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Inhibition of PKC in basolateral amygdala and posterior parietal cortex impairs consolidation of inhibitory avoidance memory

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Abstract

Hippocampal α- and βI/βII protein kinase C (PKC) are crucial for the formation of different types of memory in several species, including that for a one trial inhibitory avoidance (IA) task in rats. Many studies, however, have shown that other brain structures besides the hippocampus, notably the basolateral amygdala (BLA) and posterior parietal cortex (PC) are also necessary for memory consolidation. Here, we examine the role of α- and βI/βII PKC in the BLA and PC on the consolidation of the memory for IA in rats. The selective inhibitor of α- and βI/βII-PKC Gö 6976 and the nonselective PKC inhibitor Gö 7874 were administered into these structures at different times after training at concentrations known to inhibit PKC and to produce retrograde amnesia when given into the hippocampus. Gö 7874 blocked consolidation of IA memory when infused into BLA immediately and 30 min or into PC 180 to 360 min posttraining. Gö 6976 caused amnesia when given into the BLA also immediately or 30 min posttraining but in the PC hindered memory retention only when infused 270 and 360 min after the training session. Our data indicate that α- and βI/βII-PKC are critical for consolidation of IA memory shortly after training in BLA and that, first other isoforms and subsequently the α- and βI/βII PKC are required 3 or more hours after training in the PC. The findings on BLA are similar to those previously reported in the hippocampus, but those on PC suggest an entirely different molecular dynamics for memory formation in that area.

Keywords: Memory; Learning; Inhibitory avoidance; PKC; Parietal cortex; Amygdala

1. Introduction

Protein kinase C (PKC) is a family of phospholipid-dependent kinases (Nishizuka, 1995) that regulate synaptic transmission and neuronal function at various levels, including neurotransmitter release (Malenka et al., 1986; Majewski and Iannazzo, 1998), membrane properties (Hoffman and Johnston, 1998; Manseau et al., 1998), receptor functionality (Macek et al., 1998; Suen et al., 1998), gene expression (Routtenberg et al., 2000), and the early stages of hippocampal long-term potentiation (LTP) (Abeliovich et al., 1993; Ben-Ari et al., 1992). Molecular cloning studies have revealed the existence of at least 12 isozymes of PKC, which are divided into calcium-dependent conventional (α, βI, βII and γ isozymes) and calcium-independent subfamilies. PKC isoforms are differentially distributed in brain cells and are also differently activated by second messengers. It is generally assumed that intracellular calcium mobilization and stimulation of phospholipid turnover promote the translocation and activation of PKC. A key role for PKC in memory consolidation has been envisaged since the pioneering work of Routtenberg et al. (see Routtenberg et al., 2000 for references). Activation of PKC following learning (Farley and Auerbach, 1986; Sunayashiki-Kusuzaki et al., 1993; Bernabeu et al., 1995; Cammarota et al., 1997; Van der Zee et al., 1997a,b;

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Paratcha et al., 2000), as well as translocation of the enzyme to the membrane (Routtenberg et al., 2000), has been described. The isoforms involved include PKC α and βI/βII (Paratcha et al., 2000), which are mostly postsynaptic, and γ (Abeliovich et al., 1993; Routtenberg et al., 2000), which is also presynaptic (Tang et al., 2004; Van der Zee et al., 1997a) Bilateral inhibition of hippocampal PKC by the nonspecific inhibitors staurosporin and CGP 41231 in the first 1–2 h after training causes retrograde amnesia for step-down inhibitory avoidance (IA) memory (Jerusalinsky et al., 1994); in addition, the selective PKC α/βI inhibitor Gö 6976 and the non-isoform selective PKC inhibitor Gö 7874 block consolidation of the memory for the IA task when infused into the dorsal CA1 region at the time of training or up to 110 min later (Vianna et al., 2000).

It has been shown that consolidation of IA memory requires other cerebral regions besides the hippocampus, including the basolateral amygdala (BLA) and the posterior parietal cortex (PC; Bonini et al., 2003; Lorenzini et al., 1996; Rosatto et al., 2004). The same happens in several other simple paradigms, including fear conditioning and spatial learning tasks (Bontempi et al., 1999; Frankland et al., 2004; Maviel et al., 2004). This has been established through pharmacological manipulations, inactivation and diverse biochemical techniques. The dynamics of the participation of different types of glutamate receptors or of the ERK subfamily of mitogen-activated protein kinases is different in each of these brain regions. This indicates that they are all necessary for memory consolidation, but each uses a different sequence of molecular mechanisms for that. The sequences of molecular events used by all structures studied except the hippocampus is not analogous to that of LTP (Rosatto et al., 2004).

Here, we examine the effect of infusion in the BLA and PC of the general PKC inhibitor Gö7874 and of the selective inhibitor of α- andβI/βII-PKC, Gö 6976 at various times after training, on memory consolidation of one-trial avoidance in rats.

2. Materials and methods

2.1. Animals

Male Wistar rats (3 months of age, 250–280 g of weight) from our own breeding stock were used. The animals were housed in plastic cages under a 12 h light/dark cycle (lights on at 7:00 AM), with water and Purina lab chow freely available and at a constant temperature of 23 °C. To deliver the pharmacological agents to be tested, rats were bilaterally implanted under deep thionembutal anesthesia with 27-gauge guides aimed 1.0 mm above the lower border of the basolateral amygdala nucleus or to the posterior parietal cortex, in accordance with coordinates (A −4.2, L ±3.0, V 1.4 and A −2.8, L ±5.0, V 2, respectively) taken from the atlas of Paxinos and Watson (2000). Animals were allowed to recover for 4 days before submitting them to any other procedure. In all experiments, the "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1996) were strictly followed.

2.2. Inhibitory avoidance training

After recovery from surgery, rats were trained in a one trial, step-down, inhibitory avoidance task, a fear motivated learning paradigm much used for the pharmacological and biochemical analysis of memory (Bevilaqua et al., 1999, 2003; Cammarota et al., 2000, 2003, 2004). In order to do that, animals were gently put on a 2.5-cm-high, 7.0-cm-wide wood platform placed inside and at the leftmost extreme of a 50×25×25 cm acrylic training box whose floor was made of a grid of parallel bronze bars. At the very moment, the animal stepped down from the platform and put its four paws on the grid, it received a 0.5-mA, 2-s scrambled footshock. After that, it was immediately removed from the training box. At the time of drug delivery, a 30-gauge cannula was tightly fitted into the implanted guide with its tip protruding 1.0 mm beyond that of the guide. The infusion cannula was linked by an acrylic tube to a microsyringe and infusions (0.5 μl/side) were carried out over 60 s, first on the right and then on the left side; the 30-gauge cannula was left in place for 30 additional seconds to minimize backflow. Drugs (Gö 6976, 4.6 nM and Gö 7874, 8.0 nM) were obtained from Calbiochem (La Jolla, CA, USA) and dissolved in 2% DMSO. These concentrations have been previously found to inhibit α- and βI//βII-PKC and all PKC activity by 90%, respectively, and to cause retrograde amnesia for the memory associated with the IA task when given into the hippocampus (Paratcha et al., 2000; Vianna et al., 2000). To evaluate memory retention, latency to step down onto the grid during the training session was compared to that obtained in a test session performed 24 h later. In the test session, the procedure was identical to that used during training except that the electric footshock was omitted. Cannula placement was verified postmortem by an experimenter blind to the behavioral data as described previously (Bonini et al., 2003). Briefly, 2–4 h after the behavioral test, 0.5 μl of a 4% methylene blue solution was infused as described above and the extension of the dye 30 min thereafter was taken as indicative of the presumable diffusion of the vehicle or drug previously given to each animal. Infusions spread with a radius of less than 1 mm, as described before (Bonini et al., 2003) and were found to be correct (i.e., the cannulas were in the intended sites and diffusion of the dye was equal or less than 1 mm^3) in 97% of the animals. This will not be illustrated here since the extension of the infusions was exactly as in many previous papers from our laboratories and as that of experiments using radio-labeled compounds of molecular weight comparable to those used here (Martin, 1991). Data were analyzed using non-parametric statistics due to the introduction of a ceiling of 180 s in the duration of the retention test session.

3. Results

Fig. 1 shows the effect of Gö 6976 (4.6 nM) and Gö 7874 (8.0 nM) given bilaterally into BLA either immediately (0 min), or 30, 90, 180, 270 or 360 min after IA training on retention test latency as measured 24 h posttraining. Retrograde amnesia was observed in the animals that received the drugs either immediately or 30 min posttraining in the BLA. Drug infusions at later times were ineffective. Fig. 2 shows the effect of the same doses of the two PKC inhibitors given bilaterally into the PC at 0, 30, 90, 180, 270 or 360 min after training. Gö 6976 blocked memory consolidation when infused in the PC 270 or 360 min posttraining, but not at earlier times. Gö 7874 had a similar effect but in addition was also amnesic when given 180 min after training.

4. Discussion

The present findings illustrate the need for α- and βI/βII-PKC activity in BLA and PC at different times: in the first half-hour after training in the BLA, and 4.5–6.0 h after training in the PC. In addition, other PKCs besides the α- and βI/βII isoforms are necessary for consolidation at 180 min from training in the PC in order for memory consolidation to take place. This differs from earlier observations on the need for hippocampal PKC isoforms in the hippocampus. In that structure α- and βI/βII-PKC, and perhaps also other isoforms of the enzyme (Jerusalinsky et al., 1994; Vianna et al., 2000) are required for consolidation between the time of training and 110 min later, but not beyond that. This further exemplifies two facts concerning the physiology of memory consolidation that

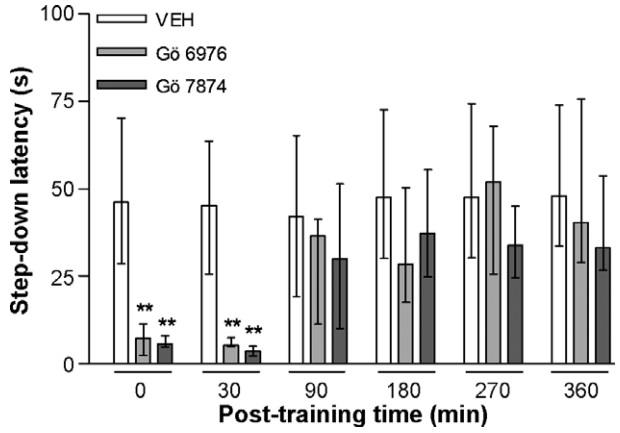

Fig. 1. Infusion of Gö 6976 or Gö 7874 into the basolateral amygdala impairs consolidation of inhibitory avoidance (IA) memory. Gö 6976 or Gö 7874 (4.6 and 8 nM, respectively) were bilaterally infused (0.5 μl) into the basolateral amygdala at different times after IA training. Bars represent median (± interquartile range) of the step-down latencies measured in a memory retention test session carried out 24 h after training. n=12–17 per group. *p<0.05 and **p<0.01 vs. vehicle (VEH) in Dunn's comparison after Kruskal–Wallis test.

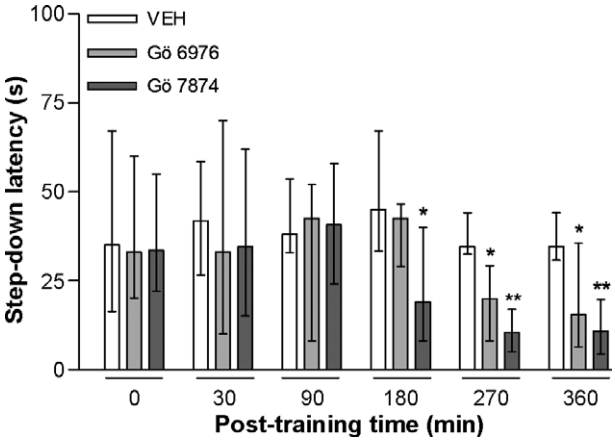

Fig. 2. Infusion of Gö 6976 or Gö 7874 into posterior parietal cortex impairs consolidation of inhibitory avoidance (IA) memory. Gö 6976 or Gö 7874 (4.6 and 8 nM, respectively) were bilaterally infused (0.5 μl) into the posterior parietal cortex at different times after IA training. Bars represent median (± interquartile range) of the step-down latencies measured in a memory retention test session carried out 24 h after training. n=11–16 per group. *p<0.05 and **p<0.01 vs. vehicle (VEH) in Dunn's comparison after Kruskal–Wallis test.

have been overlooked by most of the literature in the field. As stated in the Introduction, a large number of techniques have shown quite unequivocally that, in many tasks, memory consolidation requires the BLA, PC and several other neocortical regions. The techniques involve pharmacological (Bonini et al., 2003; Rosatto et al., 2004), biochemical (Bernabeu et al., 1995; Frankland et al., 2004), brain imaging (Maviel et al., 2004), reversible inactivation experiments (Lorenzini et al., 1996), regional brain deoxyglucose uptake (Bontempi et al., 1999) and many others, applied both to IA and to other simple tasks. Much of the literature purporting to show that one or other region is necessary for consolidation relies on old lesion studies, which failed to analyze lesions in other brain areas (Izquierdo and Medina, 1997) and suffered from the fact that a retrieval effect could never be ruled out since the lesions are also present at the time of retrieval (Bontempi et al., 1999). In contrast, pharmacological, biochemical, reversible inactivation, glucose uptake and image techniques are applied at specific times after training and prior to retrieval and thus permit a clear distinction both between early and late phases of consolidation and between consolidation and retrieval.

The role of α- and βI/-PKC in hippocampus (Vianna et al., 2000), BLA and PC (Figs. 1 and 2) during IA memory consolidation, does not imply that other PKC isoforms are not important for this process. Mice with lacking γ-PKC are deficient both in spatial learning tasks that require the hippocampus and in hippocampal LTP (Abeliovich et al., 1993). Both membrane-bound total PKC activity and phosphorylation of the presynaptic substrate of γ-PKC, B50/GAP 43 (Routtenberg et al., 2000; Zhao et al., 1995) increase after one-trial avoidance with a peak at 30 min posttraining (Cammarota et al., 1997). The posttraining

increase of hippocampal PKC activity is largely restricted to α and βI/βII PKC isoforms (Paratcha et al., 2000); but the phosphorylation of GAP43 suggests the additional activation of a presynaptic form of PKC, presumably PKCγ.

In conclusion, the present findings further illustrate the participation of more than one PKC isoforms during the consolidation process at different times after training. Like in the hippocampus (Paratcha et al., 2000; Vianna et al., 2000), α and βI/βII PKCs are crucial for consolidation early after training in the BLA. In contrast, in the PC, other PKC isozymes are crucial for consolidation 3 h after training, and α and βI/βII-PKC is required 4.5 and 6 h after training. Clearly, the consolidation of inhibitory avoidance learning requires several brain structures acting in a concerted faction (Izquierdo and Medina, 1997). It is definitely not a one-structure task using an LTP-like process (Rosatto et al., 2004).

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