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Spatial memory in aged rats is related to PKCγ-dependent G-protein coupling of the M1 receptor

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Abstract

In the present study, individual differences in spatial memory in aged Fischer 344 (F344) rats were associated with the extent of G-protein coupling of the M1 muscarinic receptor and the dendritic-to-somal ratio of hippocampal PKCγ (d/sPKCγ) immunogenicity. Following testing in the eight-arm radial maze task, 7 young and 13 aged rat brains were sectioned through the dorsal hippocampal formation (HF). G-protein coupling of the M1 receptor was assessed autoradiographically using competition binding studies in the presence and absence of a G-protein uncoupler to determine high (K_H) and low (K_L) affinity states for agonist in the HF, neocortex, and amygdala. In aged animals, a relationship between choice accuracy in the maze and K_H, a measure of M1 receptor–G-protein coupling was seen in the dentate gyrus, CA3, CA1, and neocortex. Furthermore, choice accuracy and d/sPKCγ immunogenicity showed a significant relationship in CA1. Lastly, a correlation was seen in the CA1 of aged animals between K_H and d/sPKCγ. These relationships did not hold for the amygdala. Thus, individual differences in a naturally occurring age-dependent disruption of cholinergic-PKCγ signal transduction is associated with spatial memory dysfunction.

Keywords: Hippocampus; Amygdala; Neocortex; Cholinergic; Aging; Radial arm maze

1. Introduction

The ability of acetylcholine (ACh) and other full agonists to stimulate postsynaptic muscarinic receptor-mediated phosphoinositide (PI) signaling has been shown to be important for memory function mediated by the hippocampal system [11,28,43,48,53]. The hippocampal formation (HF) is one of the brain regions most vulnerable to the aging process [13,16,51]. In keeping with the human data [8,19,45], cholinergic pharmacotherapy in aged rats with a hippocampal-dependent spatial memory deficit has often met with only limited success [8,24,29]. One explanation for this finding is that the transduction and persistence of a molecular signal along the initial segment of the cholinergic PI cascade may be defective to varying degrees in aged, memory-impaired animals.

Five muscarinic cholinergic receptor subtypes are currently known to exist in the CNS as demonstrated by molecular cloning [7]. The m1, m3, and m5 subtype receptor cloned gene products[1] are linked by G-proteins to PI-dependent phosphorylation and membrane calcium mobilization [26]. Of these receptor subtypes, the m1 is not only the most abundant in the HF, but has been implicated in neural plasticity [28,30,50]. Hippocampal cholinergic innervation may help mediate neuronal excitability by modulating calcium-dependent signal transduction. Hippocampal pyramidal neurons demonstrate decreased excitability as a function of aging, in part due to an enhanced calcium-dependent postburst afterhyperpolarization (AHP) [61]. Age-associated G-protein uncoupling of the pharmacologically defined M1 muscarinic receptor may disrupt this calcium-dependent signal transduction pathway and consequently impair neuronal plasticitiy.

[1] The nomenclature m1–m5, designated by lower case letters refer to the cloned gene products of the muscarinic cholinergic genes. These molecular subtypes are distinguished by their primary nucleotide or amino acid sequences using complementary nucleotide probes or subtype specific antibodies. Muscarinic receptor subtype ligand binding identified in the rat using pharmacological antagonists are designated by uppercase letters, M1–M5.

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In both rodents and humans, G-protein mediated high (K_H) and low (K_L) affinity states for agonist of the M1 muscarinic cholinergic receptor coexist. The high affinity agonist state (K_H) of the M1 receptor is the physiologically relevant conformation of the receptor [18,44]. The ability of several full muscarinic agonists, including carbachol to form a guanine nucleotide-dependent high affinity state of the muscarinic receptor has been shown to correlate with the efficacy of the agonist to stimulate phosphatidyl inositol breakdown and calcium mobilization [18,23]. The K_L/K_H ratio has been previously used as an index of receptor–G-protein coupling to indicate the efficacy of signal transduction of the PI pathway as mediated by muscarinic receptor activation [18,22,27,44]. Hence, the K_L/K_H ratio, and not the absolute K_H or K_L values, has been related with agonist efficacy and functional responsiveness of M1 muscarinic receptors for agonist.

Immediately downstream from the M1 receptor–G-protein complex, the PI intermediate effector, protein kinase C (PKC), has been strongly implicated in hippocampal-dependent learning and memory [1,12,13,17,38,39,53,54,56]. Muscarinic cholinergic receptors are highly expressed in hippocampal neurons containing one PKC subtype in particular, the calcium-activated, phospholipid-sensitive PKCγ isoform.

Numerous lines of research have implicated PKCγ in memory function dependent on the hippocampal system [12,13,17,53,54,57]. Stimulation of muscarinic cholinergic receptors is associated with an increase in PKCγ immunoreactivity [54], suggesting a link between muscarinic cholinergic receptor stimulation and PKCγ function. Van der Zee et al. [53] showed an increase in immunoreactivity for both PKCγ and muscarinic ACh receptors in principal hippocampal neurons of young rats following training in a hole board task. PKCγ likely phosphorylates substrate proteins that are implicated in a persistent subcellular change outlasting the initial stimulus, reminiscent of a memory trace.

Alterations in PKCγ translocation from the soluble to particulate fraction of brain tissue homogenates have been demonstrated in aged rodents [6,13,41]. Reduced phosphorylation of certain PKC substrates, including GAP-43/B-50/F1, have been documented to selectively occur in the HF of aged rats [5,49]. Furthermore, age-related learning and memory deficits show an enhanced postburst AHP [61]. These observations suggest an age-dependent impact on PKC-mediated signal transduction important in certain memory processes.

The experiments reported here were designed to test whether a naturally occurring age-dependent disruption of cholinergic signal transduction is associated with an alteration in the immunogenicity of PKCγ across somal-dendritic spatial domains of hippocampal principal neurons. These neurochemical changes were assessed in the hippocampal formation and neocortex of young (5–6 months old) and old (27–28 months old) Fischer 344 (F344) rats. Since the amygdala is not involved in spatial memory [9,58], we used this region as an internal control for muscarinic cholinergic binding.

2. Methods

2.1. Subjects

Thirty F344 rats, 12 young (4 months old at the start of the experiment) and 18 aged (26 months old) retired breeders were obtained from the National Institutes on Aging (NIA) colony. They were individually housed in a climate-controlled animal facility and maintained on a 12 h/12 h light/dark cycle. Subsequent to a 2-week acclimatization period following arrival from the NIA colony, the animals were food restricted to 80% of their ad lib body weights over a 14-day period. The weights of the young animals were adjusted for growth. Seven young and thirteen old animals were then shaped and tested in the eight-arm radial maze task as described below. To control for possible effects that training itself may have on neurochemical markers, the remaining five young and five old animals were handled in the same manner and placed in the eight-arm radial maze without exposure to training or testing trials.

2.2. Behavioral testing

The eight-arm radial maze task used to test spatial memory was similar to that described by Olton and Samuelson [40]. The arms were separated from the central stage by Plexiglas® guillotine-like doors. The maze was located in a laboratory room, with external cues placed close to each arm [14]. After adaptation to the maze and shaping to enter the arms over three days, all animals were given one trial per day and trained to a stringent criterion of three consecutive trials with no errors (i.e., no repeated entries into a given arm) or for a maximum of 30 trials. Each entry into an already visited arm constituted an error. Each animal was allowed to make a maximum of 10 choices per trial (eight if all were correct), or remain in the maze until a maximum of 15 min elapsed, whichever came first. Rats that did not reach criterion within 30 trials were considered memory impaired and assigned a score of 33, since, by definition, at least three more trials would have been required to potentially attain three consecutive trials without errors. Those animals that reached behavioral criterion were referred to as memory intact.

Memory capacity was quantified using the following measures: (1) number of trials to criterion, and (2) a choice accuracy score which takes into account the number of errors made on each trial, as well as where in the sequence of choices these errors occurred. The choice accuracy score was determined for each animal for choices 2–8 of each trial using the formula: $(P(\text{correct})_{\text{observed}} - P(\text{correct})_{\text{expected}})/(100 - P(\text{correct})_{\text{expected}}) \times 100$, where $P(\text{correct})_{\text{observed}} =$ (number of correct responses/total num-