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The Pharmacology of Mechanogated Membrane Ion Channels

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I. Introduction

More than 30 years ago, A.S. Paintal (1964) reviewed in this Journal the pharmacology of vertebrate mechanoreceptors. His review summarized drug effects on a variety of specialized mechanosensory receptors, including cutaneous, muscle and visceral receptors. His main conclusion was that the drugs then known to inhibit or stimulate mechanosensation did not act on mechanotransduction itself but rather on ancillary processes such as the action potential or muscular/vascular tone. He further concluded that mechanotransduction usually has a low susceptibility to chemical influence. In subsequent years, voltage clamp studies confirmed that specific drugs reviewed by Paintal such as local anesthetics and veratrum alkaloids were indeed potent blockers and activators, respectively, of the voltage-gated Na^+ channel (Hille, 1992). Since Paintal's review, a vast number of new drugs and toxins have been discovered that selectively act on various voltage- and ligand-gated channels (Hille, 1992). This wealth of new drugs has provided a means of classifying, purifying and studying the function of many of these channels. Unfortunately, the large majority of these drugs has not been systematically studied for possible effects on mechanotransduction. This may be partially because of the practical problem of direct recording from specialized sensory mechanotransducers. However, it is also clear that the discovery of drugs that act on mechanosensors, such as those mediating various cardiovascular reflexes or specific forms of visceral pain, would have profound clinical significance.

Over the last 10 years, there has been a resurgence of interest in mechanotransduction because of a number of technical and conceptual advances. In particular, patch clamp recording, with its inherent ability to mechanically stimulate a membrane patch while simultaneously measuring the current response (Hamill et al., 1981), has revealed the existence of a class of mechanogated (MG) membrane ion channels. This class appears distinct from voltage- and ligand-gated channels (Sachs, 1988), although recent studies indicate that mechanical stimulation can also modulate voltage- and ligand-gated channels (see Section II, B). For practical reasons related to the inaccessibility of tissue embedded or fine ciliated endings of specialized mechanoreceptors, these

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MG channels have been studied mainly in nonsensory cell types and have been shown to be expressed in cell types representative of all five living kingdoms (Martinac, 1992). MG channels form a complex class, displaying different mechanosensitivities, gating dynamics and modalities, and open channel properties (Howard et al., 1988; Sachs, 1988; Morris, 1990; French, 1992; Petrov and Usherwood, 1994; Sackin, 1995; Hamill and McBride, 1995a). Presumably, the different properties reflect different roles played by MG channels in the various cell types. Although the role of MG channels in mechanosensory cells is obvious, they have also been implicated in such basic functions as cell volume regulation (Hamill, 1983a; Christensen, 1987; Sackin, 1989) and development (Medina and Bregestovski, 1988; however, see Wilkinson et al., 1996a, b). Nevertheless, their exact role(s) in many nonsensory cells remains unknown (Morris, 1992). For this reason, high specificity inhibitors or stimulators of MG channels would be extremely useful for implicating them in specific cellular processes. Furthermore, the existence of high affinity ligands that could label specific MG channels would facilitate protein purification procedures (Sukharev et al., 1993). Although no "ideal" high specificity/affinity drug for MG channels has yet been found, recent studies have begun to reveal a number of agents that do affect different MG channel activities. The purpose of this review is to discuss this emerging pharmacology.

II. Classifications of MG Channels

To better appreciate the possible mechanisms and sites of drug action on MG channels, the various classifications and mechanisms of MG channel activation will be summarized. In general, membrane ion channels tend to be classified in terms of either their open channel properties and/or their gating properties. These two properties can be considered independent in the sense that knowledge of one does not determine the other.

A. Open Channel Properties

MG channels, like voltage-gated and ligand-gated channels, display a variety of open channel properties with different ion selectivities (e.g., cation, anion, K^+ and nonselective) and single channel conductances (~20 to 2000 pS). However, in general, the ion selectivity and conductance of biological channels are not mechanosensitive (but see Opsahl and Webb, 1994). Although some cells express only one type of MG channel, there are also cells that express as many as five types, which can be distinguished by their open channel properties (Berrier et al., 1992; Ruknudin et al., 1993). Presumably, such differences reflect variations in the pore structure (i.e., binding sites and geometry) of the channels. In terms of drugs that act by binding to specific pore lining residues, it is conceivable that channels that share open channel properties (e.g., K^+ or cation selectivity) might be susceptible to pore occlusion or open channel block by the same drug or class of drugs.

B. Gating Properties

In terms of gating, MG channels have tended to be divided into two broad groups, namely stretch-activated (SA) and stretch-inactivated (SI), depending upon whether they are opened or closed by mechanical stimulation, respectively (Guharay and Sachs, 1984; Morris and Sigurdson, 1989). However, this basic distinction may be complicated by dynamic or nonstationary kinetic properties of the SA channel. For example, certain SA channels show rapid and complete adaptation in response to sustained stimulation (i.e., appear to inactivate) (Hamill and McBride, 1992). Furthermore, SA channel activity can shift irreversibly from an adapting to nonadapting mode during the course of patch clamp recording (Hamill and McBride, 1992). Another possible complication concerns the SI channel terminology and relates to a recent demonstration that specific MG channels can respond differentally, depending on the direction of patch curvature. For example, in rat aortic endothelial cells, suction applied to the patch decreases channel activity, whereas applied pressure increases channel activity (Marchenko and Sage, 1996). Whether this phenomenon is specific to the MG channel of this cell type or a general property of the so-called SI channel remains to be determined.

Another aspect of MG channel gating relates to their sensitivity to the magnitude of mechanical stimulation (see Hamill and McBride, 1994a). Some MG channels are so sensitive that they can be gated by random fluctuations in thermal noise (Denk and Webb, 1989) or sub-Angstrom displacements (Brownell and Farley, 1979), whereas other MG channels are relatively insensitive and require stimulation that often causes membrane patch (Vandorpe et al., 1994) or cell rupture (Morris and Horn, 1991). However, the large majority of MG channels appear to be activated by modest stimuli, above the thermal noise level yet well below the level that causes membrane damage (Hamill and McBride, 1994a). Apart from the magnitude or quantitative aspects of the mechanical stimulus, there are also qualitative aspects concerning its form. For example, cell stretch and compression, osmotic swelling, fluid shear stress, and suction or pressure applied to the membrane patch are all forms of mechanical stimulation, yet each may be expected to perturb, at the molecular level, the membrane in different manners. Indeed, specific membrane channels have been found that selectively respond to one but not another form of mechanical stimulation

Abbreviations: MG, mechanogated; SA, stretch-activated; SI, stretch-inactivated; MS, mechanosensitive; IP_3, inositol trisphosphate; IC_{50}, concentration that inhibits 50%; *E. coli*, *Escherichia coli*; TTX, tetrodotoxin; TEA, tetraethylammonium; K_d, dissociation constant; TC, tubocurarine; RGDS, Arg-Gly-Asp-Ser; GS, *Grammostola spatulata*; RVD, regulatory volume decrease; I-NMBA, 6-iodide-2-methoxy-5-nitrobenzamil.

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(Olesen et al., 1988; Burton and Hutter, 1990; Sasaki et al., 1992). On the other hand, there are channels that respond to multiple forms of mechanical stimulation (Christensen, 1987; Ubl et al., 1988; Sackin, 1989; Oliet and Bourque, 1993). In this review, attention is focused almost exclusively on MG channels gated by suction/pressure applied to the membrane patch. In most cases, pressure and suction are equally effective in activating the MG channel (Sachs, 1988; Morris, 1990; McBride and Hamill, 1992), which is consistent with the channel responding to membrane tension rather than pressure itself. However, as stated above, some MG channels may be sensitive to the direction of membrane curvature (Bowman et al., 1992; Bowman and Lohr, 1996; Marchenko and Sage, 1996). Presumably, these different MG channel gating sensitivities depend upon specific extracellular/membrane/cytoskeleton interactions.

Finally, some MG channels, rather than being exclusively gated by membrane stretch, are also gated by nonmechanical stimulation such as ligands (Kirber et al., 1992; Vandorpe and Morris, 1992; Van Wagoner, 1993; Paoletti and Ascher, 1994; Vandorpe et al., 1994) and/or membrane voltage (Hisada et al., 1991; Kirber et al., 1992; Chang and Loretz, 1992; Davidson, 1993; Langton, 1993; Ben-Tabou et al., 1994; Hamill and McBride, 1996a). In specific cases, membrane stretch may only modulate activity rather than directly gate the channel (Kirber et al., 1992; Paoletti and Ascher, 1994). Such polymodal activation of a membrane ion channel complicates, from a biophysical viewpoint, the characterization and classification of the channel but nevertheless may be critical in its role(s) under different physiological and/or pathological conditions. Clearly, recognition of the normal gating behavior of a channel is essential for proper interpretation of drug effects on that channel.

C. Molecular Mechanisms

Mechanisms of mechanosensitivity can be classified into either direct or indirect, according to the way mechanical energy is coupled to the gating mechanism of the channel. In the direct mechanism, mechanical energy is directly coupled to the MG channel protein without the intervention of biochemical reactions, although energy may be focused onto the channel via cytoskeletal and/or extracellular elements. In the indirect mechanism, there are intervening biochemical steps between the initial mechanical event and channel gating such as a membrane bound mechanosensitive (MS) enzyme, which regulates a second-messenger and, in turn, influences sensitive channels. For instance, activation of a mechanosensitive phospholipase C (Brophy et al., 1993) would elevate inositol trisphosphate (IP_3), in turn releasing Ca^{2+} from IP_3-sensitive internal Ca^{2+} stores (Boitano et al., 1994) and, in this way, stimulating Ca^{2+}-sensitive channels in the plasma and possibly organelle membranes. In this example, both Ca^{2+}-release and Ca^{2+}-activated channels contribute to the MS response without themselves being directly MS. One criterion for distinguishing between direct and indirect mechanisms involves measuring the latency of channel turn-on after a step change in stimulation (Corey and Hudspeth, 1979; Ordway et al., 1991; McBride and Hamill, 1993). Another criterion is to compare mechanosensitivities in cell-attached and cell-free patches to determine the role, if any, of cytoplasmic second-messengers. Unfortunately, for many channels that display mechanosensitivity, this most basic distinction between direct and indirect mechanisms of activation has not always been made.

Both direct and indirect mechanisms may be subdivided into intrinsic or extrinsic mechanisms according to bilayer versus cytoskeletal (or extracellular) involvement in the gating mechanism. In the intrinsic mechanism, mechanosensitivity is dependent upon interactions exclusively within the membrane bilayer (Martinac et al., 1990; Sukharev et al., 1993; Opsahl and Webb, 1994). For example, intrinsic mechanisms may involve tension-dependent protein subunit recruitment or realignment within the bilayer (Opsahl and Webb, 1994; Hamill and McBride, 1994a). In the extrinsic mechanism, mechanical stimulation is applied to the channel or membrane enzyme via cytoskeletal or extracellular elements (Guharay and Sachs, 1984; Howard et al., 1988; Hamill and McBride, 1992). In this mechanism, mechanosensitivity might be lost if mechanical coupling between the channel and cytoskeletal/extracellular elements is disrupted without rupturing the patch itself (Assad et al., 1991; Hamill and McBride, 1992). In fact, such disruption can arise during the course of routine patch recordings and can result in modified MG channel properties (Hamill and McBride, 1992).

In considering the site of action of a drug, the type of mechanism underlying mechanosensitivity may be critical. For example, in the indirect mechanism, a drug could block mechanosensitivity by acting along the biochemical pathway with or without a direct action on the channel or MS enzyme. Similarly, with the extrinsic mechanism, a drug could be active because of its action on the cytoskeleton or extracellular matrix without having any direct effect on the channel itself.

III. MG Channel Drugs

The drugs that affect MG channel activity will be considered under two general categories involving either channel block or channel activation. Within these two classes, further distinctions may be made depending upon, for example, the drug's exact mechanism of action (e.g., open versus closed channel block) or its likely site of action (e.g., protein, bilayer or cytoskeleton).

A. Blockers

Of the various chemicals and drugs that block MG channels, three groups have received the most attention. These include amiloride and its analogs, aminoglycoside

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antibiotics and the lanthanides, in particular, gadolinium. In addition to these three groups, other less well characterized blocking agents will also be considered.

1. Amiloride and analogs. Amiloride is discussed first because it has been the most rigorously studied and is the only drug that has been quantitatively modeled in terms of its blocking mechanism (Lane et al., 1991; Ruesch et al., 1994). Amiloride is a member of a group of over 1000 structurally related compounds known as pyrazinecarboxyamides (Kleyman and Cragoe, 1988, 1990). Amiloride and many of its analogs are potent diuretics that act by blocking, in nanomolar to micromolar concentrations, the epithelial Na^+ channel (Benos, 1982). Although the native epithelial Na^+ channel has not been directly demonstrated to be mechanosensitive, recent cloning studies of the rat epithelial Na^+ channel indicate that the three subunits ($\alpha\beta\gamma$) that compose it show strong sequence homology to *Caenorhabditis elegans* genes, cloned from touch-insensitive mutants and believed to encode MG channel proteins (Canessa et al., 1993, 1994; Driscoll and Chalfie, 1991; Huang and Chalfie, 1994; Hong and Driscoll, 1994). Furthermore, a recent report indicates that the α-subunit of the bovine renal epithelial Na^+ channel may form a stretch-sensitive Na^+ channel when reconstituted into painted lipid bilayers in which tension was altered hydrostatically (Awayada et al., 1995). However, this result must be considered tentative, because with painted lipid bilayers, the membrane tension is not simply a function of the hydrostatic pressure gradient (e.g., see Fettiplace et al., 1971; Fettiplace personal communication). In fact, membrane tension will be determined by the equilibrium distribution of lipids between the bilayer itself and the surrounding meniscus. On this basis, the result should be confirmed under conditions in which membrane tension can be better controlled (e.g., by using the patch clamp tip-dip method (Opsahl and Webb, 1994)). Perhaps it is even more relevant to determine whether the heteroligomeric ($\alpha\beta\gamma$) or native Na^+ channel displays mechanosensitivity. Unfortunately, two recent studies attempting to directly address this important issue have not resolved it. In one case, the inability to obtain consistent results regarding the stretch sensitivity or insensitivity of the epithelial Na^+ channel in cells of the rat cortical collecting tubule precluded a resolution (Palmer and Frindt, 1996). In the other case, the lack of controls with regard to both the magnitude and form (i.e., compression versus stretch) of the mechanical stimulation casts doubt on the reported stretch sensitivity of the Na^+ currents measured in human β lymphocytes (Achard et al., 1996).

The original indication that amiloride might act on MG channels arose from observations that amiloride blocked mechanosensitivity in both the lateral line organ of *Necturus* and the skin of *Xenopus* (Jorgensen, 1985). Subsequently, amiloride was shown to directly block MG currents in dissociated audiovestibular hair cells of the chick (Jorgensen and Ohmori, 1988) and mouse (Ruesch et al., 1994) inner ear. The first study of amiloride block at the single MG channel level was carried out on the SA cation channel of *Xenopus* oocytes (Lane et al., 1991, 1992, 1993; Hamill et al., 1992). This SA channel shows basic similarities to the hair cell channel in terms of its cation selectivity (i.e., conducts Na^+, K^+ and Ca^{2+}) conductance (~50 pS) and rapid activation and adaptation kinetics (Howard et al., 1988; Taglietti and Toselli 1988; Yang and Sachs, 1989; Hamill and McBride, 1992, 1994b). Furthermore, external amiloride causes a similar voltage-dependent block of both the hair cell and oocyte MG channels. In both cases, inward current recorded at negative potential is reduced, but outward current recorded at positive potentials is almost unaffected (Jorgensen and Ohmori, 1988; Lane et al., 1991).

a. MECHANISM OF AMILORIDE BLOCK. At the single channel level, amiloride block involves brief interruptions in the inward current events that increase in frequency with amiloride concentration but decrease with depolarization (Lane et al., 1991). Because the interruptions are too brief to resolve fully, they result in an apparent reduction in single channel current amplitude with associated increased open channel noise. This type of voltage-dependent "flickery" block is often taken to reflect open channel or pore block with intermediate kinetics (e.g., see Hamill, 1983b). According to this model, amiloride, which is positively charged at physiological pH, would be driven by negative potentials into the open channel, where it would occlude or "plug" the channel. Positive potentials would reverse this block by driving the impermeant blocker back out of the channel. Modifications of the simple plug model include the partial and permeant block models (see Lane et al., 1991).

Although the various plug models are intuitively attractive, a number of observations argue against their relevance to amiloride block in both the oocyte and hair cell (Lane et al., 1991; Ruesch et al., 1994). In the first place, the channel conductance in amiloride reaches a voltage-independent value at hyperpolarized potentials. Second, the concentration dependence of amiloride block yields a Hill coefficient of 2, which indicates two amiloride molecules are required to block the channel; this seems unlikely within a single file permeation pathway. Third, amiloride block does not depend on current flux through the MG channel, thus ruling out a mechanism by which permeating ions are able to knock amiloride off the channel.

In contrast to plug models, a "conformational" model can explain both the voltage and concentration dependence of external amiloride block (Lane et al., 1991; Ruesch et al., 1994). This model assumes that the open channel can exist in one of two different voltage-dependent conformations. The open channel conformation favored at negative potentials reveals two amiloride binding sites, whereas the open conformation favored at

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positive potentials is such that these sites are inaccessible to amiloride. Thus, at positive potentials, amiloride has no effect, whereas at negative potentials, amiloride binds to the exposed sites in a cooperative, voltage-independent manner to block the channel. Furthermore, at least in the hair cell, relaxation measurements directly demonstrate that amiloride analogs block the open rather than closed channel conformation at negative potentials. Although most attention has focused on external block, in the oocyte, internally applied amiloride produces a low potency voltage-independent block that was not observed in the hair cell.

b. IONIC EFFECTS ON AMILORIDE BLOCK. External amiloride is 10 times less effective in blocking the oocyte channel (concentration that inhibits 50% (IC_{50}) = 500 μM) compared with the hair cell channel (IC_{50} = 50 μM). This difference in potency was shown not to be caused by the different ionic recording conditions used in the two preparations (Lane et al., 1993). To be specific, in the original oocyte study, amiloride block was measured in the absence of external Ca^{2+} as well as other divalent cations and in the presence of high (100 mM) external K^+ and low (5 mM) external Na^+. In contrast, hair cell measurements were made in the presence of divalent cations (2 mM) with high external Na^+ and low K^+ (Jorgensen and Ohmori, 1988; Ruesch et al., 1994). Furthermore, it has been suggested that amiloride block requires the presence of external Ca^{2+} (Cuthbert and Wong, 1972; but see Desmedt et al., 1991) and is influenced by changes in external Na^+ concentration (Benos, 1982). However, direct experiments with the oocyte indicate that inclusion of 2 mM Ca^{2+} in fact reduced (by a factor of 2) rather than increased amiloride potency. On the other hand, substitution of external Na^+ with K^+ had no effect (Lane et al., 1993). The potency reduction by Ca^{2+} was shown to occur without altering the voltage dependence of block and could be modeled by a screening of surface negative charges (Lane et al., 1993). If Ca^{2+} has a similar effect in the hair cells, then amiloride may be even more potent in situ (i.e., $IC_{50} < 50$ μM) because Ca^{2+} concentration in the normal endolymph is only ~50 μM (Crawford et al., 1991).

c. STRUCTURE-ACTIVITY STUDIES. The difference in amiloride potency for MG channels in hair cells compared with oocytes presumably reflects real differences in the binding affinities for sites on the two channels. Although these difference may indicate structural differences in the sites, "potency-sequence-fingerprinting" indicates that the binding sites may be structurally related. For example, examination of the order of blocking potency of a series of amiloride analogs indicates both channels display identical potency sequences (Lane et al., 1992; Ruesch et al., 1994). In contrast, other transport pathways that are blocked by amiloride analogs, including the epithelial Na^+ channel, show different analog potency sequences (table 1). Therefore sequence fingerprinting with amiloride analogs can compensate for amiloride's low specificity. For example, table 1 indicates that amiloride and only one appropriate analog need be tested to implicate MG channels over other suspected transporters in a specific cellular function.

Examination of the structure-activity relation in table 1 indicates that analog potency in both the oocyte and hair cell can be increased by adding hydrophobic side chains to the amiloride core structure (Lane et al., 1992; Ruesch et al., 1994). However, the hair cell studies indicate a limit where potency decreases when the bulkiness of the side chains becomes too great. This may

TABLE 1

Amiloride analog[a] potency (IC_{50} amiloride / IC_{50} analog) of MG channels and other transport pathways.

	Amiloride (IC_{50} μM)	DMA	Phenamil	PBDCB	Benzamil	HMA	I-NMBA	Ref.
MG channel mouse hair cell (cation)	1 (53)	1.3	4.4	5	9.6	12.3	29	Ruesch et al., 1994
MG channel frog oocyte (cation)	1 (500)	1.4	—	—	5.3	14.7 [BrHMA]	—	Lane et al., 1992
Epithelial Na^+ channel (high affinity)	1 (0.34)	0.04	17	—	9	0.04	7	Kleyman and Cragoe, 1988
Epithelial Na^+ channel (low affinity)	1 (10)	2.2	2.9	—	3.8	—	—	Kleyman and Cragoe, 1988
Ca^{2+} channel L-type	1 (100)	3	—	400	4	—	28	Garcia et al., 1990
Na^+-Ca^{2+} exchanger	1 (1100)	2	5.5	300	11	11	28	Kleyman and Cragoe, 1988
Na^+-H^+ exchanger	1 (84)	20	0.01	—	0.08	524	—	Kleyman and Cragoe, 1988
MG channel snail (K^+)	1 (2000)	—	—	—	—	—	—	Small and Morris, 1995
Ca^{2+} channel T-type	1 (30)	—	—	—	—	—	—	Tang et al., 1988
Voltage-gated Na^+ channel	1 (600)	—	—	—	37	—	—	Velly et al., 1988
Voltage-gated K^+ channel (delayed rect.)	1 (5)	—	—	—	—	—	—	Bielefeld et al., 1986
Nicotinic AChR cation channel	1 (100)	—	—	—	—	—	—	Kleyman and Cragoe, 1990

[a] Dimethylamiloride (DMA), hexamethyleneamiloride (HMA), 6-iodide-2-methoxy-5-nitrobenzamil (I-NMBA), bromohexamethylene-amiloride (BrHMA), 5-(N-propyl-N-butyl)-dichlorobenzamil (PBDCB).

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indicate the amiloride binding sites are located within sterically restricted hydrophobic pockets. The most potent amiloride analog that has been found to date is 6-iodide-2-methoxy-5-nitrobenzamil (I-NMBA), which blocks the hair cell MG channel with an IC_{50} of ~2 μM. However, this drug may be even more potent when measured in the absence of external divalent cations (see Section III.A.1.b).

d. SUMMARY. Although amiloride has not proven a highly potent/specific MG channel blocker, it has provided valuable information regarding one type of MG channel. First, amiloride sensitivity of the MG cation channel provided the initial clue that this channel may somehow be related to the amiloride-sensitive epithelial Na^+ channel. This idea has subsequently been reinforced by (*a*) apparent immunological cross-reactivity between the epithelial Na^+ channel and the hair cell MG channel for an antibody raised against the former (Hackney et al., 1992) and (*b*) sequence homology between the epithelial Na^+ channel and putative MG channel proteins (Canessa et al., 1994). Second, detailed analysis of amiloride block indicates multiple, voltage-sensitive open channel conformations of the MG cation channel. This feature must be incorporated into any gating model of the channel. Third, potency sequence fingerprinting using amiloride analogs provides a way of identifying MG channel involvement in specific cellular processes. Finally, because all amiloride analogs so far tested have proven more potent than amiloride, future screening of the ~1000 available analogs may reveal even more potent/specific MG channel blockers.

2. Aminoglycoside antibiotics. Antibiotics of the aminoglycoside family (e.g., gentamicin, neomycin and streptomycin) also block MG cation channels in hair cells (Kroese et al., 1989) and skeletal muscle (Sokabe et al., 1993). However, compared with amiloride, less detailed information exists on their mechanism of action, ionic interactions and structure-activity requirements. Aminoglycosides consist of two or more amino sugars in glycosidic linkage to a hexose nucleus, and most have several positive charges because each sugar may have up to two positively charged amino residues (Daniels, 1978). In contrast to amiloride analogs, aminoglycosides are quite soluble (> 500 mM) in aqueous solution, which from a practical standpoint, makes them more convenient for experimental use (e.g., see Jaramillo and Hudspeth, 1991). In terms of MG channel block, most attention has focused on actions on hair cells because chronic exposure to aminoglycosides causes hair cell death, resulting in clinically significant and irreversible hearing impairment (Rybak, 1986). Although the exact mechanism of antibiotic ototoxicity is unknown, one possibility is an action involving the MG channel (Denk et al., 1992).

The first acute studies of aminoglycoside action were on the hair cells of the amphibian lateral line organ, where gentamicin was shown to cause a reversible, dose-dependent block of microphonic potentials and afferent fiber activity (Kroese and Van den Bercken, 1980, 1982). Subsequently, voltage-clamp studies of frog vestibular hair cells indicated a rapid (< 100 μsec), voltage-dependent block of the MG currents by gentamicin and a number of other aminoglycoside antibiotics (Kroese et al., 1989; and see table 2). Comparison of tables 1 and 2 indicates the potency of aminoglycosides and amiloride analogs are comparable. In addition, block by both classes of drugs is decreased by both depolarization or external Ca^{2+} (Kroese et al., 1989; Lane et al., 1993). However, despite these similarities, the two drugs most likely involve different blocking mechanisms (Kroese et al., 1989). To begin with, the concentration dependence of aminoglycoside block indicates a Hill coefficient of 1, rather than the coefficient of 2 seen for amiloride. Second, with aminoglycosides, MG conductance and currents approach zero with hyperpolarization (e.g., see Kimitsuki and Ohmori, 1993) instead of approaching a nonzero value, as seen with amiloride. Taken together, these observations are consistent with the idea that aminoglycosides block the open channel according to a simple plug model (see Section III.A.1.a). The voltage dependence of the block indicates that external aminoglycosides binding site senses 20 to 40% of the membrane field (Kroese et al., 1989). In addition to blocking hair cell MG channels, aminoglycosides may also have direct effects on the adaptation mechanism that changes the channel's mechanosensitivity in response to steady state stimulation (Kimitsuki and Ohmori, 1993). A molecular mechanism has been put forward to explain adaptation in the hair cell (Howard et al., 1988; Hudspeth and Gillespie, 1994), and the availability of drugs that affect adaptation should prove useful in analyzing such mechanisms. Another practical use of aminoglycosides has been focal application of gentamicin to localize the MG channels on the tips of the hair cell cilia (Jaramillo and Hudspeth, 1991).

Aminoglycosides also block two different types of SA cation channels in chick skeletal muscle, namely a 60 pS voltage-independent channel and a 190 pS voltage-dependent channel. These two SA channels can further be distinguished pharmacologically by their sequence-potency-fingerprint using various aminoglycosides (Sokabe et al., 1993; and see table 2). Aminoglycosides also block a variety of other MS processes (see Section III.A.5), including the slow adapting activity of cat cutaneous mechanoreceptors (Baumann et al., 1988), hair cell activity in squid statocysts (Williamson, 1990), mechanically induced nematocyte discharge in hydrozoans (Gitter et al., 1993) and stretch-induced increase in intracellular Ca^{2+} in guinea pig ventricular myocytes (Gannier et al., 1994). Although the aminoglycoside sensitivity of these processes points to an underlying MG channel mechanism, a complication in interpretation exists because aminoglycosides, like amiloride analogs, lack specificity and have been shown to also block voltage-gated Ca^{2+} channels (Nakagawa et al., 1992), Ca^{2+}-

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TABLE 2
Aminoglycoside antibiotic block of MG and other channels

	Aminoglycoside	IC_{50}	Reference
MG channels		(μM)	
Frog hair cell cation:	verdamicin	2.0	Kroese et al., 1988
	gentamicin C2	2.5	
	gentamicin C1	5.5	
	sisomicin	6.0	
	gentamicin	7.6	
	dihydrostreptomycin	8.0	
	netilmicin	25.0	
	streptomycin	**25.0**	
	amikacin	95.0	
Chick skeletal muscle SA cation:			
60 pS	neomycin	2.4	Sokabe et al., 1993
	streptomycin	**20.7**	
	ribostamycin	32.4	
	dibekacin	47.5	
	kanamycin	54.7	
190 pS	neomycin	2.4	Sokabe et al., 1993
	dibekacin	17.5	
	kanamycin	22.1	
	streptomycin	**25.4**	
	ribostamycin	55.9	
Other channels			
Voltage-gated Ca^{2+} (N-type)			Redman and Silinsky, 1994
frog nerve terminals:	**neomycin**	**<50**	
	streptomycin	**~50**	
	gentamicin	>50	
Voltage-gated Ca^{2+} (L-type)			Nakagawa et al., 1992
guinea pig outer hair cells:	**neomycin**	**300**	
	gentamicin	1000	
	kanamycin	~1000	
	streptomycin	**1000**	
Ca^{2+}-activated K^+ channel			Nomura et al., 1990
rat brain:	**neomycin**	**195**	
	dibekacin	600	
	ribostamycin	2390	
	kanamycin	2830	
ATP-activated K^+ current			
Guinea pig outer hair cells:	**neomycin**	**90**	Lin et al., 1993

activated K^+ channels (Nomura et al., 1991) and adenosine triphosphate-sensitive channels (Lin et al., 1993) as well as increasing desensitization of acetylcholine receptor channels (Okamoto et al., 1991).

3. *Gadolinium.* Gadolinium (Gd^{3+}) is the most commonly used blocker of MG channels and is often used as a pharmacological tool for testing the putative role of MG channels in various MG processes. It is a member of the lanthanide series, which is composed of the 15 elements, inclusively, between lanthanum (La, atomic number 57, ionic radius 1.061 Å) and lutetium (Lu, atomic number 71, ionic radius 0.85 Å), with Gd in the middle (atomic number 64, ionic radius 0.938 Å). All the lanthanides are trivalent ions in aqueous solution and have proven useful in biochemical studies because of their remarkable similarity to Ca^{2+} in terms of size (ionic radius 0.99 Å), bonding, coordination geometry and donor atom preference (Moeller, 1973; Evans, 1990).

Millet and Pickard were the first to focus attention on Gd^{3+} as a possible MG channel blocker when they reported that Gd^{3+} (10 to 250 μM) blocked both thigmotropism and geotropism in plants, whereas similar concentrations of La^{3+} were without effect (Millet and Pickard, 1988). They hypothesized that Gd^{3+} blocked these mechanosensitive processes by specifically inhibiting Ca^{2+}-permeable MG channels (see also Edwards and Pickard, 1987). Subsequent patch clamp studies confirmed that Gd^{3+} did indeed block single MG channel currents in not only plant cells (Alexandre and Lassalles, 1991; Ding and Pickard, 1993a; Garrill et al., 1993) but also in fungi (Zhou et al., 1991), bacteria (Berrier et al., 1992; Martinac, 1992; Cui et al., 1995; Hase et al., 1995) and a variety of animal cells (see table 3). In particular, Yang and Sachs (1989) showed that 10 μM Gd^{3+} in solutions perfused onto outside-out patches of *Xenopus* oocytes completely and reversibly blocked MG cation channel activity. In contrast, in the same

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study, much higher concentrations (i.e., > 10 ×) of either La^{3+} or Lu^{3+} were required to cause MG channel block.

Generally, Gd^{3+} blocks MG channels in the concentration range of 1 to 100 μM, independently of either their single channel conductance or ion selectivity of the MG channel (table 3). However, there are exceptions. For instance, in one study of *Escherichia coli* (*E. coli*), Gd^{3+} in lower concentrations (20 μM) was shown to *increase* the activity of a 350 pS and a 1100 pS channel but inhibit their activity in higher concentrations (> 20 μM) (Cui et al., 1995). Similar concentration-dependent excitatory and inhibitory effects were reported for MG cation channel activity in onion protoplasts by Ding and Pickard (1993a). However, as noted by these authors (Ding and Pickard, 1993a), these mixed Gd^{3+} effects seen at low concentrations (0.1 to 2 μM) are highly variable. In another *E. coli* study, 100 μM Gd^{3+} was shown to block larger conductance (300 to 2300 pS) MG channels without affecting lower conductance (100 to 220 pS) MG channels (Berrier et al., 1992). Other exceptions include the relatively low potency (~1 mM) block of an SA K^+ channel in human myelinated nerve (Quastoff, 1994) and a cation MG channel in one strain of yeast (c.f., Zhou and Kung, 1992; Zhou et al., 1991) and its apparent ineffectiveness in blocking SA cation channels in atrial heart muscle (Kim, 1993) as well as SA K^+ channels in astrocytes (Yang and Sachs, 1989), lymphocytes (Schlicter and Sakellaropoulos, 1994) and molluscan neurons (Small and Morris, 1995). However, in some studies, the presence of anions such as bicarbonate and phosphate or the Ca^{2+} chelator, ethylene glycol-bis-N,N′-tetraacetic acid (EGTA), may contribute to the observed low potencies. These chemicals interact with or chelate Gd^{3+} and therefore change its effective concentration (Boland et al., 1991).

TABLE 3
Gadolinium block of MG and other channels

Species cell type	Ion selectivity	Channel conduct. (pS)	Blocking concent. [μM]	Reference(s)
MG channels				
E. coli	nonselective	300–2300	100	Berrier et al., 1992
E. coli	nonselective	350 and 1100	100	Cui et al., 1995
Saccharomyces (yeast)	nonselective	40	10	Gustin et al., 1988
Schizosacchar (yeast)	cation	180	1000	Zhou and Kung, 1992
Uromyces (fungi)	cation	600	50	Zhou et al., 1991
Hyphae (fungi)	cation	—	100	Garrill et al., 1993
Red beet vacuole	cation	70	10	Alexandre and Lasalles, 1991
Onion protoplast	cation	35	10	Ding and Pickard, 1993
Necturus kidney	cation	18	20	Filipovic and Sackin, 1991
Xenopus oocyte	cation	50	10	Yang and Sachs, 1989
Xenopus kidney	cation	70	10	Kawahara and Matsuzaki, 1993
Bone cell line	cation	40	20	Duncan et al., 1992
Guinea pig bladder	cation	80	20	Wellner and Isenberg, 1993; 1995
Mouse skeletal muscle	cation	20–50	>10	Franco and Lansman, 1990
Mouse skeletal muscle	cation (SI)	20–50	>10	Franco et al., 1991
Rat hepatoma cells	cation	40	50	Bear and Li, 1991
Rat supraoptic neurons	cation	~30	100	Oliet and Bourque, 1994
Mouse BC3H1 muscle	cation	30	100	Hamill and McBride, 1993
Rat cardiocytes	cation	42	1	Sandoshima et al., 1992
Chick cardiocytes	cation	25 and 50	20	Ruknudin et al., 1993
Chick cardiocytes	K^+	100 and 200	20	Ruknudin et al., 1993
Human demyelinated axon	K^+	52	1000	Quasthoff, 1994
E. coli	anion	100–250	No effect 100	Berrier et al., 1992
Rat astrocyte	K^+	70	No effect 10	Yang and Sachs, 1989
Mouse Ehrlich ascites	cation	15–40	No effect 20	Christensen and Hoffmann, 1992
Rat atrial cells	cation	20	No effect 100[a]	Kim, 1993
Rat atrial cells	K^+	50–100	No effect 100	Kim et al., 1995
Snail (*Lymnaea*) neur.	K^+	44	No effect 100[a]	Small and Morris, 1995
OTHER CHANNELS				
Species, cell type	Channel type		[μM]	Reference
Guinea pig ventricul. myocytes	Ca^{2+} (L-type)		10	Lacampagne et al., 1994
Rat pituitary	Ca^{2+} (L-type)		<0.2	Biagi and Enyeart, 1990
Rat pituitary	Ca^{2+} (T-type)		2.5	Biagi and Enyeart, 1990
Xenopus myelinated nerve	K^+ (delayed rec.)		100	Elinder and Arhem, 1994
Xenopus myelinated nerve	Na^+ (voltage-gated)		100	Elinder and Arhem, 1994
E. coli	Colicin A, N		30,100	Bonhivers et al., 1995
Bryonica dioica (plant)	Ca^{2+}-release channel ER membrane		10	Kluesener et al., 1995

[a] EGTA present with Gd^{3+}.
All channels are SA unless indicated SI.

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a. MECHANISM OF GD^{3+} ACTION. To date, no detailed mechanism for Gd^{3+} block of any MG channel has been determined. One reason for this, at least for SA channels, may be that Gd^{3+} action is complex and most likely has multiple mechanisms and sites of action depending upon its concentration. In addition to any specific interactions with membrane channel proteins, Gd^{3+}, as well as other lanthanides, has been shown to exhibit strong interactions with lipid bilayers. For example, lanthanides bind strongly to both charged and neutral bilayers (Lehrmann and Seelig, 1994). Furthermore, differential scanning calorimetric and freeze-fracture studies have shown that lanthanides are capable of increasing phase transition temperatures, decreasing membrane fluidity and promoting phase separations or domains and membrane dipole potentials (Li et al., 1994; Yu et al., 1996). Such effects may alter MG channel activity by changing the physical environment of the membrane channel protein (Yu et al., 1996). In relation to these ideas, temperature studies of MG channel activity in plant cells indicate a membrane phase transition that affects MG channel activity (Ding and Pickard, 1993b).

The most detailed analysis of Gd^{3+} block has been carried out on the *Xenopus* oocyte SA cation channel (Yang and Sachs, 1989). The properties of the various Gd^{3+} effects on this channel and the extremely narrow concentration range (5 to 10 μM) over which they occur seem to rule out a simple open channel block mechanism. Instead, Yang and Sachs (1989) proposed a combination of three different mechanisms that are evident at different Gd^{3+} concentrations. First, at low concentrations (1 to 5 μM), the Gd^{3+} reduced single channel current amplitude, seen as an almost parallel shift of the single channel current-voltage curve along the voltage axis, was proposed to be caused by Gd^{3+} screening of negative surface charges in or near the vestibule of the channel. Second, also at low concentrations, the observed voltage-independent reduction in open channel lifetime was proposed to be caused by Gd^{3+} interacting with an external allosteric site (i.e., outside the membrane field) that caused transition of the channel to a short-lived closed state. Finally, at 10 μM but not 5 μM, channel activity completely disappeared. This last effect was believed to occur because of a Gd^{3+}-induced, highly cooperative transition of the channel to a long-lived closed state. Such highly cooperative behavior would be consistent with Gd^{3+}-induced shifts in phase transitions or promotion of phase domains in the lipid bilayer, as discussed above.

Although most Gd^{3+} studies have been on SA channels, there is at least one detailed report describing Gd^{3+} block of the so-called SI cation channel in mouse (*mdx*) muscle (Franco et al., 1991). This study found, as with the SA cation channel, that Gd^{3+} block can occur with no shift in the mechanosensitivity (i.e., the pressure-response relation) of the channel (Franco et al., 1991; Yang and Sachs, 1989). Nevertheless, comparison of the voltage- and concentration-dependent properties of Gd^{3+} block of SI and SA channels indicates notable differences. In particular, the block of the SI channel involves brief, yet resolvable, channel closures with no change in single channel current amplitude. Furthermore, the voltage and concentration dependence of the open-close transitions indicates a blocking mechanism consistent with Gd^{3+} being an open pore, permeant blocker. To be specific, although the blocking rate (i.e., Gd^{3+} entry into the channel) is diffusion-limited and displays no voltage dependence, the rate of unblocking increases with hyperpolarization, presumably as Gd^{3+} is driven through the channel.

The differences in Gd^{3+} block of SI and SA cation channels may reflect real differences in the nature of Gd^{3+} interactions with the two channels. However, strict comparison between the two studies is complicated because of the different ionic conditions. In the SI case, Gd^{3+} block was measured in the presence of divalent cations (2 mM Ca^{2+} and 1 mM Mg^{2+}) but in their absence in the SA case. This difference undoubtedly contributes to the different blocking effects because Ca^{2+} also permeates and blocks both channel types (Taglietti and Toselli, 1988; Yang and Sachs, 1989; Franco et al., 1991; Lane et al., 1993). Clearly, more detailed experiments on Gd^{3+} action under similar recording conditions would be helpful. Furthermore, a more systematic study of the blocking effect of the other 15 lanthanides of SA and SI channels may provide a means of dissecting or distinguishing different mechanisms and sites of action. For example, one possible physical basis for Gd^{3+}'s higher potency compared with other lanthanides is the "gadolinium break," a term that refers to the drastic change in the stability of organic ion complexes that occurs around the middle of the lanthanide series (Nieboer, 1975). On the other hand, it is interesting that in one systematic study of lanthanides, Tm^{3+}, Er^{3+}, Dy^{3+} Tb^{3+}, Eu^{3+} were found to be as equally potent as Gd^{3+} in blocking the touch-induced action potential of the plant *Chara* (Staves and Wayne, 1993).

b. GD^{3+} BLOCK OF NON-MG CHANNELS. Although Gd^{3+} was initially thought to be selective for MG channels, it has subsequently been shown to be a particularly potent blocker of voltage-gated L-type Ca^{2+} channels (complete block with 0.2 μM) as well as T- and N-type Ca^{2+} channels (Biagi and Enyeart, 1990; Boland et al., 1991; Docherty, 1988; Song et al., 1992; Lacampagne et al., 1994; Romano-Silva et al., 1994). Furthermore, it also blocks, although less potently (~100 μM), voltage-gated Na^+ channels and K^+ channels as well as voltage-independent leak channels in myelinated nerves (Elinder and Arhem, 1994a). A multisite mechanism of block similar to that of Yang and Sachs has been proposed for Gd^{3+} block of voltage-gated Na^+ and K^+ channels (Elinder and Arhem, 1994b). On the other hand, a de-

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tailed analysis of the block of L-type Ca^{2+} channels by Gd^{3+} and other lanthanides indicates a mechanism similar to that used to explain Gd^{3+} block of the SI channel (Lansman, 1990).

c. SUMMARY. Gd^{3+} is a potent blocker of a wide variety of MG channels and, as predicted by Millet and Pickard, has proven to be the inhibitor of choice for testing the putative role of MG channels in MG processes, not only in plants, but in a wide range of animal cells (see table 4). Its action, at least on the SA cation channel, is complex, and more detailed studies taking advantage of the other lanthanides and their known physical chemistry should be helpful in distinguishing different mechanisms and sites (i.e., lipid or protein) of action.

4. Other blockers. In this section, we list a number of less characterized blockers of MG channels. Some of the drugs listed are better known as blockers of other voltage- and transmitter-gated channels.

a. NA^{+} CHANNEL BLOCKERS. Tetrodotoxin (TTX) is best known for its ability to block the voltage-gated Na^{+} channel. However, whereas some studies of the Pacinian corpuscle indicate that TTX only blocks the action potential, other studies indicate it may also reduce the mechanoreceptor potential (see Bell et al., 1994). It remains unresolved whether this reduction occurs because of direct block of MG channels or block of voltage-gated channels that may contribute to the receptor potential. At least in chick cardiomyoctes, TTX does block a 25-pS SA cation channel without affecting four other classes of SA channels also expressed in the myocyte (Ruknudin et al., 1993). Whereas TTX has no effect on the microphonic potential recorded from goldfish saccula, procaine, another Na^{+} channel blocker, produces a partial (35%) reduction at a concentration of 2×10^{-3} g/ml (Matsuura et al., 1971). In a quite different preparation, the ciliated protozoan, 1 mM procaine blocks the mechanoreceptor K^{+} current (Deitmer, 1992). However, further down the evolutionary scale, 0.5 mM procaine activates the anion MG channel in *E. coli* (see Section III, B.1, and table 5). Given these divergent effects, it would seem worthwhile to screen procaine action on other MG channels.

b. CA^{2+} CHANNEL BLOCKERS. As indicated above, amiloride, aminoglycosides and Gd^{3+} all block Ca^{2+} channels as well as MG channels. This complicates functional studies, because reports indicate that the L-type Ca^{2+} channel may be mechanosensitive as well as voltage-sensitive (Langton, 1993; Ben-Tabou et al., 1994). One strategy to discriminate its role has been to test more specific Ca^{2+} channel blockers such as dihydropyridines (see Naruse and Sokabe, 1993). However, some caution is required with this approach because such Ca^{2+} channel blockers also affect MG channel activities, including diltiazem, which blocks the 25-pS SA, TTX-sensitive cation channel in chick cardiomyoctes (Ruknudin et al., 1993). Other known Ca^{2+} blockers, including Co^{2+}, La^{3+}, heptanol and octanol, block mechanotransduction in hair cells (Ohmori, 1985).

c. K^{+} CHANNEL BLOCKERS. To date, blocker pharmacology has been mostly focused on the SA cation-selective channel, and the major blockers of this channel have also been shown to act on Na^{+} and Ca^{2+} channels. By analogy, one might expect that SA K^{+} channels would be sensitive to the various toxins and drugs that act selectively on K^{+} channels. Indeed, a number of K^{+} channel blockers do act on SA K^{+} channels but appear to be ineffective against the SA cation channels (Morris, 1990). However, in general, these blockers are of relatively low potency and overall low specificity for K^{+} channels. For example, the SA K^{+} channel in frog renal proximal tubule can be blocked by external Ba^{2+} (5 mM) (Filipovic and Sackin, 1991; Cemerikic and Sackin, 1993), whereas the SA K^{+} channel in molluscan neurons is blocked by external tetraethylammonium (TEA) (K_d of 10 mM) and quinidine (K_d = 0.8 mM) but unaffected by either of these internally. Also ineffective on the latter channel when applied externally are Ba^{2+} (50 mM), apamin (1 μM) and 4-aminopyridine (10 mM) (Small and Morris, 1995). In contrast, Ba^{2+} (2 mM) blocks the SA K^{+} channels in rat brain neurons, but external TEA, 4-aminopyridine or quinidine apparently does not (Kim et al., 1995). On the other hand, in the ciliated protozoan, *Stylonychia*, external 4-aminopyridine (0.5 mM) and TEA (1 mM) but not Cs^{+} block the MG K^{+} current (Deitmer, 1992). Neither external TEA (1 mM) nor 4-aminopyridine (1 mM) blocks the MG cation channel in crayfish stretch receptor (Erxleben, 1989).

d. CALCIUM IONS. Ca^{2+} has been shown to block MG cation channels in *Xenopus* oocytes (Taglietti and Toselli, 1988; Yang and Sachs, 1989; Lane et al., 1993) and vertebrate hair cells (Crawford et al., 1991). Detailed analysis of this Ca^{2+} block by Taglietti and Toselli (1988) indicates a permeant ion block mechanism in which Ca^{2+}, like Na^{+} and K^{+}, enters and binds to the channel but displays a much smaller K_d (50 μM) and much longer occupancy time (400 nsec) compared with Na^{+} (2 mM and 20 nsec) or K^{+} (3.2 mM and 12 nsec). The higher affinity for Ca^{2+} results in greater channel occupancy so that inward flow of Na^{+} and K^{+} is competitively inhibited.

e. PROTONS. The open probability of SA cation channels is reduced to near zero when external pH is reduced from 7.2 to 4.5 in onion cell epidermis (Ding et al., 1993) and from 7.2 to 5.8 in *E. coli* (Cui et al., 1995). In *E. coli*, acidic pH was also shown to reduce the sensitivity of the MG channel (i.e., shifted the pressure-response curve to the right). The mechanism of the acid effects on MG channel gating remains unknown but may be due to protonation of amino groups or a proton-induced conformation change (Cui et al., 1995). In the case of plants, the pH effect has been proposed to underlie the inhibitory effect of acid soil syndrome on plant root tip growth (Ding et al., 1993). In contrast to the strong inhibitory effects of acid pH, alkaline pH up to 8.6 has only a slight inhibitory affect on the *E. coli* channels (Cui et al., 1995).

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TABLE 4
Processes tested for MG channel drug sensitivity

Process Stimulation used	Cell type	Gd^{3+}	Amilor.	Amino-glycoside	Conc. tested μM except when in ( )	Reference
Stimulated increase in internal Ca^{2+}						
Touch	Cultured chick heart	Y			20	Sigurdson et al., 1992
Touch	Rabbit airway epithelial	Y			50–100	Boitano et al., 1994
Touch	Rabbit osteoclasts	N			200	Xia and Ferrier, 1995
Cell-stretch	Pig vascular smooth muscle	Y			10	Davis et al., 1992
Cell-stretch	Guinea pig ventricular myocyt.			Y	40	Gannier et al., 1994
Substrate-stretch	Human umbilical endothelial	Y			10	Naruse and Sokabe, 1993
Substrate-stretch	Pulmonary arterial smooth musc.	Y			10	Bialecki et al., 1992
Substrate-stretch	Rat lung cell	Y			10	Liu et al., 1994
Osmotic swelling	Guinea pig outer hair cells	Y			50	Harada et al., 1993
Osmotic swelling	Human dystrophic myotubes	Y			50	Pressmar et al., 1994
Osmotic swelling	Human fibroblasts	Y			50	Bibby and McCulloch, 1994
Osmotic swelling	Human T lymphocytes	Y			200	Schlichter and Sakellaropoulos, 1994
Osmotic swelling	Rat renal collecting duct cells	N			40	Mooren and Kinne, 1994
Fluid shear stress	Bovine aortic endothelial cells	Y			10	Oliver and Chase, 1992
Volume regulatory events						
Volume regulatory decrease in response to osmotic swelling	Human blood lymphocytes	Y			1	Deutsch and Lee, 1988
	Mouse neuroblastoma cells	Y			10	Lippmann et al., 1995
	Guinea pig cochlear hair cells	Y			10	Crist et al., 1993
	Frog kidney proximal cells	Y			10	Robson and Hunter, 1994
	Mouse Ehrlich ascite cells	Y			10	Christensen and Hoffmann, 1992
	Human fibroblasts	Y			50	Bibby and McCulloch, 1994
	Human astrocytoma cells	Y			100	Medrano and Gruenstein, 1993
	Human leukemic cells lines	N			10–100	Gallin et al., 1994
Hypoosmotic-induced						
Cation current	Rat osteosarcoma cell	Y			10	Duncan et al., 1992
Cation current	Frog kidney proximal cells	Y			10	Robson and Hunter, 1994
$K^+_{(Ca)}$ current	Guinea pig outer hair cell	Y			50	Harada et al., 1993
$Cl^+_{(Ca)}$ current	Rat lacrimal acinar cells	Y			20	Kotera and Brown, 1993
Cl^- current	*Xenopus* oocytes	Y			>250	Ackerman et al., 1994
Osmolyte efflux	*E. coli*	Y			1000	Berrier et al., 1992
Hyperosmotic-induced						
Cation current	Human airway epithelial cells	Y			10	Chan and Nelson, 1992
Cl^- currents	Rat osteoblastic cells	N			100	Chesnoy-Marchais and Fritsch, 1994
Growth/development/metabolism					(mM)	
Oocyte maturation	*Xenopus* oocytes	N	N	N	(.1,2,1)	Wilkinson et al., 1996a,b
Fertilization	*Xenopus* oocytes	N	N	N	(.1,2,1)	Wilkinson et al., 1996a,b
Embryogenesis	*Xenopus* oocytes	N	N	N	(.1,2,1)	Wilkinson et al., 1996a,b
Sperm motility	*Xenopus* sperm	N	N	N	(.1,2,1)	Wilkinson et al., 1996a,b
Embryogenesis	*Xenopus* and ascidian oocytes	N			100	Steffensen et al., 1991
Stretch-induced						
Myoblast fusion	Chick skeletal muscle	Y			10	Shin et al., 1996
Cell alignment	Cultured endothelial cells	Y			10	Naruse et al., 1993
Cell proliferation	Rat lung cells	Y			10	Liu et al., 1994
DNA synthesis	Rat lung cells	Y			10	Liu et al., 1994
Phospholipase C	Rabbit aortic smooth musc.	N			100	Matsumoto et al., 1995

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TABLE 4 *continued*

Process Stimulation used	Cell type	Gd^{3+}	Amilor.	Amino-glycoside	Conc. tested μM except when in ( )	Reference(s)
Actin polymerization	Human fibroblasts	N			10	Pender and McCulloch, 1991
Hypertrophy	Rat cardiac myocytes	N			50	Sadoshima and Izumo, 1993
Genes activation	Rat cardiac myocytes	N			10	Sadoshima et al., 1992
Strain-activated						
Depolarization	Rat osteosarcoma cells	Y			10	Duncan and Hruska, 1994
Specialized mechanosensory receptors						
Pressure-induced						
Baroreceptor discharge	Rat arterial	N			400	Andresen and Yang, 1992
Baroreceptor discharge	Rabbit carotid	Y			10	Hajduczok et al., 1994
Stretch-induced						
Depolarization	Frog muscle spindles	Y	Y		(1 mM, 1 mM)	Ito et al., 1990
Depolarization	Crayfish stretch receptor	Y			400	Swerup et al., 1991
Touch-induced						
Calcium increase	Rat nodose sensory neurons	Y			10	Sharma et al., 1995
Depolarization	Cat cutaneous mechanoreceptors			Y	(i.v. 2.5 mg/min)	Baumann et al., 1988
Depolarization	Squid statocyst			Y	(10 mM)	Williamson, 1990
Cation inward current	Rat nodose sensory neurons	Y			20	Cunningham et al., 1995
Cation inward current	Rat supraortic neurons	Y			100	Oliet and Bourque, 1994
Voltage-induced						
Hair cell motion	Guinea pig outer hair cells	Y			500	Santos-Sacchi, 1991
Muscle						
Stretch-induced						
Force development	Guinea pig papillary muscle	Y			10	Lab et al., 1994
Contracture	Rat uterine smooth muscle	Y	N		100, 3000	Kasai et al., 1995
Arrhythmias	Canine ventricular muscle	Y			10	Hansen et al., 1990
Depolarization	Canine ventricular muscle	Y			10	Stacy et al., 1992
Depolarization	Guinea pig urinary bladder myocytes	Y			40	Wellner and Isenberg, 1994
Peptide secretion	Rat atrial myocytes	Y			5–80	Laine et al., 1994
Transmitter release	Frog neuromuscular junction	N			100	Chen and Grinnell, 1995
Plants and some lower invertebrates						
Touch-induced action potential	*Chara corallina*	Y			0.0001	Staves and Wayne, 1993
Touch-induced tendril coiling	*Byronia dioica*	Y			10,000	Kluesener et al., 1995
Ca^{2+} efflux from ER vesicles	*Byronia dioica*	Y			20	
Gravity-induced						
Thigmotropism	*Zea mays*	Y			10	Millet and Pickard, 1988
Orthogeotropism	*Zea mays*	Y			250	Millet and Pickard, 1988
Tip growth	*Saprolegnia ferax* (Fungi)	Y			100	Garrill et al., 1993
Rise in $[Ca^{2+}]_i$	Fungi growing tip	Y			100	Garrill et al., 1993
Wind-induced						
Rise in $[Ca^{2+}]_i$	*Nicotiana plumbaginifolia*	N			(10 mM)	Knight et al., 1992
Touch-induced						
Nematocyte discharge	*Hydra vulgaris*			Y	50	Gitter et al., 1993
Nematocyte discharge	*Cnidaria* (Coelenterates)	Y			1	Salleo et al., 1994a,b

However, for the SA cation channel in chick skeletal muscle, raising pH from 7.4 to 10 significantly *increases* the channel's stretch sensitivity as well as its voltage sensitivity without affecting single channel conductance (Guharay and Sachs, 1985). These last effects were proposed to occur because a titratable site, possibly involving a lysine group of an N-terminal amino acid, may be responsible for controlling voltage and stretch sensitivity (Guharay and Sachs, 1985).

f. ALUMINUM IONS. Aluminum ions (Al^{3+}) (10 to 100 μM) also inhibit the SA Ca^{2+} channel in the plasma membrane of onion cells. This inhibition might also contribute to acid soil syndrome because low pH elevates free Al^{3+} in the soil (Ding et al., 1993).

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g. TUBOCURARINE. (+)-Tubocurarine (TC) and other bisquarternary amines (gallamine, decamethonium and succylcholine) selectively depress (TC: IC_{50} = 19 μM) the mechanoreceptor current of the ciliate protozoan *Stentor coeruleus* without affecting the resting potential and the action potential (Wood, 1985). The mechanoreceptor current, in the absence of TC, is increased by depolarization (i.e., MG channel open probability increases with depolarization), whereas TC block is relieved by depolarization. To explain these observations, Wood (1985) proposed that the MG channel can exist in two voltage-dependent closed conformations (R and U) and one open conformation. One of the closed conformations (R), predominates at depolarized potentials and can be mechanically activated to open. The other closed conformation (U), predominates at hyperpolarized potentials and cannot be opened by mechanical stimulation. TC blocks the channel by selectively binding to the U form and thus prevents the channel from being mechanically activated. This model is similar to the conformation model proposed for amiloride block of the MG channel in *Xenopus* oocytes (Lane et al., 1991), except the oocyte model proposes two open conformations. In both cases, drug binding occurs to the conformation that predominates at hyperpolarized potentials.

h. HALOTHANE AND OTHER INHALATION ANESTHETICS. Halothane reduces the mechanoreceptor potential of crustacean stretch receptors: TTX was present to block Na^+ channels. The mechanism of this block may involve effects on cable properties because neither the time course nor amplitude of the mechanoreceptor current appeared to be altered (see fig. 8 in Swerup and Rydqvist, 1985). In a whole lung preparation halothane, enflurane and isoflurane were shown to raise the pressure threshold for recruitment of slowly adapting stretch receptors in the tracheobronchial system and inhibit pulmonary irritant receptors (Nishino et al., 1994). Again, in this situation, the exact mechanism of drug action remains unknown.

i. QUININE. Quinine, a cinchona alkaloid used for the treatment of malaria, is known to induce a reversible hearing loss in mammals in millimolar concentrations (see references cited in Matsuura et al., 1971). Quinine causes an irreversible suppression of microphonic potentials recorded from fish saccular hair cells (Matsuura et al., 1971) and an apparent increase in stiffness of the hair bundle of the fish lateral line organ (Van Netten et al., 1994). These results are similar to the effects of aminoglycosides on hair cells and may indicate a direct blockage of the MG channel by quinine.

j. FATTY ACIDS. A group of positively charged (medium to long chain) fatty acid analogs (table 5) has been shown to suppress a SA K^+ channel in toad gastric smooth muscle (Petrou et al., 1994). In contrast, negatively charged (medium to long chain) fatty acids activate the same channel and will be considered in more detail in the next section (Petrou et al., 1994). Short chain fatty acids, whether positively or negatively charged, have no effect on the channel activity (Petrou et al., 1994). In addition to the fatty acids, the naturally occurring positively charged amino alcohol, sphingosine, also suppresses channel activity. These positively charged compounds do not appear to act as open channel blockers because they do not reduce either channel open time or single channel conductance (Petrou et al., 1994). Instead, their inhibitory actions has been proposed to arise through an allosteric mechanism (Petrou et al., 1994).

k. INTEGRIN-BLOCKING PEPTIDES AND ANTIBODIES. A specific model of mechanotransduction proposes that mechanical energy distorting the extracellular matrix is focused through to the cytoskeleton via integrins that span the plasma membrane (Wang et al., 1993; Ingber, 1993). In this model, one would expect that agents, such as blocking peptides or antibodies, that inhibit integrin binding to extracellular matrix proteins would reduce or block mechanosensitivity. Two studies, although not directly on MG channels, lend support to this idea. In one study (Wayne et al., 1992), it has been demonstrated that the integrin blocking peptide Arg-Gly-Asp-Ser (RGDS) inhibits both the gravitational and hydrostatic pressure-induced polarity of cytoplasmic streaming in the plant *Chara*. Both processes have been proposed to be mediated by MG channels. In another study (Chen and Grinnell, 1995), it has been demonstrated that the integrin-blocking peptide RGD, as well as integrin antibodies, suppress stretch-induced release of transmitter from frog nerve terminals. Stretch-induced release still occurs in the absence of external Ca^{2+}, is not blocked by 100 μM Gd^{3+} and may involve either MG release of internal Ca^{2+} or shift in Ca^{2+} sensitivity of the release process. Unfortunately, for patch clamp studies, a practical problem in testing integrin function using blocking peptides is that the extracellular matrix must typically be removed before the plasma membrane can be patch clamped.

l. CISPLATIN. Cisplatin is the major antineoplastic agent used to treat solid tumors such as ovarian, testicular and bladder cancers (Rosenberg, 1985; Seymour, 1993). Unfortunately, cisplatin has a number of dose-limiting side effects that include ototoxicity with consequent hearing loss (see McAlpine and Johnstone, 1990) and distal sensory neuropathy, indicated by early decreased vibratory sensibility (Thompson et al., 1985). Based on acute studies on guinea pig cochlear microphonic potentials, it has been proposed that cisplatin acts by blocking the MG channel in hair cells in a manner analogous to aminoglycoside antibiotics (McAlpine and Johnstone, 1990). However, voltage clamp studies of hair cells have not been carried out to confirm this action. In our own studies to test this proposed action, we have found that, at up to 100 μM, neither cisplatin nor transplatin blocks the MG cation channel in *Xenopus* oocytes (L. Hu, D.W.M and O.P.H, unpublished observations).

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TABLE 5
Amphipathic and amphiphilic actions on MG channels

Cell Type	Channel Ion Selectivity Conductance	Compound Tested	Action[a]	Reference
Rat brain neurons	SA K^+-selective 45, 52, and 143 pS	Arachidonic acid	A	(Kim et al., 1995)
		Linoleic acid	A	
		γ-Linolenic acid	A	
		Docosahexaenoic acid	A	
		Myristic acid	NE	(Kim et al., 1995)
		Oleic acid	NE	
		Elaidic acid	NE	
		Palmitic acid	NE	
		Stearic acid	NE	
		Palmitoleic acid	NE	
		Arachidic acid	NE	
		Erucic acid	NE	
		Nervonic acid	NE	
		(Negative compounds)		
Toad (*Bufo marinus*)	SA K^+ selective 50 pS	Arachidonic acid	A	(Petrou et al., 1994)
		Myristic acid	A	
		Oleic acid	A	
		Tetradecanesuphonate	A	
		(Positive compounds)		
		Decylamine	I	
		Dodecylamine	I	
		Tetradecylamine	I	
		Oleylamine	I	
		Sphingosine	I	
		(Neutral compounds)		
		Octanol	NE	
		Decanol	NE	
		Dodecanol	NE	
Rabbit pulmonary artery smooth muscle	SA $K^+_{(Ca)}$ 270 pS	Arachidonic acid	A	(Kirber et al., 1992)
		Myristic acid	A	
		Linoelaidic acid	A	
E. coli	SA Anion selective ~700 pS	Chlorpromazine (+ve)	A	(Martinac et al., 1990)
		Procaine (+ve)	A	
		Tetracaine (+ve)	A	
		Trinitrophenol (−ve)	A	
		Lysolecithin (neutral)	A	
Chick skeletal muscle	SA cation 60 pS Voltage-independent	Chlorpromazine	A	(Sokabe et al., 1993)
		Trinitrophenol	A	
Canine ventricular myocytes	Swelling activated Cl^- conductance	Chlorpromazine	I	(Tseng, 1992)
		Trinitrophenol	A	

[a] A, activate; NE, no effect; I, inhibit.

m. TARANTULA SPIDER (*GRAMMOSTOLA SPATULATA*) VENOM. Preliminary reports indicate that the venom from the tarantula spider (*Grammostola spatulata*) (GS) blocks SA channel currents in *Xenopus* oocytes and chick heart cells (Niggell et al., 1996). GS venom also blocks the hypotonic swelling-induced elevation of intracellular Ca^{2+} (sensed with Fura-2) in GH3 cells proposed to be mediated by SA cation channels (Chen et al., 1996). The blocking concentrations of venom used were 1000 to 15,000 times dilutions in saline. Identification of the MG channel active components of the venom has yet to be made. Concerning the venom's specificity for MG channels, it was demonstrated in GH3 cells that the crude venom did not block L-type Ca^{2+} channels (Chen et al., 1996). However, in another study on rat hippocampal neurons, it has been shown that a purified GS venom component (*w*-grammotoxin) does block in the micromolar range N-, P- and Q-type but not L-type Ca^{2+} channels (Piser et al., 1995).

n. COLCHICINE AND VINBLASTINE. The antimiotic drugs, colchicine and vinblastine, which act by disrupting microtubules (Borgers et al., 1975), abolish mechanotransduction in the nematode *Caenorhabditis elegans* (Chalfie and Thomson, 1982) and the cricket *Acheta domesticus* (Erler, 1983), respectively. The concentrations and incubation times used were 0.5 to 1mM for 12 hours for colchicine and 10 mM for up to 21 hours for vinblastine. In each case, the specific microtubule struc-

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ture that is disrupted is proposed to be part of a supramolecular complex that underlies touch sensitivity (Thurm et al., 1983; Huang et al., 1995; for review see Hamill and McBride, 1996b). In contrast to the blocking effects of these drugs on lower invertebrates, neither colchicine nor vinblastine reduces MG channel activity in chick skeletal muscle (see Sachs, 1988) or in *Xenopus* oocytes (OPH and DWM unpublished observations). This lack of sensitivity in the oocyte may indicate that other types of non-microtubule cytoskeletal structures can focus mechanical energy onto MG channels (see Sachs, 1988; Hamill and McBride, 1995a).

5. Blocker sensitivity of mechanosensitive processes. Table 4 lists the wide variety of mechanosensitive processes that have been tested at the tissue or whole cell level for MG channel blocker sensitivity. Clearly, Gd^{3+} has proven the most popular agent in such studies. A likely reason for this is that Gd^{3+} causes a complete and voltage-independent block (i.e., "knock out" effect) of many types of SA channels, often at relatively low concentrations (1 to 20 μM). In contrast, amiloride and aminoglycosides only produce a partial and highly voltage-dependent block, even with relatively high drug concentrations (1 to 2 mM). However, even if Gd^{3+}'s action is all-or-none, additional control experiments should be carried out before either accepting or rejecting an MG channel role in a specific process. For example, a false positive could arise if the process involved a voltage-gated Ca^{2+} channel or another process that was Gd^{3+} blocked. Conversely, a false negative may arise if an underlying MG channel is Gd^{3+} insensitive (see table 3). Notwithstanding these caveats, the breadth of physiologically important mechanosensitive processes that display, at the tissue and/or whole cell level, the same Gd^{3+} sensitivity and ionic requirements as single MG channels recorded in the membrane patch is highly impressive. Presumably, it is the functional versatility of MG channels that underlies their ubiquitous expression in cells spanning the full evolutionary spectrum. Other notable highlights, trends and exceptions indicated in table 4 are discussed below (see Section III.A.5.a-f).

a. MECHANICALLY INDUCED ELEVATION OF INTERNAL Ca^{2+}. Internal Ca^{2+} is well recognized as a second-messenger in a wide range of cellular processes. In table 4, the various forms of mechanical stimulation that cause elevation of $[Ca^{2+}]_i$ in various cell types are listed. In general, $[Ca^{2+}]_i$ elevation may come about from one or a combination of the following mechanisms: increased Ca^{2+} influx from the external solution; decreased Ca^{2+} efflux from the cell and/or increased Ca^{2+} mobilization from internal stores. In many of the cases listed in table 4, the MS $[Ca^{2+}]_i$ increase is blocked by Gd^{3+}. This is consistent with a Ca^{2+} influx via Gd^{3+}-sensitive SA cation channels (Sigurdson et al., 1992; Oliver and Chase, 1992; Naruse and Sokabe, 1993; Harada et al., 1993; Sharma et al., 1995). However, there is also pharmacological evidence for L-type Ca^{2+} channel involvement (e.g., nifedipine and nimodipine sensitivity) (McCarty and O'Neil, 1991; Boitano et al., 1994; Mooren and Kinne, 1994, but see Naruse and Sokabe, 1993), which is significant given the recently demonstrated mechanosensitivity of this channel (Ben Tabou et al., 1994; Langton, 1993). In cases in which the mechanically induced increase in $[Ca^{2+}]_i$ occurs via release from internal stores, external Ca^{2+} is not necessary (Boitano et al., 1994; Charles et al., 1991; Demer et al., 1993; but see Sigurdson et al., 1992). For example, in airway epithelial cells, internal Ca^{2+} release is proposed to be mediated by a mechanosensitive phospholipase C that modulates, via IP_3, Ca^{2+} release from internal Ca^{2+} stores (Boitano et al., 1994). Interestingly, Gd^{3+} addition, in the absence of external Ca^{2+}, can cause a larger MS increase in $[Ca^{2+}]_i$, presumably because Gd^{3+} blocks Ca^{2+} efflux via MG channels in the plasma membrane (Boitano et al., 1994).

b. VOLUME REGULATORY EVENTS. Many cell types show a regulatory volume decrease (RVD) when swollen in hypotonic solutions. Table 4 lists examples of RVD that can be blocked by low (10 μM) Gd^{3+}. In general, RVD is mediated by an increase in K^+ and Cl^- efflux and consequent net water efflux and may involve either Ca^{2+}-dependent or Ca^{2+}-independent mechanisms (for critical review see Foskett, 1994). Therefore, Gd^{3+} could presumably block RVD either by blocking Ca^{2+} influx into the cell (e.g., via a SA cation channel), thereby indirectly blocking $K^+_{(Ca)}$ and/or $Cl^-_{(Ca)}$ channels (table 4) or by directly blocking swelling-activated K^+ and/or Cl^- channels (Deutsch and Lee, 1988; Berrier et al., 1992; Medrano and Gruenstein, 1993; Ackerman et al., 1994). Many cells also show a regulatory volume increase (RVI) when shrunken in hypertonic solution. RVI may arise through net influx of Na^+ and Cl^- and consequent net water influx. In this regard, Gd^{3+} also blocks a cation channel activated by osmotic shrinkage (Chan and Nelson, 1992), although it remains unclear at this stage whether the channel is MG.

c. GROWTH AND DEVELOPMENTAL EVENTS. It is plausible that as cells grow in size, develop and divide that mechanical signals play a regulatory role. In this regard, MG channels would seem attractive candidates to sense and transduce tension changes in the cell membrane during cell growth and development. This idea has received some experimental support in the form of Gd^{3+} sensitivity of stretch-induced endothelial cell alignment, lung cell proliferation and myoblast fusion (see table 4). In the case of *Xenopus* oocytes, which do express a relatively high and uniform density of MG channels, the lack of MG channel blocker sensitivity seems to rule out MG channels playing a critical role in oocyte maturation, fertilization or embryogenesis (see Wilkinson et al., 1996a, b). However, a possible role in oocyte growth and differentiation has not been excluded. The proposal that SA cation channels are involved in stretch-induced cardiac hypertrophy (Bustamante et al., 1991) seems unlikely, given that Gd^{3+} does not block stretch-induced hypertrophy of rat cardiac myocytes (Sadoshima et al., 1992).

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d. SPECIALIZED MECHANOSENSORY RECEPTORS. An obvious question concerning the various MG channel blockers is whether they also act on specialized mechanoreceptors such as those studied by Paintal (1964). In the case of arterial baroreceptors, although an initial study indicated little or no Gd^{3+} sensitivity (Andresen and Yang, 1992), more recent studies indicate that 10 μM Gd^{3+} is sufficient to block baroreceptor discharge in carotid receptors (Hajduczok et al., 1994) as well as mechanotransduction in the nodose sensory neurons that project to the carotid (Sharma et al., 1995; Cunningham et al., 1995). In the case of central osmoreception, which is important in regulating fluid balance and thirst, it has been demonstrated that Gd^{3+} blocks (IC_{50} = 20 μM) the hypertonically induced MG cation conductance that underlies osmosensitivity in rat brain supraoptic neurons (Oliet and Bourque, 1994, 1996). However, in the most recent study, examination of the single channel data indicates that Gd^{3+} only partially blocked the channel, even at 100 μM. Unfortunately, a complicating factor in these measurements was the presence of the chelator ethylenediamine-tetraacetic acid (1 mM) in the pipette recording solution. In the case of vertebrate muscle stretch receptors, relatively high (~1 mM) Gd^{3+} or high (2 mM) amiloride concentrations only produce partial block (Ito et al., 1990). Similarly, high (400 μM) Gd^{3+} is required to block the crayfish muscle stretch receptor (Swerup et al., 1991). Unfortunately, no reports exist on the Gd^{3+} sensitivity of specialized cutaneous mechanoreceptors such as the Pacinian corpuscle. Finally, although amiloride and aminoglycosides are well established blockers of mechanotransduction in the vertebrate audiovestibular hair cells, there are no published reports on the Gd^{3+} sensitivity of mechanotransduction in this preparation, although high Gd^{3+} concentrations (500 μM) have been reported to block voltage-dependent outer hair cell motility (Santos-Sacchi, 1989, 1991; however, see Gale and Ashmore, 1994).

e. MUSCLE. MG channel blocker sensitivity supports a role for SA cation channels in stretch-induced contraction in smooth muscle (table 4; see also Kirber et al., 1988) and stretch-induced depolarizations and arrhythmias in ventricular muscle (Hansen et al., 1991; Stacy et al., 1992).

f. PLANTS. Perhaps the most notable feature of plant studies is the wide range of Gd^{3+} concentrations required to block different processes. For example, whereas only 100 picomolar Gd^{3+} is required to block the touch-induced action potential in *Chara*, 10 mM Gd^{3+} only partially blocks tendril coiling in *Bryonia* (table 4).

B. Activators

A diverse group of compounds including lipid metabolites, free fatty acids, lipids and amphipathic molecules are able to activate certain MG channels. It is believed that these compounds themselves directly affect channel activity by either interacting with the channel protein or the lipid environment rather than by producing active metabolites through various enzymatic pathways (e.g., lipoxygenase or cyclo-oxygenase pathways) that then act on the channel (Meves, 1994). A number of possible mechanisms have been proposed: (*a*) partitioning into the bilayer to alter membrane tension (Martinac et al., 1990; Markin and Martinac, 1991), (*b*) changing membrane deformation energy (Lundbaek and Andersen, 1994) or (*c*) interacting with allosteric sites on the channel protein (Petrou et al., 1994; Kirber et al., 1992; Kim et al., 1995).

1. Amphipathic molecules. Amphipathic molecules have both hydrophilic and hydrophobic groups and may be either positive, negative or have no net electric charge (e.g., chlorpromazine, trinitrophenol and lysolecithin, respectively). Martinac et al. (1990) demonstrated that amphipathic molecules, when introduced into the external bathing solution, could reversibly increase the open probability of an SA anion channel in *E. coli*. When a particular amphipath, either cationic or anionic, was used alone, the effects were always stimulatory (i.e., open probability increased). However, cationic and anionic amphipaths, used in succession, first stimulated, then neutralized, this stimulatory effect and eventually resulted in a stimulatory effect. The effects were typically slow, sometimes taking up to an hour to increase open probability to unity. Amphipaths appeared to act by shifting the sigmoidal stimulus-response relation to the left so that lower pressures activated the channel without affecting the slope of the Boltzmann (i.e., stimulus-response curve). These effects were interpreted in terms of the bilayer couple hypothesis, in which cationic amphipaths insert into the positive inner monolayer and anionic species partition into the negative outer leaflet of the *E. coli* membrane (Sheetz and Singer, 1974). According to this hypothesis, the introduction of molecules into one leaflet increases the membrane tension in the adjacent leaflet and thereby activates the MG channel. However, it remains unclear why neutral amphipaths have a stimulatory effect unless steric factors also result in unequal partition within the different monolayers (Martinac et al., 1990; Markin and Martinac, 1991). A notable feature of the bilayer couple model is that mechanosensitivity derives entirely from interactions within the bilayer. This basic idea has received strong support, at least for the *E. coli* MG channel, in the form of recent studies showing that mechanosenitivity is retained when the MG channel protein, either purified from membranes or in vitro transcribed from the cloned gene, is reconstituted into artificial lipid bilayers (Sukharev et al., 1993; 1994). It will be interesting to determine whether amphipath sensitivity is retained when channels are reconstituted into bilayers formed from only pure neutral phospholipid.

The bilayer couple model contrasts with models that depend upon cytoskeletal (Guharay and Sachs, 1984) or extracellular protein interactions (Howard et al., 1988; Wang et al., 1993). Although different mechanisms of

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mechanosensitivity need not be mutually exclusive, it is interesting that chlorpromazine and trinitrophenol also stimulate SA cation channels in chick skeletal muscle preparation (Sokabe et al., 1993) where the initial cytoskeleton model was developed (Guharay and Sachs, 1984). On the other hand, lipid molecules have been shown to trigger changes in the elasticity of the cytoskeletal network in plant cells (Grabski et al., 1994), raising the possibility that amphiphiles and amphipaths have multiple sites of action capable of influencing MG channel activity.

2. Fatty acids and lipids (amphiphilic molecules). Amphiphilic molecules have polar heads attached to a long hydrophobic tail and include such compounds as fatty acids and lipid molecules. Recent studies indicate that fatty acids activate various MG channels independent of fatty acid metabolic pathways (i.e., cyclo-oxygenase or lipoxygenase pathways) (Kiber et al., 1992; Kim, 1992; Petrou et al., 1994; Kim et al., 1995). In particular, Kirber et al. (1992) demonstrated that arachidonic acid increases or modulates the activity of a large conductance, SA $K^+_{(Ca)}$ channel in rabbit arterial smooth muscle. This effect does not depend on the generation of arachidonic acid metabolites because saturated fatty acids such as myristic and linoelaidic acids, which are not substrates for the enzymes that convert arachidonic acid to active metabolites, also activate the channel. Further evidence of a direct effect was indicated by cell-free patch experiments in which activation by fatty acids was retained in the absence of soluble cytoplasmic enzymes (Kirber et al., 1992). Another SA K^+ channel, in this case in gastric smooth muscle, also displays fatty acid activation (Petrou et al., 1994). However, although negatively charged fatty acids stimulate MG channel activity, positively charged fatty acids inhibit activity (see above in Section III, A.4.j), and neutral analogs are without effect (table 5). This SA K^+ channel seems likely to be indirectly mechanosensitive (see mechanisms above) because stretch activation occurs with latencies of seconds rather than milliseconds (Ordway et al., 1991), persists for seconds after removal of stretch, and activation can be suppressed by perfusion with albumin, which is known to remove fatty acids from membranes (Ordway et al., 1995). These observations have led to the proposal that membrane stretch alters channel activity by generation of fatty acids, possibly via a stretch sensitive membrane phospholipase (Jukka et al., 1995).

In a third cell type, involving neurons from the mesencephalic and hypothalamic regions of the rat brain, there are a number of fatty acid-activated SA K^+ channels that can be distinguished by their open channel properties. In all cases, albumin fails to reduce stretch sensitivity, indicating that stretch and fatty acids may activate this channel by different mechanisms (Kim et al., 1995). A similar conclusion was made for the arachidonic sensitive SA K^+ channel in heart muscle (Kim, 1992). Another difference between SA K^+ channels in neurons and smooth muscle is that the saturated fatty acids myristic acid and oleic acid, which activate the smooth muscle K^+ channels, have no effect on the neuronal channels. Taken together, the evidence indicates that the SA K^+ channels in muscle and neurons involve direct fatty acid activation of the channel protein itself or an associated regulatory protein on smooth muscle and neurons. The fact that the fatty acid activation profile of K^+ channels in muscle and neurons differs indicates there may be a number of cell type-specific fatty acid binding sites, which may or may not be involved in conferring mechanosensitivity on the channel. Arachidonic acid and other fatty acids, apart from effecting the SA channels described above, also have actions on a wide range of other non-MG channels involving a variety of mechanisms (for review see Meves, 1994).

A number of features distinguish amphipathic from amphiphilic activation. First, the time course of activation differs. Whereas amphipaths activate the channel slowly (~1 hour), fatty acid activation is relatively fast and occurs in seconds. Second, at least for the $K^+_{(Ca)}$, fatty acids are not capable of activating the channel in the absence of basal activity (i.e., in zero external Ca^{2+} or at very negative potentials), indicating a modulatory role rather than primary activation (Kirber et al., 1988). In contrast, amphipaths can activate the *E. coli* MG channel in the absence of mechanical stimulation (Martinac et al., 1990). Third, the dependence of charge polarity of fatty acid effects differs from the polarity-independent stimulatory effect of amphipaths (Martinac et al., 1990). Finally, although the $K^+_{(Ca)}$ channel is stretch-sensitive, it is not clear to what extent stretch and fatty acids can act independently of one another. For example, it may be stretch sensitivity does not reside in the channel itself but instead resides in a stretch-sensitive phospholipase that generates fatty acids (Brophy et al., 1993; Jukka et al., 1995). In contrast, with the *E. coli* channel, it is quite clear from reconstitution experiments, using the purified channel protein, that mechanosensitivity arises purely from interactions between the protein and its surrounding bilayer environment (Sukharev et al., 1994).

3. Other activators. As in the case with blockers, there are a number of chemicals that have been reported to have activating effects on specific MG channels.

a. ALUMINOFLUORIDE. In contrast to blocking effects of Al^{3+} described above, it has been reported that aluminofluoride (20 μM $AlCl_3$ plus 20 mM KF) activates a SA cation channel in gastric smooth muscle cells (Hisada et al., 1993). However, AlF_3 effects were studied on SA channel activity activated by membrane hyperpolarization rather than by stretch (see Hisada et al., 1991). Interestingly, in contrast to the plant study in which Al^{3+} was reported to have an inhibitory effect (Ding et al., 1993), application of 20 μM $AlCl_3$ had no effect on SA channel activity when applied in the absence of F^- (Hisada et al., 1993). The mechanism of action of AlF_3

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remains unknown but it has been proposed that this activation may play a role in smooth muscle contraction (Hisada et al., 1993).

b. ETHYL-N-PHENYLCARBAMATE. The herbicide, ethyl-N-phenylcarbamate, which is known to interfere with gravitropism in plants, has been shown to gradually stimulate MG channel activity in onion protoplasts over the concentration range of 10 to 1000 μM (Ding and Pickard, 1993a). Although the mechanism of action has not been determined, this compound is known to alter the plant cytoskeleton (Nick et al., 1991).

c. CYTOCHALASINS. A specific theory of SA channel activation is based on cytoskeletal involvement in focusing mechanical energy onto the SA channel. Because several classes of compounds interact with specific cytoskeletal proteins, a reasonable assumption is that at least one or more of these drugs may have affects on channel activities (see Mills and Mandel, 1994). However, as it turns out, the results so far have been either negative or ambiguous. For example, the microtubule disrupting drugs, colchicine and vinblastine, while blocking mechanotransduction in lower invertebrates, have been shown to have no effect on SA channels activity in at least two different vertebrate preparations (see Section III, A.4.n). In the case of cytochalasins, which are known to selectively disrupt actin filaments, the reports on SA channels have been contradictory between laboratories; even conflicting reports have arisen from a single laboratory. The basis of the discrepancies is that some studies report that cytochalasins increase the SA channel's sensitivity to stretch (Guharay and Sachs, 1984; Small and Morris, 1994), whereas others report no effect on stretch sensitivity (Sokabe et al., 1991; O.P.H. and D.W.M., unpublished observations). Despite the apparent failure of pharmacological means to clearly implicate specific cytoskeletal proteins in SA channel function, other studies, using nonpharmacological means, have clearly implicated some form of cytoskeletal involvement (Hamill and McBride, 1995a). These studies involve conditions in which the membrane in the patch is physically decoupled from the underlying cytoskeleton (Hamill and McBride, 1992) or in which cytoskeleton free vesicles of plasma membrane have been studied (Hamill et al., 1995; Zhang et al., 1996). Under both conditions, mechanosensitivity is either lost entirely or significantly reduced.

IV. Summary and Conclusions

In this article, the actions, mechanisms and applications of various ions and drugs that interact with MG channels have been discussed. At present, no compound has been found that displays the high specificity and affinity exhibited by tetrodotoxin or α-bungarotoxin that proved so useful in the functional and structural characterization of the voltage-gated Na^+ channel and the acetylcholine receptor channel, respectively. Nevertheless, three different classes of compounds have been discovered since Paintal's review that clearly block MG channels. These compounds, represented by amiloride, gentamicin and gadolinium, act mainly on the SA cation channel, which appears to be shared by many nonsensory and some mechanosensory cells. Each class of compound can be distinguished by the voltage and concentration dependence of the block and most likely involves different mechanisms of blocking action. In general, the MG channel blocker pharmacology indicates a variety of "receptor sites" on MG channels. The recognition and acceptance of such receptors should provide added impetus for continued screening for more potent drugs, venoms and toxins.

In the case of activators, little is understood of the mechanisms by which the various amphipathic and amphiphilic compounds stimulate MG channels, although different bilayer and protein mechanisms have been evoked. Even less is understood of the role the new class of MG K^+ channel and their modulation by fatty acids plays in physiological and perhaps pathological processes. However, given that K^+ channels in general tend to reduce the excitability of nerve and muscle, plausible roles include fatty acid regulation of vascular tone and control of neuronal network excitability. In both cases, more detailed understanding is required regarding the physiological stimuli that modulate these channels through their fatty acid receptors. It may turn out that recognition and/or development of cell-type specific agents that activate such MG channels will possess high therapeutic potential. In any case, the observation that MG channels can be chemically blocked and/or activated by a wide range of compounds requires revision of the long-standing conclusion of Paintal that mechanotransduction is a process that has a low susceptibility to chemical influence.

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