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A regulatory role for protease-activated receptor-2 in motivational learning in rats

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ABSTRACT

Serine proteases such as tissue plasminogen activator (tPA), thrombin and neuropsin influence hippocampal plasticity involved in learning and memory by facilitating both synaptic remodelling and long-term potentiation. Given our previous findings that trypsin and its receptor, protease-activated receptor-2 (PAR2), are both highly expressed in pyramidal neurons of the hippocampus and that activation of PAR2 attenuates 'pathogenic' plasticity related to epilepsy, we wished to determine the role for PAR2 in normal, non-pathological hippocampal plasticity related to learning and memory. In a strain of rat that show high basal levels of anxiety, the Genetic Absence Epilepsy Rats from Strasbourg (GAERS), peripheral administration of the PAR2 peptide agonist, SLIGRL (1.5 mg/kg s.c.), induced distinct deficits in experience-dependent learning both in the test–retest paradigm of the elevated-plus maze and in the Morris water maze. In separate, conscious rats with indwelling intra-cerebroventricular cannulae, SLIGRL rapidly appeared in cerebrospinal fluid (CSF) following peripheral administration and had a half-life in CSF of approximately 25 min. These results suggest that activation of central PAR2 with brain accessible peptide agonists causes a temporary deficit in the formation and/or recollection of experience-dependent learning and memory.

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1. Introduction

The serine proteases tPA (Calabresi et al., 2000; Centonze et al., 2002; Komai et al., 2000; Meighan, Meighan, Davis, Wright, & Harding, 2007; Nagy et al., 2006; Tomimatsu, Idemoto, Moriguchi, Watanabe, & Nakanishi, 2002), neuropsin (Tamura et al., 2006) and thrombin (Almonte et al., 2007; Ito et al., 2007) are implicated in NMDA-receptor mediated long-term potentiation (LTP) associated with cognition (Madani et al., 1999, 2003; Mhatre et al., 2004; Nagy, Bozdagi, & Huntley, 2007; Schmitt, Hiemke, Fahrenholz, & Schroeder, 2006), emotional-reward behaviour (Bahi & Dreyer, 2007; Ito et al., 2007; Nagai et al., 2006) and the development of stress-induced anxiety-like behaviours (Matys & Strickland, 2003; Pawlak, Magarinos, Melchor, McEwen, & Strickland, 2003; Pawlak et al., 2005). These behaviours appear to not only depend on the enhancement of NMDA receptor signalling in the hippocampus, but also synaptic remodelling and structural changes, such as dendritic spine loss (Bennur et al., 2007; Mitra, Jadhav, McEwen, Vyas, & Chattarji, 2005; Vyas, Jadhav, & Chattarji, 2006). Thus, serine proteases like tPA, thrombin and possibly neuropsin may facilitate cellular 'learning' via multiple mechanisms (Samson & Medcalf, 2006) and, as such, be involved in cognition and the pathogenesis of cognitive disorders like Alzheimer's disease (Cacquevel et al., 2007; Lee, Hwang, Im, Koh, & Kim, 2007; Melchor & Strickland, 2005; Mhatre et al., 2004; Wang, Luo, & Reiser, 2008), addiction (Bahi & Dreyer, 2007; Ito et al., 2007; Nagai et al., 2006) and anxiety (Almonte et al., 2007; Pawlak et al., 2003; Pawlak et al., 2005).

Whether these effects of serine proteases are mediated via centrally expressed protease-activated receptors (PARs) is unknown. PAR1 signalling, however, appears to mediate the potentiating effects of thrombin (Gingrich, Junge, Lyuboslavsky, & Traynelis, 2000) and plasmin (Mannaioni et al., 2008) on NMDA receptors rather than through a direct interaction of thrombin with NR1/2A and NR1/2B subunits of the NMDA receptor. Also, fear-mediated emotional learning in response to painful stimuli is reduced in mice lacking the PAR1 gene (Almonte et al., 2007) and PAR1 appears to be involved in the regulation of nicotine and morphine reward behaviours (Ito et al., 2007; Nagai et al., 2006). Thus, PAR1 may have important roles in protease-mediated acquisition, consolidation and/or retrieval of fear-motivated 'associative' memories. Although little research has investigated the involvement of PAR2 in such cognitive processes, it has been shown that neuronal PAR2 may be neuroprotective in a model of HIV-associated dementia (Noorbakhsh et al., 2005), against amyloid-β toxicity and endoplasmic reticulum stress in hippocampal pyramidal neurons in a model of Alzheimer's disease

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(Afkhami-Goli et al., 2007) and prevents trypsin over-expression in a model of epilepsy (Lohman, O'Brien, & Cocks, 2008); all neurodegenerative conditions ultimately affecting cognition.

If proteases are involved in memory formation (Centonze et al., 2002; Madani et al., 1999, 2003; Pawlak et al., 2005) and considering the colocalisation of trypsin with tPA in PAR2-positive pyramidal neurons in the hippocampus (Lohman, O'Brien, & Cocks, 2008), then both trypsin and PAR2 may have roles in plastic processes related to normal learning and memory. Here we tested this hypothesis by investigating the effects of SLIGRL in normal hippocampal-dependent cognition and on anxiety- and depressive-like behaviours associated with motivational learning using well-validated behavioural assays including the elevated-plus maze test–retest paradigm, the open field habituation test, the Morris water maze and the sucrose-consumption test. We also assessed brain permeability of SLIGRL following peripheral administration using liquid-chromatography mass spectrometry (LCMS) to validate this route of injection.

2. Materials and methods

2.1. Animals

Experiments were performed on Genetic Absence Rats of Strasbourg (GAERS), a strain of Wistar rat that show elevated anxiety-like behaviours (Jones et al., 2008), which provide a good model to study any changes in anxiety-like behaviours induced by PAR2. GAERS were bred and housed in the Ludwig Institute of Cancer Research/Department of Surgery, Royal Melbourne Hospital Biological Research Facility. Rats of both sexes (12–14 weeks of age) were housed in single-sex boxes in the facility maintained at 21–24 °C on a 12 h light/dark cycle, with food and water supplied *ad libitum*. Male rats were used for the CSF extraction experiments, whereas female rats were used in the behavioural studies as these were used in previous studies investigating central roles for PAR2 agonists (Lohman et al., 2008) and known to be responsive to SLIGRL. There have been no reported differences in the permeability of the blood–CSF barrier to peptides or other small molecules between male and female rats (Haqqani et al., 2005; Li et al., 2008).

2.2. Drugs and peptides

Ketamine (Parnell Laboratories) and xylazine (Troy Laboratories; 75 mg/kg and 10 mg/kg, respectively), diluted in 0.9% sterile saline for anaesthesia, were injected intraperitoneally (i.p.). Carprofen (Rimadyl, Pfizer, UK; 5 mg/kg in saline (1 ml)) for post-surgical analgesia was delivered subcutaneously (s.c.) prior to anaesthesia. Angiotensin II (AngII) (1 μM/μl in 0.9% sterile saline, Auspep, Australia) was injected intra-cerebroventricularly (i.c.v.) for cannula placement validation. Pentobarbitone sodium (Lethabarb, Virbac Animal Health; 300 mg/kg, i.p.) was used for euthanasia. Carboxyl-terminal amidated PAR2-activating (Ser-Leu-Iso-Gly-Arg-Leu-NH2; SLIGRL) and inactive 'control' (Leu-Arg-Gly-Iso-Leu-Ser-NH2; LRGILS) peptides (AusPep, Australia) in 0.9% sterile saline were administered (1.5 mg/kg s.c.) for behavioural tests.

3. Pharmacokinetic analysis of SLIGRL in CSF

3.1. Surgery

Male adult rats (200–250 g) were implanted with an indwelling stainless steel cerebroventricular cannula (9.0 mm length, 22G, Plastics One, Australia; stereotaxic coordinates; 1.5 mm lateral, 0.8 mm caudal to bregma, 3.0 mm ventral to brain surface (Paxinos & Watson, 2005)) as previously described (Lohman et al., 2008). The lateral ventricle was chosen as a source of CSF due to the ease of access during serial collection from an unanaesthetised animal (Malhotra, Lemaire, & Sawchuk, 1994; Yang, Huang, Gan, & Sawchuk, 2005) and the fact the CSF here mainly originates from the choroid plexus (Smith, Johanson, & Keep, 2004), where the immediate concentrations of compounds which cross from plasma to CSF are best assessed (Sussmuth, Brettschnieder, Ludolph, & Tumari, 2008).

3.2. Confirmation of i.c.v. cannula placement

Seven days post-surgery, rats implanted with i.c.v. cannulae were subjected to experiments which confirm the correct placement of the cannula into the lateral ventricle. When injected directly i.c.v., AngII induces excessive drinking behaviour in rats (DiNicolantonio et al., 1982; Stroud, O'Brien, Jupp, Wallengren, & Morris, 2005). Rats were placed individually in an experimental cage and water (of known weight/volume) supplied for 30 min, following which the water was reweighed. Rats were then injected with AngII (2 nM in 2 μl in saline, i.c.v. over 1 min) and returned to their boxes with the water bottles replaced for a further 30 min. Only rats that consumed $\geqslant$5-times more water after injection of AngII than before (Stroud et al., 2005) were used to further experimentation.

3.3. Cerebrospinal fluid (CSF) extraction

Twenty-four hours following cannula placement confirmation, unanaesthetised rats were subject to the CSF extraction protocol under manual restraint. A polyethylene injection line (i.d. 0.28 mm, o.d. 0.61 mm, length 20 cm) connected to a 10 μl glass Hamilton syringe an internal (injection) needle (28G, i.d. 0.18 mm, o.d. 0.36 mm with 0.5 ml projection (i.e. 9.5 mm length), Plastics One, Australia) was used for CSF extraction at approximately 2 μl/min. CSF samples were rapidly transferred to 500 μl microfuge (PCR) tubes and frozen on dry ice. Samples were serially collected from each rat at the following time points; 5 min prior to, and 10, 20, 45, 60 and 90 min post-SLIGRL injection (1.5 mg/kg, i.p.). No more than 8 μl was extracted per sample collection (4 μl average, $n = 3$). It was not specifically assessed whether LRGILS crosses the blood–CSF barrier, but as it has very similar chemical attributes (i.e. structure, molecular weight, H-bond donors and acceptors, cLogP) to SLIGRL, similar bioavailability would be expected (Lipinski, Lombardo, Dominy, & Feeney, 2001).

3.4. Preparation of CSF samples for liquid-chromatography mass spectroscopy (LCMS) analysis

CSF samples were thawed and transferred to fresh microfuge tubes placed on ice. A volume of acetonitrile twice that of each collected CSF sample was added to precipitate any blood products and large proteins and centrifuged at 32,500 rpm for 5 min at 4 °C. Supernatants were removed and transferred to fresh microfuge tubes. The samples were then diluted further with an equivalent volume of milliQ-filtered water, effectively increasing the volume of original CSF samples sixfold. Samples were then frozen on dry ice for LCMS analysis.

3.5. LCMS analysis of CSF samples

LCMS (Agilent 1100 LCMS ion-trap SL) analysis was performed at the Bio21 Institute (Melbourne, Victoria, Australia). The detection of SLIGRL in 'spiked' (i.e. known concentration) saline and neat (clean) CSF samples had previously been confirmed, and the elution 'finger-print' of SLIGRL determined (657.5 MW, elution time

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