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Inhibition of mGluR1 and IP_3Rs impairs long-term memory formation in young chicks

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ABSTRACT

Calcium (Ca^{2+}) is involved in a myriad of cellular functions in the brain including synaptic plasticity. However, the role of intracellular Ca^{2+} stores in memory processing remains poorly defined. The current study explored a role for glutamate-dependent intracellular Ca^{2+} release in memory processing via blockade of metabotropic glutamate receptor subtype 1 (mGluR1) and inositol (1,4,5)-trisphosphate receptors (IP_3Rs). Using a single-trial discrimination avoidance task developed for the young chick, administration of the specific and potent mGluR1 antagonist JNJ16259685 (500 nM, immediately post-training, ic), or the IP_3R antagonist Xestospongin C (5 μM, immediately post-training, ic), impaired retention from 90 min post-training. These findings are consistent with mGluR1 activating IP_3Rs to release intracellular Ca^{2+} required for long-term memory formation and have been interpreted within an LTP2 model. The consequences of different patterns of retention loss following ryanodine receptor (RyR) and IP_3R inhibition are discussed.

1. Introduction

Calcium (Ca^{2+}) is an ubiquitous signal transduction molecule, active in a diverse range of cellular functions in the brain including the regulation of neuronal excitability, neurotransmitter release and gene transcription (Berridge, 1998). Many studies have revealed that Ca^{2+} has an integral role in memory processing (Bauer, Schafe, & LeDoux, 2002; Blackwell & Alkon, 1999; Deyo, Nix, & Parker, 1992; Gibbs, Gibbs, & Ng, 1979; Quevedo et al., 1998; Woodside, Borroni, Hammonds, & Teyler, 2004). A vital role of Ca^{2+} can also be identified in long-term potentiation (LTP) and long-term depression (LTD), the putative cellular correlates of learning and memory (Bliss & Collingridge, 1993; Cavazzini, Bliss, & Emptage, 2005; Lynch, 2004).

The capacity of Ca^{2+} to regulate cellular functions is dependent on levels in the cytosol. Intracellular Ca^{2+} levels can be raised by Ca^{2+} influx from the extracellular environment or through release from intracellular stores. The mechanisms of Ca^{2+} influx though plasma membrane channels are well established (Catterall, 2000). Ca^{2+} can also be released from intracellular stores in the endoplasmic reticulum upon activation of either ryanodine receptors (RyRs) or inositol (1,4,5)-trisphosphate (IP_3) receptors (IP_3Rs; Berridge, 2002; Meldolesi, 2001), but less is known about the role of such stores in memory processing. Nevertheless, a number of studies using both genetic knockout techniques and pharmacological methods have demonstrated that RyR activation contributes to LTP and LTD (Balschun et al., 1999; Futatsugi et al., 1999; O'Mara, Rowan, & Anwyl, 1995; Obenaus, Mody, & Baimbridge, 1989; Reyes & Stanton, 1996; Wang, Wu, Rowan, & Anwyl, 1996). In particular, RyRs appear to be involved in LTP1, a form of LTP induced by weak stimulation (Raymond & Redman, 2002, 2006). Several behavioural studies have also demonstrated that the inhibition of RyRs impairs retention for spatial (Ohnuki & Nomura, 1996) and discrimination avoidance learning (Edwards & Rickard, 2006; Salinska, Bourne, & Rose, 2001). In addition, mice with genetic knockout of RyR subtype 3 also display deficits for contextual fear conditioning and spatial memory (Balschun et al., 1999; Kouzu, Moriya, Takeshima, Yoshioka, & Shibata, 2000).

IP_3R-dependent calcium stores have received much less attention with respect to their role in memory processing. Interestingly, inhibition studies with the drug Xestospongin C (5 μM) suggest that IP_3Rs appear to underlie LTP2, a translation-dependent, but transcription-independent form of LTP induced by moderate stimulation (Raymond, 2007; Raymond & Redman, 2002, 2006). Behavioural studies have tended to investigate the receptors or enzymes that lead to IP_3 production rather than via direct blockade of IP_3Rs. As such only broad statements about the possible role of IP_3Rs in memory processing can be made. For example, group I metabotropic glutamate receptors (mGluRs), which include subtype 1

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(mGluR1) and 5 (mGluR5), are coupled to the enzyme phospholipase C (Pin & Duvoisin, 1995). Stimulation of group I mGluRs leads to the formation of IP_3 and diacylglycerol (Hermans & Challiss, 2001), and in turn intracellular Ca^{2+} release (Cui, Bernier, Harnett, & Morikawa, 2007; Kawabata et al., 1996; Kawabata et al., 1998; Power & Sah, 2007). There is a substantial body of evidence supporting a role for group I mGluRs in synaptic plasticity (Anwyl, 1999; Bashir et al., 1993; Ugolini, Corsi, & Bordi, 1997; Volk, Daly, & Huber, 2006; Yang, Wu, Liu, & Tung, 1998) and long-term memory processing (Aiba et al., 1994; Balschun & Wetzel, 2002; Conquet et al., 1994; Riedel, Platt, & Micheau, 2003; Simonyi, Schachtman, & Christoffersen, 2005; Simonyi et al., 2007), and by broad inference IP_3Rs. Interestingly, the retention loss produced by group I mGluR inhibition is consistent with the findings of Buckley and Caldwell (2004) and Weeber and Caldwell (2004) in which phospholipase C activity was observed in the hippocampus and medial frontal cortex of rats during long-term memory formation of a fear conditioning task. Nevertheless, these findings provide only limited and indirect support for a role for IP_3Rs in memory processing.

The present study sought to directly investigate the activation of IP_3Rs in memory processing using a single-trial discrimination avoidance task developed for the young chick. This task has several advantages for studying the role of IP_3Rs in memory formation. The task is considered to be ecologically valid and is temporally precise (Gibbs & Ng, 1977). In addition, previous studies using this task have yielded relevant findings related to intracellular Ca^{2+} release. For example, inhibition of RyR-dependent intracellular Ca^{2+} release with dantrolene (5 mM, ic) resulted in a persistent retention loss from 40 min post-training (Edwards & Rickard, 2006). In addition, Rickard and Ng (1995) demonstrated that 500 μM (RS)-α-methyl-4-carboxyphenylglycine (MCPG), a non-specific group I mGluR antagonist, administered intracranially 5 min post-training, impaired retention during long-term memory formation from 90 min post-training. In a non-discrimination variant of this task, Salinska (2006) demonstrated that the inhibition of mGluR5 using 8 mM (ic) 2-methyl-6-(phenyl)-pirydyne (MPEP), administered immediately post-training, resulted in an impairment of retention evident from 15 min post-training. Given that group I mGluRs are likely to activate IP_3Rs, but that mGluR5 inhibition does not coincide with long-term memory, the current study explored the possibility that memory processing requires mGluR1-activated Ca^{2+} release from IP_3Rs.

2. Materials and methods

2.1. Animals

Each week, day-old white-Leghorn × black-Australorp chicks were obtained from a local hatchery. Upon arrival, chicks were allocated randomly into two batches, those to be used in experiments that day and those that were to be used the following day. Chicks for the following day's experiments were housed in a purpose-built brooder (2.5 × 1.5 m). Crushed poultry food and water were supplied *ad libitum* to chicks housed in the brooder with heating maintained at 30–38 °C beneath four suspended heat lamps. The following day, water was removed from brooder several hours prior to testing.

All behavioural experiments were conducted in a single-purpose behavioural laboratory. Chicks undergoing experimentation were randomly sorted into pairs at the start of the experimental day and housed in open-topped wooden pens (18 × 25 × 20 cm) for the duration of the experiment. Pairing chicks avoided isolation stress which is known to affect the memory trace (DeVaus, Gibbs, & Ng, 1980). Twenty chicks were allocated to each treatment group (one data-point). Prior to occupation, the floor of each pen was scattered with crushed poultry food. For identification, one chick per pair was marked with a black felt-tip pen on the back of the head. The laboratory was maintained at 23–26 °C and additional warmth was provided by a series of single 15 W light globes suspended above the pens. Chicks were left undisturbed for approximately 30 min before the task began to allow acclimatisation.

2.2. Task procedure

All procedures were approved by the Monash University Animal Ethics Committee and conducted in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (7th edition, 2004). Chicks were trained using a single-trial discrimination avoidance task, described elsewhere in detail (Gibbs, 1991; Gibbs & Ng, 1977). In brief, the task is a bead discrimination task consisting of three stages: pre-training, training and testing. During four pre-training trials chicks were encouraged to peck at small beads. The first two pre-training trials involved the presentation of a small (2 mm) chrome bead for as long as it took to get a short series of pecks from both chicks in each pair. The remaining pre-training trials presented a 4 mm red (trial 3), then a 4 mm blue glass bead (trial 4), each for 10 s. All pre-training trials used beads dipped in water to encourage pecking.

The training trial occurred approximately 1 h after the pre-training trial. A red bead, similar to that used in pre-training, was dipped in the non-toxic taste aversant methyl anthranilate (neat; Sigma–Aldrich, NSW) and presented to each pair of chicks for 10 s. Upon pecking, chicks typically demonstrated a disgust response which included head shaking and/or beak wiping. Chicks were tested for retention of this aversive experience at various times post-training depending upon the experiment being performed. A dry red bead was first presented and then a dry blue bead. Both beads were similar to those presented at pre-training and each bead was presented for 10 s to each pair of chicks.

A custom-made, hand-held electronic device recorded the number of pecks by each chick at the various beads across the trials. The latency to first peck was also recorded. Chicks were excluded from the subsequent data analysis if they did not train or if they did not peck at the non-aversive blue bead at test. In particular, generalised avoidance of the non-aversive blue bead at test may indicate single-day effects or that an administered drug had attentional or sensory–motor effects unrelated to memory (Gibbs, 1991; Gibbs & Ng, 1977). Exclusions due to blue bead avoidance at test were both rare and randomly distributed amongst the various data-points, indicating that the drugs did not have sensory–motor effects unrelated to memory processes. A discrimination ratio was calculated for each chick as a measure of retention. The discrimination ratio was the number of pecks to the blue bead at test divided by the sum of the pecks to red and blue beads at test. A discrimination ratio of 1.0 indicated perfect retention, whereas 0.5 indicated total amnesia. For each group a mean discrimination ratio was also calculated.

2.3. Drugs and administration

(3,4-Dihydro-2*H*-pyrano[2,3-*b*]quinolin-7-yl)-(*cis*-4-methoxycyclohexyl)-methanone (JNJ16259685) is considered a potent and specific non-competitive mGluR1 antagonist (Lavreysen et al., 2004). Xestospongin C was used as a potent and specific competitive IP_3R antagonist (Gafni et al., 1997). Both drugs have been used *in vivo* in behavioural studies (Galeotti, Bartolini, & Ghelardini, 2006; Steckler et al., 2005). JNJ16259685 (Tocris Bioscience, Ellisville, USA) was initially diluted in ethanol and Xestospongin C (Caymen Chemical, Ann Arbor, USA) in dimethyl sulfoxide (DMSO) and both were stored as stock solutions at −20 °C. On each experimental day, one aliquot of stock was brought to room temperature and diluted in sterile 154 mM saline to working concentrations within an hour of use. For JNJ16259685, the final concentration of ethanol was <.004% (v/v). For Xestospongin C, the final concentration of DMSO for the time of administration and retention function studies was 4.5% (v/v). For the dose response study, all but one group of chicks administered Xestospongin C (those administered 15 μM; 13.4% DMSO) received a final concentration of DMSO ⩽10% (v/v). Chicks did not show any sensory–motor effects associated with the DMSO and similar concentrations of DMSO have been used with other drugs without observable behavioural side-effects (Edwards & Rickard, 2006; Ohnuki & Nomura, 1996; Salinska et al., 2001). Vehicles contained the same concentration of ethanol or DMSO (v/v) as JNJ16259685 and Xestospongin C.

Drugs (or vehicles) were administered intracranially by free-hand injection. A 10 μl volume was administered into each hemisphere using a 27.5-gauge needle attached to a Hamilton Repeating Dispenser syringe. A plastic sleeve around the needle shaft controlled injections to a depth of 3.5 mm. Injections were made into the region of the intermediate medial mesopallium (IMM), previously known as the intermediate medial hyperstriatum ventrale (IMHV; Reiner et al., 2004). This region is critical for memory processing for this task (Gibbs & Summers, 2002; Patterson & Rose, 1992; Rose & Stewart, 1999). The drug dose and time of administration varied with each type of experiment.

3. Results

3.1. The effect of the mGluR1 antagonist JNJ16259685 on retention

3.1.1. Dose response study for JNJ16259685

Various concentrations of JNJ16259685 ranging from 0.1 to 500 nM or the vehicle, were administered immediately (within 10 s) after the training trial. This range was based upon the work