Commentary

Delineating muscarinic receptor functions

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Acetylcholine, as the first chemical molecule to be established as a neurotransmitter, has attracted enduring interest from researchers over several decades. However, deciphering all of the physiological manifestations associated with actions of this molecule has been difficult, and many mysteries of cholinergic neurotransmission still remain to be resolved. Like many other neurotransmitters, the actions of acetylcholine are mediated by both ionotropic and metabotropic receptors. Within these two classes of receptors, a large amount of heterogeneity has been discovered that further complicates the assignment of specific physiological functions (1, 2). Five different subtypes (M1–M5) of G protein-coupled muscarinic receptors have been identified and characterized. The M1, M3, and M5 subtypes couple to the G_q/G_{11} family of G-proteins whereas M2 and M4 preferentially interact with the G_i/G_o family (2, 8). Aside from being involved in the control of many peripheral cholinergic responses, muscarinic receptors significantly contribute to a variety of centrally mediated functions, such as locomotion, learning and memory, regulation of circadian rhythm, antinociception, generation of epileptic seizures, and thermoregulation (1, 2).

The involvement of muscarinic transmission in a variety of physiological responses has accelerated the pace for the development of muscarinic drugs as the pharmacotherapeutic strategies for treatment of several disorders, such as Parkinson's and Alzheimer's diseases (1). However, most common muscarinic pharmacological agents have significant drawbacks because of their high degree of unwanted side effects. These limitations are mainly attributable to a lack of subtype selectivity of the available agents for the individual muscarinic receptors, and this is compounded by the lack of information about the functional specificity of these receptor subtypes as well as their overlapping pattern of expression in the many tissues of the body. The study by Gomeza *et al.* (5), which uses the approach of inactivating an individual gene in the mouse by homologous recombination, should greatly help in resolving not only the issue of receptor subtype specificity but also the complex issue of neuronal circuit interaction. It was convincingly demonstrated that M4 muscarinic receptor deficient mice do not show altered responses to muscarinic agonist-induced receptor activation such as analgesia, tremor, hypothermia, and salivation. Surprisingly, though, these mice showed an increase in basal locomotor activity and greatly enhanced locomotor responses after activation of D1 dopamine receptors. Thus, to date, genetic deletion of the M1 (6), M2 (4), and M4 (5) receptor genes have been reported. The muscarinic receptor functions found to be affected in the mutants are summarized in Table 1.

Although information from the genetic deletion approach is not yet available for the M3 and M5 muscarinic receptor subtypes, interesting generalizations can already be made about the physiological actions of various subtypes. Particularly, the classic central responses to muscarinic agonists, such as tremor, analgesia, a major part of hypothermic responses, as well as cardiac responses can be decisively attributed to M2 subtype stimulation. Muscarinic agonist-induced seizures and M-currents in sympathetic ganglion neurons have been associated with the M1 subtype; however, studies on other receptor subtype knockout mice will be required to confirm an exclusive role for this subtype in these functions. Pharmacological investigations have connected the M3 receptor with salivation responses (1, 2), and, indeed, muscarinic agonist-induced salivation was found to be preserved in M1, M2, and M4 receptor knockout mice (4–6). Although this connection may also need direct confirmation from a similar approach with the M3 receptor subtype, the current map of muscarinic receptor function has already provided invaluable information, which will undoubtedly facilitate the development and characterization of more specific pharmacological agents.

Besides clarifying the specific role of the receptor proteins in the whole animal, knockout models are increasingly valuable in assessing neuronal circuitry and neurotransmitter interactions. An important observation presented by Gomeza *et al.* in mice lacking M4 muscarinic receptors is supersensitivity of dopamine receptors, particularly the D1 subtype (5). This supersensitivity was accompanied by a mild locomotor hyperactivity. From multiple pharmacological, anatomical, and electrophysiological studies, it has become evident that muscarinic cholinergic and dopaminergic functions might interact (3, 7, 9). There are several potential levels of these interactions, determined by both pre- and postsynaptic localization of muscarinic receptors and an important role of intrastriatal cholinergic interneurons in striatal neuronal organization (3). A cellular colocalization of the dopaminergic and muscarinic receptors on striatal medium spiny γ-aminobutyric acid neurons and cholinergic interneurons has been well established. In clinical practice, anticholinergic drugs are commonly used as a therapy to treat Parkinson's disease symptoms and extrapyramidal side effects associated with chronic neuroleptic treatment (1, 3). However, despite decades of research, the precise mechanism that underlies this cholinergic-dopaminergic interaction is still unknown.

The paper by Gomeza *et al.* (5) sheds light on this relatively poorly understood phenomenon. The authors suggest that, in normal animals, M4 muscarinic receptors located on striatonigral projection neurons can counteract the increase in neuronal activity after D1 receptor activation (5). Thus, in these mice, the observed hyperactivity and D1 receptor supersensitivity may be caused by increased activity of the striatonigral pathway because of the lack of inhibitory striatal M4 receptors. These findings highlight a putative interaction between these two neurotransmitter systems. Although the molecular events underlying this interaction are not obviously apparent, many possibilities exist. First, colocalization of the receptors on the same cellular components in the striatum suggests the possibility of a direct interaction between muscarinic and dopaminergic receptors at the level of G-proteins and/or intracellular signaling. Another possibility might involve the well established role of cholinergic interneurons in the striatum, the major locomotor

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Table 1. Summary of muscarinic receptor functions affected in genetically altered mice

Genetic model	Functional responses to muscarinic agonist	Other phenotypic manifestations
M1 muscarinic receptor knockout mice (6)	Disrupted pilocarpine-induced seizures Absence of M-current potassium channel modulation in sympathetic ganglion neurons Preserved pilocarpine-induced salivation Preserved pilocarpine-induced tremor	
M2 muscarinic receptor knockout mice (4)	Disrupted oxotremorine-induced tremor Diminished oxotremorine-induced analgesia Attenuated oxotremorine-induced hypothermia Altered muscarinic regulation of heart rate Preserved oxotremorine-induced salivation	
M4 muscarinic receptor knockout mice (5)	Preserved hypothermia, salivation, tremor, analgesia induced by oxotremorine	Hyperactivity and D1 dopamine receptor supersensitivity

output region (3, 7). The cholinergic interneurons could mediate intrastriatal communication, and several investigators have proposed the existence of a local feedback loop to dopaminergic neurons involving these interneurons (9). If this assumption is correct, one might expect that elimination of muscarinic receptors could indirectly lead to an altered functional state of dopaminergic neurons, which, in turn, could determine the responsiveness of postsynaptic DA receptors. Another possibility for these complex interactions could involve a long striatonigral feedback pathway with cholinergic interneurons as intermediates between dopaminergic and γ-aminobutyric acid neurons (3, 9). Perturbations in muscarinic output could also indirectly modulate dopaminergic transmission and therefore induce alterations in receptor sensitivity. Additionally, the possible involvement of glutamate transmission as an intermediate for this interaction cannot be excluded (3). The assessment of steady-state neurotransmitter and metabolite levels in the striatal tissue is obviously not enough to address either of these possibilities. Future in-depth investigations using functional approaches of neurotransmitter homeostasis such as extracellular dopamine and acetylcholine dynamics and detailed assessment of activity of pre- and postsynaptic receptors is warranted in these animals.

Previous studies by Gomeza *et al.* established that oxotremorine-induced tremor in mouse is mediated by M2 muscarinic receptors (4). Based on this observation, the authors concluded that these receptors might be involved in the muscarinic-dependent modulation of motor control, particularly the Parkinsonian-like symptoms. The present find-ings (5) highlight another aspect of the involvement of muscarinic receptors in motor behavior and implicate the M4 muscarinic receptor in the modulation of dopaminergic responses. Taken together, these observations suggest that cholinergic–dopaminergic interactions in the control of locomotor function can occur at several levels and that distinct muscarinic receptor populations are likely involved in different aspects of the movement control.

The observations recently reported by Gomeza *et al.* (5) on the M4 receptor knockout mice are extremely valuable and timely. Although one should be mindful of the enormous task involved in acquiring and validating physiological data in these animal models, the availability of the M4 knockout mice should facilitate examination of several other interesting issues. A potentially more sensitive reflection of postsynaptic dopaminergic supersensitivity in the apomorphine-induced response paradigm is the climbing response, and such observations could further strengthen the model. Another important confirmation of D1 dopamine receptor supersensitivity would be the use of other drugs that stimulate dopamine receptors indirectly, such as cocaine and amphetamine. Moreover, this idea raises the interesting issue of the assessment of the rewarding properties of psychoactive drugs such as cocaine in these mice. Supersensitivity of dopamine receptors is considered to be an important component involved in acquisition of the drug-taking behavior, and altered rewarding properties of these drugs in M4 receptor knockout mice might be anticipated.

Overall, the study by Gomeza *et al.* (5) further illustrates the usefulness of the genetic deletion approach, in understanding of the function of proteins inaccessible or poorly assessable pharmacologically. Although common limitations of this approach are often attributed to compensatory changes in response to the lack of a functional gene, this study shows how such changes can represent valuable clues to understanding complex neurotransmitter interactions. This study further demonstrates the value of a thorough physiological and pharmacological characterization of such genetically derived animal models. Definitive delineation of a map of specific muscarinic receptor functions could potentially provide an opportunity to develop novel pharmacotherapies, based on selective targeting of affected functions with minimal side-effects.

1. Brown, J. H. & Taylor, P. (1996) in *The Pharmacological Basis of Therapeutics*, eds. Hardman, J. G. & Limbird, L. E. (McGraw–Hill, New York), pp. 141–160.
2. Caulfield, M. P. (1993) *Pharmacol. Ther.* **58,** 319–379.
3. Di Chiara, G., Morelli, M. & Consolo, S. (1994) *Trends Neurosci.* **6**, 228–233.
4. Gomeza, J., Shannon, H., Kostenis, E., Felder, C., Zhang, L., Brodkin, J., Grinberg, A., Sheng, H. & Wess, J. (1999) *Proc. Natl. Acad. Sci. USA* **96,** 1692–1697.
5. Gomeza, J., Zhang, L., Kostenis, E., Felder, C., Bymaster, F., Brodkin, J., Shannon, H., Xia, B., Deng, C.-X. & Wess, J. (1999) *Proc. Natl. Acad. Sci. USA* **96,** 10483–10488.
6. Hamilton, S. E., Loose, M. D., Qi, M., Levey, A. I., Hille, B., McKnight, G. S., Idzerda, R. L. & Nathanson, N. M. (1997) *Proc. Natl. Acad. Sci. USA* **94,** 13311–13316.
7. McGinty, J. F. (1999) *Ann. N. Y. Acad. Sci.* **877,** 129–139.
8. Nathanson, N. M. (1987) *Annu. Rev. Neurosci.* **10,** 195–236.
9. Potter, P. E., Hadjiconstantinou, M., Rubenstein, J. S. & Neff, N. H. (1984) *Eur. J. Pharmacol.* **106,** 607–611.