Mini-Review

Mouse models to investigate the function of spermine

Anthony E. Pegg* and Xiaojing Wang

Department of Cellular and Molecular Physiology; Milton S. Hershey Medical Center; Pennsylvania State University College of Medicine; Hershey, PA USA

Key words: polyamines, spermine, spermidine, Kir channels, glutamate receptors

Many functions have been ascribed to polyamines, but there has been no clear identification of a unique role for spermine. The Gy mouse has a deletion of part of the X chromosome that includes the SMS gene encoding spermine synthase. Tissues from male Gy mice have no spermine but increased spermidine. They have multiple abnormalities including a tendency to sudden death, small size, circling behavior and other neurological symptoms, sterility and deafness. These changes are reversed by breeding with mice expressing a spermine synthase transgene. Detailed studies of hearing in Gy mice show that the absence of spermine synthase leads to loss of the endocochlear potential. Since this potential requires the cochlear lateral wall-specific Kir4.1 channel, regulation by spermine of transport via these channels appears to be an essential function. A similar spermine-related defect in the functioning of cardiac Kir channels could account for arrhythmias leading to sudden death. The effect of the absence of spermine on glutamate receptor ion channels in the brain may account for the neurological symptoms and could contribute to the lack of fertility and normal growth but more direct effects on gene expression are also possible. Advantages and limitations of the Gy model are discussed.

The structures of the polyamines (putrescine, spermidine and spermine) and the enzymes responsible for their synthesis and interconversion are shown in Figure 1. Polyamines are clearly essential components of mammalian cells since inactivation of genes encoding early steps in the pathway for their biosynthesis is lethal. However, these studies do not provide a clear explanation of the need for spermine. Polyamine function has been the topic of a myriad of publications and numerous roles have been described. Briefly, these can be summarized as affecting the gene expression, either directly or by altering the expression of proteins that act as transcription and translation factors, modifying signaling pathways including kinases and phosphatases and acting as regulators of ion channels (reviewed in refs. 1–5). A multitude of secondary effects arise from these sources. A unique function of spermidine in eukaryotes is that it is used as the precursor of hypusine, a post-translational modification of eIF-5A. This modification is essential for eIF-5A function(s). Although these functions are not well understood, the modification is essential for viability and at least partially accounts for the absolute requirement for spermidine.6,7 However, spermine can only be used for hypusine synthesis after back-conversion into spermidine. All of the other polyamine functions that have been described can be fulfilled by either spermidine or spermine albeit with different affinity and potency.

Furthermore, many microorganisms do not contain spermine and yeast is viable and grows normally even when the spermine synthase gene (SMS) is deleted.8 In plants, SMS is also dispensable although there is another gene encoding the synthesis of the isomer, thermospermine, and this product may substitute for spermine.9 It seems unlikely that genes leading to the production of spermine would have been retained without their imparting some benefit and it is possible that a requirement may be seen in yeast and plants if placed under a stress but this have not yet been demonstrated.

In mammals however, there is more compelling evidence that spermine plays a unique function. A strain of mouse called Gy has a deletion of the X chromosome that eliminates the SMS gene.10,11 Male mice with this deletion have a severe phenotype as described below (Fig. 2). A rare X-linked recessive human disease termed Snyder-Robinson syndrome (SRS) is caused by SMS gene mutations that greatly reduce, but do not eliminate all spermine synthase activity.12–14 Affected males have mental retardation, hypotonia and movement disorders as well as bone-related abnormalities including a marfanoid habitus, skeletal defects, osteoporosis and facial asymmetry. Also, although the mechanism is unclear, it has been reported that patients with Alzheimer’s disease have reduced spermine and increased spermidine levels in the brain.15

Tissues in Gy mice have no spermine and cultured cells from SRS patients have a reduction in spermine. This is certainly consistent with the concept of a unique function for spermine. This is the current working hypothesis but it should be pointed out there are two other less likely explanations that have not been completely ruled out. Firstly, there is substantial increase in spermidine and in total polyamine content. (It appears that the reduction in spermine leads to a stimulation of the earlier steps in the pathway, and thus the rise is spermidine is greater than the decrease in spermine). Secondly, there is huge rise in the content of the aminopropyl-donor deAdoMet (see Fig. 1) due to an increase in its synthesis and lack of conversion into spermine. It is possible that the rise in deAdoMet or spermidine affects the phenotype.

The Gy mouse model provides an opportunity to probe unique functions of spermine but it is not an ideal model. The phenotype...
of these mice includes reduced bone density, a tendency to sudden
dehth, small size, a tendency to circling behavior (hence the name
Gyro) and other neurological symptoms, sterility and deafness
(Fig. 2). We have shown that all of these attributes, except for the
effect on bone, are reversed by breeding with mice expressing a
spermine synthase transgene (CAG-SMS).16,17 The primary cause of
the poor bone development in Gy mice is that the X chromosomal
deletion also inactivates a gene called Phex, which regulates phos-
phate metabolism. Other deletions of Phex that do not extend into
the SMS or inactivating point mutations of Phex also cause similar
changes in bone but none of the changes in other organs described
above that are reversed by provision of spermine synthase. Thus, it
cannot be concluded from this model that spermine is required for
normal bone development and maturation but this cannot really be
ruled out and requires further work since the human SRS phenotype
suggests a skeletal involvement.

Our recently published studies demonstrate clearly that normal
synthesis of spermine is needed for hearing.17 The Gy mice are
totally deaf; distortion product otoacoustic emission testing showed
no difference between the response of Gy mice and the noise floor.
There was an almost complete loss of the endocochlear potential.
All of these changes were reversed by breeding with the CAG-SMS mice,
which express spermine synthase under the control of a composite
CMV-IE enhancer/chicken beta-actin promoter that gives ubiqui-
tous but unregulated expression of spermine synthase.18 Although
it was not possible to measure the spermine levels in the inner ear
due to the small amount of tissue, it is likely, based on our measure-
ments of many other organs,18,19 that spermine was absent in this
organ in the Gy mice and restored to normal levels by the expression
of the transgene. A plausible explanation for these results is that the
cochlear lateral wall-specific Kir4.1 channel, which is known to be one
of the channels subject to strong inward rectification by polyamines
with spermine being the most potent,4,20,21 is critical to maintain
the endocochlear potential and that spermine is essential for this
purpose. These results provide strong support for the physiological
importance of regulation of inward rectification of Kir channels by
spermine and establish a clear function for normal polyamine levels
in hearing.17

Another phenotype of the Gy mice is a tendency to sudden
death. The reason for this is not fully understood at present but
a reasonable hypothesis is that cardiac arrhythmias lead to sudden
death. We have preliminary evidence that the Gy mice show an
altered electrocardiogram indicating abnormal cardiac electrical
activity. This could also be due to an altered activity of the inwardly
rectifying Kir channels located in the myocytes.22,23 Further study
is needed to establish the importance of normal spermine levels in
ion movement across the membrane in these cells but this is an
important area for further research.

Interactions with other types of ion channels may underlie some
of the other defects in Gy mice since spermine is the most potent
polyamine in these reactions. Polyamines have been shown to affect
glutamate receptor ion channels (NMDA, AMPA and kainate
receptors), certain connexin-linked gap junctions and some other
channels that are involved in intracellular calcium signaling or Na+
transport.5,24-27 The neurological deficiencies in Gy mice (and in
SRS patients) could also be related to a lack of effects of spermine on
glutamate receptors that mediate synaptic plasticity and excitatory
synaptic transmission in the mammalian brain. However, it is certainly
possible that some effects are related to altered gene expression due
to the changed polyamine content altering metabolites in the brain
and elsewhere. In preliminary experiments using magnetic resonance
spectroscopy, we have observed a decrease in the taurine:creatine +
phosphocreatine ratio in the hippocampus region of the brains of Gy
mice that is reversed in the Gy/CAG-SMS crosses.
The sterility of the Gy mice is not due solely to their small size. There is a striking alteration in the testicular morphology with most of the cells in the seminiferous tubules remaining as spermatogonia or early stage primary spermatocytes with an almost total absence of mature spermatozoa. There is independent evidence that strict control of polyamine levels is needed for testicular function. Overexpression of ODC is associated with a loss of fertility and there are haploid germ cell specific forms of both antizyme and antizyme inhibitor, which provide tight regulation of ODC. However, it is not certain that the defect in sperm development in the Gy mice is solely due to local lack of correct polyamine levels. There appears to be a reduction in the number of Leydig cells in the testes and brain-related endocrine defects may also contribute to developmental defects. Cell cultures from Gy and rescued CAG-SMS/Gy mice are a promising tool to attack this problem.

Direct studies of ion channel activity and proteomic analysis of altered protein content in the Gy mice are promising avenues for the further identification of spermine functions. However, this model has many disadvantages in addition to the deletion of the Phex gene described above and the tendency to sudden death. A serious problem with the Gy mouse model is that the mice are only viable on the mixed B6C3H background and attempts to transfer the genotype by back-crossing or to generate clean knock outs by inactivating only the spermine synthase have not led to any viable offspring. Such mixed backgrounds pose significant problems for experimentation and greatly increase individual variability. The studies already carried out show that spermine deficiency affects a number of organs including reproductive tissue, the inner ear, the brain and heart. It should be possible to generate more useful experimental models to address the critical function of spermine by selectively inactivating the spermine synthase gene in particular cell types. Although there is some concern that these models may be compromised by uptake of spermine from other cells, the studies with Gy mice suggest that this is not likely to occur to any major extent. All tissues from Gy mice that have been examined so far have undetectable levels of spermine despite a diet that contains this polyamine. Even after increasing the spermine content in the diet or administration of spermine by injection of the maximally tolerated dose there was only a very limited uptake.

The CAG-SMS mice were originally derived on the B6D2 background but have been backcrossed to inbred B6 strain. Since the crosses of these mice with Gy mice are viable and fertile, it should be possible by continued backcrossing to B6 to identify the gene(s) that are present in the B6C3H strain that allow the male Gy spermine synthase mice to survive even in the absence of spermine.

Finally, the results showing a lack of spermine in the tissues of Gy mice, even after feeding them with diets that contain substantial amounts of spermine or injection of spermine, raise major questions concerning the polyamine transport system. Such systems have been identified for the movement of polyamines across the intestinal wall and uptake into many different cell types. The reason that this system does not contribute significantly to spermine content is unclear but may be related to the elevated spermidine content. Overcoming the uptake block would enable experimentation to reverse the Gy mouse phenotype to be carried out by providing spermine rather than a spermine synthase transgene and, more importantly, might suggest a therapeutic approach to treatment of SRS patients, particularly if uptake into the brain can be achieved.

Acknowledgements

Research on polyamines in the authors’ laboratory is supported by grants CA-018138 and GM-26290 from the National Institutes of Health, Bethesda, MD.

References

1. Gerber EW, Meykens FL Jr. Polyamines and cancer: old molecules, new understanding. Nat Rev Cancer 2004; 4:781-92.
2. Tsurui Y, Higashi K, Taniguchi S, Shigemasa A, Nishimura K, Yamamoto K, et al. Enhancement of the synthesis of Rspo4, Cra and H-NS by polyamines at the level of translation in Escherichia coli cultured with glucose and glutamate. J Bacteriol 2007; 189:2359-68.
3. Ray RM, Bhattacharya S, Johnson LR. EGFR plays a pivotal role in the regulation of polyamine-dependent apoptosis in intestinal epithelial cells. Cell Signal 2007; 19:2519-27.
4. Kurata HT, Diraviyam K, Marton LJ, Nichols CG. Blocker protection by short spermine analogs: refined mapping of the spermine binding site in a Kir channel. Biophys J 2008; 95:3827-39.
5. Jin L, Miyazaki M, Mizuno S, Takigawa M, Hirose T, Nishimura K, et al. The pore region of N-methyl-D-aspartate receptors differentially influences stimulation and block by spermine. J Pharmacol Exp Ther 2008; 327:68-77.
6. Hyvonen MT, Keinanen TA, Cerrada-Gimenez M, Sinervirta R, Grigorenko N, Khomutov AR, et al. Role of hypusinylated eukaryotic translation initiation factor 5A in polyamine depletion-induced cytostasis. J Biol Chem 2007; 282:34700-6.
7. Chatrhopadhyay MK, Park MH, Tabor H. Hypusine modification for growth is the major function of spermidine in Saccharomyces cerevisiae polyamine autoregulated growing in limited spermidine. Proc Natl Acad Sci USA 2008; 105:195-20.
8. Hamaoki-Katagiri N, Katagiri Y, Tabor CW, Tabor H. Spermine is not essential for growth of Saccharomyces cerevisiae: identification of the SPE1 gene (spermine synthase) and characterization of a spe1 deletion mutant. Gene 1998; 210:195-210.

Copyright and Reuse

This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.
26. Fleidervish IA, Libman L, Katz E, Gutnick MJ. Endogenous polyamines regulate cortical neuronal excitability by blocking voltage-gated Na⁺ channels. Proc Natl Acad Sci USA 2008; 105:18994-9.

27. Shin J, Shen F, Huguenard J. PKC and polyamine modulation of GluR2-deficient AMPA receptors in immature neocortical pyramidal neurons of the rat. J Physiol 2007; 581:679-91.

28. Halmekytö M, Alhonen L, Wahlfors J, Sinervirta R, Eloranta T, Jänne J. Characterization of a transgenic mouse line overexpressing the human ornithine decarboxylase gene. Biochem J 1991; 278:895-8.

29. Christensen GL, Ivanov IP, Wooding SP, Arkins JF, Miernik A, Schlegel PN, et al. Identification of polymorphisms and balancing selection in the male infertility candidate gene, ornithine decarboxylase antizyme 3. BMC Med Genet 2006; 7:27.

30. Larque E, Sabater-Molina M, Zamora S. Biological significance of dietary polyamines. Nutrition 2007; 23:87-95.

31. Seiler N, Delcros JG, Moulinoux JP. Polyamine transport in mammalian cells. An update. Int J Biochem 1996; 28:843-61.