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Pharmacology of A-type K^+ channels

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Abstract

Transient outward potassium currents were first described nearly 60 years ago, since then major strides have been made in understanding their molecular basis and physiological roles. From the large family of voltage-gated potassium channels members of 3 subfamilies can produce such fast-inactivating A-type potassium currents. Each subfamily gives rise to currents with distinct biophysical properties and pharmacological profiles and a simple workflow is provided to aid the identification of channels mediating A-type currents in native cells. Their unique properties and regulation enable A-type K^+ channels to perform varied roles in excitable cells including repolarisation of the cardiac action potential, controlling spike and synaptic timing, regulating dendritic integration and long-term potentiation as well as being a locus of neural plasticity.

Keywords: A-type K^+ channel, Voltage-clamp, potassium channel, Kv4, Kv1.4, Kv3.4

1. Introduction

A-type refers to neuronal K^+ currents generating a transient outward current in response to depolarising voltage-clamp steps. This stereotypical phenotype was first described in neurons from sea slug *Onchidium* (Hagiwara et al., 1961). These early experiments described the now stereotypical hallmarks of A-type currents. They identified a transient outward voltage-gated K^+ current activated by depolarising steps from a hyperpolarised potential (Fig. 1 C & D). In current clamp when hyperpolarising steps were used to remove A-current inactivation, they observed an increase in the latency to the first spike in response to depolarising current steps (Fig. 1 A & B). Such transient outward currents were later designated as I_A, a term which has persisted (Connor and Stevens, 1971). Work over the subsequent decades has described A-type channels in the heart (the corresponding current fraction is called I_{to} in the heart; see Chapter 4 for more details) where they contribute to the early repolarisation phase (Dixon et al., 1996; Yeola and Snyders, 1997) and in many different types of neurons where they contribute to such varied functions as controlling spiking, neurotransmitter release, dendritic integration and long term potentiation (Molineux et al., 2005; Imai et al., 2019; Watanabe et al., 2002; Frick et al., 2004; Chen et al., 2006; Shevchenko et al., 2004; Kim et al., 2005; Kuo et al., 2017).

The transient nature of A-type currents is due to rapid voltage-dependent inactivation, with inactivation decay time constants typically in the 10s of milliseconds. The subunit composition and presence of accessory subunits or other modulatory factors determine the exact biophysics and pharmacological profile of these currents. Alpha subunits from three subfamilies of voltage-gated K^+ channel generate rapid inactivating K^+ currents when expressed as homomers: Kv1.4, Kv3.3, Kv3.4 and all three Kv4 members, Kv4.1, Kv4.2 & Kv4.3 (Coetzee et al., 1999). The presence of the ß subunit Kvß1 can also confer A-type kinetics to other, normally delayed rectifier subunits from the Kv1 family (Rettig et al., 1994). These different subfamilies have distinct biophysical properties, enabling the subfamilly mediating a native A-type current to be narrowed down with simple voltage-clamp protocols and confirmed with their pharmacological profiles.

Figure 1 near here

2. Identifying native A-type channels

2.1 Biophysics

2.1.1 Activation range. Canonical A-type K^+ currents have sub-threshold activation ranges, i.e. they begin to activate at voltages more negative than that required to generate an action potential (Fig. 1). A hall mark of the Kv3 subfamily is their supra-threshold activation range, they typically do not begin to activate until voltages greater than -30 mV with half activation voltages around +10 mV (Rudy and McBain, 2001). This

property restricts their physiological activation window to that of the action potential, in which Kv3 channels act to rapidly repolarise the cell to the resting state (Rudy and McBain, 2001; Johnston et al., 2010). A-type K^+ currents mediated by channels containing Kv3.3 and/or Kv3.4 can therefore be distinguished from other A-type subunits by their positively shifted activation curve (Fig. 2 B).

2.1.2 Inactivation properties. The molecular basis of rapid K^+ channel inactivation has been extensively studied with Kv1.4 used as the prototype. Two forms of inactivation have been demonstrated; fast N-type or "ball and chain" inactivation involves the N-terminal inactivation peptide of the alpha subunit binding within the central cavity to occlude the inner pore (Hoshi et al., 1990; Zhou et al., 2001). The rate of this type of inactivation is directly related to the number of N-terminal peptides, e.g. Kv1.4 homomers, possessing 4 N-terminal peptides, show faster inactivation than Kv1.4/Kv1.1 heteromers, which have 3 of less depending on the stoichiometry (Ruppersberg et al., 1990; MacKinnon et al., 1993). The presence of Kvß1 subunits confer a similar N-terminal inactivation peptide (one per subunit), which further accelerates the inactivation rate of Kv1.4 or can endow Kv1.1 with fast A-type inactivation (Rettig et al., 1994). Kv3.3 and Kv3.4 are thought to inactivate through a similar mechanism showing a similar dose dependency of N-terminal inactivating peptide on inactivation rate (Rudy et al., 1999; Weiser et al., 1994). In addition to the described N-type inactivation, Kv1.4 channels also inactivate by a slower C-type inactivation attributed to partial collapse of the selectivity filter / outer pore. N and C-type inactivation in Kv1.4 are allosterically coupled (Hoshi et al., 1990; Hoshi et al., 1991; Bett and Rasmusson, 2004) and the rate of recovery from inactivation is controlled by the slower C-type inactivation (Rasmusson et al., 1995). As a result Kv1.4 channels recover from inactivation with a time constant of seconds (Bett and Rasmusson, 2004; Roeper et al., 1997).

In contrast, the molecular details concerning inactivation of Kv4 channels have not been determined with such precision. Kv4 subunits use neither the N nor C-type inactivation processes identified for Kv1.4, instead inactivation seems to involve the inner vestibule of the pore and readily occurs through the closed state (Jerng et al., 1999; Bähring et al., 2001; Beck and Covarrubias, 2001; Shahidullah and Covarrubias, 2003). Kv4 channels possess a N-terminal inactivating domain but rather than contributing to inactivation this region is the binding site of the ß-subunits of Kv4 channels known as K^+ channel interacting proteins (KChIPs). These accessory proteins appear to be obligatory subunits for native channels (Rhodes et al., 2004). KChIPs and DPPX, another group of accessory proteins, dramatically increase the surface expression of Kv4 subunits and alter the kinetics of and recovery from inactivation of Kv4 subunits (Beck et al., 2002; Jerng et al., 2004).

The kinetics of inactivation cannot reliably distinguish between Kv1.4 and Kv4 subunits as their decay time constants can overlap, largely depending on the composition of accessory subunits. However, the recovery from inactivation of Kv4 channels is usually much more rapid, with time constants around 10s of milliseconds (Kim et al., 2005; Beck et al., 2002; Johnston et al., 2008; Amadi et al., 2007), compared to Kv1.4 with recovery rates of 100s of milliseconds to seconds (Ruppersberg et al., 1990; Rasmusson et al., 1995; Roeper

et al., 1997).

Two simple voltage protocols can therefore help to determine the subfamily responsible for native A-type currents. First the activation curve should be attained with a simple I/V protocol with test steps from ~-100 mV, to ensure removal of inactivation (Fig. 2 A). The peak current soon after the test step (red arrow in Fig. 2 A) should be corrected for the non-linear driving force before being normalised and fit with a Boltzmann function (Clay, 2000). With the protocol shown in Fig. 2 A the steady-state inactivation curve can also be constructed by plotting the current amplitudes measured at the yellow arrow vs the voltage of the preceding test step. The activation curve will distinguish between Kv3 mediated A-type currents with positive shifted activation ranges and Kv4 or Kv1 mediated A-type currents which have more negative sub-threshold activation ranges (Fig. 2 B). If necessary, the A-type current can be isolated from other endogenous currents with a subtraction protocol as shown in (Johnston et al., 2008). If the A-current has a sub-threshold activation range, the rate of recovery from inactivation can be determined with a protocol similar to that shown in Fig. 2 B right; rapid recovery from inactivation, a tau <~100 ms, is indicative of Kv4 subunits whereas currents displaying time constants of several hundreds of milliseconds or longer are likely to be mediated by Kv1.4 subunits.

2.2 Pharmacology

Pharmacology of ion channels can serve multiple purposes as illustrated by the holy grail of pharmacology, a selective antagonist, a molecule that will antagonise one kind of channel and no other. Such a molecule can be used to unambiguously determine the presence of a channel, its contribution to total current and the role of this channel in normal physiology. There are some striking examples of this with biologically derived toxins, e.g. Dendrotoxin-K is selective for any channel containing Kv1.1 subunits and blocks such channels in the 10 nM range (Wang et al., 1999; Robertson et al., 1996; Dodson et al., 2002). Unfortunately, such molecules are not always available particularly for the large diversity of Kv channels. However, less selective antagonists can still serve a useful role; when combined with other antagonists or biophysical characteristics they can aid identification of the subfamily mediating an A-type current. With these considerations in mind the following discussion largely focuses on molecules that fit these criteria.

2.2.1 Kv3 A-type currents. All Kv3 channels are blocked by 1 mM tetraethylammonium (TEA) which is readily washed on and off tissue (Johnston et al., 2010). TEA has an IC50 for Kv3.3 and Kv3.4 in the 100-200 µM range and acts by occluding the pore (De Miera et al., 1992; Rettig et al., 1992). TEA sensitivity in combination with a positive activation range provides a strong indication that a Kv3 subunit mediates an A-type current (Fig. 2 B). Block of an A-type current by 1 mM TEA rules out Kv4 subunits which are insensitive to even 100 mM (Johnston et al., 2008; Johnston et al., 2010), though the presence of DPPX can increase their sensitivity to TEA somewhat. Kv1.4 homomers are also insensitive to TEA (Ruppersberg et al., 1990) however heteromeric Kv1.1/Kv1.4 channels are endowed with a mix of properties from their homomeric

counterparts; they display A-type kinetics with slower inactivation rates and have moderate TEA sensitivity with an IC50 of ~10 mM (Ruppersberg et al., 1990). Although 1 mM TEA can be used to unambiguously identify a high-voltage activated A-type currents, it is of limited use in exploring their functional role as many other non-A-type channels are sensitive to TEA e.g. some Kv7 subunits and BK channels (Johnston et al., 2010). The sea anemone toxins BD-I and BDS-II were thought to be selective antagonists of Kv3.4 channels which would provide a powerful tool for elucidating the functional role Kv3.4 A-type currents in physiology (Diochot et al., 1998). However, later detailed investigation of the interaction of BDS toxins with Kv3 subunits revealed them to act as gating modifiers and with little selectivity between Kv3 subunits (Yeung et al., 2005). BDS-I is also highly potent for Nav1.7 channels, slowing inactivation rates with a consequent broadening of action potentials and increased resurgent currents during repolarisation (Liu et al., 2012). Consequently, BDS toxins have limited potential for revealing the physiological role of Kv3 A-type currents. This also highlights a pitfall of using poorly characterised pharmacological tools; it took 14 years after their first characterisation as selective Kv3.4 blockers to realise a more complete pharmacological profile of BDS toxins. To date there are no sufficiently selective antagonists of Kv3.3 or Kv3.4 channels suitable for studying their physiological roles.

Figure 2 near here

2.1.2 Kv1.4 A-type currents. Kv1.4 channels are relatively insensitive to TEA (Ruppersberg et al., 1990) and have a lower sensitivity (IC50 ~12.5 mM) to the broad spectrum K^+ channel blocker 4-AP (Stühmer et al., 1989). In fact, there is a paucity of pharmacological tools available for Kv1.4 channels, they are somewhat unique in their resistance to biological toxins which are very potent against many of the other Kv1.x members. At 1 µM the small molecule CP-339,818 shows comparative selectivity for Kv1.4 and Kv1.3 over other Kv channels, including Kv3 and Kv4 subfamilies (Nguyen et al., 1996). CP-339,818 preferentially binds to the C-type inactivated state and therefore shows a use dependent block. At concentrations greater than 1 µM off-target effects become a problem with many other channels being inhibited e.g. Kv2.1, HCN1, Kv3.2 (Lee et al., 2008; Nguyen et al., 1996; Sforna et al., 2015). Another small molecule inhibitor, UK-78,282, has a very similar pharmacological profile to CP-339,818 (Hanson et al., 1999). To date there are no well-established selective antagonists of Kv1.4 channels suitable for studying its physiological roles, though if a contribution from Kv1.3 can be ruled out, CP-339,818 may serve this purpose.

2.1.3 Kv4 A-type currents. The anti-arhythmic drug, flencainide, at 10 µM inhibits Kv4.2 & Kv4.3 leaving Kv1.4 unaffected and has been used to determine the contribution of these channels to the cardiac I_{to} current (Dixon et al., 1996; Yeola and Snyders, 1997). Although flencainide can be used to distinguish between A-type currents mediated by Kv1.4 and Kv4 subunits it also inhibits a number of other channels including the

cardiac Nav1.7 channel (Ramos and O'leary, 2004), ether-a-go-go related gene (ERG) K^+ channels (Paul et al., 2002) and ryanodine receptors (Mehra et al., 2014). The spider toxins, phrixotoxin 1 & 2, are potent and selective inhibitors of Kv4.2 and Kv4.3 with IC50s between 5 - 70 nM (Diochot et al., 1999), these toxins are gating modifiers stabilising the closed state (Chagot et al., 2004) which shifts the activation curve to more positive potentials (Diochot et al., 1999).

Kv4.2 and Kv4.3 show strong expression in many areas of the brain and heart (Serodio et al., 1996) and consequently have received the most attention. The first member of this family to be cloned, Kv4.1, shows much weaker expression in both heart and brain (Serodio et al., 1996) and as a result the pharmacological profiles of many molecules have mostly omitted this subunit. However, Kv4.1 is expressed in the suprachiasmatic nucleus (Hermanstyne et al., 2017), striatum (Song et al., 1998), basolateral amygdala (Dabrowska and Rainnie, 2010) and nociceptive neurons in the dorsal root ganglion (Phuket and Covarrubias, 2009). The spider toxin Heteroscodra maculata 1 (HmTx-1) is reported to inhibit all 3 Kv4 members but also inhibits Kv2.1 (Escoubas et al., 2002). Phrixotoxin-1, although only initially tested on Kv4.2 and Kv4.3, also inhibits Kv4.1 but with 3 times lower affinity; this block seems to be voltage-independent (Yunoki et al., 2014). At present phrioxtoxin-1 provides a useful tool for identifying the presence of Kv4 currents and exploring their physiological roles, without being able to distinguish between different Kv4 subunits.

3. Physiological roles of A currents

3.1 Neural excitability and spike properties

A-type channels are rapidly activated upon depolarisation and therefore contribute to action potential repolarisation (Kim et al., 2005; Carrasquillo et al., 2012; Rudy and McBain, 2001), including in the heart (Dixon et al., 1996; Yeola and Snyders, 1997). Kv4 subunits are predominantly located in the somato-dendritic compartments of neurons (Trimmer, 2015) , which regulates back-propagation of action potentials into the dendritic tree. The sub-threshold activation range of Kv4 and Kv1.4 channels enables them to regulate intrinsic firing/bursting of neurons in many areas including the: suprachiasmatic nucleus (Hermanstyne et al., 2017), hypothalamus (Mendonça et al., 2018; Imai et al., 2019), dorsal root ganglion (Zemel et al., 2018), hippocampus (Bourdeau et al., 2007), substantia nigra (Liss et al., 2001) and cortex (Carrasquillo et al., 2012). A key factor determining the ability of A-type channels to exert influence is the availability of channels in their non-inactivated state, for example fast spiking will result in more inactivated A-type channels and consequent spike broadening (Kim et al., 2005). The steady-state inactivation curve (Fig. 2A yellow) describes the proportion of channels available at resting membrane potentials. This property is influenced by the presence of accessory subunits (Jerng et al., 1999) and can be dynamically regulated by phosphorylation (Rosenkranz et al., 2009) and Ca^{2+} (Anderson et al., 2010a). Cav3 T-type

channels which are also activated at sub-threshold potentials form complexes with Kv4 channels and provide a source of Ca^{2+} that shifts the inactivation curve of Kv4 channels to more positive potentials (Anderson et al., 2010a; Anderson et al., 2010b), increasing their availability. The presence of Cav3 channels can therefore alter the contribution that A-type channels make to neural excitability (Heath et al., 2014).

Figure 3 near here

Due to the lack of specific inhibitors of Kv1.4, Kv3.3 and Kv3.4 there are few concrete demonstrations of their physiological roles. Throughout the brain Kv1.4 is often found in axons and presynaptic terminals (Veh et al., 1995; Cooper et al., 1998) and may therefore play a role in regulating transmitter release. Kv1.4's slow recovery from inactivation means that with repetitive firing the repolarisation rate of presynaptic action potentials will reduce as Kv1.4 accumulates in its inactivated state. Slower repolarisation of the action potential causes larger presynaptic Ca^{2+} influx and an increase in release probability (Yang and Wang, 2006), suggesting that Kv1.4 may play a role in short term synaptic plasticity. Consistent with this idea, knockdown of Kv1.4 in the hippocampus reduced paired pulse facilitation (Meiri et al., 1998), a form of presynaptic short-term plasticity. Ca^{2+} can also regulate the rate of recovery from inactivation of Kv1.4 in a CamKinase II dependent manner (Roeper et al., 1997), providing scope for regulation of such short-term plasticity. Kv3.3 and Kv3.4 are also located in axons and synaptic terminals (Laube et al., 1996; Ishikawa et al., 2003; Trimmer, 2015) and could have similar roles.

3.2 Dendritic integration and LTP

In hippocampal pyramidal neurons the density of Kv4 channels increases from the soma towards the distal dendrites (Fig. 3A), this gradient depends on expression of the accessory subunit DPP6 (Sun et al., 2011). This gradient of Kv4 channels controls the ability of action potentials to back-propagate and invade the dendritic tree (Hoffman et al., 1997; Johnston et al., 2000) (Fig. 3B), a phenomenon required for induction of synaptic plasticity in these cells (Magee and Johnston, 1997; Buchanan and Mellor, 2007). Consistent with this idea, action potentials more readily back-propagate into the dendritic tree when Kv4.2 channels are knocked down (Fig. 3 B) and consequently the threshold for LTP induction is lowered (Chen et al., 2006). Kv4 channels are also a locus of plasticity in CA1 dendrites being targeted by mitogen-activated protein kinase (Rosenkranz et al., 2009). Induction of LTP results in a shift in the steady-state inactivation of Kv4 channels to more negative-potentials (Fig. 3C) (Frick et al., 2004) and internalisation of channels (Fig. 3D) (Kim et al., 2007), effects which are localised to the site of synaptic input. Both of these changes reduce the available A-type current and consequently increase dendritic excitability and therefore the likelihood of further plasticity.

A similar attenuation of back propagating action potentials by A-type currents is observed in the lateral dendrites of mitral cells of the olfactory bulb (Margrie et al., 2001; Christie and Westbrook, 2003), this influences the extent of lateral dendrodendritic interactions with inhibitory granule cells. A-type K^+ channels also influence the inhibitory side of the reciprocal synapse between granule and mitral cell dendrites. Granule cells receive excitation from mitral cell dendrites via AMPA and NMDA receptors and provide GABAergic feedback inhibition. The A-type current in granule cells attenuates excitation for the fast AMPA component; spiking and feedback inhibition then occur with a delay, following the time course of the NMDA component (Schoppa and Westbrook, 1999).

The varied biophysics, sub-cellular distributions and dynamic modulation of A-type K^+ channels enable them to perform a multitude of roles in regulating cellular excitability. Large strides have been made in the 60 years since their discovery but much still remains to be elucidated.

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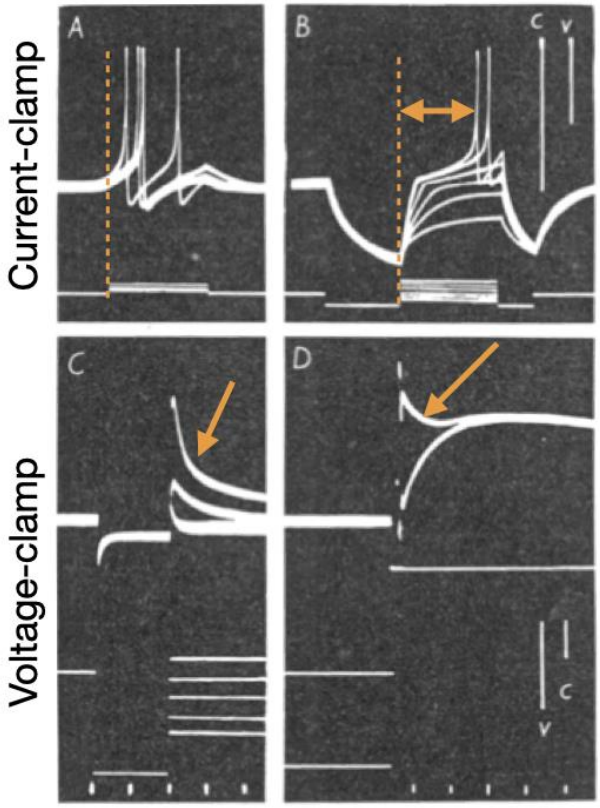

Figure 1. Initial observations of A-type currents in *Onchidium.* A) Current clamp recording of action potentials evoked from rest with short spike latencies. **B)** A preceding hyperpolarising step removes steady-state inactivation of the A-type current and results in a lag to the first spike as the A-current inactivates (yellow arrow). **C)** Voltage-clamp recording showing a fast inactivating outward current activated at voltage around resting membrane potentials after hyperpolarising steps, yellow arrow. **D)** The A-current is present at more positive potentials only with a hyperpolarising pre-step, yellow arrow. The delayed rectifier can be isolated by inactivating the A-current. Adapted from Hagiwara et al., (1961) reproduced with permission.

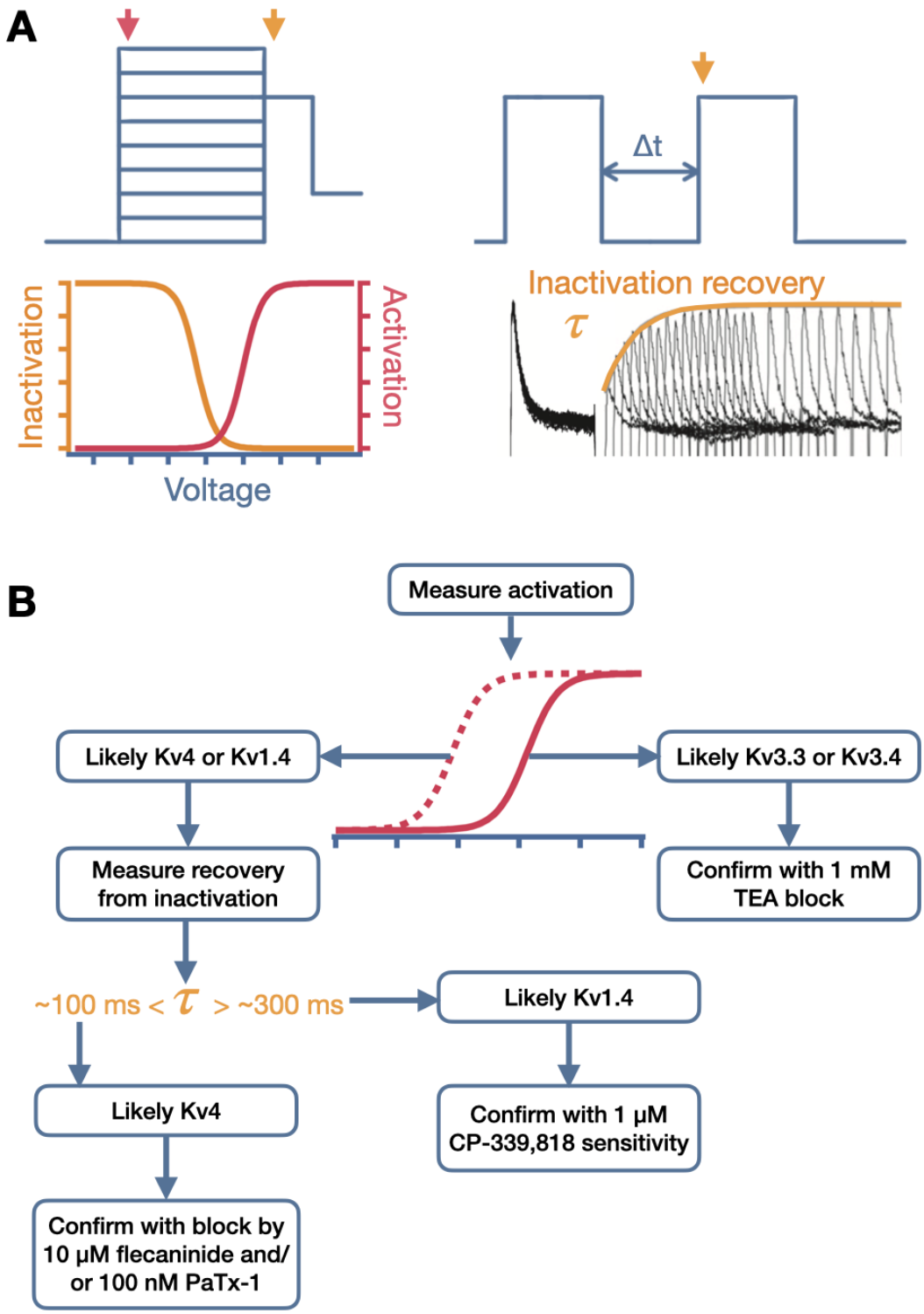

Figure 2. Biophysical and pharmacological identification of channels mediating native A-type K^+ currents. A) *Left:* Voltage protocol to determine the activation and inactivation curves for A-type channels. The activation curve can be constructed by measuring the peak current soon after the test step (red arrow) and correcting it for the non-linear driving force (see text). The steady-state inactivation curve can be obtained by measuring the current at the yellow arrow as a function of the preceding test step. Note: the duration of the initial hyperpolarising pre-pulse and the test step should be adjusted depending on the kinetics of that current being measured, e.g. the duration pre-pulse should ensure complete recovery from inactivation. *Right:* The rate of recovery from inactivation can be determined with a pair of test steps of

sufficient duration to allow complete inactivation of the A-current and separated by a variable delay (Δt). The time constant of recovery can then be determined by fitting an exponential to the current amplitude of the second step as a function of Δt. Note the current amplitude of the first step should be constant. Adapted from Johnston *et al,* (2008). **B)** A biophysical and pharmacological procedure to aid identification of the channel mediating a native A-current (see text for further description).

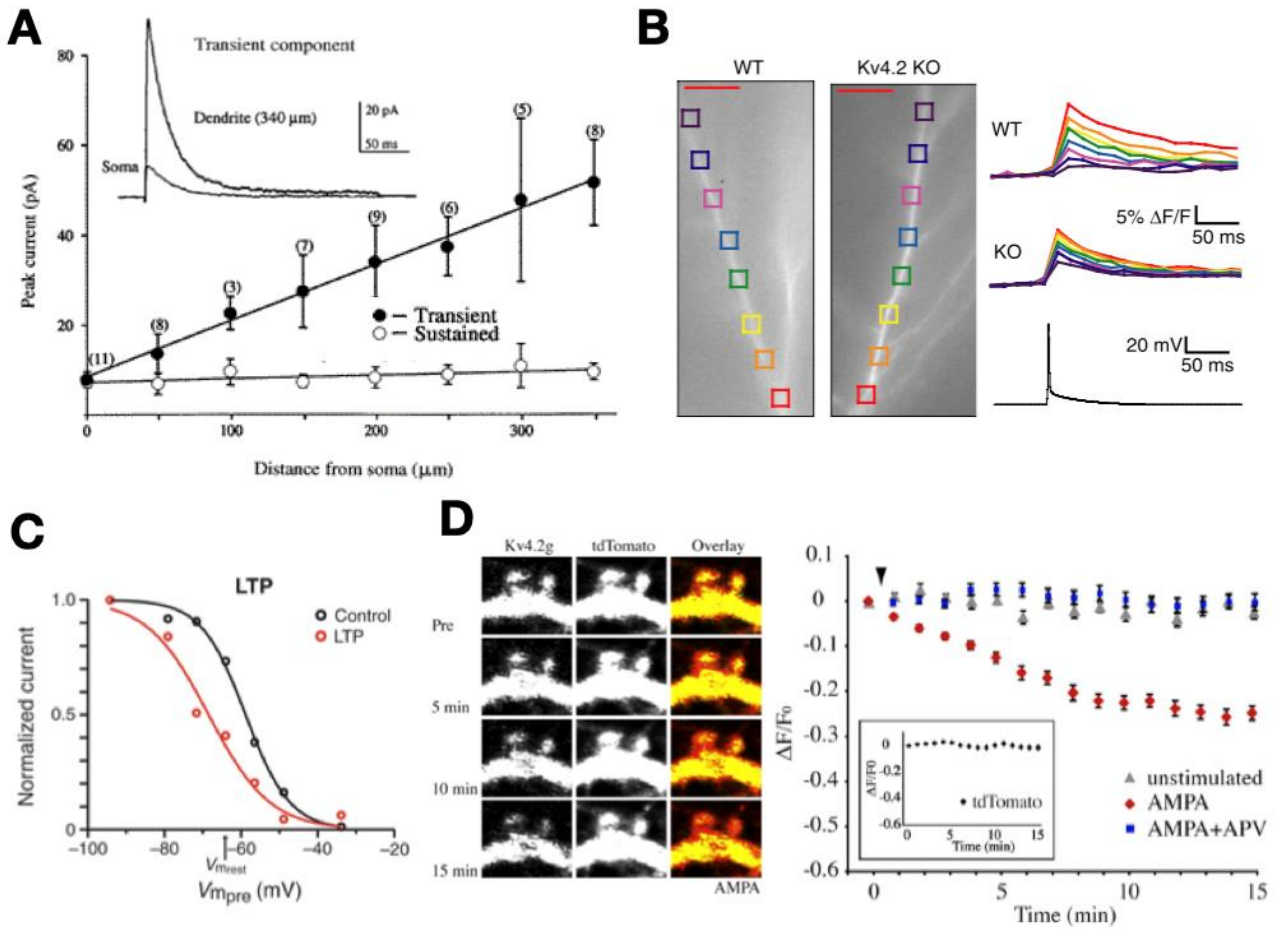

Figure 3. Dendritic Kv4 A-type channels regulate and are a locus of plasticity in hippocampal CA1 neurons. A) Dendritic recordings of Kv4 A-type K^+ currents demonstrate a gradient of current that increases with distance from soma adapted from Hoffman et al., (1997) with permission. **B)** Imaging of fura-2 loaded CA1 neurons reveal attenuation of back-propagating action potential evoked Ca^{2+} transients. This attenuation is dramatically reduced when Kv4.2 is absent, adapted from Chen, *et al.* (2006, Copyright 2006, Society for Neuroscience) with permission. **C)** The steady-state inactivation curve of dendritically located Kv4 channels is shifted to more hyperpolarised potentials after induction of LTP with theta burst stimuli. Note that this decreases the available Kv4 current at resting membrane potentials by ~50%, adapted from Frick et al., (2004) with permission. **D)** Fluorescently tagged A-type channels (Kv4.2g) are trafficked from the membrane following activation of synaptic receptors, adapted from Kim et al., (2007) with permission.