K⁺ Currents Generated by NMDA Receptor Activation in Rat Hippocampal Pyramidal Neurons

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Shah, Mala M. and Dennis G. Haylett. K⁺ currents generated by NMDA receptor activation in rat hippocampal pyramidal neurons. *J Neurophysiol* 87: 2983–2989, 2002; 10.1152/jn.00802.2001. Long lasting outward currents mediated by Ca²⁺-activated K⁺ channels can be induced by Ca²⁺ influx through N-methyl-D-aspartate (NMDA)-receptor channels in voltage-clamped hippocampal pyramidal neurons. Using specific inhibitors, we have attempted to identify the channels that underlie these outward currents. At a holding potential of −50 mV, applications of 1 mM NMDA to the soma of cultured hippocampal pyramidal neurons induced the expected inward currents. In 44% of cells tested, these were followed by outward currents (average amplitude 60 ± 7 pA) that peaked 2.5 s after the initiation of the inward NMDA currents and decayed with a time constant of 1.4 s. In 43% of those cells exhibiting an outward current, SK channel inhibitors, UCL 1848 (100 nM) and apamin (100 nM) abolished the outward current. In the remainder of the cells, the outward currents were either insensitive or only partly inhibited (44 ± 4%) by 100 nM UCL 1848. In these cells, the outward currents were reduced by the slow afterhyperpolarization (sAHP) inhibitors, muscarine (3 μM; 43 ± 9%), UCL 1880 (3 μM; 34 ± 10%), and UCL 2027 (3 μM; 57 ± 6%). Neither the BK channel inhibitor, charybdotoxin (100 nM), nor the Na⁺/K⁺ ATPase inhibitor, ouabain (100 μM), reduced these outward currents. Irrespective of the pharmacology, the time course of the outward current did not differ. Interestingly, no correlation was observed between the presence of a slow apamin-insensitive afterhyperpolarization and an outward current insensitive to SK channel blockers following NMDA-receptor activation. It is concluded that an NMDA-mediated rise in [Ca²⁺], can result in the activation of apamin-sensitive SK channels and of the channels that underlie the sAHP. The activation of these channels may, however, depend on their location relative to NMDA receptors as well as on the spatial Ca²⁺ buffering within individual neurons.

**INTRODUCTION**

An afterhyperpolarization comprising fast (fAHP), medium (mAHP), and slow (sAHP) components follows a train of action potentials in hippocampal pyramidal neurons (Sah 1996; Storm 1990). The fAHP lasts for <20 ms and is due to the opening of large conductance Ca²⁺-activated K⁺ channels (BK channels) (Sah 1996; Storm 1987). The mAHP activates within 50 ms and can last ≤1 s. A variety of K⁺ channels, including apamin-sensitive, small-conductance Ca²⁺-activated K⁺ channels (SK channels), may contribute to the mAHP (Shah and Haylett 2000b; Stocker et al. 1999; Storm 1989). The sAHP peaks approximately 500 ms after a train of action potentials and can last several seconds (Sah 1996; Storm 1990). A Ca²⁺-dependent K⁺ conductance, which is insensitive to apamin, tetaethylyammonium (TEA), and 4-aminopyridine but inhibited by neurotransmitters such as acetylcholine and noradrenaline, underlies the sAHP (Sah 1996). Recently, two simple analogs of clortimazole, UCL 1880 and UCL 2027, have been shown to have some selectivity as blockers of the sAHP current (sIAHP) in hippocampal pyramidal neurons (Shah et al. 2001). However, it is unclear which K⁺ channel(s) underlie the sAHP (Castle 1999).

Afterhyperpolarizations can be generated by activation of voltage-gated Ca²⁺ channels (either by triggering action potentials or by a depolarizing step). Alternatively, Ca²⁺ entry through N-methyl-D-aspartate (NMDA) receptor channels should allow their activation. Indeed, in both hippocampal pyramidal and cortical neurons, synaptically released glutamate has been shown to induce a hyperpolarization resembling the sAHP in its kinetics (Nicoll and Alger 1981; Yu et al. 1999). Furthermore, in the absence of Mg²⁺, glutamatergic excitatory postsynaptic potentials (EPSPs) are broadened in the presence of isoprenaline (Lancaster et al. 2001). Since isoprenaline inhibits the sAHP, this provides some evidence that the K⁺ channels underlying the sIAHP may be activated by Ca²⁺ influx during the EPSP. Additionally, in hippocampal pyramidal neurons (Zorumski et al. 1989) and cortical pyramidal neurons (Mistry et al. 1990; Yu et al. 1999), NMDA applications, under voltage-clamp conditions, result in a very slow outward current following the inward current. In hippocampal pyramidal neurons, the outward current is reported to be insensitive to apamin but can be partially inhibited by 10 mM TEA and 500 μM d-tubocurarine (Zorumski et al. 1989). It is known that activation of NMDA receptors results in Ca²⁺ influx throughout the cell (Segal and Manor 1992) and it is therefore surprising that the apamin-sensitive SK channels are not reported to be activated. In lamprey spinal cord neurons, apamin-sensitive SK channels have been suggested to be activated by Ca²⁺ entry via NMDA receptor channels (Grillner et al. 2001; Hill et al. 1989). It has also been recently reported that Ca²⁺ entry exclusively through NMDA receptors can activate BK channels in olfactory bulb granule cells (Isaacson and Murphy 2001). Hence, it is possible that a variety of Ca²⁺-activated K⁺ channels can contribute to the outward

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current reported to be generated via NMDA receptor activation in hippocampal neurons.

The present study aims to further characterize the outward current generated via activation of NMDA receptors in cultured hippocampal neurons using the novel selective sAHP blockers, UCL 1880 and UCL 2027. In particular, we wished to see whether its properties match those of the sAHP. Since apamin-sensitive SK channels and BK channels are present in hippocampal neurons and contribute, respectively, to the mAHP and fAHP that follow action potentials, it was of additional interest to determine the extent of their activation by Ca\(^2+\) influx through NMDA receptors. Some of this work has previously been presented in abstract form (Shah and Haylett 2000c).

**METHODS**

Hippocampal neurons (from CA1 and CA3 regions) were isolated from 4-day-old Sprague-Dawley rats and maintained in culture using neurobasal medium supplemented with 0.25 mM l-glutamine, 2% B27 serum free supplement, and 0.02 mg ml\(^{-1}\) gentamicin (Shah and Haylett 2000b). Electrophysiological recordings were obtained from cells in culture for 8–15 days. Pyramidal cells were identified by their morphology.

For electrical recordings, cells were superfused with a bathing solution of the following composition (in mM): 130 NaCl, 3 KCl, 2.5 CaCl\(_2\), 5 HEPES free acid, 10 glucose, 10 glycine, 26 NaHCO\(_3\), and 1 \(\mu\)M tetrodotoxin (TTX) and 5 \(\mu\)M 6,7-dinitroquinoxaline-2,3-dione (DNQX), pH maintained at 7.2 by continuously gassing with 95% O\(_2\)–5% CO\(_2\). In some experiments, the superfusion solution contained (DNQX), pH maintained at 7.2 by continuously gassing with 95% O\(_2\)–5% CO\(_2\). In some experiments, the superfusion solution contained 0.43 mM Ca\(^2+\) and 0.5 mM EGTA to yield a free Ca\(^2+\) concentration of 0.92 \(\mu\)M, calculated using the program REACT (G. L. Smith, Dept. of Physiology, University of Glasgow, UK). Perforated patch recordings were obtained from a holding potential of 7.2 mV, using the discontinuous voltage-clamp mode (sampling rate 3–5 kHz) of an Axoclamp 2A amplifier (Axon Instruments). NMDA was applied every 30 s for 300 ms using a puffer pipette (tip diameter 1–2 \(\mu\)m; applied pressure, 5 psi) with the pipette positioned <5 \(\mu\)m from the cell soma. AHP currents were activated when required by applying a 60 mV depolarizing step from a holding potential of −50 mV every 10 s. Drugs were applied by switching to a superfusion fluid containing the drug. The inlet tube was positioned such that the flow was directed onto the patched cell. All signals were filtered using the Axoclamp 2A low-pass filter at 3 kHz and were recorded on a chart recorder and an oscilloscope. Signals were also digitized at 48 kHz (VR-10 digital data recorder; Instutech Corp.) and recorded continuously on a video recorder. Digitized signals were also acquired on a computer using pClamp6 software (Axon Instruments).

**Data analysis**

Data were analyzed using pClamp6. Only cells that demonstrated a stable baseline current and a reproducible outward current in response to NMDA applications for 5 min prior to drug applications were used for analytical purposes (data not shown). The inhibitory action of drugs was calculated as the percentage change in the control net outward current at the time of the outward current peak. [In some cases, the current in the presence of the blocker at this time was inward because of a persistent NMDA current (see Fig. 1D). Since the change in current caused by a drug might then exceed the peak outward current, an apparent inhibition in excess of 100% could be recorded.] Values were expressed as the mean ± SE and significance levels were determined by appropriate \(t\)-tests. The traces shown in the figures were subjected to smoothing using adjacent averaging (20 points) in Microcal Origin 6.0.

The time course of the outward current was estimated by fitting the outward current alone to the following empirical exponential equation

\[
y = y_0 + A_1 e^{-\tau_1 t} + A_2 e^{-\tau_2 t}
\]

where \(\tau_1\) and \(\tau_2\) are the respective growth and decay time constants of the outward current, \(t\) represents the time from the development of the net outward current, and \(y_0\) is the holding current at a potential of −50 mV. \(A_1\) and \(A_2\) are respective amplitudes of the growth and decay phases.

Precise measurement of the time course and amplitude of the outward current was difficult as the outward current often began to decline before the end of the 300 ms application of 100 \(\mu\)M N-methyl-D-aspartate (NMDA; holding potential, \(V_h = −50\) mV). B: response to 10 s application of 100 \(\mu\)M NMDA; \(V_h = −20\) mV (notice the much greater holding current needed). C: current response of hippocampal pyramidal cell to 300 ms application of 100 \(\mu\)M N-Methyl-D-aspartate (NMDA; holding potential, \(V_h = −50\) mV). C: effect of low (0.92 \(\mu\)M) Ca\(^2+\), (C) and 1 mM Ba\(^2+\) (D) on currents produced by 1 mM NMDA; \(V_h = −50\) mV. Note that in the presence of 1 mM Ba\(^2+\), the outward holding current was reduced and to aid comparison the baseline currents have been superimposed. Accordingly, the zero current level is not indicated. E: effects of 7-chlorokynurenic acid (7-CK) on the currents produced by NMDA. Control and recovery traces were recorded in the presence of 10 \(\mu\)M glycine, whereas 7-CK was applied in a glycine-free solution. The inset shows the effect of 7-CK on the outward current on an expanded current scale. The traces have been superimposed in the inset.
during the inward NMDA response and influenced its decline (see, for example, Fig. 1D). To discern the characteristics of the current inhibited by the application of drugs, the current in the presence of a drug was subtracted from that in its absence. This difference current was also fitted with the above equation.

Materials

All drugs were obtained from Sigma except for neurobasal medium, B27 serum-free supplement, L-glutamine, gentamicin, and FCS, which were obtained from Gibco. KMeSO₄ was purchased from Pfaltz and Bauer (UK). UCL 1880 and UCL 2027 were synthesized by M. Javedzadeh-Tabatabaie and Dr. Z. Miscony under the supervision of Prof. C. R. Gannellin (Dept. of Chemistry, University College London; Shah et al. 2001).

RESULTS

Characterization of the NMDA-induced outward current

Application of 100 μM NMDA, at a holding potential of −50 mV, produced inward currents of 0.55 ± 0.17 nA (n = 9), which developed rapidly and decayed quickly after the NMDA pulse (Fig. 1A). However, with this concentration of NMDA, no outward current followed the inward current. Changing the holding potential to −20 mV and extending the pressure application to 10 s (n = 4) produced an inward current that rapidly declined to a steady lower level (Fig. 1B) but still with no clear evidence of a subsequent outward current. In three of these cells a depolarizing step from −50 to +10 mV was also applied and in each case both a mI_AHP and a sI_AHP were produced. This suggested Ca²⁺-activated K⁺ channels were present in these cells and that given a sufficient rise in [Ca²⁺], an outward K⁺ current might have been expected.

Increasing the NMDA concentration to 1 mM on average increased the inward current to 0.65 ± 0.05 nA (n = 135), but the difference was not significant (P > 0.05). The rate of decline of the NMDA inward current varied between cells, with some cells showing a very fast rate (Fig. 1C), whereas in others the decline was slower or biphasic (data not shown). In 60/135 cells an outward current followed the inward current produced by 1 mM NMDA (e.g., Fig. 1C). The outward current activated by NMDA increased with successive applications (at 30-s intervals). It peaked 2.5 ± 0.2 s (n = 60) after the initiation of the inward NMDA current and decayed slowly with a time constant of 1.4 ± 0.2 s (n = 60). The amplitude of the outward current varied substantially and was on average 60.0 ± 6.5 pA (n = 60). The amplitude of the inward current induced by NMDA receptor activation in cells that failed to develop an outward current (0.60 ± 0.05 nA, n = 75) was not significantly different to that in cells which did develop an outward current (0.69 ± 0.08 nA; n = 60; P > 0.05).

A concentration of 1 mM NMDA might be expected to have effects on other glutamate receptors. However, application of 1 mM NMDA in a glycine-free solution containing the glycine site antagonist 7-chlorokynurenic acid (7-CK, 10 μM), which will prevent activation of NMDA receptors but leave α-amino-3 hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), kainate, and metabotropic receptors functional (Moroni et al. 1992), failed to produce either inward or outward currents (inhibition of control currents were 98.6 ± 1.4 and 106.2 ± 16.7%, respectively, n = 5; Fig. 1E). The effect was reversible within 2 min. This shows that the inward current was produced by activation of NMDA receptors and that the outward current was a consequence of the activation.

The outward current was abolished by lowering the bath Ca²⁺ concentration to 0.92 μM (n = 5; Fig. 1C) or by exposure to 1 mM Ba²⁺ (n = 6; Fig. 1D). The inward current amplitude was little affected by applications of the low Ca²⁺ and 1 mM Ba²⁺ solutions (% inhibition = 2.4 ± 14.0, n = 5; and −2.5 ± 9.3%, n = 6, respectively). These findings confirm earlier reports of a Ca²⁺-dependent K⁺ conductance activated by Ca²⁺ influx via NMDA receptors (Mistry et al. 1990; Yu et al. 1999; Zorumski et al. 1989).

At a holding potential of −50 mV, a standing outward current is also present. This current was significantly reduced in the presence of 1 mM Ba²⁺ (% inhibition = 113 ± 28%, P < 0.01; n = 3), suggesting that a K⁺ conductance(s) underlies it.

Pharmacology of the NMDA-induced outward current

UCL 1848, a potent and rapidly reversible blocker of apamin-sensitive SK channels (Benton et al. 1999; Chen et al. 2000; Shah and Haylett 2000a), was used to examine the role of these particular channels in the NMDA-induced outward current. UCL 1848, at a supramaximal concentration of 100 nM, abolished the outward current in 16/37 (43%) cells tested. Because of the overlapping time courses of the inward and outward currents, the decay of the net inward current became slower in the presence of UCL 1848. Consequently, the procedure used to quantify the inhibition (as explained in methods) gave values in excess of 100% (% inhibition of current = 177 ± 37%, n = 16; Fig. 2A). The effects of UCL 1848 occurred within 2 min of bath application and were reversible within 5 min of washout (Fig. 2C). Not surprisingly, in these cells apamin (100 nM) also completely inhibited the outward current produced (% inhibition of peak current = 132 ± 16%, n = 6; Fig. 2B). The effect of apamin did not, however, reverse within 15 min of washout. In these cells, application of 3 μM muscarine and 3 μM UCL 1880, inhibitors of the sAHP, had little effect on the outward current generated by NMDA [% inhibition = 9.9 ± 8.9% (n = 3) and −9.5 ± 20.3% (n = 5), respectively]. In all cells tested, both UCL 1848 and apamin had negligible effects on the inward current amplitude (−0.19 ± 3.8 and 4.3 ± 6.3% inhibition, respectively; for example, see Fig. 2C).

In 9/37 (24%) cells on which 100 nM UCL 1848 was tested, the outward current was only partially inhibited, by 44.3 ± 4.4% (n = 9). In the remaining 12/37 cells, UCL 1848 had very little effect on the outward current (% inhibition = −13.5 ± 4.4%, n = 12). In those cells in which the outward current was either partially or wholly insensitive to UCL 1848, 3 μM muscarine, 3 μM UCL 1880, and 3 μM UCL 2027 partially reduced the outward current by 42.6 ± 8.9% (n = 6; Fig. 3A), 33.5 ± 9.7% (n = 4; Fig. 3B), and 57.0 ± 5.8% (n = 5; Fig. 3C), respectively. Muscarine, UCL 1880, and UCL 2027 had only small effects on the peak inward current due to NMDA receptor activation [% inhibition = −14.2 ± 11.2% (n = 9, P > 0.05), 18.7 ± 5.5% (n = 9; P > 0.05), and 3.2 ± 6.8% (n = 5; P > 0.05), respectively].

As muscarine, UCL 1880, and UCL 2027 only partially inhibited the UCL 1848-insensitive outward current, it is possible that a third type of Ca²⁺-activated K⁺ conductance
contributes to the generation of the current. BK channels are present in these neurons (see INTRODUCTION) and might conceivably be activated by Ca\(^{2+}\)/H11001 influx through the NMDA channels.

Their possible involvement in the generation of the outward current was tested using charybdo toxin, a potent blocker of BK channels (McManus 1991). Application of 100 nM charybdotoxin to UCL 1848-insensitive cells had little effect on the inward current due to NMDA receptor activation (% inhibition = 9.8 ± 5.2%, n = 5, P = 0.1) and the subsequent outward current (% inhibition = 11.2 ± 4.8%, n = 5, P = 0.1, Fig. 3D).

In dopaminergic neurons (Johnson et al. 1992; Mercuri et al. 1996), an outward current that is attributed to the activity of the Na\(^+/K\(^+/\)-ATPase, and which can be reduced by ouabain, is generated following NMDA receptor activation. To test the possible contribution of such a current in hippocampal pyramidal neurons, a maximal concentration of ouabain (100 \(\mu\)M) (Munakata et al. 1998) was applied to cells that exhibited a UCL 1848-insensitive current. It was found that this concentration of ouabain, rather than reducing, on average increased the outward current generated by NMDA receptor activation by 67.6 ± 26.1% (n = 5, Fig. 3E), although the difference was not significant (P > 0.05). The inward NMDA current was little affected by ouabain (% inhibition = 3.9 ± 5.3%, n = 5).

Since it is possible that the activation of different channels might result in currents with different time courses, the currents sensitive to SK and sAHP inhibitors were determined by subtracting the blocked currents from the controls. To further aid the analysis, it was assumed that apamin and UCL 1848 inhibit only SK channels and that muscarine, UCL 1880, and UCL 2027 inhibit only the current responsible for the sAHP, so that results can be combined. Blocker-sensitive outward currents obtained in this way are shown in Fig. 4. In each case, the outward difference currents peaked significantly earlier (in the presence of SK channel inhibitors, 1.1 ± 0.2 s, n = 16; P < 0.05; in the presence of sAHP inhibitors, 1.4 ± 0.2 s, n = 14; P < 0.05) than the absolute peak (2.5 ± 0.2 s, n = 30; Fig. 4) of the outward current generated by 1 mM NMDA. The decay time constants of the subtracted UCL 1848- and apamin-sensitive currents (1.3 ± 0.1 s, n = 16, Fig. 4A) and the muscarine-, UCL 1880-, and UCL 2027-sensitive currents

![Figure 2](http://jn.physiology.org/)

**FIG. 2.** Effects of 100 nM UCL 1848 (A, applied for 1 min) and 100 nM apamin (B, applied for 2 min) on the outward current produced by 1 mM NMDA. The location of the traces shown in (A) are indicated in (C). The traces have been superimposed. C: continuous recording of NMDA responses before, during, and after washout of UCL 1848. (Note the apparent reduction in the outward holding current by UCL 1848 is probably artifactual since it was not observed in other cells. The same applies to the apparent fluctuation of the inward currents.) V_H = −50 mV. Traces in (A) and (B) are superimposed and maximum recoveries within 15 min of washout are shown. The calibration bars shown in (A) also apply to (B).

![Figure 3](http://jn.physiology.org/)

**FIG. 3.** Effects of (A) muscarine, (B) UCL 1880, (C) UCL 2027, and (D) charybdo toxin on outward currents which were not inhibited by 100 nM UCL 1848. The data are from different cells. The traces in each panel have been superimposed. E: effects of a 3-min application of 100 \(\mu\)M ouabain on the currents generated by NMDA. Each trace is the average of 3 consecutive recordings. The traces have been superimposed. V_H = −50 mV.
induced outward current as well as both the mI AHP and the nM UCL 1848. Only 3/11 cells displayed a sIAHP and a current exhibited an outward current that was totally inhibited by 100 nM UCL 1848-insensitive outward current component to NMDA also pressure-applied 1848-insensitive outward current when 1 mM NMDA is applied. The outward current was abolished by 1 mM Ba2+, in keeping with the activation of a K+ current. It should be noted that although Ba2+ permeates NMDA receptor channels and can reduce inward currents (although this is not apparent in Fig. 1), it is unlikely that Ba2+ would have significantly affected the elevation of [Ca2+]. (Segal and Manor 1992). Lowering the extracellular [Ca2+] to just under 1 μM also abolished the outward current, suggesting that the generation of the outward current was dependent on Ca2+ influx. [It is important also to consider the possibility that somatic [Ca2+]i could be raised by Ca2+ influx through Ca2+ channels activated by NMDA-induced depolarization of poorly clamped distal dendrites. This seems unlikely since 1) for slow responses the voltage clamp is likely to extend well into the dendrites (Spruston et al. 1993); 2) the small tip diameter of the puffer pipette allowed some localization of the NMDA application to the soma. Thus fast inward currents, in excess of 0.5 nA, were only obtained with the pipette positioned close to the soma, suggesting that the NMDA concentration declined quite steeply away from the point of application; 3) in the presence of TTX, Ca2+ spikes in the dendrites are unlikely to propagate to the soma (Schwindt and Crill 1997); and 4) there was no evidence of spike activity on the current or voltage records.]

A number of Ca2+-activated K+ channels could potentially underlie the outward currents induced by NMDA receptor activation. In 43% of the pyramidal cells exhibiting an outward current, both UCL 1848 and apamin abolished the outward current, implicating apamin-sensitive SK channels. However, in the remainder of the cells tested, the outward current was either only partially sensitive or completely insensitive to UCL 1848. In these cells, the outward current was partly inhibited by the sI AHP inhibitors, muscarine, UCL 1880, and UCL 2027, suggesting that a current with the properties of the sI AHP can also result from NMDA receptor activation. It should be noted that although in some cases the outward current was totally insensitive to inhibition by UCL 1848, it was only partially reduced by the sI AHP inhibitors, raising the possibility that unidentified Ca2+-activated K+ channels may also contribute to the generation of the outward current in these cells. Contrary

**DISCUSSION**

As expected, NMDA application induced a substantial inward current. The current normally peaked within 300 ms, in keeping with the close apposition of the puffer pipette to the cell. An outward current followed 1 mM NMDA, but not 100 µM NMDA inward currents in approximately 44% of the cells. This is in agreement with previous studies (Zorumski et al. 1989) and is consistent with the report that Ca2+ entry is maximal when 300 µM NMDA is applied (Reichling and MacDermott 1993).

The outward current evoked by 1 mM NMDA was entirely due to the activation of NMDA receptors since it was abolished (along with the inward current) by the use of 7-CK in conjunction with a glycine-free solution to prevent NMDA receptor activation (Fig. 1). This, in particular, rules out an important role for metabotropic glutamate receptors which would not be blocked by 7-CK (Moroni et al. 1992) [(AMPA) and kainate receptors were blocked throughout with DNQX.] The outward current was abolished by 1 mM Ba2+, in keeping with the activation of a K+ current. The traces have been superimposed. $V_{\text{mem}} = -50$ mV. Calibration bars apply to both (A) and (B).

(1.4 ± 0.4 s, n = 9, Fig. 4B) were not significantly different from those of the controls (1.1 ± 0.1 s, n = 16; Fig. 4A).

**Correlation between the presence of a sI AHP and a UCL 1848-insensitive outward current when 1 mM NMDA is pressure-applied**

We also examined whether cells that displayed a UCL 1848-insensitive outward current component to NMDA also exhibited a sI AHP and vice versa. The sI AHP was evoked using a 170-ms depolarizing step from −50 to +10 mV. An NMDA-induced outward current as well as both the mI AHP and the sI AHP were seen in 11/26 cells tested. Of these cells, 8/11 exhibited an outward current that was totally inhibited by 100 nM UCL 1848. Only 3/11 cells displayed a sI AHP and a current that was partially or completely insensitive to UCL 1848. A sI AHP but no outward current could be recorded in 13/26 cells. In the remainder of the cells (2/26), an NMDA-induced outward current insensitive to UCL 1848 was generated by NMDA receptor activation but no sI AHP could be detected.

![Graph](image-url)
to the recent evidence that BK channels in olfactory bulb granule cells are tightly coupled to NMDA receptors (Isaacsnon and Murphy 2001), our results suggest that BK channels are not involved in the outward current resulting from NMDA receptor activation in these hippocampal pyramidal neurons (Fig. 3D). A role for IKCa channels is also ruled out by the lack of effect of charybdotoxin, which is a potent blocker of these channels also (McManus 1991). The possibility that charybdotoxin-insensitive BK channels (see, for example, Behrens et al. 2000 and Meera et al. 2000) may contribute to the outward current remains to be explored. Similarly, although it has been demonstrated that the Na+/K+ ATPase inhibitor, ouabain, can inhibit an NMDA-induced outward current in mid-brain dopaminergic neurons (Johnson et al. 1992; Mercuri et al. 1996), ouabain had no significant effect on the outward current in these hippocampal neurons. Thus of the known Ca2+-activated K+ channels, only SK channels and sAHP channels contribute to the generation of the outward current following NMDA applications. Whether SK channels or sAHP channels are activated is presumably dependent on their localization relative to the NMDA receptors and on the degree of spatial buffering of Ca2+ within any particular neuron.

A surprising finding was that the outward currents sensitive either to SK channel or to sAHP blockers had very similar time courses. When AHPs in rat hippocampal pyramidal cells are evoked using a train of action potentials or a depolarizing step, the apamin-sensitive AHP peaks usually within 50 ms and lasts for a period of <1 s, whereas the apamin-insensitive, muscarine- and UCL 1880-sensitive sAHP has a slow rising phase and lasts for several seconds (Shah and Haylett 2000b; Stocker et al. 1999). In contrast, all NMDA-induced outward currents, irrespective of the underlying channels, displayed a slow rising phase and lasted for several seconds. Cloned SK channels (Xia et al. 1998) as well as native hippocampal SK channels (Hirschberg et al. 1999; Selyanko et al. 1998) respond rapidly to Ca2+. The slow kinetics of the outward current, therefore, could reflect the time course of the Ca2+ transient due to NMDA receptor activation, although Ca2+ imaging studies would be needed to confirm this. In this context, it should also be noted that the time course of the apamin-sensitive mIAHP corresponds to the time course of the Ca2+ transients that follow action potential generation (Sah and Clements 1999). Ca2+ entry via NMDA receptors can also induce Ca2+ release from ryanodine-sensitive stores (for example, see Emptage et al. 1999) and this could conceivably delay the peak of the Ca2+ transient and contribute to the slow time course of the outward current.

A sIAHP can be activated in only approximately 60% of these cultured cells (see Shah and Haylett 2000b). It was, therefore, of interest to investigate whether a UCL 1848-insensitive outward current in response to NMDA was restricted to this subset of cells. No such correlation was, however, discovered. Application of NMDA to cells that had both a sIAHP and a mAHP usually produced an outward current that was totally sensitive to UCL 1848. The presence of the sIAHP and a UCL 1848-insensitive NMDA activated outward current could only be demonstrated in 3/26 cells. Also, in half of the cells, although the sIAHP could be detected, NMDA-receptor activation did not generate an outward current. These results suggest that there is compartmentalization of Ca2+ in hippocampal pyramidal neurons and that the channels underlying the sIAHP are more likely to be activated by Ca2+ entry through voltage-gated Ca2+ channels than through NMDA receptor channels. By extension, these findings also suggest that Ca2+ entry via voltage-gated Ca2+ channels did not significantly contribute to the generation of the outward currents following NMDA applications.

In conclusion, Ca2+ influx through NMDA receptors can certainly activate SK channels and most probably also the sAHP channels. The resulting hyperpolarization will enhance the normal voltage-dependent block by Mg2+-activated K+ channels and hence affect temporal and spatial integration of synaptic activity. Thus the activation of Ca2+-activated K+ channels by an NMDA receptor-mediated rise in intracellular Ca2+ is likely to contribute to the physiological mechanisms controlling cell excitability.

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