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The M_4 muscarinic acetylcholine receptor plays a key role in the control of murine hair follicle cycling and pigmentation

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Abstract

Cholinergic receptors of the muscarinic class (M1–M5) are expressed in epidermal keratinocytes and melanocytes as well as in the hair follicle. Knockout (KO) mice of all five receptors have been created and resulted in different phenotypes. KO mice with a deletion of the M4 muscarinic acetylcholine receptor (M4R) present a striking hair phenotype, which we have analyzed here in greater detail by quantitative histomorphometry. Earlier studies revealed a retarded hair follicle morphogenesis in M4R KO mice, compared to age-matched wild type controls. On day 17, when mice enter the first hair growth cycle, the KO mice still showed a slightly retarded catagen phase. Subsequently, hair follicles of the KO mice stayed in a highly significantly prolonged telogen phase, while wild type mice had already far progressed in the hair cycle by entry into anagen. Most strikingly, the M4R KO mice did not engage in follicular melanogenesis and failed to produce pigmented hair shafts. The current pilot study suggests that the M4R plays a fundamental role in the control of the murine hair follicle cycling and is an essential signaling element in the control of hair follicle pigmentation.

Keywords: M4 muscarinic acetylcholine receptor; Hair cycle; Hair follicle; Pigmentation; Keratinocytes; Melanocytes

Introduction

The class of muscarinic acetylcholine receptors (mAChRs) consists of five subtypes, M1–M5. Within the epidermis, all five subtypes of mAChRs, including M4 mAChR (M4R), are expressed in both keratinocytes and melanocytes (Ndoye et al., 1998; Buchli et al., 2001). Even-numbered mAChRs, M2 and M4, selectively couple pertussis toxin-sensitive G_i-proteins that consequently inhibit adenylate cyclase (AC), weakly stimulate protein kinase C, open inwardly rectifying K^+ channels, and inhibit Ca^{2+} flux (reviewed by Grando et al., 2006).

Keratinocytes of the stratum spinosum show the highest expression of M4R within the epidermis (Ndoye et al., 1998). Recently an important role of M4R in keratinocyte migration was realized by comparing wound healing in M4R wild type and knockout (KO) mice: M4R KO mice showed significantly decreased epithelialization rate concomitant with a reduced migration distance of keratinocytes (Chernyavsky et al., 2003, 2004). The M4R KO mice exhibited changes in the integrin expression, which shifted from migratory integrins, such as α_5, α_V, β_5, in wild type towards the sedentary integrins α_2 and α_3 (Chernyavsky et al., 2004).

In epidermal melanocytes, muscarinic stimulation increases the intracellular free Ca^{2+} concentration. Regulation of this ion plays a fundamental role in the control of melanocyte dendricity (Meyer zum Gottesberge, 1995). Moreover, the uptake of the essential amino acid L-phenylalanine, which serves as substrate for melanin biosynthesis, involves the Ca^{2+}-dependent Phe-Na^+/ATPase antiporter (Schallreuter and Wood, 1999). Activation of M2R and M4R is thought to inhibit melanogenesis via the inhibition of AC and reduced cAMP synthesis. This represents a negative feedback regulation to the αMSH/MC1R and catecholamine/β2 adrenergic receptor (β2AR)

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response in melanocytes (Gillbro et al., 2004; Kurzen and Schallreuter, 2004).

The mAChRs are also expressed in the inner root sheath and the central cell layer of human hair follicles (Kurzen et al., 2004). In an earlier study, distinct hair follicle development phenotypes had been noted in 1 day old M3R KO and M4R KO mice: while the first displayed advanced hair follicle morphogenesis, the latter appeared significantly retarded compared to their wild type littermates (Chernyavsky et al., 2004).

Exploiting murine hair follicle cycling as a model for exploring the role of mAChR in a complex neuroectodermal–mesodermal interaction system *in vivo* (Paus and Cotsarelis, 1999; Slominski and Paus, 1993; Schmidt-Ullrich and Paus, 2005; Peters et al., 2006), we have now examined the hair phenotype of selected mAChR KO mice more closely, and have concentrated here on M4R.

In this pilot study, we show that the M4R-coupled pathway plays a fundamental role in murine hair follicle cycling and pigmentation.

Materials and methods

The M4R KO mice were kindly provided by Dr. J. Wess (Molecular Signaling Section, LBC-NIDDK, NIH, Bethesda, MD, USA). Skin samples of M4R KO mice and their wild type littermates were harvested from the back at days 17, 34 and 42 post partum (dpp). This allows one to reliably assess differences in the entry in to hair follicle cycling (dpp 17 = first catagen), and in subsequent hair cycling activity (dpp 34 = first anagen, dpp 42 = second telogen) (Paus et al., 1999; Müller-Röver et al., 2001). For cryosectioning, skin samples were embedded as described elsewhere (Paus et al., 1999), and 6 μm sections were prepared. At each time point, histomorphometric analysis was performed on 25 to 50 hair follicles in Giemsa-stained skin sections. Hair cycle stages were determined and grouped as described elsewhere (Paus et al., 1999; Müller-Röver et al., 2001). In addition, melanin was visualized histochemically, using Masson–Fontana stain (silver nitrate deposition). Mice skin of early developmental

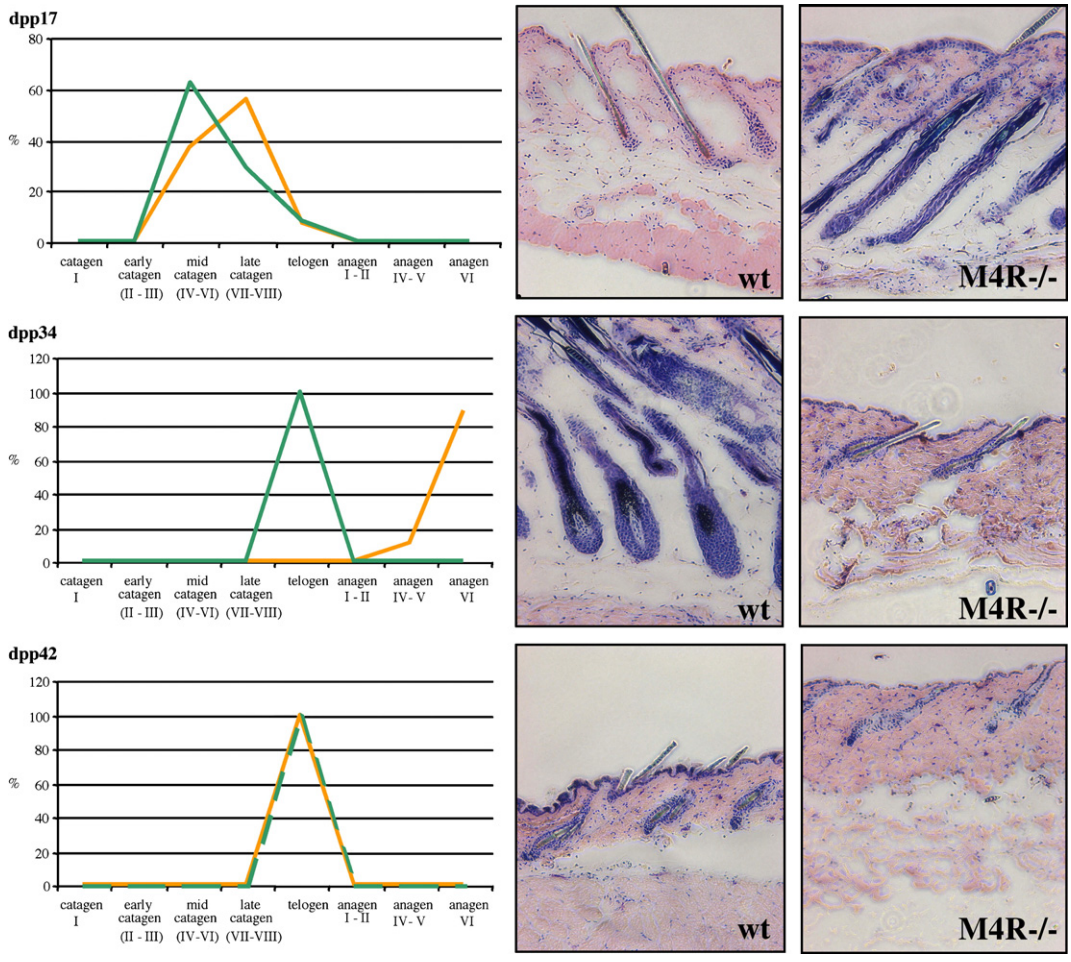

Fig. 1. Histomorphometric analyses of hair follicles of wild type and M4 mAChR KO mice. About 25 to 50 hair follicles per group were included in the analyses and the percentage of hair follicles per stage of hair cycle (dpp 17, 34, 42) was summarized in the graphs. At day 17 (dpp 17), when the first genuine hair cycle started, a slight retardation of catagen was visible in the M4R KO mice (green lines) compared to wild type (yellow lines). The retardation becomes more prominent on dpp 34 when wild type mice progresses into anagen IV–VI while M4 mAChR KO animal stay in telogen. On dpp 42, only telogen hair follicles can be found in both groups. Representative images of hair follicles in the skin of M4R wild type and KO mice supplement the graphic presentation. Magnification ×100.