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Noradrenergic Modulation of the Hyperpolarization-Activated Cation Current (I_h) in Dopamine Neurons of the Ventral Tegmental Area

Francisco Arencibia-Albite¹, Carlos Paladini², John T. Williams³, and Carlos A. Jiménez-Rivera⁴

¹Department of Physiology, Universidad Central del Caribe, Bayamón, P.R.

²Department of Biology, University of Texas, San Antonio

³Vollum Institute, Portland, Oregon

⁴Department of Physiology, University of Puerto Rico, Medical Sciences Campus, San Juan, P.R.

Abstract

Alterations in the state of excitability of midbrain dopamine (DA) neurons from the ventral tegmental area (VTA) may underlay changes in the synaptic plasticity of the mesocorticolimbic system. Here, we investigated norepinephrine's (NE) regulation of VTA DA cell excitability by modulation of the hyperpolarization-activated cation current, I_h in whole cell recordings. Current clamp recordings show that NE (40 μ M) hyperpolarizes spontaneously firing VTA DA cells (11.23 ± 4 mV; $n = 8$). In voltage clamp, NE (40 μ M) induces an outward current (100 ± 24 pA; $n = 8$) at -60 mV that reverses at about the Nernst potential for potassium (-106 mV). In addition, NE (40 μ M) increases the membrane conductance ($179 \pm 42\%$; $n = 10$) and reduces I_h amplitude ($68 \pm 3\%$ of control at -120 mV; $n = 10$). The noradrenergic α -1 antagonist prazosin (40 μ M; $n = 5$) or the α -2 antagonist yohimbine (40 μ M; $n = 5$) did not block NE effects. All NE-evoked events were blocked by the D_2 antagonists sulpiride (1 μ M) and eticlopride (100 nM) and no significant reduction of I_h took place in the presence of the potassium channel blocker BaCl₂ (300 μ M). Therefore, it is concluded that NE inhibition of I_h was due to an increase in membrane conductance by a non-specific activation of D_2 receptors that induce an outward potassium current and not a result of a second messenger system acting on h-channels. The results also suggest that I_h channels are mainly located at dendrites of VTA DA cells and thus their inhibition may facilitate the transition from single spike firing to burst firing and vice versa.

Keywords

VTA; I_h ; Norepinephrine; Dopamine receptors; Adrenergic receptors

Author for correspondence: Carlos A. Jiménez-Rivera, Ph.D., Department of Physiology, P.O.Box 365067, University of Puerto Rico, Medical Sciences Campus, San Juan, Puerto Rico, 00936.

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Introduction

The ventral tegmental area (VTA) is the source of dopaminergic projections that innervate structures in the ventral forebrain and cortical areas, a system collectively known as the mesocorticolimbic (Dahlstrom and Fuxe, 1964; Ungerstedt, 1971). The pattern of activity of VTA dopamine cells ranges from single action potential pacemaker-like firing to multiple action potential burst firing (Cooper, 2002). Bursting activity causes a greater increase in extracellular dopamine (DA) concentration per action potential than does the low frequency pacemaker-like firing (Kitai et al., 1999; Overton and Clark, 1997; Grace and Bunney, 1984). Therefore, transitions between these two states of excitability may provide a physiological mechanism by which DA neurons modulate the mesocorticolimbic system.

The state of excitability of midbrain DA cells depends on the modulation of the conductances that generate and affect the different firing modes of these neurons (Kitai et al., 1999; Overton and Clark, 1997; Grace and Bunney, 1984). Among these conductances is the hyperpolarization-activated cation current, I_h (Cathala and Paupardin-Tritsch, 1999; Jiang et al., 1993; Mercuri et al., 1995; Watts et al., 1995). The I_h is a depolarizing current that affects cell excitability in three interrelated ways: it helps to determine the resting potential and firing frequency; reduces the amplitude and duration of hyperpolarizing inputs; and generates pacemaker activity when coupled to other voltage-dependent conductances (e.g., I_T , a voltage dependent calcium current) (Luthi and McCormick, 1998; Pape, 1996). In the lateral geniculate nucleus of the thalamus (McCormick and Pape, 1990), in primary sensory neurons of the dorsal root ganglia (Yagi and Sumino, 1998), and in neurons of the medial nucleus of the trapezoid body (Banks et al., 1993), norepinephrine (NE) affects the state of excitability by modulation of the voltage sensitivity of I_h . The possibility of noradrenergic modulation of I_h in VTA DA cells has not been studied. Here we explore the effects of NE in I_h voltage and time dependence in this nucleus.

Methods

Preparation of horizontal midbrain slices

Male Sprague-Dawley rats (15–20 days postnatal) were anesthetized with a chloral hydrate intraperitoneal injection (15mg/Kg) and then decapitated. The brain was removed from the skull in less than 1.5 minutes and put into ice-cold (2°C – 4°C) dissection solution of composition (mM): NaCl 126; KCl 2.5; NaH₂PO₄ 1.2; MgCl₂ 7.0; CaCl₂ 0.5; Glucose 25; NaHCO₃ 25; saturated with 95% O₂ and 5% CO₂. A brain section containing the ventral and rostral half of the brainstem was obtained as follows. Two coronal cuts were performed to remove the cerebellum and the rostral half of the cerebral hemispheres. The remaining piece was placed with the caudal end up and a transverse cut was made through the cerebral aqueduct. The portion containing the ventral half of the cerebral aqueduct was positioned with the ventral surface up and two parasagittal cuts were made to remove the temporal lobes. The resulting block was glued with cyanoacrylate (Krazy Glue™) to the stage of a Leica VT 1000S vibratome (Nussloch, Germany) immersed in ice-cold dissection solution and cut into horizontal slices (thickness: 220 μm) starting from the ventral surface. To reduce shear forces on the tissue, a block of agar was glued to the stage and placed against the caudal end of the tissue (opposite to the vibratome blade). Slices were cut and discarded until the floor of the interpeduncular fossa was reached. The next three slices were placed in an incubation chamber containing oxygenated artificial cerebrospinal fluid (ACSF) (mM: NaCl 126; KCl 2.5; NaH₂PO₄ 1.2; MgCl₂ 1.0; CaCl₂ 2.0; Glucose 25; NaHCO₃ 25; saturated with 95% O₂ and 5% CO₂) at 35° C. Slices remained in the chamber between 30 to 60 minutes before recording. One half of the slice was placed in the recording chamber (500 °L) and superfused with ACSF (35°C) at a rate of 2 mL/min. Slices not used for recording were kept in the incubation chamber until the end of the experiments.

Whole cell recordings

The recording site in the brain slice was visually identified with an Olympus BX51WI microscope equipped with infrared light and Nomarski optics. The VTA was identified as the region lateral to the fasciculus retroflexus and medial to the medial terminal nucleus of the accessory optic tract (Paxinos and Watson 1986; Johnson and North, 1992). The substantia nigra compacta (SNc), identified as the regions rostral and caudal to the medial terminal nucleus of the accessory optic tract, was avoided. Whole cell recordings were made using borosilicate glass pipettes pulled in two stages by a vertical pipette puller (PP-830; Narishige, Japan). When the recording pipette was filled with the internal solution ((mM) K-methyl sulfate 115; KCl 20; MgCl₂ 1; HEPES 10; EGTA 0.1; ATP 2; GTP 0.3; creatine phosphate 10), the tip of the pipette had a resistance of 2–3 MΩ. The recording pipette was advanced towards the brain slice and a seal was obtained by applying a pulse of suction. After gigaseal formation between the pipette and the cell membrane the holding potential was set to –60 mV and the fast capacitance was compensated. In order to obtain the whole cell configuration the membrane was ruptured by further suction. Once in whole cell configuration the slow capacitance was corrected and the access resistance compensated. Recordings with high access resistance values (10–20 MΩ) were rejected if the access resistance changed by more than 15%.

Whole cell recordings were done using the Axopatch 1-D amplifier (Axon Instruments, CA, USA). Membrane currents were filtered at 10 kHz and digitized at 5 kHz (Digidata 1322A; Axon Instruments, CA, USA). The digitized current was displayed and analyzed on a MacIntosh G4 computer using Axograph 4.6 software (Axon Instruments, CA, USA).

Data Analysis

I_h was evoked by family of 6 hyperpolarizing voltage steps (step amplitude: –10 mV; step duration: 1 s) from the holding potential of –60 mV to –120 mV. The steps were applied in 7 s intervals. I_h was calculated as the difference between the membrane current at the end of the voltage step (I_{ss}) and the instantaneous current (I_{inst}) measured after the settling of the capacitive transient. To determine I_h voltage dependence, activation curves were constructed from I_h tail currents, which were calculated by subtracting the pre-step holding current from the peak of the tail current. The activation curves were fitted by the Boltzmann function to estimate the potential of half-activation ($V_{1/2}$) and the slope factor (k):

$$\frac{I_{tail}}{I_{tailmax}} = \frac{1}{1 + \exp\left(\frac{V - V_{1/2}}{k}\right)}$$

I_h time dependence was studied by fitting current traces (from –90 to –120 mV) with an exponential function of the form:

$$I_h(t) = I_{h,SS} \left[1 - \exp\left(-\frac{t}{\tau_h}\right) \right]$$

where τ_h is the activation time constant that gives the time (ms) required to reach 63% of $I_{h,SS}$, which is the steady state I_h amplitude for the fitted current trace. Membrane conductance was estimated by the slope obtained from linear fits to the plot of I_{ins} vs. command voltage. Averaged data are expressed as means \pm S.E.M. Statistical significance of data from different drug conditions was assessed with 1-way or 2-way ANOVA as appropriate.

Compounds

Drugs were applied (2 min.) to the slice by superfusion. Complete exchange of the bath solution occurred in less than 1 min. All drugs and salts were acquired from Sigma (St. Louis, Missouri).

Results

The data presented here were based on *in vitro* whole cell recordings from 78 putative VTA dopaminergic neurons. Recorded cells were located lateral to the fasciculus retroflexus and medial to the medial terminal nucleus of the accessory optic tract (Paxinos and Watson 1986; Johnson and North, 1992). They were considered putative VTA dopamine cells if they exhibited a pronounced I_h (i.e., the smallest I_h amplitude was 500 pA at -120 mV) and a slow spontaneous activity (Mercuri et al., 1994; Paladini and Williams, 2004; Paladini et al., 2004).

Margolis et al. (2006) clearly shows that if a VTA cell is dopaminergic then it has I_h , although the converse is not necessarily true since according to some researchers approximately 1/3 of VTA cells that have I_h are not DA cells. Therefore, immunohistochemistry is the only reliable method for identifying I_h containing cells as dopaminergic (Margolis et al. 2006). Nevertheless, our electrophysiological criteria for identification VTA DA cell is satisfactory by current standards (Paladini and Williams, 2004; Paladini et al., 2004; Neuhoff et al., 2002). Indeed, convincing evidence had been presented about the cellular physiology of DA cells base only on this criteria (Fiorillo and Williams, 1998; Fiorillo and Williams, 2000; Paladini et al., 2001; Morikawa et al., 2003; Paladini and Williams, 2004; Paladini et al., 2004).

Noradrenergic effects on VTA dopamine cells

Norepinephrine (NE; 40 μ M) application evokes an outward current (100 ± 24 pA; $n = 8$) at -60 mV ($n = 11$; Fig. 1B). The activation of this current was associated with an increase in membrane conductance ($179 \pm 42\%$; $n = 10$, data not shown) and membrane hyperpolarization (11.23 ± 4 mV; $n = 8$; Fig. 1A and 1C). The NE-evoked current reversed at -96 ± 8 mV ($n = 6$). This value was close to the reversal potential of K^+ (-106 mV) as predicted by the Nernst equation (Fig. 1D). These effects were blocked by 300 μ M of external barium (data not shown). The NE effects on membrane conductance and membrane potential were not seen in the presence of the D_2 antagonist eticlopride (100 nM) or sulpiride (1 μ M), ($n = 19$; data not shown). These results are in agreement with those of Grenhoff et al. (1995) who have shown that NE induced hyperpolarization in VTA DA cells was due to activation of D_2 receptors.

NE inhibits I_h

The effects of NE on I_h were studied with whole cell voltage clamp recordings in a total of 35 putative VTA DA cells.

Concomitant to the NE evoked increase in potassium conductance there was a decrease in I_h amplitude ($n = 10$; Fig. 2A and 2C). This inhibition was apparent from the reduction in the difference between I_{SS} and I_{ins} , which resulted mainly from an increase in I_{ins} (Fig. 2A and 2B). The increased I_{ins} was seen at all potentials where I_h inhibition was present and the time course of I_h inhibition was parallel to the time course of the increase in I_{ins} (Fig. 2B). Therefore, the time course of I_h inhibition is parallel to the time course of the increase in potassium conductance (Fig. 1 and Fig. 2B).

To permit the comparison of the responses of the recorded neurons, I_h was expressed as a percentage of the maximal I_h during the control condition and plotted against the command potentials. As seen in Figure 2C, the percentage of I_h inhibition increases with the amplitude of the hyperpolarizing voltage step and reaches a maximal value of 32% at -120 mV.

I_h inhibition is not mediated by alpha adrenergic receptors

The amplitude of I_h can be reduced by activation of protein kinase C (PKC) (Zhaoping et al, 2003; Cathala and Paupardin-Tritsch, 1997) or by a decrease in cytoplasmic levels of cAMP

(Ulens and Siegelbaum, 2003). The adrenergic metabotropic receptors alpha-1 and alpha-2 are known to exert these actions. For example, all subtypes of alpha-1 receptors are known to activate PKC (Zhong and Minneman, 1999), whereas alpha-2 receptors tend to decrease intracellular levels of cAMP (Raymond et al, 1994). In order to determine if the NE-evoked I_h inhibition was caused by the activation of one of these receptors, the following experiments were performed. First, I_h was recorded in the presence of NE plus the alpha-1 antagonist prazosin ($n = 5$). Under these conditions I_h inhibition was significant and not different from the inhibition evoked by NE alone (Fig. 3C, 3E and 3G). Second, I_h was recorded in the presence of NE plus the alpha-2 antagonist yohimbine ($n = 5$). Nevertheless in the presence of yohimbine, I_h inhibition was similar to the inhibition evoked by NE alone (Fig. 3D, 3F and 3G). Therefore, the inhibition of I_h does not appear to result from the activation of these adrenergic receptors.

The non-specific activation of D_2 receptors by NE inhibits I_h

The effects of NE on membrane potential and holding current were caused by the non-specific activation of D_2 receptors and not by the activation of adrenergic receptors (Fig. 1). Furthermore, the time course of the D_2 mediated effects was parallel to that of I_h inhibition (Fig. 2A and 2B). These findings suggest that I_h inhibition could have resulted from the activation of D_2 receptors. To determine if this was the case, I_h was recorded in the presence of NE plus the specific D_2 antagonist eticlopride. Under these conditions, NE was unable to evoke I_h inhibition ($n = 10$; Fig. 4). It is therefore concluded that I_h inhibition resulted from the non-specific activation of D_2 receptors by NE.

NE inhibits I_h without affecting its voltage and time dependence

Dopamine D_2 receptors are known to lower the intracellular concentration of cAMP (Kebabian and Calne, 1979). Hence the sensitivity of h-channels to hyperpolarization should be diminished when these receptors are activated (Ulens and Siegelbaum, 2003). If this is the case for the D_2 mediated I_h inhibition, then the I_h activation curve should be shifted to the left on the potential axis, and the activation time constants at the interval of potentials where the inhibition is present should be increased. This type of inhibition is called voltage dependent inhibition because at maximal I_h activation, the inhibition is not present. However, if the inhibition is present without a negative shift in the activation curve and increase in the activation time constants, the inhibition is called voltage independent.

To determine if I_h inhibition was voltage dependent or independent, activation curves were constructed from the tail currents recorded in both the presence and absence of NE for five cells. A control activation curve was constructed by averaging the five curves in the absence of NE. A treatment curve was constructed by averaging the five curves in the presence of NE. Both curves were fitted by the Boltzmann function to estimate the average potential of half-activation ($V_{1/2}$) and the average slope factor (k) under each condition. This analysis revealed that $V_{1/2}$ and k were not affected by the presence of NE and that there was no significant difference between the two curves (2-way ANOVA) (Fig. 5A). The same cells used in the previous analysis were also inspected for changes in time dependence. For each cell, current traces from -90 to -120 mV were well fitted by single exponential functions in the presence and absence of NE. Current traces at -70 and -80 mV were not used for curve fitting since the time dependence at these potentials was not exponential for all cells. A control and treatment graph of the activation time constants vs. command potentials was constructed for each cell. Control and treatment graphs were averaged into single plots and statistically compared (2-way ANOVA). This analysis showed that the activation time constants were not significantly affected by the presence of NE (Fig. 5B).

These results show that I_h inhibition was voltage independent since in the presence of NE the activation curve was similar to the curve in the control condition and there was no significant change in the activation time constants at all tested potentials (Fig. 5A and 5B).

I_h inhibition is dependent upon the increase in membrane conductance

The results presented show that I_h inhibition is voltage independent (Fig. 5). However, recordings showed that I_h inhibition and the increase in conductance share a parallel time course (Fig. 2B). Thus the inhibition could be dependent upon the D_2 mediated increase in potassium conductance. In midbrain DA cells the D_2 mediated increase in potassium conductance has been shown to be blocked by barium at 300 μM (Cathala and Paupardin-Tritsch, 1999). Therefore, to test if I_h inhibition was dependent on the increase in potassium conductance, I_h was recorded in the presence of NE plus 300 μM of external barium. Under these conditions NE failed to inhibit I_h ($n = 5$) suggesting that the inhibition is dependent upon the increase in membrane conductance (Fig. 6).

Discussion

The present study shows that NE hyperpolarizes VTA DA cells by increasing the membrane conductance to potassium through a non-specific activation of dopamine D_2 receptors. Parallel to the time course of the D_2 -mediated hyperpolarization, NE also inhibits I_h . This effect of NE does not appear to arise from a specific modulatory action of second messengers on h-channels since the inhibition is present without a hyperpolarizing shift in the activation curve nor an increase in the activation time constants. I_h inhibition appears to be dependent upon the D_2 -mediated opening of potassium channels; that is, the inhibition probably resulted from inefficient space clamp of the neuron caused by the increase in conductance. A similar mechanism has been reported in SNc DA cells where I_h inhibition by NE or baclofen results from the increase in conductance and is not caused by a distinct effect of these agonists on I_h (Cathala and Paupardin-Tritsch, 1999; Watts et al, 1995). Our results differ from the above in two important ways. First, the VTA is functionally different to the SNc in terms of synaptic inputs, functional specificity and gene expression profiles (Sesack and Orr, 2002; Grace et al. 2007 and Greene, 2006). For example, burst firing in VTA DA cells is triggered by glutamatergic afferents that originate from the prefrontal cortex, pedunculopintine tegmentum and the lateral preoptic-rostral hypothalamic area. In contrast, burst firing in SNc DA cells is evoked by glutamatergic afferents from the subthalamic nucleus (Grace et al., 2007). Hence, VTA DA cells are exposed to a neurochemical environment quite different from that of DA cells in SNc. In addition, concerted differences in gene expression appear to underlie the unique properties of distinct dopamine neurons. VTA DA cells prominently express genes related to synaptic plasticity and neuropeptides, suggesting a role of VTA dysfunction in addiction and mood disorders. On the other hand, SNc DA neurons which have a tendency to degenerate in Parkinson's disease, express high levels of transcripts related to energy metabolism, mitochondria and phosphate signalling pathways (Greene, 2006). Thus, the need to identify differences and similarities at the cellular level between these anatomical structures is imperative. Second, the present work clearly shows that NE evoked I_h inhibition is voltage independent.

Ideal space clamp is achieved when the charge density imposed by the command potential is uniform across the cell capacitance. This means that the membrane potential equals the command potential at any location of the neuron. In reality, due to the cable-like geometry of neurons, the charge deposited by the command potential escapes across the membrane conductance as one moves away from the electrode, leading to a non-uniform charging of the cell capacitance and thus poor voltage clamp of sites distal to the electrode. Therefore, during a significant increase in membrane conductance, the voltage control of dendrites is severely

reduced because of the increase in electrotonic length of these neural processes (Spruston et al., 1994).

If the channels that generate the whole cell I_h have a dendritic location, then the attenuation of voltage control of dendrites as a consequence of the increase in conductance will underlay the apparent I_h inhibition without NE having any modulatory action on h-channels. In support of this hypothesis is the finding that I_h inhibition in VTA DA cells cannot be isolated from the increase in conductance when barium is used to block potassium channel opening. Blocking the increase in conductance with barium abolishes I_h inhibition. It can be argued that external barium may prevent the binding of NE to the D_2 receptor and hence blocks I_h inhibition. Notwithstanding, barium blocks the pore of potassium channels (Kubo et al., 1993) and is not known to affect the activation of the D_2 receptors (Cathala and Paupardin-Tritsch, 1999). Taken together, these results make it difficult to support the possibility that I_h inhibition by NE is caused by a distinct mechanism acting on h-channels. They rather suggest that I_h inhibition is a consequence of inefficient space clamp caused by the increase in potassium conductance.

Norepinephrine is known to modulate the I_h of thalamic relay neurons (McCormick and Pape, 1990), neurons from the medial nucleus of the trapezoid body (MNTB) (Banks et al., 1993) and primary sensory neurons of the dorsal root ganglia (Yagi and Sumino, 1998). In the thalamus, NE activates beta-adrenergic receptors elevating intracellular cAMP levels and shifting I_h voltage dependence to more depolarized potentials. Similarly, in neurons of MNTB, NE also evokes a cAMP-dependent positive shift on the I_h activation curve. On the other hand, activation of alpha-2 adrenergic receptors in dorsal root ganglion (DRG) neurons evokes a significant reduction in I_h amplitude. In view of this data the lack of noradrenergic modulation of I_h in VTA DA cells suggests the absence of both types of adrenergic receptors. In support of this contention is the *in vitro* intracellular recording study of Grenhoff et al. (1995) which did not find alpha-2 or beta noradrenergic responses to NE bath application in VTA and SNc DA cells.

A more recent study in SNc DA cells has shown the functional presence of alpha-2 adrenergic receptors (Cathala et al., 2002). However, the activation of these receptors was not associated with I_h inhibition as in DRG neurons. A possible explanation for this paradox (i.e., alpha-2 receptors that do not affect I_h) is that alpha-2 receptors in DA cells activate a second messenger pathway that does not interact with h-channels. That is, although the activation of alpha-2 receptors may result in I_h inhibition (e.g., reduction in cAMP levels), these metabotropic receptors could be localized in a cell compartment distant or isolated from h-channels. A similar argument would also explain our results, i.e., the activation of dopamine D_2 receptors does not affect I_h voltage and time dependence in VTA DA cells. Therefore, the spatial separation of alpha-2 and D_2 receptors from h-channels may explain why I_h in midbrain DA cells is insensitive to the activation of these receptors.

The noradrenergic effects on VTA DA cells described here differ from the effects observed by others in that we did not record alpha-1 adrenergic responses to NE bath application, i.e. inward current (Grenhoff et al., 1995; Paladini et al., 2001; Paladini and Williams, 2004). Such a discrepancy could be the result of different experimental conditions. For example, the DA cells used in our study were obtained from rats of different age range and species than those used in other studies. On the other hand, studies have revealed a specific role of alpha-1B receptors in the modulation of activity of nigrostriatal DA neurons (Battaglia et al., 2003). Due to the lack of specific antagonists for alpha-1 receptor subtypes, it is possible that some of the observed NE effects could still be mediated by alpha-1 receptors. Prazosin, being a non-subtype selective antagonist, might not be able to block such actions.

All subtypes of alpha-1 adrenergic receptors are known to activate PKC (Zhong and Minneman, 1999). The activation of PKC in VTA and SNc DA cells inhibits I_h (Zhaoping et al., 2003; Cathala and Paupardin-Tritsch, 1997). Interestingly, studies that clearly show the functional presence of alpha-1 receptors in the midbrain do not report alpha-1 effects on I_h (Grenhoff et al., 1995; Paladini et al., 2001; Paladini and Williams, 2004). Since PKC is known to exist in numerous isoforms (Zhaoping et al., 2003), it is possible that the absence of alpha-1 effects on I_h results from the activation of a PKC isoform that does not phosphorylate h-channels or interacts with them indirectly.

In addition, it is worth noting that the DA cell response to the activation alpha-1 adrenergic receptors is sensitive to the duration of the activation. For instance, an outward current is observed when these receptors are activated by a transient iontophoretic pulse but an inward current is seen when a prolonged activation is evoked by bath application of an agonist. This dual action is due to fact that alpha-1 receptors are couple to phosphoinositide hydrolysis (Paladini and Williams, 2004). Here we show that bath application of NE activates D_2 receptors evoking an outward current identical to the outward current evoked by iontophoretic activation of D_2 receptors reported by Paladini and Williams (2004). Thus, suggesting that extrasynaptic DA may have similar effects as those evoked by rapid synaptic release of the neurotransmitter.

The reduction in I_h amplitude due to a significant drop in input conductance suggests that h-channels are mainly located at the dendrites of DA cells in comparison to the soma. This places the functional role of I_h in the excitability of midbrain DA cells in a position quite different from the function most commonly ascribed to this conductance (i.e., a positive shift in the voltage dependence of I_h increases DA cell excitability). In particular, burst firing in DA cells evoked by activation of a dendritically located high threshold calcium spike (HTCaS) may be prevented by a depolarizing shift of I_h activation curve and vice versa.

In a DA cell the low threshold sodium spike is initiated in the axon hillock, whereas the HTCaS is generated somewhere in the dendrite. Somatic sodium spikes propagate forward into the axon and back into the dendrites. During back-propagation somatic spikes interact with dendritic synaptic potentials, leading to the temporal summation of both events. If this summation reaches the threshold of HTCaS then the activation of this calcium spike provides a long lasting somatic supra-threshold depolarization that initiates bursts of sodium action potentials in the axon hillock (Overton and Clark, 1997). Consequently, the interaction of back-propagating somatic spikes with dendritic synaptic inputs may facilitate the transition from single spike firing to burst firing in DA cells.

The temporal summation of dendritic synaptic inputs with back-propagating action potentials depends on the electrotonic properties of the dendrites. For example, the presence of leak conductances such as potassium channels or I_h makes the temporal summation of these events more difficult (Berger et al., 2001). I_h activation in layer V pyramidal neurons of the somatosensory cortex may prevent the back-propagation of somatic action potentials and thereby their ability to initiate burst firing when temporally coupled to synaptic inputs (Berger et al., 2003). Moreover, pyramidal bursting neurons in the Subiculum of the Hippocampus, which had a large I_h , exhibit a reduced temporal summation of synaptic inputs when stimulated at high frequency (van Wilie et al., 2006). This property provides the neuron with the ability to discriminate between inputs in terms of their frequencies. It is possible that dendritically located I_h in midbrain DA cells may play similar roles. Therefore, neuromodulators that negatively shift the voltage dependence of I_h may facilitate burst firing whereas those that evoke a positive shift may prevent burst firing.

Finally, the mesocorticolimbic system is a brain region rich in DA receptors and DA is a more potent agonist of D_2 receptors than NE. For example, GTPase activity evoked by D_2 activation

in rat striatal membranes has an EC_{50} of 3.7 μ M versus 14 μ M for NE (Odagaki et al., 1995). This may suggest that NE plays a minor role in the physiology of this complex neural circuit; however, empirical evidence indicates a much bigger role for this catecholamine. Experiments have demonstrated that the loss of a trophic influence of neurons from the locus coeruleus might be crucial in Parkinson's disease (Gesí et al., 2000). On the other hand, NE may decrease DA cell excitability through synaptic activation of alpha-1 adrenergic receptors in the VTA (Paladini and Williams, 2004). Also, the rewarding properties of cocaine and morphine are reduced in mice lacking alpha-1 adrenergic receptors (Drouin et al., 2002). In addition, blockade of alpha-adrenergic receptors with prazosin protects against methamphetamine toxicity (Battaglia et al., 2003). In light of this evidence, our results should not be viewed as validation of the idea that the principal neuromodulator of the mesocorticolimbic system is DA. Instead, they are a reminder that in the same way DA can modulate the mesocorticolimbic system by activation of receptors that are not their pharmacological counterparts (Paladini et al., 2001), NE may modulate this circuit through activation of DA receptors. In turn, this requires a reevaluation of a popular model in receptor physiology that states that there is strict correspondence between an agonist and its receptor.

In conclusion, it appears that I_h inhibition by NE in VTA DA cells resulted from inefficient space clamp rather than arising from a modulatory action of second messengers on h-channels. This result also suggests that h-channels are mostly located in the dendrites and thus their activation may prevent the transition from single spike firing to burst firing.

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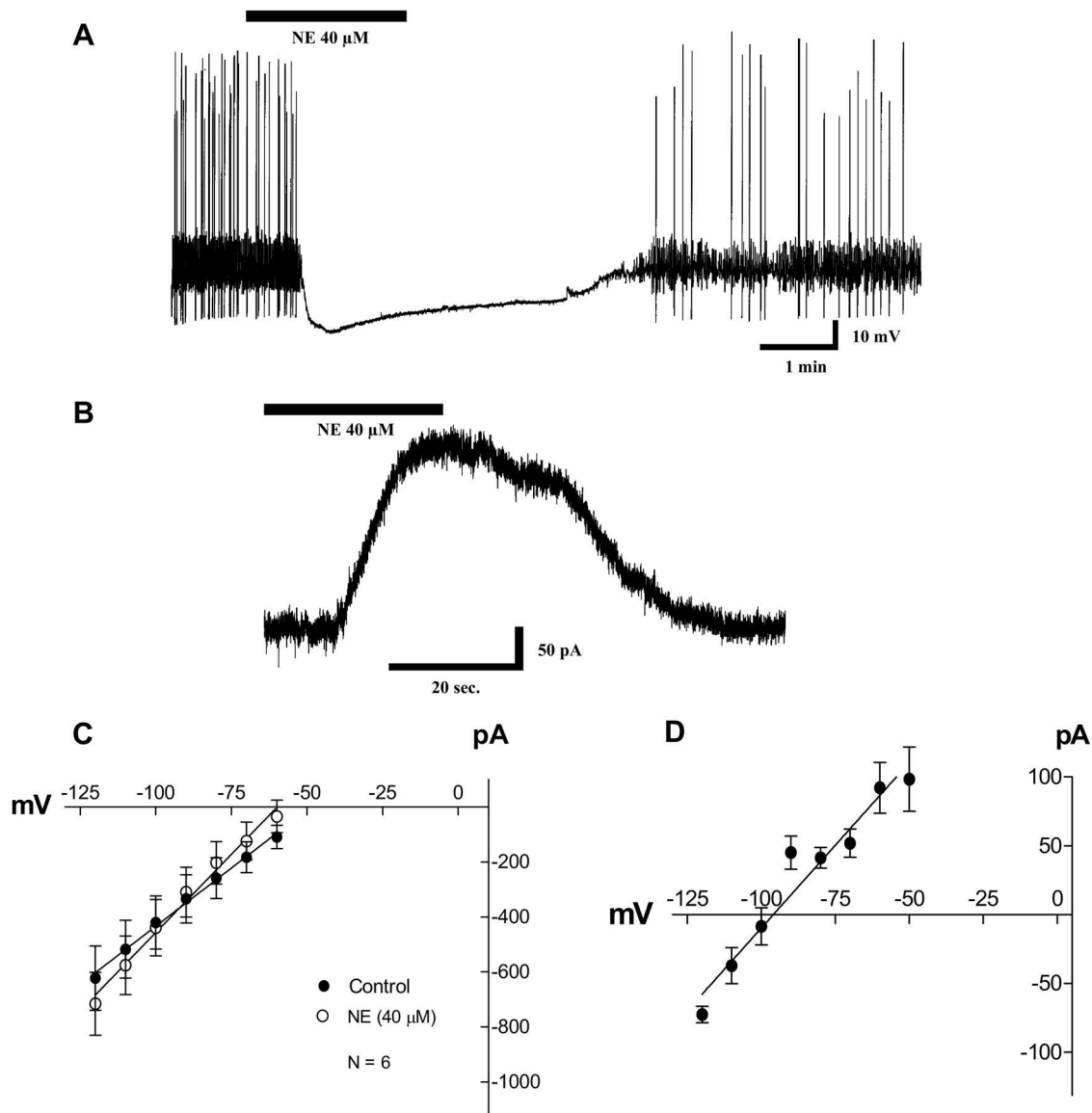


Fig. 1. Effects of norepinephrine (NE; 40 μ M) on membrane potential, holding current and membrane conductance of VTA dopamine (DA) cells

A. Bath application of NE hyperpolarizes spontaneously firing VTA DA cells.

B. NE evokes an outward current on VTA DA cells when held at -60 mV.

C. Current vs. voltage curves showing the effect of NE on membrane conductance.

Observe that NE increases the membrane conductance (increased slope). The curves also show what is evident from parts A and B. That is, NE evokes an outward current at -60 mV (reduction in the current amplitude) and hyperpolarizes (change of intercepts on the potential axis) the probed cells. The membrane potential coordinate of the point of intersection of the two IV-curves represents the reversal potential of the conductance activated by NE (see part D). The curves were constructed by plotting the instantaneous membrane current against the command potentials. Points on each curve represent the average \pm s.e.m. from a sample of six cells. The smooth lines show linear fits.

D. Current vs. voltage curve obtained by subtracting the NE curve from the control curve for each of the six cells in part C. Points on the curve represent average \pm s.e.m.. This curve represents the current vs. voltage relation of the conductance activated by NE. The reversal

potential (intercept on the potential axis) for this conductance is -96 ± 8 mV ($n = 6$), which is very close to the predicted Nernst potential for K^+ (-106 mV). The smooth line shows a linear fit.

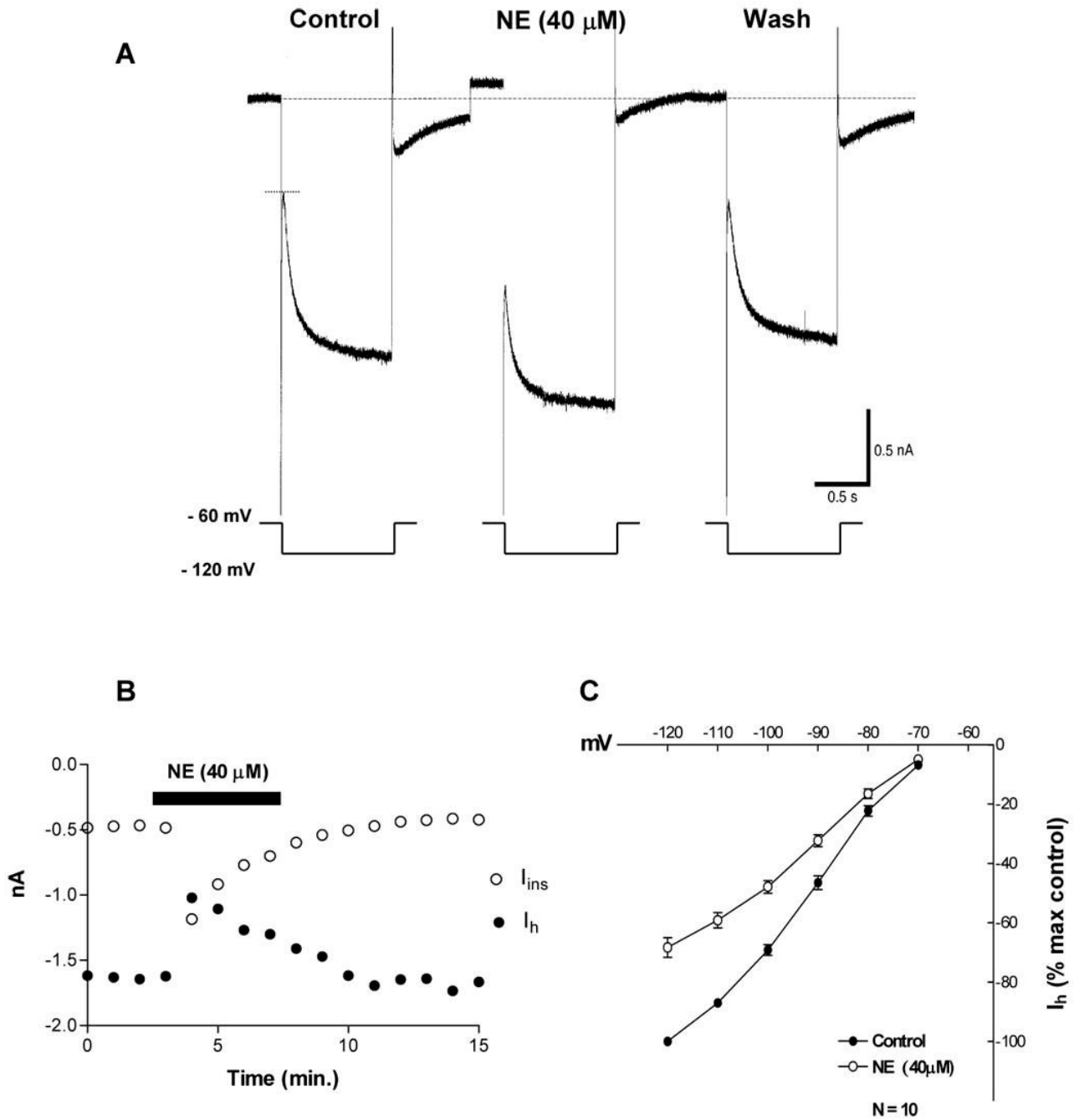


Fig. 2. NE (40 μ M) inhibits I_h

A. Voltage clamp recording showing the current response of a VTA DA cell when held at -60 mV and commanded to -120 mV for 1 second. Bath application of NE (2 min.) induced an outward current of 96 pA (change in holding current), a 1.7-fold increase in membrane conductance and a 30% reduction in I_h amplitude. The membrane conductance was calculated from the amplitude of the instantaneous current (between and ----).

B. The time course of I_h inhibition is parallel to that of the NE-induced increase in membrane conductance (increase in instantaneous current (I_{ins}) amplitude). The data used to plot the graph is from a recording similar to the one shown in part A.

C. NE significantly reduced I_h amplitude (2-way ANOVA, $F = 249$, $df = 1, 108$; $P < 0.0001$). Data points represent average \pm s.e.m. of ten cells.

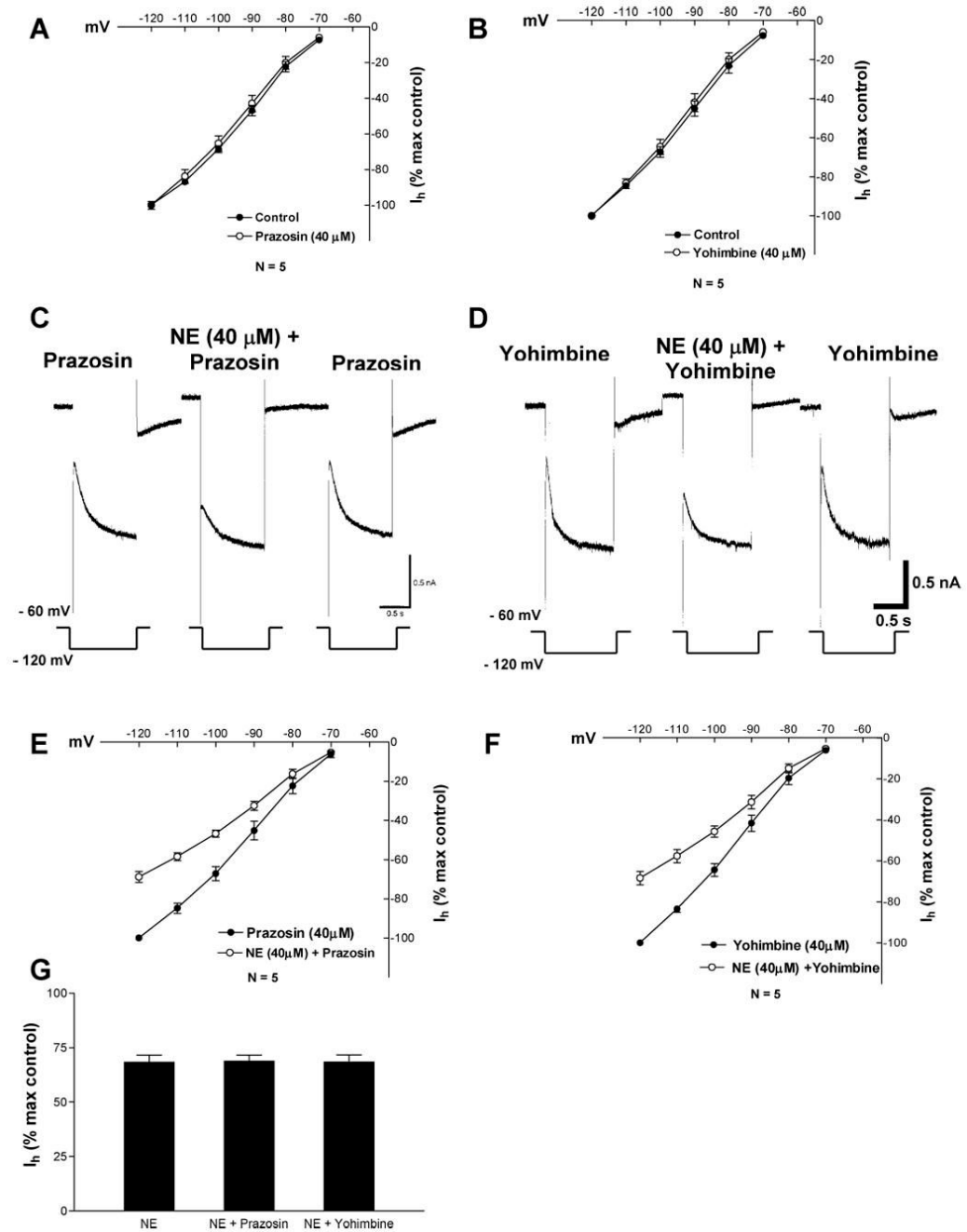


Fig. 3. Alpha adrenergic antagonists did not block the NE-induced outward current, increase in membrane conductance or I_h inhibition

Data points on each graph represent average \pm s.e.m. of five cells.

A. At 40 μ M alpha-1 antagonist prazosin did not affect I_h amplitude.

B. At 40 μ M alpha-2 antagonist yohimbine did not affect I_h amplitude.

C. In the presence of prazosin, NE evokes an outward current, increases the membrane conductance and inhibits I_h .

D. In the presence of yohimbine, NE evokes an outward current, increases the membrane conductance and inhibits I_h .

E. NE significantly reduced I_h amplitude in the presence of prazosin (2-way ANOVA, $F=534.9$, $df = 1, 48$; $P < 0.0001$).

F NE significantly reduced I_h amplitude in the presence of yohimbine (2-way ANOVA, $F=99.72$, $df = 1, 48$; $P < 0.0001$).

G. Maximal I_h inhibition (-120 mV) in the presence of NE and prazosin or NE and yohimbine was not different from that of NE alone (1-way ANOVA, $MS= 0.3481$, $df = 2, 19$; $P = 0.9956$).

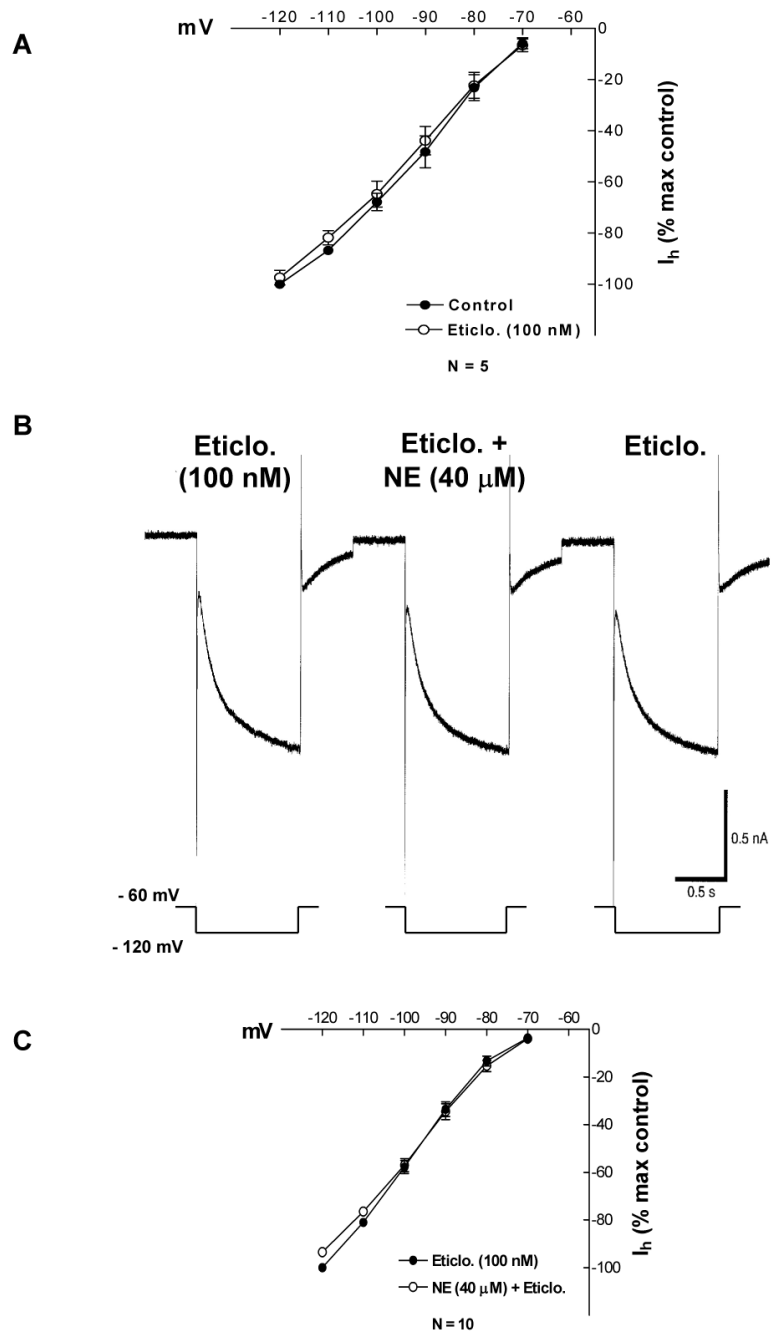


Fig. 4. In presence of D₂ antagonist eticlopride (100 nM) NE did not affect I_h
 Data points in part A and C represent average \pm s.e.m. of five and ten cells, respectively.
A. At 100 nM eticlopride has no effect on I_h amplitude.
B. Eticlopride blocks the NE-induced outward current, increase in membrane conductance and I_h inhibition.
C. Summary of NE effects on I_h in the presence of eticlopride.

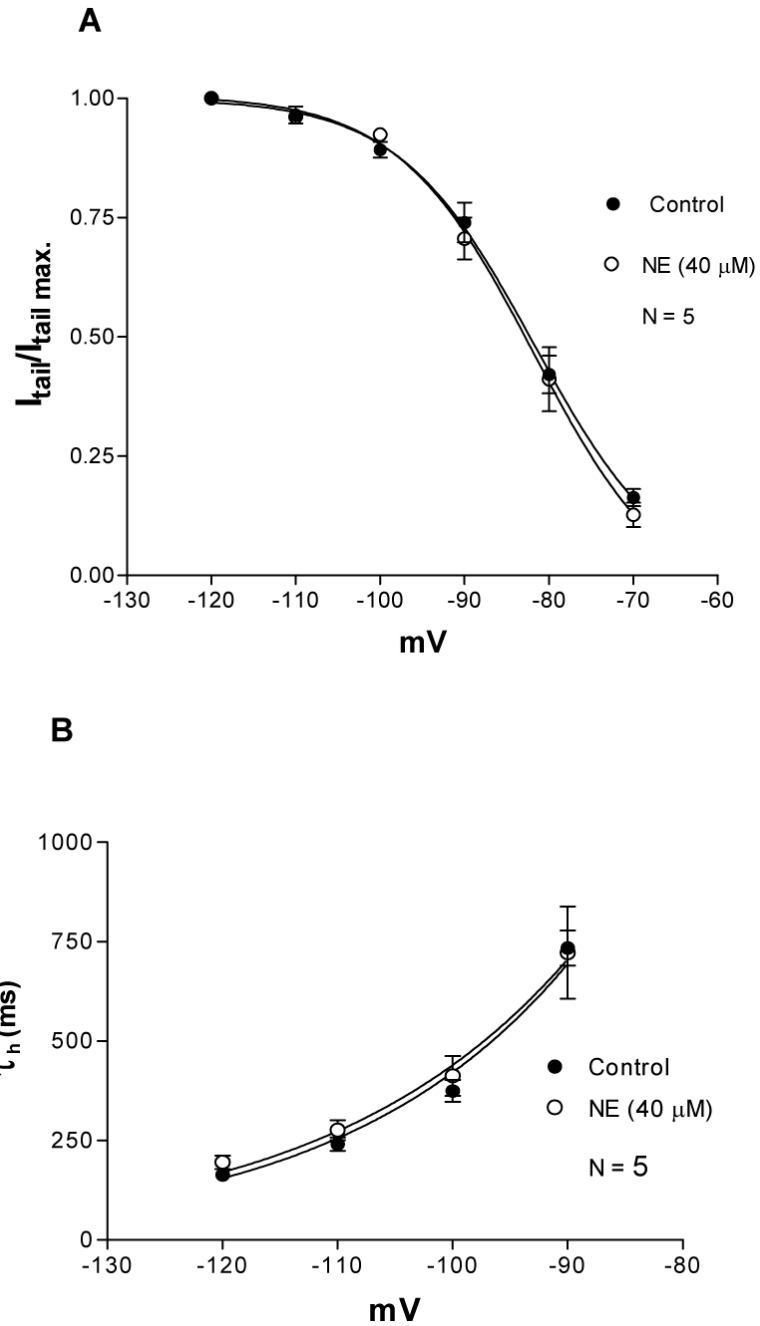


Fig. 5. NE (40 μM) inhibits I_h without affecting its voltage and time dependence

A. NE inhibits I_h without affecting its activation curve, indicating a voltage independent inhibition (2-way ANOVA, $F = 0.2384$, $df = 1, 48$; $P = 0.6276$). The level of I_h activation for a given command potential and condition were calculated as the ratio between the I_h tail (I_{tail}) current at the command step and the tail current at -120 mV (I_{tail max.}). The half activation voltage and slope factor (k) were -82 mV and k = 8 mV in control and in NE. The smooth curves show the Boltzmann fit. Data points represent average ± s.e.m. of five cells.

B. The time course of I_h was fitted by single exponential function. The activation time constants (τ_h) obtained from the fit are plotted against command potentials. NE did not affect the time dependence of I_h, suggesting a voltage independent inhibition (2-way ANOVA, $F = 0.4951$, df

= 1, 32; $P = 0.4868$). The smooth curves show a single exponential fit. Data points represent average \pm s.e.m. of five cells.

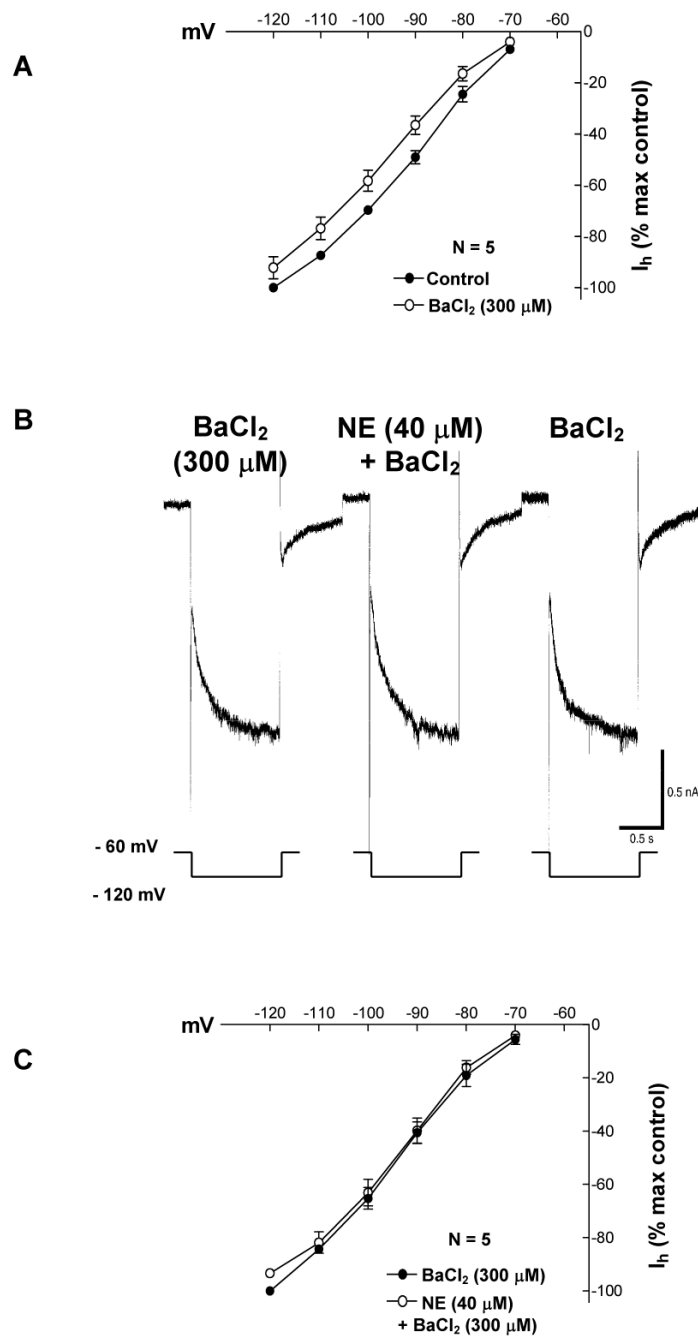


Fig. 6. In the presence of the K⁺ channel blocker BaCl₂ (300 μM), NE did not affect I_h
 Data points in part A and C represent average ± s.e.m. of five cells.
A. At 300 μM BaCl₂ causes a small diminution of I_h amplitude.
B. BaCl₂ blocks the NE-induced outward current, increase in membrane conductance and I_h inhibition.
C. Summary of NE effects on I_h in the presence of BaCl₂.