study that the TNF-α inhibitory effects on LTP are dependent on mGluR activation. Activation of the mGluR receptors do not by themselves lead to an inhibition of LTP. It is only in the presence of pathological levels of the cytokine TNF?α that their activation becomes negative in LTP induction. It is thus possible that this rise in intracellular Ca^{2+} caused by TNF-α may lead to the activation of calcineurin which antagonizes the action of PKA in the phosphorylation/dephosphorylation status of Ca^{2+}/calmodulin dependent protein kinase II, a key kinase involved in LTP induction mechanisms. A full understanding of the inhibitory effects of TNF-α on LTP awaits further clarification but this paper presents findings that will undoubtedly focus future studies on the mGluR-intracellular Ca^{2+} pathway, providing further insight into the complex nature of TNF-α signaling in the brain.

4. Experimental procedures

Adult male Wistar rats (50–80 g) were decapitated under sevoflurane anaesthesia, in accordance with UCD and the Department of Health regulations. Transverse hippocampal slices (350 μm) were prepared by standard methods. Slices were equilibrated for at least 1 h in a holding chamber at room temperature, pH 7.4, in oxygenated artificial cerebrospinal fluid (aCSF; composition in mM: NaCl, 120; KCl, 2.5; $MgSO_4$, 2; $CaCl_2$, 2; $NaHCO_3$, 26; NaH_2PO_4, 1.25; D-glucose, 10) before being transferred to a recording chamber perfused with aCSF at a

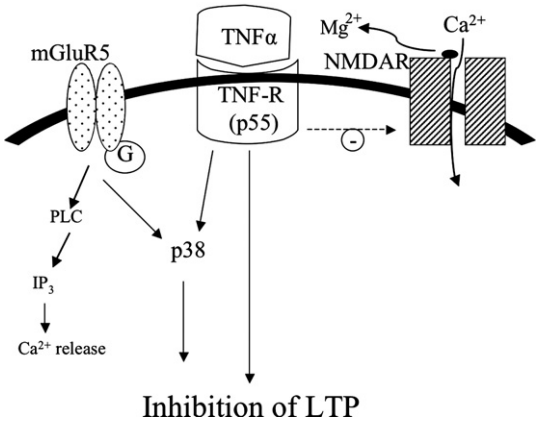

Fig. 5 – Schematic diagram of the role of the mGluR and TNFR1 in LTP TNF-α inhibits the early phase of LTP by activation of TNFR1 (p55) and is dependent on p38 activation. Activation of the G-protein-linked mGluRs leads to inositol-1,4,5-trisphosphate (IP3) receptor-mediated calcium release via phospholipase C (PLC). TNF-α may also alter calcium homeostasis. Combined activation of TNFR1 and mGluR5 may lead to the impairment of LTP. TNF-α may also have modulatory effects on the NMDA receptor.

flow rate of 5 ml/min at 29–30 °C. Extracellular recordings of field excitatory post-synaptic potentials (fEPSP) were elicited by stimulation of the medial perforant path of the dentate gyrus by a monopolar glass electrode at a frequency of 0.05 Hz. Responses were recorded by a glass electrode placed in the middle third of the molecular layer in the presence of 100 μM of the $GABA_A$ receptor antagonist picrotoxin (Sigma- Aldrich Ireland Ltd., Dublin, Ireland) and stimulus strength was adjusted to give a response 35% of maximal amplitude. Stable baseline recordings were made for at least 20 min prior to application of drugs. LTP was induced by high frequency stimulation (HFS) consisting of eight trains of eight pulses at 200 Hz at 2 s intervals at a stimulus strength corresponding to 75% of maximal amplitude. Recordings were analyzed off-line using the Strathclyde electrophysiology software (J. Dempster, Glasgow, UK).

Recombinant rat TNF-α (R&D systems, UK) was prepared in sterile PBS containing 0.1% BSA (Sigma-Aldrich Ireland Ltd.). Stable baseline recordings (20 min) were made before application of TNF-α (5 ng/ml). This concentration is known to be in the pathophysiological range. Additional drugs used included (RS)-*a*-methyl-4-carboxyphenylglycine (MCPG; Tocris Cookson Ltd., UK) dissolved in 1 eq. NaOH to 100 mM. Methyl-6-(phenylethynyl) pyridine (MPEP; Sigma, UK) and ryanodine all dissolved in distilled H_2O.

Final concentrations of the respective drugs applied to the slice preparation in the extracellular aCSF were as follows; TNF-α, 5 ng/ml, (RS)-MCPG, 500 μM, MPEP, 5 μM, Ryanodine, 1 μM. Vehicle controls for these drugs were carried out in all cases.

All data are expressed as mean ± S.E.M. In all experiments examining LTP, all data points represent 5 min averages. For statistical analysis of data, two-tailed paired student's *t*-test was employed to test significance between baseline and post-treatment values, and two-tailed unpaired student's *t*-test was employed for comparisons between different test and control slices. In Figs. 1 and 3 single factor ANOVA with post hoc Dunnett's was carried out to test the significance between the mGluR antagonists (MCPG and MPEP), ryanodine and TNF-α and control LTP, respectively at the time interval, 100 to 120 min post tetanus.

Acknowledgment

This work was supported by the Higher Education Authority of Ireland (PRTLI cycle 3).

REFERENCES

Agostinho, P., Oliveira, C.R., 2003. Involvement of calcineurin in the neurotoxic effects induced by amyloid-beta and prion peptides. Eur. J. Neurosci. 17, 1189–1196.

Amrani, Y., Krymskaya, V., Maki, C., Panettieri Jr., R.A., 1997. Mechanisms underlying TNF-alpha effects on agonist-mediated calcium homeostasis in human airway smooth muscle cells. Am. J. Physiol. 273, L1020–L1028.

Anwyl, R., 1999. Metabotropic glutamate receptors: electrophysiological properties and role in plasticity. Brain Res. Brain Res. Rev. 29, 83–120.

Bashir, Z.I., Bortolotto, Z.A., Davies, C.H., Berretta, N., Irving, A.J., Seal, A.J., Henley, J.M., Jane, D.E., Watkins, J.C., Collingridge, G.L., 1993. Induction of LTP in the hippocampus needs synaptic activation of glutamate metabotropic receptors. Nature 363, 347–350.

Benzing, T., Brandes, R., Sellin, L., Schermer, B., Lecker, S., Walz, G., Kim, E., 1999. Upregulation of RGS7 may contribute to tumor necrosis factor-induced changes in central nervous function. Nat. Med. 5, 913–918.

Benzing, T., Kottgen, M., Johnson, M., Schermer, B., Zentgraf, H., Walz, G., Kim, E., 2002. Interaction of 14-3-3 protein with regulator of G protein signaling 7 is dynamically regulated by tumor necrosis factor-alpha. J. Biol. Chem. 277, 32954–32962.

Bolshakov, V.Y., Siegelbaum, S.A., 1994. Postsynaptic induction and presynaptic expression of hippocampal long-term depression. Science 264, 1148–1152.

Bolshakov, V.Y., Carboni, L., Cobb, M.H., Siegelbaum, S.A., Belardetti, F., 2000. Dual MAP kinase pathways mediate opposing forms of long-term plasticity at CA3-CA1 synapses. Nat. Neurosci. 3, 1107–1112.

Bortolotto, Z.A., Bashir, Z.I., Davies, C.H., Taira, T., Kaila, K., Collingridge, G.L., 1995. Studies on the role of metabotropic glutamate receptors in long-term potentiation: some methodological considerations. J. Neurosci. Methods 59, 19–24.

Butler, M.P., O'Connor, J.J., Moynagh, P.N., 2004. Dissection of tumor-necrosis factor-alpha inhibition of long-term potentiation (LTP) reveals a p38 mitogen-activated protein kinase-dependent mechanism which maps to early-but not late-phase LTP. Neuroscience 124, 319–326.

Camodeca, N., Breakwell, N.A., Rowan, M.J., Anwyl, R., 1999. Induction of LTD by activation of group I mGluR in the dentate gyrus in vitro. Neuropharmacology 38, 1597–1606.

Chen, C.C., Sun, Y.T., Chen, J.J., Chiu, K.T., 2000. TNF-alpha-induced cyclooxygenase-2 expression in human lung epithelial cells: involvement of the phospholipase C-gamma 2, protein kinase C-alpha, tyrosine kinase, NF-kappa B-inducing kinase, and I-kappa B kinase 1/2 pathway. J. Immunol. 165, 2719–2728.

Chen, Q.S., Wei, W.Z., Shimahara, T., Xie, C.W., 2002. Alzheimer amyloid beta-peptide inhibits the late phase of long-term potentiation through calcineurin-dependent mechanisms in the hippocampal dentate gyrus. Neurobiol. Learn. Mem. 77, 354–371.

Chinestra, P., Aniksztejn, L., Diabira, D., Ben-Ari, Y., 1993. (RS)-alpha-methyl-4-carboxyphenylglycine neither prevents induction of LTP nor antagonizes metabotropic glutamate receptors in CA1 hippocampal neurons. J. Neurophysiol. 70, 2684–2689.

Conn, P.J., Pin, J.P., 1997. Pharmacology and functions of metabotropic glutamate receptors. Annu. Rev. Pharmacol. Toxicol. 37, 205–237.

Coogan, A., O'Connor, J.J., 1997. Inhibition of NMDA receptor-mediated synaptic transmission in the rat dentate gyrus in vitro by IL-1 beta. NeuroReport 8, 2107–2110.

Cunningham, A.J., Murray, C.A., O'Neill, L.A., Lynch, M.A., O'Connor, J.J., 1996. Interleukin-1 beta (IL-1 beta) and tumour necrosis factor (TNF) inhibit long-term potentiation in the rat dentate gyrus in vitro. Neurosci. Lett. 203, 17–20.

Ferreiro, E., Oliveira, C.R., Pereira, C., 2004. Involvement of endoplasmic reticulum Ca^{2+} release through ryanodine and inositol 1,4,5-triphosphate receptors in the neurotoxic effects induced by the amyloid-beta peptide. J. Neurosci. Res. 76, 872–880.

Fitzjohn, S.M., Kingston, A.E., Lodge, D., Collingridge, G.L., 1999. DHPG-induced LTD in area CA1 of juvenile rat hippocampus; characterisation and sensitivity to novel mGlu receptor antagonists. Neuropharmacology 38, 1577–1583.

Fleischer, S., Inui, M., 1989. Biochemistry and biophysics of excitation–contraction coupling. Annu. Rev. Biophys. Biophys. Chem. 18, 333–364.

Hotta, K., Emala, C.W., Hirshman, C.A., 1999. TNF-alpha upregulates Gialpha and Gqalpha protein expression and function in human airway smooth muscle cells. Am. J. Physiol. 276, L405–L411.

Iida, R., Saito, K., Yamada, K., Basile, A.S., Sekikawa, K., Takemura, M., Fujii, H., Wada, H., Seishima, M., Nabeshima, T., 2000. Suppression of neurocognitive damage in LP-BM5-infected mice with a targeted deletion of the TNF-alpha gene. FASEB J. 14, 1023–1031.

Jia, Z., Lu, Y., Henderson, J., Taverna, F., Romano, C., Abramow-Newerly, W., Wojtowicz, J.M., Roder, J., 1998. Selective abolition of the NMDA component of long-term potentiation in mice lacking mGluR5. Learn. Mem. 5, 331–343.

Kassiotis, G., Kollias, G., 2001. Uncoupling the proinflammatory from the immunosuppressive properties of tumor necrosis factor (TNF) at the p55 TNF receptor level: implications for pathogenesis and therapy of autoimmune demyelination. J. Exp. Med. 193, 427–434.

Lu, Y.M., Jia, Z., Janus, C., Henderson, J.T., Gerlai, R., Wojtowicz, J.M., Roder, J.C., 1997. Mice lacking metabotropic glutamate receptor 5 show impaired learning and reduced CA1 long-term potentiation (LTP) but normal CA3 LTP. J. Neurosci. 17, 5196–5205.

Malherbe, P., Kratochwil, N., Zenner, M.T., Piussi, J., Diener, C., Kratzeisen, C., Fischer, C., Porter, R.H., 2003. Mutational analysis and molecular modeling of the binding pocket of the metabotropic glutamate 5 receptor negative modulator 2-methyl-6-(phenylethynyl)-pyridine. Mol. Pharmacol. 64, 823–832.

Manzoni, O.J., Weisskopf, M.G., Nicoll, R.A., 1994. MCPG antagonizes metabotropic glutamate receptors but not long-term potentiation in the hippocampus. Eur. J. Neurosci. 6, 1050–1054.

Marchetti, L., Klein, M., Schlett, K., Pfizenmaier, K., Eisel, U.L., 2004. Tumor necrosis factor (TNF)-mediated neuroprotection against glutamate-induced excitotoxicity is enhanced by N-methyl-D-aspartate receptor activation. Essential role of a TNF receptor 2-mediated phosphatidylinositol 3-kinase-dependent NF-kappa B pathway. J. Biol. Chem. 279, 32869–32881.

Meissner, G., 1994. Ryanodine receptor/Ca^{2+} release channels and their regulation by endogenous effectors. Annu. Rev. Physiol. 56, 485–508.

Mulkey, R.M., Endo, S., Shenolikar, S., Malenka, R.C., 1994. Involvement of a calcineurin/inhibitor-1 phosphatase cascade in hippocampal long-term depression. Nature 369, 486–488.

Murray, C.A., Lynch, M.A., 1998. Evidence that increased hippocampal expression of the cytokine interleukin-1 beta is a common trigger for age- and stress-induced impairments in long-term potentiation. J. Neurosci. 18, 2974–2981.

O'Mara, S.M., Rowan, M.J., Anwyl, R., 1995. Metabotropic glutamate receptor-induced homosynaptic long-term depression and depotentiation in the dentate gyrus of the rat hippocampus in vitro. Neuropharmacology 34, 983–989.

Pascual, R.M., Billington, C.K., Hall, I.P., Panettieri Jr., R.A., Fish, J.E., Peters, S.P., Penn, R.B., 2001. Mechanisms of cytokine effects on G protein-coupled receptor-mediated signaling in airway smooth muscle. Am. J. Physiol.: Lung Cell. Mol. Physiol. 281, L1425–L1435.

Plata-Salaman, C.R., Ffrench-Mullen, J.M., 1992. Interleukin-1 beta depresses calcium currents in CA1 hippocampal neurons at pathophysiological concentrations. Brain Res. Bull. 29, 221–223.

Plata-Salaman, C.R., ffrench-Mullen, J.M., 1994. Interleukin-1 beta inhibits Ca^{2+} channel currents in hippocampal neurons through protein kinase C. Eur. J. Pharmacol. 266, 1–10.

Pollock, J., McFarlane, S.M., Connell, M.C., Zehavi, U., Vandenabeele, P., MacEwan, D.J., Scott, R.H., 2002. TNF-alpha receptors simultaneously activate Ca^{2+} mobilisation and stress kinases in cultured sensory neurones. Neuropharmacology 42, 93–106.

Richter-Levin, G., Errington, M.L., Maegawa, H., Bliss, T.V., 1994. Activation of metabotropic glutamate receptors is necessary for long-term potentiation in the dentate gyrus and for spatial learning. Neuropharmacology 33, 853–857.

Riedel, G., Casabona, G., Reymann, K.G., 1995. Inhibition of long-term potentiation in the dentate gyrus of freely moving rats by the metabotropic glutamate receptor antagonist MCPG. J. Neurosci. 15, 87–98.

Selig, D.K., Lee, H.K., Bear, M.F., Malenka, R.C., 1995. Reexamination of the effects of MCPG on hippocampal LTP, LTD, and depotentiation. J. Neurophysiol. 74, 1075–1082.

Smith, I.F., Boyle, J.P., Vaughan, P.F., Pearson, H.A., Peers, C., 2001. Effects of chronic hypoxia on Ca(2+) stores and capacitative Ca(2+) entry in human neuroblastoma (SH-SY5Y) cells J. Neurochem. 79, 877–884.

Snyder, E.M., Nong, Y., Almeida, C.G., Paul, S., Moran, T., Choi, E.Y., Nairn, A.C., Salter, M.W., Lombroso, P.J., Gouras, G.K., Greengard, P., 2005. Regulation of NMDA receptor trafficking by amyloid-beta. Nat. Neurosci. 8, 1051–1058.

Snyder, E.M., Philpot, B.D., Huber, K.M., Dong, X., Fallon, J.R., Bear, M.F., 2001. Internalization of ionotropic glutamate receptors in response to mGluR activation. Nat. Neurosci. 4, 1079–1085.

Sriram, K., Matheson, J.M., Benkovic, S.A., Miller, D.B., Luster, M.I., O'Callaghan, J.P., 2002. Mice deficient in TNF receptors are protected against dopaminergic neurotoxicity: implications for Parkinson's disease. FASEB J. 16, 1474–1476.

Stellwagen, D., Malenka, R.C., 2006. Synaptic scaling mediated by glial TNF-α. Nature 440, 1054–1059.

Tancredi, V., D'Arcangelo, G., Grassi, F., Tarroni, P., Palmieri, G., Santoni, A., Eusebi, F., 1992. Tumor necrosis factor alters synaptic transmission in rat hippocampal slices. Neurosci. Lett. 146, 176–178.

Wang, Q., Walsh, D.M., Rowan, M.J., Selkoe, D.J., Anwyl, R., 2004. Block of long-term potentiation by naturally secreted and synthetic amyloid beta-peptide in hippocampal slices is mediated via activation of the kinases c-Jun N-terminal kinase, cyclin-dependent kinase 5, and p38 mitogen-activated protein kinase as well as metabotropic glutamate receptor type 5. J. Neurosci. 24, 3370–3378.

Wang, Q., Wu, J., Rowan, M.J., Anwyl, R., 2005. Beta-amyloid inhibition of long-term potentiation is mediated via tumor necrosis factor. Eur. J. Neurosci. 22, 2827–2832.