Mg$^{2+}$ Inhibition of ATP-Activated Current in Rat Nodose Ganglion Neurons: Evidence That Mg$^{2+}$ Decreases the Agonist Affinity of the Receptor

CHAÖYING LI, ROBERT W. PEOPLES, AND FORREST F. WEIGHT

Laboratory of Molecular and Cellular Neurobiology, National Institute on Alcohol Abuse and Alcoholism, National Institutes of Health, Bethesda, Maryland 20892-8205

Li, Chaoying, Robert W. Peoples, and Forrest F. Weight. Mg$^{2+}$ inhibition of ATP-activated current in rat nodose ganglion neurons: evidence that Mg$^{2+}$ decreases the agonist affinity of the receptor. J. Neurophysiol. 77: 3391–3395, 1997. The effect of Mg$^{2+}$ on ATP-activated current in rat nodose ganglion neurons was investigated with the use of the whole cell patch-clamp technique. Mg$^{2+}$ decreased the amplitude of ATP-activated current in a concentration-dependent manner over the concentration range of 0.25–8 mM, with a 50% inhibitory concentration value of 1.5 mM for current activated by 10 μM ATP. Mg$^{2+}$ shifted the ATP concentration-response curve to the right in a parallel manner, increasing the 50% effective concentration value for ATP from 9.2 μM in the absence of added Mg$^{2+}$ to 25 μM in the presence of 1 mM Mg$^{2+}$. Mg$^{2+}$ increased the deactivation rate of ATP-activated current without changing its activation rate. The observations are consistent with an action of Mg$^{2+}$ to inhibit ATP-gated ion channel function by decreasing the affinity of the agonist binding site on these receptors.

INTRODUCTION

Extracellular adenosine 5′-triphosphate (ATP) has been reported to mediate fast excitatory neurotransmission in the CNS (Edwards et al. 1992) and the peripheral nervous system (Evans et al. 1992; Galligan and Bertrand 1994; Silinsky et al. 1992; Sneddon et al. 1982) via activation of a class of ATP-gated ion channels designated P2X purinoreceptors. Recent studies have demonstrated that these receptor channels possess modulatory sites for several endogenous substances. For example, Zn$^{2+}$ potentiates ATP-activated inward current in neurons (Cloues et al. 1993; Li et al. 1993) and in Xenopus oocytes expressing the P2X$_2$ (Brake et al. 1994) or P2X$_4$ (Séguela et al. 1996) ATP receptor subunits. Cu$^{2+}$, at physiological concentrations, has also recently been found to enhance the function of ATP-gated ion channels in sensory neurons (Li et al. 1996a). In addition, extracellular protons enhance the function of ATP-gated ion channels in sensory neurons (Li et al. 1996b, 1997) and of recombinant P2X$_2$ subunits expressed in Xenopus oocytes (King et al. 1996). In contrast, Mg$^{2+}$ has been reported to inhibit ATP-mediated responses. In pheochromocytoma PC12 cells, Mg$^{2+}$ block of ATP-activated channels was proposed to be due to the binding of Mg$^{2+}$ to a specific binding site on the receptor; however, the mechanism of that block was not established (Nakazawa and Hess 1993). More recently, the Mg$^{2+}$ inhibition of ATP-gated channel function in PC12 cells was attributed to a decrease in the concentration of ATP$^+$ (Choi and Kim 1996; Kim and Rabin 1994). Although inhibition of ATP-gated receptor channels in neurons by Mg$^{2+}$ has been observed (Krishtal and Marchenko 1984), the mechanism for this effect has not been determined. In the present study, we used the whole cell patch-clamp technique to investigate the effect of Mg$^{2+}$ on the function of ATP-gated ion channels in freshly isolated rat nodose ganglion neurons.

METHODS

Isolation of rat nodose ganglion neurons and whole cell patch-clamp recording were carried out as described previously (Li et al. 1993). Neurons were voltage clamped at −60 mV. The external solution contained (in mM) 150 NaCl, 5 KCl, 1 CaCl$_2$, 1 MgCl$_2$, 10 N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid (HEPES), and 10 n-glucose, pH adjusted to 7.4, osmolality adjusted to 340 mosmol/kg. The patch pipette (internal) solution contained (in mM) 140 CsCl, 1 CaCl$_2$, 2 MgCl$_2$, 11 ethylene glycol-bis(β-aminoethyl ether)-N,N,N′,N′-tetraacetic acid, 10 HEPES, and 2 ATP, pH adjusted to 7.4, osmolality adjusted to 310 mosmol/kg. Agonist solutions were delivered by gravity flow from a linear barrel array consisting of fused silica tubes (~200 μm ID) connected to independent reservoirs; solutions were exchanged by shifting the pipette horizontally with the use of a micromanipulator. In studies of activation and deactivation, rapid solution changes were achieved with the use of a modification of a system described previously (Lin and Stevens 1994). With the use of this system, the 10–90% rise time of the junction potential at an open pipette tip was <5 ms.

Virtually all nodose ganglion neurons tested exhibited ATP-activated current (>95%). A subset of neurons (~21%) was relatively insensitive to Mg$^{2+}$ (Li et al. 1996b); data used for analysis in this study were from neurons in which the current activated by 10 μM ATP was inhibited by ≥25% by 1 mM Mg$^{2+}$. Analysis of concentration-response curves was performed with the use of the program ALLFIT (DeLean et al. 1978), and concentrations of different forms of ATP were estimated with the use of the program “Bound and Determined” (Brooks and Storey 1992). Statistical significance of results was assessed with the use of Student’s t-tests or analysis of variance (ANOVA) as noted. Average values are reported as mean ± SE. Activation and deactivation time constants were determined by fitting the data to a single-exponential function with the use of the program NFIT (Island Products, Galveston, TX).

RESULTS

Figure 1A illustrates that, compared with current in the absence of added Mg$^{2+}$, the application of 1 mM Mg$^{2+}$ de-
The effect of Mg$^{2+}$ on the ATP concentration-response curve might be due to a decrease in the concentration of one or more active forms of ATP, such as ATP$^{4-}$, as has been proposed for Mg$^{2+}$ inhibition of P2 purinoceptor function in PC12 cells (Kim and Rabin 1994). To test this possibility, we held the concentrations of all forms of ATP (except for MgATP$^2$- and Mg$^2$ATP) constant while the Mg$^{2+}$ concentration was changed from 0 to 1 mM. This was done by increasing the total ATP concentration from 5.5 $\mu$M in 0 mM Mg$^{2+}$ to 10 $\mu$M in 1 mM Mg$^{2+}$ (Fig. 2). Under these conditions, the current activated by 5.5 $\mu$M ATP in Mg$^{2+}$-free solution was much greater in amplitude than that activated by 10 $\mu$M ATP in normal external solution (980 ± 110 vs. 620 ± 101 pA, respectively; Student’s t-test, $P < 0.01$; $n = 8$).

The possible mechanism of Mg$^{2+}$ action on ATP-gated channels was investigated further by studying the activation and deactivation time constants for ATP-activated current in solutions with different Mg$^{2+}$ concentrations. Figure 3 shows that the addition of 1 or 2.5 mM Mg$^{2+}$ substantially decreased the deactivation time constant ($\tau_{OFF}$) of ATP-acti-

FIG. 1. Inhibition of ATP-activated current by extracellular Mg$^{2+}$. A: records of current activated by 10 $\mu$M ATP in solutions containing 0, 1, and 2.5 mM Mg$^{2+}$. B: concentration-response curve for Mg$^{2+}$ reduction of current activated by 10 $\mu$M ATP. Each point is the average of 4–6 cells. Curve shown is best fit of data to the logistic equation $y = E_{max}/1 + (x/IC_{50})^n$, where $E_{max}$ is the maximal response, $IC_{50}$ is the concentration inhibiting 50% of the response, and n is the slope factor. Values obtained were: $E_{max}$, 99% inhibition of control response, $IC_{50}$, 1.5 mM, and n 1.5. C: concentration-response for ATP-activated current in the absence (○) and presence (●) of 1 mM Mg$^{2+}$. Amplitude is normalized to current activated by 500 $\mu$M ATP in normal external solution. Each data point is an average of 5–8 cells; error bars not visible are smaller than size of symbols. Curves shown are best fits of data to the logistic equation given for B. Mg$^{2+}$ (1 mM) significantly increased the 50% effective concentration ($EC_{50}$) for ATP from 9.2 $\mu$M to 25 $\mu$M ($P < 0.01$).

FIG. 2. Evaluation of whether Mg$^{2+}$ inhibition of ATP-activated current is due to a decrease in the concentrations of ≥1 active forms of ATP. Records are currents activated by 10 $\mu$M ATP in normal external solution and by 5.5 $\mu$M ATP in Mg$^{2+}$-free solution in a single neuron. Under these conditions, the concentrations of all forms of ATP, except for MgATP$^2-$ and Mg$^2$ATP, were held constant. Note that the current activated by 5.5 $\mu$M ATP in Mg$^{2+}$-free solution was much greater in amplitude than that activated by 10 $\mu$M ATP in normal external solution ($P < 0.01$).
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FIG. 3. Effect of Mg\(^{2+}\) on activation and deactivation time constants (\(\tau_{on}\) and \(\tau_{off}\), respectively) of ATP-activated current. A: currents activated by 5 \(\mu\)M ATP in solutions containing 0, 1, and 2.5 mM Mg\(^{2+}\). \(\tau_{on}\) and \(\tau_{off}\) were determined by fitting data to single-exponential equations. B: \(\tau_{on}\) (●) and \(\tau_{off}\) (○) as a function of Mg\(^{2+}\) concentration. Note that increasing Mg\(^{2+}\) concentration had no significant effect on \(\tau_{on}\) (\(P > 0.05\)) but greatly decreased \(\tau_{off}\) (\(P < 0.01\)).

Discussion

The observations reported here show that physiological concentrations of extracellular Mg\(^{2+}\) can inhibit ATP-activated current in rat nodose ganglion neurons. In the present study ATP had an EC\(_{50}\) of 25 \(\mu\)M in the presence of 1 mM Mg\(^{2+}\). This compares to EC\(_{50}\) values of 24–30 \(\mu\)M for ATP in our previous experiments on acutely isolated nodose ganglion neurons in the presence of Mg\(^{2+}\) (Li et al. 1993, 1996a,b). An EC\(_{50}\) value of 3 \(\mu\)M for ATP in cultured nodose ganglion neurons has been reported by other investigators (Khakh et al. 1995). One possible explanation for this difference in sensitivity to ATP could be differences in the expression of ATP receptor subunits in different conditions (e.g., acute isolation vs. cell culture). It should also be noted that the properties of recombinant P2X receptor subunits often do not correspond well to the properties of P2X receptors in native neurons (Collo et al. 1996).

One mechanism considered for Mg\(^{2+}\) inhibition of ATP-activated current in PC12 cells was an open-channel block by Mg\(^{2+}\) (Nakazawa and Hess 1993). Open-channel block is a typical form of noncompetitive inhibition, in that it is characterized by a decrease in \(E_{\text{max}}\) of the agonist concentration-response curve without a change of EC\(_{50}\). For ATP-activated current in rat nodose ganglion neurons, however, we found that Mg\(^{2+}\) did not affect the \(E_{\text{max}}\) of the agonist concentration-response curve, but rather shifted the concentration-response curve to the right, increasing the EC\(_{50}\) value. Thus this observation is not con-
sistent with a classical noncompetitive type of inhibition. A shift to the right of the agonist concentration-response curve without a change of $E_{\text{max}}$ is generally attributed to competitive antagonism, but could also be due to a decrease in the concentration of the active form of agonist, as has been proposed for Mg$^{2+}$ inhibition of ATP-activated responses in PC12 cells (Kim and Rabin 1994). In regard to these possible mechanisms, it has been found that a competitive antagonist (Clements and Westbrook 1994) or a decrease in the agonist concentration (Bean 1990; Khakh et al. 1995; Li et al. 1997) will decrease the activation rate of the receptor ion channel without changing its deactivation rate. However, we found that for Mg$^{2+}$ inhibition of ATP-activated current in rat nodose ganglion neurons, Mg$^{2+}$ did not affect the activation rate but rather increased the deactivation rate, which is not consistent with either competitive antagonism or a decrease in the agonist concentration. In contrast, it has been found that decreasing the affinity of the agonist binding site on a receptor increases the deactivation rate without changing the activation rate (Gu and Huang 1994; Li et al. 1997; Wang and MacDonald 1995). Moreover, a decrease in the affinity of the agonist binding site on the receptor shifts the agonist concentration-response curve to the right without changing $E_{\text{max}}$. Thus our observations on Mg$^{2+}$ inhibition of ATP-activated current in rat nodose ganglion neurons are consistent with Mg$^{2+}$ decreasing the affinity of these receptors for ATP. Interestingly, a similar effect of Ca$^{2+}$ on the deactivation rate of ATP-gated ion channels in bullfrog dorsal root ganglion neurons has been reported previously (Bean 1990); whether Ca$^{2+}$ and Mg$^{2+}$ share a common mechanism and site of action on ATP-gated channels should be addressed in future studies. Mg$^{2+}$ produces a voltage-dependent open-channel block of N-methyl-D-aspartate receptors (Nowak et al. 1984), and has also recently been demonstrated to increase the affinity of these receptors for the coagonist glycine (Wang and MacDonald 1995). The results of the present study are consistent with a novel mechanism by which Mg$^{2+}$ can inhibit the function of a ligand-gated ion channel by decreasing the agonist affinity of the receptor.

ATP can be released from damaged cells and activated platelets (for review, see Gordon 1986), as well as mast cells (Osipchuk and Cahalan 1992) in injured or inflamed tissue. Because P2X purinergic receptors are present in afferent fibers of sensory neurons (Chen et al. 1995), locally released ATP may stimulate these sensory afferents. In addition, ATP has been demonstrated to mediate neurotransmission in peripheral ganglia (Evans et al. 1992; Galligan and Bertrand 1994; Silinsky et al. 1992). Approximately 50% of the total Mg$^{2+}$ in the body is located intracellularly in soft tissues (Elin 1994). Cell lysis in areas of tissue damage could therefore presumably result in release of Mg$^{2+}$. Furthermore, Mg$^{2+}$ concentrations in blood and extracellular fluid can be altered in a number of physiological and pathophysiological states, such as pregnancy, hypertension, and myocardial infarction, as well as by changes in dietary intake of Mg$^{2+}$ (Altura and Altura 1996). Thus modulation of the function of ATP-gated ion channels by Mg$^{2+}$ may represent an important physiological mechanism for regulation of sensory neurotransmission in the periphery.

Address for reprint requests: C. Li, Laboratory of Molecular and Cellular Neurobiology, NIAAA, National Institutes of Health, 12501 Washington Ave., Rockville, MD 20852.

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