Specific Enhancement of SK Channel Activity Selectively Potentiates the Afterhyperpolarizing Current $I_{AHP}$ and Modulates the Firing Properties of Hippocampal Pyramidal Neurons

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SK channels are Ca2+-activated K+ channels that underlie after hyperpolarizing (AHP) currents and contribute to the shaping of the firing patterns and regulation of Ca2+ influx in a variety of neurons. The elucidation of SK channel function has recently benefited from the discovery of SK channel enhancers, the prototype of which is 1-EBIO. 1-EBIO exerts profound effects on neuronal excitability but displays a low potency and limited selectivity. This study reports the effects of DCEBIO, an intermediate conductance Ca2+-activated K+ channel modulator, and the effects of the recently identified potent SK channel enhancer NS309 on recombinant SK2 channels, neuronal apamin-sensitive AHP currents, and the excitability of CA1 neurons. NS309 and DCEBIO increased the amplitude and duration of the apamin-sensitive afterhyperpolarizing current without affecting the slow afterhyperpolarizing current in contrast to 1-EBIO. The potentiation by DCEBIO and NS309 was reversed by SK channel blockers. In current clamp experiments, NS309 enhanced the medium afterhyperpolarization (but not the slow afterhyperpolarization sAHP) and profoundly affected excitability by facilitating spike frequency adaptation in a frequency-independent manner. The potent and specific effect of NS309 on the excitability of CA1 pyramidal neurons makes this compound an ideal tool to assess the role of SK channels as possible targets for the treatment of disorders linked to neuronal hyperexcitability.

In hippocampal pyramidal neurons voltage-independent, Ca2+-activated K+ channels are responsible for the generation of two distinct afterhyperpolarizing currents, $I_{AHP}$3 and $sI_{AHP}$ (1–4). $I_{AHP}$ is characterized by a time constant of decay of ~100 ms and by its sensitivity to the bee venom toxin, apamin, and to the scorpion toxins, scyllatoxin and tapamin (5–7). $sI_{AHP}$ is characterized by a slower time course (in the range of seconds), by its lack of sensitivity to apamin or any other classical K+ channel blocker, and by its modulation by several neurotransmitters (1–3, 8). Based on their kinetic and pharmacological features and on the results obtained from genetically manipulated mice, SK channels mediate $I_{AHP}$ whereas the molecular correlate for $sI_{AHP}$ is still unknown (2–4, 9, 10).

In addition to the use of selective blockers, an important contribution to the elucidation of the physiological role of SK and IK channels has arisen from the use of a small organic compound that enhances channel activity, the benzimidazolone 1-EBIO (11–15). 1-EBIO enhances the activity of SK channels in the presence of the physiological activator, intracellular Ca2+, by increasing the apparent sensitivity of SK channels to Ca2+ (14). As a consequence, 1-EBIO increases the amplitude of SK-mediated AHP currents and their duration in a variety of neurons, leading to profound changes in neuronal activity and firing patterns (14, 16–18). Although 1-EBIO has been a useful tool to elucidate the function of SK channels in their native context, it has some important limitations. First, it affects not only the SK channels but also the as yet unidentified Ca2+-dependent K+ channels underlying $sI_{AHP}$ (14). Additionally, prolonged applications of 1-EBIO have been shown to lead to a decrease in Ca2+ currents in hippocampal neurons (14). Finally, and most importantly, 1-EBIO displays a relatively low potency (EC50 on SK channels ~700 μM) (14). These limitations of 1-EBIO have prompted the development of novel, more potent SK channel enhancers. DCEBIO, a dichlorinated analogue of 1-EBIO, has been reported to enhance the activity of intermediate conductance Ca2+-activated K+ channels (IK channels) (19). Moreover, recently 6,7-dichloro-1H-indole-2,3-dione-3-oxime (NS309) (20) has been described as a more potent enhancer of the activity of recombinant SK and IK channels.

In the present study, we provide the first characterization of the effect of DCEBIO on recombinant SK channels and a quantification of the potency differences between DCEBIO, NS309, and 1-EBIO on recombinant SK2 channels, the predominant SK channel subtype in hippocampus. We have furthermore investigated the actions of DCEBIO and NS309 on the native SK channels mediating $I_{AHP}$, on the distinct Ca2+-activated K+ current $sI_{AHP}$, and on the firing behavior of CA1 pyramidal neurons in hippocampal slices.

MATERIALS AND METHODS

Electrophysiology on Recombinant hSK2 and Kv7.2/7.3 Channels—HEK293 cells stably expressing human SK2 (20) or co-expressing Kv7.2 and Kv7.3 channel subunits (21) were plated on coverslips 12–24 h prior...
to the experiments. For each experiment, a coverslip was placed in a 15-μl perfusion chamber (flow rate ~1 ml/min). All experiments were performed at room temperature (20–22 °C) using borosilicate pipettes (resistance 2–3 mehmohms) controlled by a micromanipulator (PatchMan, Eppendorf, Germany). The extracellular solution contained (in mM): 140 KCl, 2 CaCl₂, 1 MgCl₂, 10 HEPES, adjusted to pH 7.4 with KOH. The osmolarity of the extracellular solution was 285 mosM. For inside-out experiments on the hSK2 currents, three bath (intracellular) solutions were used and denoted as 0.01, 0.2, and 10 to indicate their concentration. The solutions containing 0.01 and 0.2 mmol/L DCEBIO and NS309, SK Channel Enhancers More Potent than 1-EBIO—DCEBIO and NS309 have been shown to modulate recombinant IK and SK channels, respectively, at lower concentrations than required for 1-EBIO. To compare their relative potency on recombinant SK channels, we have first characterized the effect of DCEBIO on SK2 channels, in view of the essential role played by the SK2 (KCa2.2) subunit for the control of the SK2 channel activity by increasing the apparent sensitivity to Ca²⁺, and concentration-response experiments were therefore performed in the inside-out configuration, which allows full control of the free [Ca²⁺]. Activation curves yielded an EC₅₀ of 0.42 μM for Ca²⁺ (n = 8) (data not shown) with maximal activation obtained at 10 μM free Ca²⁺. The concentration-response curves for the three enhancers were performed at 200 nm free Ca²⁺, which activated 5% (0.048 ± 0.009) of the maximal SK current. Fig. 1A shows the control currents (Ctrl) as well as the currents obtained in the presence of the enhancers upon application of voltage ramps from −80 to +80 mV. For all compounds, potentiation was concentration-dependent but not voltage-dependent. The traces shown in Fig. 1A were obtained from experiments similar to the one shown in Fig. 1B, where the current at −75 mV is depicted as a function of time. The inside-out patch was exposed first to 0.01 μM Ca²⁺ to define the background current level and subsequently to 10 μM Ca²⁺ to define the maximal resistance and of the amplitude and duration of the Ca²⁺ action current, well within the limits needed to maintain a stable amplitude of IₐHIP and IₘHIP under control conditions. Data are reported without corrections for liquid junction potentials.

**Data Acquisition and Analysis**—Data were acquired using a patch clamp EPC9 amplifier (HEKA, Lambrecht, Germany) filtered at 0.25–1 kHz, sampled at 1–4 kHz, and stored on a Macintosh G4 or Power PC. Analysis was made using the Pulse and Pulsefit (HEKA, Lambrecht, Germany), Igor Pro (Wave Metrics), SigmaPlot (SPSS, Inc.), InStat (Graphpad), and Excel (Microsoft) software. Values are presented as mean ± S.E. For statistical analysis, the Student’s t test was used, and differences were considered statistically significant when p < 0.05. Concentration-response relationships were fitted to the Hill equation I/Iₘₐₓ = [E]ⁿ/[([E]ₙ⁺ + [EC₅₀]ⁿ⁺)] to obtain EC₅₀ values and Hill-coefficients (n). [E] is the concentration of the enhancer.

**Pharmacology on Cells and Brain Slices**—Drugs were applied in the bath solution. NS309, DCEBIO, 1-EBIO, and retigabine were dissolved in Me₂SO as 500–1000-fold concentrated stock solutions and stored at −20 °C, diluted prior to use, and bath-applied in the perfusing artificial cerebrospinal fluid. All controls were performed in Me₂SO at the same final concentration as during NS309, DCEBIO, or 1-EBIO application (0.005–0.3%). NS309 was synthesized at NeuroSearch A/S; DCEBIO was from Tocris (Bristol, UK) or synthesized at NeuroSearch A/S; 1-EBIO was from Sigma-Aldrich; retigabine (N-(2-amino-4-(4-fluorobenzylamino)-phenyl) carbamic acid ethyl ester) was synthesized at NeuroSearch; tetraethylammonium, potassium gluconate, Na₂ATP, Na₂GTP, 8CPT-cAMP, and dimethyl sulfoxide (Me₂SO) were obtained from Sigma; tetrodotoxin was from Alomone Laboratories (Jerusalem, Israel); noradrenaline and d-tubocurarine were from RBI (Natick, New Jersey); apamin was from Latoxan (Rosans, France); all other salts and chemicals were obtained from Merck or Sigma.

**RESULTS**

DCEBIO and NS309, SK Channel Enhancers More Potent than 1-EBIO—DCEBIO and NS309 have been shown to modulate recombinant IK and SK channels, respectively, at lower concentrations than required for 1-EBIO. To compare their relative potency on recombinant SK channels, we have first characterized the effect of DCEBIO on SK2 channels, in view of the essential role played by the SK2 (KCa2.2) subunits in mediating IₐHIP in hippocampal pyramidal neurons (5, 7, 9). We then compared the effect of DCEBIO to that of NS309 and 1-EBIO on SK2 channels. The three enhancers augment SK channel activity by increasing the apparent sensitivity to Ca²⁺, and concentration-response experiments were therefore performed in the inside-out configuration, which allows full control of the free [Ca²⁺]. Activation curves yielded an EC₅₀ of 0.42 μM for Ca²⁺ (n = 8) (data not shown) with maximal activation obtained at 10 μM free Ca²⁺. The concentration-response curves for the three enhancers were performed at 200 nm free Ca²⁺, which activated 5% (0.048 ± 0.009) of the maximal SK current. Fig. 1A shows the control currents (Ctrl) as well as the currents obtained in the presence of the enhancers upon application of voltage ramps from −80 to +80 mV. For all compounds, potentiation was concentration-dependent but not voltage-dependent. The traces shown in Fig. 1A were obtained from experiments similar to the one shown in Fig. 1B, where the current at −75 mV is depicted as a function of time. The inside-out patch was exposed first to 0.01 μM Ca²⁺ to define the background current level and subsequently to 10 μM Ca²⁺ to define the maximal
current, which was used to normalize the currents. The concentration-response for DCEBIO was then determined at the subthreshold Ca\(^{2+}\) concentration of 200 nM, with control of maximal and background currents at the end of the experiment (Fig. 1B). The currents measured at the steady-state level of activation were plotted as a function of the DCEBIO concentration as illustrated in Fig. 1C, together with the values obtained for 1-EBIO and NS309 in similar experiments. Fig. 1C underscores the higher potency on SK2-mediated currents of DCEBIO (EC\(_{50}\) = 27 μM; n = 1.4) and even more remarkably of NS309 (EC\(_{50}\) = 0.62 μM; n = 1.4) when compared with 1-EBIO (EC\(_{50}\) = 453...
319 ± 42%. sIAHP co-exists with I_{AHP} in CA1 pyramidal neurons but is mediated by channels clearly distinct from the SK channels underlying I_{AHP} and of as yet unknown molecular identity (2–5,9). Neither the sIAHP amplitude (118 ± 10%) nor its time constant of decay (112 ± 5%) were significantly increased by DCEBIO (Fig. 2). The remarkable potentiation of I_{AHP} led to an increase of the charge transfer, estimated as the integral of the two AHP currents (I_{AHP} + sIAHP), by 154 ± 16% (n = 7) (Fig. 2C). The specificity of the DCEBIO effect on neuronal SK channels was confirmed by the full block of the enhanced I_{AHP} upon application of the SK channel blocker d-tubocurarine (curare, 100 μM) (Fig. 2A). sIAHP was instead identified by its suppression by noradrenaline (1 μM) (Fig. 2A), known to inhibit this current by activating the cAMP/protein kinase A pathway in hippocampal neurons (23, 29). These results demonstrate that DCEBIO is a more potent enhancer of both recombinant and neuronal SK currents when compared with 1-EBIO.

The rest of our study focuses on NS309, which displays an enhanced potency on recombinant SK2 channels compared with both 1-EBIO and DCEBIO (Fig. 1C). A crucial question is how effective and selective this compound is on SK channels in their native, neuronal environment. When tested on I_{AHP} and sIAHP in CA1 pyramidal neurons in hippocampal slices, 10 μM NS309 induced a marked increase of I_{AHP} amplitude with respect to the control currents recorded prior to application of the compound (Fig. 3A). I_{AHP} was measured in isolation, upon inhibition of sIAHP by the cAMP analogue 8-CPT-cAMP (50 μM). The relative increase in amplitude of the apamin-sensitive I_{AHP} was 182 ± 22% (n = 8) (Fig. 3, A and C). NS309 had an even more prominent effect on the time constant of deactivation of I_{AHP}, which was slowed by ~6-fold, changing from 119 ± 19 ms to 654 ± 77 ms after NS309 application (n = 8). As a consequence, the charge transfer of I_{AHP} measured as the integral of the current was increased by almost 10-fold (949 ± 165%; n = 8) (Fig. 3, A–C). By comparison, the application of the same concentration of DCEBIO (10 μM) resulted in an increase of the I_{AHP} amplitude by 158 ± 20% and in an increase of its decay time constant by 149 ± 20% (n = 4) (data not shown). NS309 was applied for several minutes to yield a maximal and stable potentiation of I_{AHP}, as illustrated by the time course of action of this drug (Fig. 3B). The augmentation of I_{AHP} by NS309 was only scarcely reversible, even after prolonged wash out periods (data not shown). The application of NS309 (10 μM) did not affect the input resistance of the neurons (n = 18).

At a lower concentration (1 μM), NS309 had qualitatively similar but slightly less pronounced effects on both amplitude and charge transfer of I_{AHP}. Thus, I_{AHP} amplitude was increased to 139 ± 10% (n = 3) (Fig. 3, D and F). Similar to what was previously observed at higher concentrations, 1 μM NS309 slowed the deactivation of I_{AHP} by ~4-fold, increasing its time constant of decay (τ) from 86 ± 9 ms to 361 ± 39 ms (n = 3). As a consequence, the I_{AHP} charge transfer increased by ~5-fold (500 ± 18%; n = 3) (Fig. 3, D–F). Also at 1 μM, the action of NS309 developed slowly (Fig. 3E). The effect of NS309 can be entirely ascribed to an enhancement in the activity of the SK channels underlying I_{AHP} as the NS309-enhanced current was fully blocked by the SK channel blockers d-tubocurarine (d-TC, curare; 200 μM; n = 8) (Fig. 3, A and B) and apamin (25 nM; n = 3) (Fig. 3, D and E).

Next, to investigate the effects of NS309 (10 μM) on the apamin-sensitive AHP current sIAHP, we applied the compound together with 25 nM apamin to block I_{AHP}. NS309 affected neither the amplitude (96 ± 13%; p = 0.63; n = 3) (Fig. 4) nor the charge transfer (108 ± 15%; p = 0.67; n = 3) (Fig. 4, A and C) of the sIAHP once it had reached steady state. Furthermore, after NS309 application, sIAHP was fully inhibited by 2.5 μM noradrenaline (Fig. 4, A and B). This result underscores the selective nature of NS309 as an SK channel enhancer in contrast to
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1-EBIO, which increased both $I_{\text{AHP}}$ and $sI_{\text{AHP}}$ in CA1 pyramidal neurons (14). Thus, we can conclude that NS309 is a selective enhancer of the SK channels mediating $I_{\text{AHP}}$ in hippocampal neurons and more potent than both 1-EBIO and DCEBIO.

**NS309 Alters the Firing Pattern of Hippocampal Pyramidal Neurons**

The selective potentiation of IAHP, without any effect on sIAHP, produced by NS309 prompted us to use this compound to test the specific impact of SK channel enhancement on the firing properties of hippocampal neurons. This was investigated in current clamp recordings performed in the absence and presence of NS309 ($10 \mu M$/$9262$M). Under control conditions, depolarizing current pulses elicited trains of action potentials characterized by early and late spike frequency adaptation (Fig. 5A, left panel). Application of NS309 was followed by d-tubocurarine ($d$-TC; $200 \mu M$) in B and apamin ($25 \mu M$) in E, which completely blocked the enhanced $I_{\text{AHP}}$. In B, points were omitted after $I_{\text{AHP}}$ reached steady-state level to allow for testing of the access resistance. The diagrams in B and E are from the same representative cells shown in A and D, C and F, bar diagrams summarizing the effects of $10 \mu M$ NS309 (C) and $1 \mu M$ NS309 (F) on the $I_{\text{AHP}}$ peak amplitude and charge transfer in eight (C) and three cells (F).

**Does the Potentiation of $I_{\text{AHP}}$ by NS309 Depend on Frequency?**

The slow time course of action of NS309, with time constants in the range of 6–8 min to reach the maximal effect on IAHP, raised the question as to whether the effect of this compound on IAHP is frequency-dependent. To test this hypothesis, we designed three experimental paradigms. In the first paradigm, short current injections (5 ms long) of sufficient intensity to elicit action potentials were applied in trains of eight at a frequency of 10 Hz (Fig. 6A) ($n = 5$) or 33 Hz (Fig. 6B) ($n = 5$) in current clamp experiments. This stimulation pattern mimics the physiological input from bursting CA3 neurons to CA1 neurons (30, 31). Upon appli...
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FIGURE 4. NS309 does not affect the apamin-insensitive sAHP in CA1 pyramidal neurons. A, sAHP, measured in the presence of apamin (25 nM; left panel) to block IAHP, is not affected by the application of 10 μM NS309. 2.5 μM noradrenaline completely inhibited sAHP. The right panel shows a superposition of sAHP traces before (black) and after (gray) the application of 10 μM NS309, emphasizing the lack of effect of this compound on the peak amplitude and time course of sAHP. B, lack of effect of NS309 (10 μM) on the peak amplitude (amp) of sAHP, plotted against time. NS309 was applied for 18 min after the sAHP amplitude had stabilized and produced no effect on the sAHP amplitude. The subsequent application of noradrenaline (NA; 2.5 μM) induced a complete suppression of sAHP. The time course is from the same representative cell shown in A. C, bar diagram showing that 10 μM NS309 had no effect on the sAHP peak amplitude and charge transfer.

FIGURE 5. NS309 reduces the firing frequency of CA1 pyramidal neurons. A, trains of action potentials elicited by 800 ms current injections (260 μA) from the resting membrane potential (-57 mV; no steady current injected) before and after the application of 10 μM NS309. In this representative cell, the number of action potentials elicited by the same current injection was decreased in the presence of NS309, and the late phase of spike frequency adaptation was enhanced. These effects were reversed upon application of apamin (25 nM; right panel), which produced an additional small increase in the number and initial frequency of the action potentials in the train, as previously reported (5). NS309 did not affect the resting membrane potential or the input resistance of the cell. Similar results were obtained in three cells. B, afterhyperpolarizations (medium AHP, mAHP; slow AHP, sAHP) following a burst of action potentials triggered by a 200 ms current injection (500 pA) before and after the application of 10 μM NS309. NS309 enhanced the mAHP (superimposed traces in right panel) without affecting the sAHP. The effect of NS309 on mAHP was fully counteracted upon application of apamin (25 nM), in agreement with the results obtained on the underlying currents (IaHP and sAHP) in voltage clamp recordings. Action potentials were truncated for better resolution of the afterhyperpolarizations following the bursts. Similar results were obtained in three cells.

The results obtained in this study show that, in accordance with their effects on recombinant channels, DCEBIO and even more prominently NS309 are potent and specific enhancers of the activity of neuronal SK channels. In particular, the SK-mediated IaHP was increased in amplitude and even more remarkably in duration by the application of concentrations as low as 1 μM NS309 (Fig. 3). NS309 did not influence the currents mediated by recombinant BK channels Kv7.4 (KCNQ4) (20) or Kv7.2/7.3 channels (this study) expressed in HEK293 cells. In particular, the lack of effect of NS309 on Kv7.2/7.3 channels supports the hypothesis that the enhancement of the mAHP observed in this study (Fig. 5B) is not because of an increase of IaHP, a voltage-dependent current contributing to the generation of the mAHP (26), but solely of IaHP. The selectivity of the effects of DCEBIO and NS309 on neuronal SK channels is further supported by the full suppression of the enhanced IaHP and mAHP by the SK channel blockers apamin and d-tubocurarine (Figs. 2, 3, 5, and 6). These results are in good agreement with the reported enhancement of recombinant SK channels by NS309, whereby the enhanced currents were completely blocked by apamin in a heterolo-
FIGURE 6. The action of NS309 is not frequency-dependent in CA1 neurons. A and B, action potentials elicited by trains of 5-ms-long current injections delivered at 10 (A) and 33 (B) Hz before and after the application of 10 μM NS309. NS309 increases the afterhyperpolarization following the single action potential and the trains. The enhancement of the AHPs following single action potentials and the trains is better illustrated in the right panels, showing the two last action potentials in the trains (truncated) and the AHPs on an expanded voltage and time scale. The traces in gray are before and those in black after NS309 application. The relative increase of AHP was not significantly different at 10 and 33 Hz, as shown in the inset in B (left panel), summarizing the results obtained in five (10 Hz) and three cells (33 Hz). C and F, IAHP traces obtained in response to 100-ms-long depolarizing pulses to +10 mV (holding potential, −50 mV) delivered every 30 s (C; 0.033 Hz) or 6 s (F; 0.167 Hz). Three superimposed traces are shown, illustrating the change in amplitude and time course of IAHP before (black, Control) and after the application of 10 μM NS309 (dark gray, NS309). The third set of traces (light gray) shows that, at both frequencies, the enhanced IAHP was fully blocked by d-tubocurarine (C; d-TC, 200 μM) or by apamin (F; 50 nM). D and G, time course of action of NS309 (10 μM) on the IAHP charge transfer estimated from the area under each current trace (IAHP) elicited every 30 s in D and every 6 s in G. The application of NS309 was followed by d-tubocurarine (d-TC; 200 μM) in D and by apamin (Apa; 50 nM) in G, which completely blocked the enhanced IAHP. The plots are from the same representative cells shown in C (plot D) and in F (plot G). E and H, bar diagrams summarizing the effects of 10 μM NS309 on the IAHP peak amplitude (E) and charge transfer (H) in eight and five cells stimulated at 0.033 and 0.167 Hz, respectively. No significant differences were observed in the potentiation of IAHP at these two frequencies. I, the time course of the potentiating action of 10 μM NS309 (see, for example, plots D and G) on the IAHP peak amplitude (left bars) and charge transfer (right bars) were fitted with exponential functions, and the corresponding time constants were obtained for seven cells stimulated at 0.033 Hz and four cells at 0.167 Hz (same protocol as in C and F). The time required by NS309 to have a full effect on IAHP was not significantly different at the two stimulation frequencies. J, the time course of the potentiating action of 10 μM NS309 on the IAHP peak amplitude (left bars) and charge transfer (right bars) were fitted with exponential functions, and the corresponding time constants were obtained. IAHP was elicited using a sequence of eight short (5 ms) pulses to +10 mV (holding potential = −50 mV) delivered in four cells at 10 Hz and five cells at 33 Hz. The time required by NS309 to have a full effect on IAHP was not significantly different at the two stimulation frequencies.
gous expression system (20). Additionally, we have not observed any effect of DCEBIO and NS309 on the amplitude or time course of sIAHP in CA1 neurons (Figs. 2 and 4). These results further strengthen the notion that sIAHP is mediated by a conductance clearly distinct from the SK channels, which are unlikely to contribute to its generation, as supported by differences in their kinetics, pharmacology, distribution, and by recent data obtained from genetically modified animals missing specific SK channel subunits (1–4, 9, 10). NS309 has been reported to block recombinant hERG channels with a \( K_p \) value of 1.3 \( \mu \)M (20), and hERG channels have been suggested to play a role in spike frequency adaptation in dorsal root ganglion-neuroblastoma hybrid cells (25). However, the blocking of hERG channels by NS309 would produce an effect opposite of what we observed on the firing pattern of CA1 neurons. Additionally, the changes in the firing pattern observed after the application of NS309 were fully reversed by the specific SK channel blocker apamin (Fig. 5), making it unlikely for conductances other than SK to contribute to the observed NS309 effects. Finally, even at high concentrations (10 \( \mu \)M), NS309 did not change the membrane resistance of the CA1 neurons (n = 18) (data not shown).

When compared with the first SK channel enhancer tested on neurons, 1-EBIO (14), DCEBIO and NS309 were more potent, as expected from the 17-fold difference in the potency of 1-EBIO and DCEBIO, and the 731-fold difference of 1-EBIO and NS309 on recombinant SK2 channels reported in this study. Additionally, both DCEBIO and NS309 were more selective, as they enhanced I\(_{\text{AHP}}\) without affecting sIAHP, whereas 1-EBIO produced a small but significant enhancement also of sIAHP (14), making it unlikely for conductances other than SK to contribute to the observed NS309 effects. Finally, even at high concentrations (10 \( \mu \)M), NS309 did not change the membrane resistance of the CA1 neurons (n = 18) (data not shown).

The time course of action of NS309 on 1-EBIO (14), DCEBIO and NS309 were tested at the same concentration (100 \( \mu \)M), NS309 did not change the membrane resistance of the CA1 neurons compared with 1-EBIO. However, when 1-EBIO and DCEBIO were tested at the same concentration (100 \( \mu \)M) on CA1 pyramidal neurons compared with 1-EBIO. Nonetheless, we cannot exclude that, at concentrations higher than those tested in this study, DCEBIO and NS309 could affect also sIAHP. Finally, we have observed a difference between the actions of NS309 and of 1-EBIO and DCEBIO on I\(_{\text{AHP}}\), in that NS309 has a more pronounced effect on the time course rather than the peak amplitude of I\(_{\text{AHP}}\).}

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7 G. Rogge and P. Pedarzani, unpublished observation.