Behavioural Brain Research 216 (2011) 458–462

Contents lists available at ScienceDirect

Behavioural Brain Research

journal homepage: www.elsevier.com/locate/bbr

Short communication

Blocking SK channels impairs long-term memory formation in young chicks

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ARTICLE INFO

Article history:
Received 15 March 2010
Received in revised form 21 July 2010
Accepted 25 July 2010
Available online 3 August 2010

Keywords:
Small-conductance calcium-activated potassium channel
SK channel
Apamin
Discriminative avoidance learning
Memory
Chick

ABSTRACT

The role of small-conductance calcium-activated potassium (SK) channels in memory formation was explored in chicks trained on a single-trial discrimination avoidance task. Blockade of SK channels using apamin (1 nM, 0.02 ng/hem, i.c.) impaired long-term memory retention when administered between 10 min prior to, and 30 min after, training. Apamin (1 nM, 0.02 ng/hem, immediately post-training, i.c.) resulted in persistent impairment of retention during the long-term memory stage by 90 min post-training until at least 24 h post-training, indicating SK channels contribute to long-term memory.

Intracellular calcium signalling has a fundamental role regulating numerous cascades of cellular events underlying memory processing [8]. In young chicks, a single-trial discrimination avoidance task has revealed distinct roles for various calcium channels and calcium-dependent processes in the three temporally defined stages of memory formation—short-term (STM), intermediate-term (ITM) and long-term memory (LTM) [16]. Expression of the STM stage (which decays by 15 min after learning) is prevented by the general calcium channel blocker lanthanum chloride [15]. ITM (20–50 min post-training) is dependent upon intracellular calcium release as the inhibition of ryanodine receptors (RyRs) with dantrolene (5 mM, immediately post-training) prevented expression of the second phase of ITM (ITMB) with a persistent retention loss observed by 40 min post-training [9]. Intracellular calcium release also occurs through inositol (1,4,5)-trisphosphate (IP_3) receptors (IP_3Rs) and inhibition of these channels resulted in retention loss by 90 min post-training [2]. This retention loss occurred 30 min into the expression of the protein synthesis-dependent LTM stage, which is observed from 60 min post-training [16]. However, the function of IP_3R-dependent calcium release remains unknown.

Calcium-activated potassium channels are possible targets for intracellular calcium release underlying memory processing for this discrimination avoidance task. This is indicated by the finding that the inhibition of large-conductance calcium-activated potassium (BK) channels with iberiotoxin (50 nM, immediately post-training) resulted in a transient retention loss centred at 40 min post-training, the same retention loss profile to that produced by a low dose of the RyR antagonist dantrolene (10 nM, immediately post-training) [9]. These findings suggest a relationship between RyRs and BK channels during memory retrieval.

A potential candidate for intracellular calcium release yet to be explored in this model of memory formation is small-conductance calcium-activated potassium (SK) channels. SK channels are insensitive to changes in membrane potential, being activated by elevations in cytosolic calcium levels [22,27]. There is an established role for SK channels modulating neuronal excitability by hyperpolarizing the membrane at somatic regions [10]. SK channels are also expressed in dendrites and dendritic spines [24] where they regulate dendritic excitability, synaptic transmission and synaptic plasticity [10,20]. Behavioural tasks with rodents have demonstrated SK channels are involved in memory processing, but the literature is inconsistent on their specific role. Blockade of SK channels, using apamin (i.p.), facilitated the rate of learning for appetitively motivated bar-pressing tasks [6,28] and object recognition tasks [7,39], and facilitated consolidation using an olfactory association task (apamin i.c.v.) [11,31]. However, investigations with other rodent tasks, including passive avoidance tasks and spatial memory water and Y-mazes, have yielded mixed results of either no or minimal effects [5,6,23,39,41]. In contrast, blockade of SK channels with apamin (i.c.v.) impaired retention for a bar-pressing task at 24 h [28]. In support of an IP_3R/SK channel signalling cascade in memory processing, calcium release from IP_3Rs appears to activate SK channels to regulate the neuronal firing in the prefrontal cortex during working memory [3,21].

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doi:10.1016/j.bbr.2010.07.032

The aim of the current study was to investigate whether SK channels were required for memory formation using a single-trial discrimination avoidance task developed for the young chick. Training on this task yields three temporally defined and sequentially dependent stages of memory formation [16]. The standard task using strongly reinforced training was used to determine in which stage of memory impaired retention is first observed and whether it is persistent or transient. This is fundamental for understanding the processes underlying memory formation that SK channels may be linked to. There is a weakly reinforced variant of this task which can be used to investigate whether pharmacological agents or behavioural means can promote consolidation of a labile memory trace [4]. In general, consolidation of the memory trace only occurs by agents acting on either STM, ITM or both [12,17], therefore, it is important to use the strongly reinforced training task to first determine in which memory stage SK channels are required. This task also has the advantage that drugs can be administered intracranially to chicks, which avoids possible peripheral effects of apamin on adrenaline release [11,33,34] which may alter learning via increased arousal.

Typically, day-old male and female chicks of egg-laying strains (hybrids of New Hampshire, Rhode Island Red, white Leghorn and black Australorp) were obtained on the morning of each experiment from a local poultry farm. On occasion, chicks were also housed overnight in a brooder for experiments the following day. Details of housing conditions are described elsewhere [2]. Briefly, groups of 20 chicks were housed in pairs in open-topped wooden pens scattered with crushed poultry food. The mean discrimination ratio for each group is represented by one data point. All procedures were approved by the Monash University Animal Ethics Committee and comply with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (7th Edition, 2004).

Chicks were trained on a strongly reinforced single-trial discrimination avoidance task [12,13,17]. The bead discrimination task involved pre-training, training and testing. During pre-training chicks were encouraged to peck at small chrome, red and blue beads, dipped in water, in successive trials. The training trial involved the presentation of a similar red bead to that used in pre-training, dipped in the non-toxic taste aversant methyl anthranilate (Sigma–Aldrich, Castle Hill, Australia) at 100% (v/v). Each pair of chicks was presented with the training bead for 10 s. Depending on the experiment, retention was tested at various time-points after training by presenting a dry red bead, then a dry blue bead to each pair of chicks for 10 s duration. Each group of 20 chicks was tested for retention once. A hand-held digital device recorded pecks by each chick during each trial.

Chicks were excluded from the data analysis if they failed to train or to peck the non-aversive blue bead at test. Avoidance of the blue bead at test may indicate drug-induced sensory-motor effects or other non-specific effects (e.g., fatigue) on performance. Exclusions were few and dispersed equally amongst the groups in the present study. Final group sizes are reported in each figure.

Retention was measured by a discrimination ratio which is the number of pecks to the blue bead at test divided by the total pecks to the red and blue beads at test. A discrimination ratio of 1.0 represents perfect retention while a discrimination ratio of 0.5 indicates amnesia. For each group of chicks, a mean discrimination ratio is calculated.

Apamin has high sensitivity for blocking SK2 and SK3 channel subtypes, with a lower sensitivity for the SK1 subtype [19,31]. Apamin was diluted in 154 mM saline. 10 μL of apamin, or a saline control, was administered free-hand by an experienced injector into the intermediate medial mesopallium [36] of each hemisphere using a 27 gauge × 1/2 in. needle attached to a Hamilton Repeating Dispenser syringe. A plastic sleeve around the needle shaft controlled injections to a depth of 3.5 mm. This brain region is known to be important for memory formation in the chick [17,38].

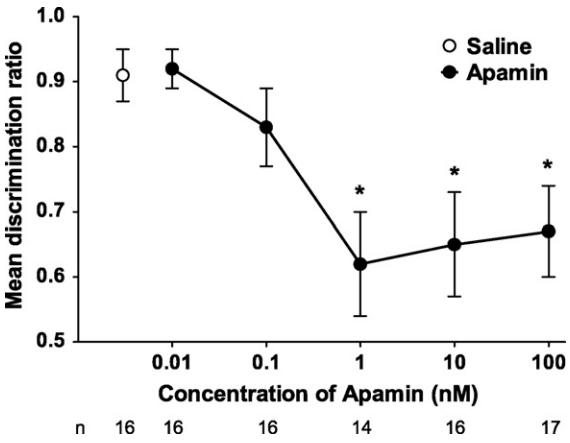

Fig. 1. Dose–response study for apamin administered immediately after strongly reinforced training with retention tested at 120 min post-training. Each data point represents the mean discrimination ratio (±SEM) for a separate group of chicks. $^{*}p < .05$.

In an initial dose toxicity study, concentrations of apamin between 0.1 and 10 μM (2.03–203 ng/hem) were tested. This concentration range was based upon previous studies [3,11,32]. Negative drug side-effects including shaking, poor righting reflex and impaired balance were observed at 1 and 10 μM within 20 min of apamin administration. These symptoms were similar to the adverse effects reported in rats with intraperitoneal (0.3–2.0 mg/kg) [28,41] and intracranial administration (>100 ng/hem) [3]. Therefore, these doses were avoided in all subsequent experiments. No adverse symptoms were observed in either the toxicity or subsequent studies for doses ≤300 nM.

A dose–response study was conducted using concentrations of apamin between 0.01 and 100 nM (0.2 pg/hem to 2.03 ng/hem) administered immediately post-training, with retention tested at 120 min post-training. This time of test was well within the LTM stage for this task [16]. Apamin resulted in impairment of retention, with the greatest inhibition observed at 1 nM (0.02 ng/hem; see Fig. 1). A one-way independent ANOVA demonstrated a significant dose effect ($F_{5,89} = 4.52$, $p < .005$, $\eta^2 = .20$), with Dunnett's post hoc tests showing concentrations of 1 nM and above significantly impaired retention compared to chicks administered the saline control (each $p < .05$).

A time of administration study was performed to determine the range of times during which memory processing was vulnerable to the administration of apamin. Apamin (1 nM, 0.02 ng/hem) or saline, was administered from 20 min before training to 60 min post-training, as there was little indication of optimum administration times from previous research. Retention testing occurred at 120 min post-training. Apamin impaired retention when administered between 10 min prior to training and 30 min post-training (see Fig. 2). A two-way independent ANOVA revealed significant main effects for drug ($F_{1,230} = 46.86$, $p < .001$, $\eta^2 = .14$) and time of administration ($F_{6,230} = 6.02$, $p < .001$, $\eta^2 = .11$). A significant interaction was also demonstrated ($F_{6,230} = 4.30$, $p < .001$, $\eta^2 = .08$). Simple main effects analyses demonstrated that administration of apamin between 10 min prior to training up to 30 min post-training significantly impaired retention (each $p < .05$).

A retention function study was performed to determine when the onset of retention loss occurred following apamin administration and to see whether this retention loss was persistent or transient as an indication of whether apamin affected either memory formation or retrieval. Chicks were administered either 1 nM (0.02 ng/hem) apamin, or saline, immediately post-training (which