Recent work has indicated that sigma receptor ligands can modulate potassium channels. However, the only sigma receptor characterized at the molecular level has a novel structure unlike any other receptor known to modulate ion channels. This 26-kDa protein has a hydropathy profile suggestive of a single membrane-spanning domain, with no apparent regions capable of G-protein activation or protein phosphorylation. In the present study patch clamp techniques and photoaffinity labeling were used in DMS-114 cells (a tumor cell line known to express sigma receptors) to investigate the role of the 26-kDa protein in ion channel modulation and probe the mechanism of signal transduction. The sigma receptor ligands N-allylnormetazocine (SKF10047), ditolylguanidine, and (±)-2-(N-phenylethyl-N-propyl)-amino-5-hydroxytetralin all inhibited voltage-activated potassium current (I_K). Iodoazidococaine (IAC), a high affinity sigma receptor photoprobe, produced a similar inhibition in I_K, and when cell homogenates were illuminated in the presence of IAC, a protein with a molecular mass of 26 kDa was covalently labeled. Photolabeling of this protein by IAC was inhibited by SKF10047 with half-maximal effect at 7 μM. SKF10047 also inhibited I_K with a similar EC50 (14 μM). Thus, physiological responses to sigma receptor ligands are mediated by a protein with the same molecular weight as the cloned sigma receptor. This indicates that ion channel modulation is indeed mediated by this novel protein. Physiological responses were the same when cells were perfused internally with either guanosine 5'-O-(2-thiotriphosphate) or GTP, indicating that signal transduction is independent of G-proteins. These results demonstrate that ion channels can be modulated by a receptor that does not have seven membrane-spanning domains and does not employ G-proteins. Sigma receptors thus modulate ion channels by a novel transduction mechanism.

Sigma receptors are widely distributed in neuronal and non-neuronal tissue and are distinguished by their ability to bind a broad range of chemically unrelated ligands, including (+)-

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†† To whom correspondence should be addressed: Dept. of Physiology, University of Wisconsin School of Medicine, Madison, Wisconsin 53706. Tel.: 608-262-9111; Fax: 608-265-5512; E-mail: majackson@macs.wisc.edu.

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Sigma Receptor Photolabeling and Sigma Receptor-mediated Modulation of Potassium Channels in Tumor Cells*

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Russell A. Wilke†‡, Rakesh P. Mehta†‡, Patrick J. Lupardus, Yuenmu Chen**, Arnold E. Ruoho**, and Meyer B. Jackson†‡‡

From the Departments of †Medicine, ‡Physiology, and **Pharmacology, University of Wisconsin School of Medicine, Madison, Wisconsin 53706

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Sigma receptors are widely distributed in neuronal and non-neuronal tissue and are distinguished by their ability to bind a broad range of chemically unrelated ligands, including (+)-

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The amino acid sequence deduced from these clones is not easily reconciled with a physiological function of ion channel modulation. The vast majority of receptors that couple to separate ion channel proteins contain seven putative membrane-spanning segments and require G-proteins for signal transduction (11). Evidence both for (1, 4) and against (12) a role for G-proteins in sigma receptor responses has been presented, but hydropathy analysis of the deduced sigma receptor sequence indicated that this protein contains a single putative membrane-spanning domain, with no regions known to interact with G-proteins. In fact, the proteins encoded for by sigma receptor cDNAs encode a 25.3-kDa protein (7–10). The protein encoded for by these cDNAs binds sigma receptor ligands, but physiological responses of expressed sigma receptors have yet to be demonstrated.

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protein is involved in the modulation of $I_K$ by sigma receptor ligands. Further experiments showed that G-proteins do not mediate this response. Thus, this novel receptor protein employs a signal transduction mechanism not yet encountered in the ligand-induced modulation of ion channels.

**EXPERIMENTAL PROCEDURES**

**Cell Culture**—DMS-114 cells were obtained from the ATCC, Manassas, VA and maintained in Waymouth Medium 752/1 (ICN Biomedicals, Costa Mesa, CA) with 10% bovine calf serum (Life Technologies, Inc.). Flasks were incubated at 37°C in 5% CO₂, 95% air and subcultured regularly by mechanical dissociation.

**Photolabeling**—[125I]IAC was prepared according to Kahoun and Ruoho (6). For photolabeling, DMS-114 cells were pelleted by low speed centrifugation (200 x g) and resuspended in phosphate-buffered saline (PBS) diluted 10-fold in distilled water. (PBS had the following composition: 140 mM NaCl, 2.7 mM KCl, 10 mM Na₂HPO₄, 1.8 mM KH₂PO₄, pH 7.3.) Recombinant DNase (1 IU) was then added, and cells were homogenized with a Teflon pestle. The homogenate was resuspended in PBS and divided into 100-μl aliquots. Sigma receptor ligands such as SKF10047 were then added, and the mixture was incubated for 30 min on ice. [125I]IAC (1 nm) was added, and the incubation was continued for an additional 7.5 min, at which point illumination was then performed for 5 s with a high pressure AH-mercury lamp. Proteins were separated by SDS-polyacrylamide gel electrophoresis (12% acrylamide) and stained for [125I]IAC photolabeling on a PhosphorImager. Permanent autoradiograms were developed on x-ray film for all experiments included in this study.

**Electrophysiology**—DMS-114 cells were plated on coverslips for voltage-clamp recording. 2 h prior to recording, coverslips were transferred from the CO₂/air incubator to a superfusion chamber containing physiological salt solution (115 mM NaCl, 4.0 mM KCl, 1.25 mM Na₂HPO₄, 26 mM NaHCO₃, 2 mM CaCl₂, 1 mM MgCl₂, and 10 mM glucose, pH 7.4) saturated with 95% O₂, 5% CO₂. Individual cells were visualized with an upright differential interference contrast microscope (Diastar, Leica Microsystems, Inc., Buffalo, NY) and a × 40 water immersion, long working distance objective.

Voltage clamp recordings were made with an EPC-9 patch clamp amplifier (InstruTECH Corp., Port Washington, NY) interfaced to a Macintosh computer. Whole-cell currents were recorded using pipettes filled with 130 mM KCl, 10 mM EGTA, 2 mM MgCl₂, 4 mM MgATP, 100 μM NaGTP, and 10 mM HEPES, pH 7.3. Pipettes were fabricated from thin walled borosilicate glass, and the pipette shanks were chemically polished with Syргard to reduce electrode capacitance (19). Prior to approaching the cell membrane, pipette resistances typically ranged from 4 to 8 megohms. Immediately after breaking in, cell capacitance and series resistance were determined with the transient cancellation capability of the EPC-9. In cases where the series resistance exceeded 15 megohms, this value was partially compensated electronically.

**Drug Application**—Sigma receptor ligands (other than IAC) were obtained from Research Biochemicals, International (Natick, MA). These compounds were dissolved in physiological buffer and added to the bathing solution by direct pipette injection or through the use of a simple gravity-feed system (rate, 1–3 μl/min). Prior to the addition of drugs, $I_K$ was recorded at 10-s intervals for 1–3 min to obtain a stable base line. $I_K$ was also recorded after the removal of any drug(s) to demonstrate viability of the cell and recovery of current to base line. In experiments conducted using highly lipophilic drugs such as (6)-2-[(N-phenethyl-N-propyl)-amino]-5-hydroxytetralin (PPHT), the agent was eluted from the CO₂/air incubator to a superfusion chamber containing physiological buffer and then injected through one shank of the pipette.

**RESULTS**

**Inhibition of Potassium Current by Sigma Receptor Ligands**—DMS-114 cells displayed an outward current in response to depolarizing test pulses under voltage clamp (Fig. 1). Voltage steps from −80 to 10 mV rapidly activated this current, which showed a slight exponential decline as the potential was held constant for 500 ms. Tail currents reversed direction at the K⁺ equilibrium potential, and this value shifted appropriately with changes in external K⁺ concentration, indicating that the outward current was carried by K⁺ (n = 3, data not shown). When DMS-114 cells were exposed to the sigma receptor agonist SKF10047, whole-cell $I_K$ was inhibited in a concentration-dependent fashion (Fig. 1A). Kinetic analysis revealed that the current amplitude was uniformly reduced by this agent and that the time constants for channel activation and inactivation remained unaltered (control: $\tau_{act} = 9.7 ± 0.6$ ms, $\tau_{inact} = 3.8 ± 1.0$ s; 10 μM SKF10047: $\tau_{act} = 9.7 ± 3.2$ ms, $\tau_{inact} = 3.8 ± 1.0$ s; n = 3).

Whole-cell $I_K$ was also potently inhibited by the sigma receptor ligand IAC (Fig. 1B). A radiolabeled version of this agent was originally developed as a photoaffinity label for neuronal sigma receptors (6, 14). Although significant inhibition of $I_K$ was seen with 0.01 μM IAC, 1 μM IAC inhibited $I_K$ by more than 90%. The observation that IAC modulates $I_K$ indicates that this compound is a potent sigma receptor agonist.

To address the issue of ligand selectivity, additional chemi-
cally unrelated sigma receptor ligands were tested for inhibition of $I_K$. SKF10047 is the ligand for which sigma receptors were initially named (22). Fig. 1 demonstrates that this compound inhibits $I_K$ in a concentration-dependent manner, and Fig. 2 shows that this effect is reversible. Like SKF10047, DTG binds to sigma receptors with a $K_D$ in the nanomolar range, and its affinity for members of other receptor families is on the order of 1000-fold lower (1). Fig. 2 demonstrates that DTG reduced $I_K$ with an efficacy and potency similar to SKF10047. Fig. 2 shows a similar result with PPHT, a drug that binds both sigma receptors and dopamine receptors (23) and that has recently been shown to modulate neurohypophysial $I_K$ (5, 20).

Thus, four compounds known to bind sigma receptors, IAC, SKF10047, DTG, and PPHT, all inhibit $I_K$ in DMS-114 cells.

Photolabeling of Sigma Receptors—$[125I]$IAC covalently labels sigma receptors upon illumination and was used to identify the receptor as a 26-kDa protein in rat brain and liver and in human placenta (6). The labeling of this protein in each tissue could be blocked by haloperidol as well as other sigma receptor ligands. As noted above, IAC inhibited $I_K$ (Fig. 1). When DMS-114 cells were homogenized and illuminated in the presence of $[125I]$IAC, subsequent SDS-polyacrylamide gel electrophoresis and phosphorimaging demonstrated labeling of a protein with an apparent molecular mass of 26 kDa (Fig. 3).

Photolabeling of the 26-kDa band in DMS-114 cells was inhibited by two sigma receptor ligands, haloperidol (lane 2) and SKF10047 (lanes 4–6). This indicates that DMS-114 cells contain a protein with a similar molecular weight and that this protein has binding properties similar to previously characterized sigma receptors.

A progressive increase in the block of $[125I]$IAC photolabeling of the 26-kDa band was evident as the SKF10047 concentration increased from 1 to 100 $\mu$M (Fig. 4, top). The inhibitory effect of SKF10047 on the photolabeling of this band was plotted simultaneously with the inhibitory effect of SKF10047 on $I_K$ (Fig. 4, bottom). These effects had similar concentration dependences. When these data were fitted to a single-site saturation equation (see "Experimental Procedures"), an $EC_{50}$ of 7.6 $\mu$M was obtained for the inhibition of photolabeling, and an $EC_{50}$ of 14.6 $\mu$M was obtained for the inhibition of $I_K$. These two values are statistically indistinguishable, indicating that the inhibition of $I_K$ is mediated by the 26-kDa sigma receptor identified by photolabeling. This is an important result because it links the 26-kDa sigma ligand binding protein to the functional response of $I_K$ modulation.

Interestingly, the overall efficacy of SKF10047 appears to be identical for the two end points of inhibition of photolabeling and inhibition of $I_K$. At 100 $\mu$M, SKF10047 reduced whole-cell $I_K$ to 24 ± 5% of control (n = 3); this same concentration of SKF10047 reduced $[125I]$IAC photolabeling to 31 ± 8% of control (n = 3). This suggests that full occupation of sigma receptor binding sites produces a nearly complete block of $I_K$.

Transduction Mechanism—Receptor-mediated modulation of voltage-gated ion channels often reflects a shift in the voltage...
dependence of the channel. Although this feature is not diagnostic of a particular transduction mechanism, it is still widely regarded as important. We therefore examined the voltage dependence of inhibition of $I_K$ by PPHT by varying test pulses used to activate $I_K$ from $-60$ to $30$ mV. Current was recorded before and after a 3-min exposure to $30 \mu M$ PPHT. At all voltages tested, PPHT reduced $I_K$ by approximately proportional amounts, suggesting that sigma receptor ligands do not produce their inhibitory effect on $K^+$ channels by shifting the voltage dependence of activation. Current was converted to conductance using the relation, $G_K = I_K / (V - e_K)$, where $G_K$ is $K^+$ conductance and $e_K$ is the Nernst potential for $K^+$ computed for the bathing and patch pipette solution compositions. The plots of $G_K$ vs. pulse potential are shown in Fig. 5 along with best fitting Boltzmann functions (see “Experimental Procedures”). Under control conditions, the conductance-voltage plot was characterized by a steepness factor ($k$) of $10 \pm 1$ mV and a voltage midpoint ($V_{1/2}$) of $-6 \pm 1$ mV. After exposure to PPHT, neither the steepness factor ($6 \pm 4$ mV) nor the voltage midpoint ($-9 \pm 4$ mV) had been altered ($n = 3$). The modulation of $I_K$ in neurohypophysial nerve terminals by sigma receptor ligands exhibits a similar voltage independence (5, 20).

DMS-114 cell $I_K$ exhibits a weak voltage-dependent inactivation, as indicated by the decay of whole-cell current (Fig. 1), and it was already noted above that the time constants for activation and inactivation remained the same during challenges with sigma receptor ligand. We also examined inactivation of $I_K$ by varying the voltage at which cells were held prior to depolarizing test pulses (to $10$ mV). PPHT produced no shifts in the voltage dependence of inactivation (data not shown). These results indicate that sigma receptor-mediated modulation of $I_K$ is not the result of shifts in voltage dependence.

Extensive literature on receptor-mediated modulation of ion

![Fig. 4. Concentration dependence of photolabeling inhibition and $I_K$ inhibition. An experimental protocol similar to that of Fig. 3 was used to examine the concentration dependence of an SKF10047 block of [$^{125}$I]IAC photolabeling (top). $I_K$ was also measured in the presence of various SKF10047 concentrations ([SKF]/μM), using the pulse protocol from Fig. 1. Left axis, the concentration dependence of current reduction, plotted as normalized $I_K$ averaged over three experiments (closed squares); right axis, the concentration dependence of photolabeling inhibition (closed circles). For both effects, the best fitting curves are shown as dotted lines (see text for parameter values).](image)

![Fig. 5. Potassium conductance versus voltage. Cells were held at $-80$ mV and interrupted at 5-s intervals with 500-ms pulses to each of the voltages shown. Current was then converted to conductance (see text) and plotted as a function of voltage (open circles). After a 3-min exposure to $30 \mu M$ PPHT (closed circles), the cells were again subjected to a repeat series of voltage pulses. Each data point represents the mean current from three experiments.](image)
Sigma Receptor-mediated Potassium Channel Modulation

DISCUSSION

These studies demonstrate that sigma receptor activation reduces voltage-dependent $I_{K}$ by binding to a protein with a molecular mass of 26 kDa. Four chemically distinct sigma receptor ligands (SKF10047, DTG, PPH, and IAC) inhibited $I_{K}$ in a reversible, concentration-dependent fashion. IAC also photolabeled a protein with a molecular mass of 26 kDa, and this photolabeling was blocked by concentrations of SKF10047 similar to those that inhibited $I_{K}$ in patch clamp recordings. These studies provide the first link of a protein with sigma ligand binding activity to $K^{+}$ channel modulation. Thus, despite the presence of a putative endoplasmic reticulum sequence and the absence of putative N-glycosylation sites (7–10), the physiological function of the sigma receptor indicates a location in or near the plasma membrane.

The modulation of voltage-dependent $K^{+}$ channels by membrane-bound receptors has been extensively studied in many systems. In general, transduction of these responses requires the activation of a G-protein (11, 24, 25). Our observation that the inhibition of $I_{K}$ is mediated by a receptor with an apparent molecular mass of only 26 kDa is difficult to reconcile with the hypothesis that G-proteins are involved in the transduction of this response. Many G-protein-coupled receptors are similar in size to rhodopsin, with molecular weights of roughly 40–50 kDa (plus carbohydrate) (29). Although G-protein-coupled receptors with higher molecular weights are quite common (30), homologous receptors with molecular masses as low as 26 kDa have yet to be identified. Furthermore, the seven transmembrane segments found in all known G-protein-coupled receptors are inconsistent with the single membrane-spanning segment implied by the hydrophathy plots constructed from the deduced sigma receptor amino acid sequence (7–10).

Efforts to establish a role for G-proteins in sigma receptor function have met with mixed results. Cholera toxin reduced responses to sigma receptor ligands in one study (4) but not in another (12). In melanotrophs, the guanine nucleotide analogue GDPβS has been shown to prevent the inhibition of $I_{K}$ by DTG (4). These results may reflect the existence of another molecular species of sigma receptor that is a member of the G-protein-coupled receptor family. In the present study, cells perfused internally with GDPβS exhibited the same response to SKF10047 as control cells perfused internally with GTP, and GDPβS is known to be a very potent inhibitor of G-protein-dependent processes (11, 24, 25, 27, 29). This result therefore indicates that the recently cloned 26-kDa receptor modulates $K^{+}$ channels without coupling to G-proteins. In this regard it is relevant that the concentration dependences of both ligand binding and $I_{K}$ inhibition by SKF10047 were similar (Fig. 4). In contrast to G-protein-coupled receptors, where spare receptors and other variations in the efficiency of coupling can cause large discrepancies between the apparent $K_{D}$ and the physiological $EC_{50}$ (29), the modulation of $K^{+}$ channel function by sigma receptor ligands appears to be tightly coupled to receptor binding.

If G-proteins do not mediate the inhibition of $I_{K}$ by sigma receptor activation, then these results may indicate that sigma receptor-mediated signal transduction depends on other molecular factors. An interesting possibility is that sigma receptors alter $K^{+}$ channel activity through a direct protein-protein interaction with the channel, analogous to the actions of auxiliary $\beta$ subunits (31) and MinK proteins (32, 33), both of which can modulate the function of voltage-gated $K^{+}$ channels without forming channels themselves. Another possibility is that sigma receptors interact with a protein kinase (by a G-protein-independent mechanism) and that this enzyme modifies channel function. These mechanisms have not been demonstrated

\[ \text{Current (\% Control)} \]

\[ \text{[SKF10047] (\mu M)} \]

\[ \text{GTP} \quad \text{GDPβS} \]

\[ \text{FIG. 6. G-protein independence of sigma receptor-mediated changes in } I_{K} \text{. } I_{K} \text{ was monitored before and after superfusion with various concentrations of SKF10047, according to the same protocol employed in Fig. 1. Control experiments were conducted with pipette solution containing 100 μM GTP (open bars). A parallel set of experiments was then conducted with GTP replaced by 100 μM GDPβS in the patch pipette solution (closed bars). At all concentrations studied, the effects of SKF10047 were identical between GTP and GDPβS. Data represent mean ± S.E. for 3–6 terminals.} \]

channels has shown that in most instances such responses are mediated by G-proteins (11, 24, 25). However, the deduced amino acid sequence of sigma receptors does not fit with a G-protein-coupled receptor motif (7–10). There have been reports that the binding activity of sigma receptors can be altered by GTP and guanosine nucleotides and that sigma receptor-mediated responses are attenuated by cholera toxin and GDPβS (1, 4), but another study showed that cholera toxin had no effect (12). Both the size (1) and deduced amino acid sequence (7–10) of sigma receptors are difficult to reconcile with that of known G-protein-coupled receptors. We therefore tested the role of G-proteins by adding GDPβS (100 μM) to the patch pipette filling solution and allowing it to diffuse into the cell interior during whole-cell recordings.

Base-line currents were first collected for at least 3 min after break in, allowing ample time for a molecule of this size to diffuse the cell interior (26). G-protein-mediated responses in neurons have been shown to be attenuated 75% by this mode of GDPβS addition 2 min after break in (27). We found that responses to SKF10047 were not reduced by GDPβS. Both 10 and 100 μM SKF10047 showed equal efficacy for the inhibition of $I_{K}$ regardless of whether cells were perfused with 100 μM GDPβS or 100 μM GTP (Fig. 6). It therefore appears that sigma receptor-mediated modulation of $I_{K}$ can occur independently of G-protein activation. Similar results have been obtained with sigma receptor-mediated modulation of $I_{K}$ in rat neurohypophysial nerve terminals. In these experiments GDPβS also failed to block the modulation of $I_{K}$ by SKF10047. The entry of the guanine nucleotide GTPγS into neurohypophysial terminals was verified by monitoring current through Ca$^{2+}$-activated $K^{+}$ channels. GTPγS triggers the G-protein-mediated dephosphorylation of this channel in the neurohypophysis (28), and 50 μM GTPγS added to the patch pipette filling solution reduced current to $79 \pm 3\%$ of the original level at break in, with a half-time of $29 \pm 4\ s (n = 11)$.

\[ \text{P. J. Lupardus, R. A. Wilke, Y. Chen, R. E. Ruoho, and M. B. Jackson, submitted for publication.} \]
previously in ligand-induced ion channel modulation, indicating that sigma receptors inhibit $I_K$ by a new transduction process.

It is noteworthy that DMS-114 cells are tumor cells derived from a neuroendocrine progenitor (34). These cells retain many properties of an excitatory cell line, including the expression of voltage-dependent ion channels (35, 36). The coupling between sigma receptors and $I_K$ described here in DMS-114 cells is very similar to that found in neuroendocrine neurohypophysial nerve terminals (5). Furthermore, DMS-114 cells exhibit ectopic release of the neurohypophysial hormone vasopressin (17, 18). The coexpression of these distinctive properties in both DMS-114 cells and the neurohypophysis may reflect a link in the mechanisms of regulation of different cellular functions that is operating both in this tumor cell line and in the native hypothalamic neurohypophysial system. In vivo, tumor-related secretion of ectopic neurohypophysial hormones is responsible for inducing derangements of fluid and sodium homeostasis in as many as 30% of patients with primary small cell lung carcinoma (37). The finding that sigma receptors are functionally linked to membrane excitability in such cells indicates that this protein may represent a useful therapeutic target for the curtailment of ectopic hormone secretion.

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