Behavioral and neurochemical effects on hippocampal noradrenergic neurotransmission caused by the lack of M₄ muscarinic acetylcholine receptors in mice

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Introduction

The muscarinic M₄ acetylcholine receptor subtype is centrally involved in regulation of dopamine release in striatal areas and it has been suggested that selective activation of the M₄ muscarinic cholinergic system may prove useful for the treatment of schizophrenia. During the past decade, studies with M₄ receptor knockout mice (M₄⁻/⁻ mice) have clarified the functional roles of the M₄ receptor subtype. However, to the best of our knowledge, the potential relevance of the M₄ receptor for cognitive functions including memory has not been investigated so far. To this end, we have used the novel object recognition and the location recognition tasks in order to assess recognition memory. The mice were also tested in the forced swim and marble burying tests. Microdialysis was used to measure noradrenalin release in the mouse striatum and hippocampus, areas important for recognition memory.

Methods

Animals

M₄⁻/⁻ mice were generated as previously described (Gomeza et al. 1999). Founder mice of mixed genetic background (129SvEv/CF1) were backcrossed to the C57BL/6Ntac strain for 11 generations. M₄⁻/⁻ mice, heterozygotes (M₄⁺/- mice) and wild type littermates (M₄⁺/+ mice) were bred at the animal facilities at the Panum Institute, University of Copenhagen.

Drugs used for the behavioural studies

Desipramine (10mg/kg s.c.)

Tail-suspension

The tail suspension test (TST) was a slightly modified version from that developed by Steru and co-workers (Steru et al. 1985). Briefly, one hour after vehicle or desipramine administration, mice (8 wildtype and 6 M₄⁻/- mice) were...
tested. Mouse immobility was scored over a period of 6 min by an observer blinded to the treatment and was defined as the absence of limb movement.

**Marble burying**

Vehicle or desipramine treated mice were placed individually in macrolon cages containing 20 clean glass marbles evenly placed on 5 cm woodchip bedding material. The number of marbles buried was counted 30 min. later. The percent inhibition of marble burying by desipramine was calculated as the difference between the number of buried marbles after vehicle treatment divided by the number of marbles buried after vehicle treatment.

**Novel object and location recognition**

M4-/− and wild type mice underwent behavioral testing in novel object recognition or location recognition tasks (Manning et al 2010). For novel object recognition two identical objects were placed in adjacent corners of the testing arena and mice were allowed to freely explore for 10 min. The mice were reintroduced to the testing arena for a retention trial following a 1 h. intertrial period for 5 min. The original objects were removed and replaced with two new objects, one identical to the objects in the training trial and one novel object. For location recognition, the training trial was identical to that used during novel object recognition. However, following a 1 h. intertrial period mice were allowed to explore the testing arena for 5 min. containing two identical copies of the object used during the training trial, one placed in its previous location and one in a novel location diagonally opposite to the other object. Each trial was recorded and the time spent interacting with the objects as well as distance moved during the trials was analyzed using EthoVision XT.

**In vivo microdialysis**

For freely moving in vivo microdialysis studies, a Ringer’s solution was perfused at a rate of 1,7 ul/min through the probe (2mm). Twenty minutes samples were collected after an acclimatization period. Three samples were collected to establish noradrenaline baseline levels. Subsequently, mice were injected with saline or desipramine. Hplc-EC measurement of noradrenaline levels was carried out as described in Weikop et al. (2004).

**Results and Discussion**

M4-/− mice displayed normal novel object recognition, but have impaired location recognition compared to wild type controls.
Figure 1. Novel object and location recognition in M₄⁻/⁻ and WT mice. A) Novel object recognition index in M₄⁻/⁻ and WT mice, n=5-7. B) Location recognition index was significantly reduced in M₄⁻/⁻ mice compared to WT mice, *p<0.05, n=9.

The M₄⁻/⁻ mice showed the same baseline levels of immobility in the tail suspension test as well as burying behavior in the marble burying test. However after administration of desipramine, a noradrenalin uptake inhibitor, the M₄⁻/⁻ mice did not, in contrast to the wild type mice, decrease these behaviors.

![Graph showing immobility percentage](image)

Figure 2. Tail suspension test in M₄⁻/⁻ (grey) and WT (white) mice. A) Time (s) spent immobile during the 6 min. test. Pre-treatment with desipramine (10 mg/kg) significantly reduced immobility time in both genotypes (p<0.05 and<0.001), and significantly more in M₄⁻/⁻ mice compared to WT mice (p<0.01).

In vivo microdialysis studies showed that M₄⁻/⁻ mice released less noradrenalin in the ventral hippocampus after administration of desipramine compared to wild type mice.

The present study demonstrates that the M₄ receptor is involved in various aspects of memory function and modulates hippocampal noradrenergic neurotransmission.

References