Profound alterations in the intrinsic excitability of cerebellar Purkinje neurons following neurotoxin 3-acetylpyridine (3-AP)-induced ataxia in rat: new insights into the role of small conductance K^+ channels

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Running title: Alteration in SK channel function causes cerebellar ataxia

Abstract

Alterations in the intrinsic properties of Purkinje cells (PCs) may contribute to the abnormal motor performance observed in ataxic rats. To investigate whether such changes in the intrinsic neuronal excitability could be attributed to the role of Ca^{2+}-activated K^+ channels (K_{Ca}^{2+}), whole cell current clamp recordings were made from PCs in cerebellar slices of control and ataxic rats. 3-AP induced profound alterations in the intrinsic properties of PCs, as evidenced by a significant increase in both the membrane input resistance and the initial discharge frequency, along with the disruption of the firing regularity. In control PCs, the blockade of small conductance K_{Ca}^{2+} channels by UCL1684 resulted in a significant increase in the membrane input resistance, action potential (AP) half-width, time to peak of the AP and initial discharge frequency. SK channel blockage also significantly decreased the neuronal discharge regularity, the peak amplitude of the AP, the amplitude of the after hyperpolarisation and the spike frequency adaption ratio. In contrast, in ataxic rats, both the firing regularity and the initial firing frequency were significantly increased by the blockade of SK channels. In conclusion, ataxia may arise from alterations in the functional contribution of SK channels, to the intrinsic properties of PCs.

Key words: Cerebellar Ataxia; Intrinsic Excitability; Neurotoxin 3-Acetylpyridine; Purkinje Neurones; Small conductance Ca^{2+}-activated K^+ channels

Introduction

Dysfunction or degeneration of Purkinje cells, the key neuronal cells of the cerebellar circuitry, causes ataxia. In our previous study, we showed that neurotoxin 3-acetylpyridine (3-AP) induced cerebellar ataxia, which was associated with motor incoordination and alterations in the morphological and intrinsic electrophysiological characteristics of Purkinje cells (Janahmadi et al., 2009). However, the cellular mechanisms underlying these changes have not yet been fully determined. Intrinsic neuronal excitability, which refers to the ability of a neuron to fire action potentials in the absence of synaptic inputs, could be directly attributed to the biochemical and electrophysiological properties of intrinsic membrane channels (Schulz, 2006; Russo et al., 2008), so that alterations in the function of these channels may lead to plasticity in neuronal excitability and in neural circuits to which they belong. Among the wide range of ion channels, potassium channels, which are the largest and most diverse group of ion channels, play a main regulatory role in excitable cells. Consequently, malfunction of these channels can result in several neurological disorders, including ataxia. In animal models of ataxia, Sausbier et al. (2004) reported that Ca^{2+}-activated K^+ channel deficiency causes dysfunction of Purkinje cells (PCs) and thereby results in cerebellar ataxia. At the cellular level, they showed that cerebellar Purkinje cells from mice lacking BK (big conductance) channels display a dramatic reduction in spontaneous activity. A remarkable reduction in the amplitude of post-stimulus after hyperpolarisation (AHP), which is linked to Ca^{2+}-activated K^+ conductance, was also recently reported in a rat model of ataxia (Janahmadi et al., 2009). In many neurons of the central nervous system, including PCs, both large conductance K^+ (BK) and small conductance K^+ (SK) channels are found (Shepard and Bunney, 1991; Sah, 1996; Shah and Haylett, 2000; Faber and Sah, 2002; Cingolani et al., 2002; Edgerton and Reinhart, 2003; Maingret et al., 2008; Haghdoost-Yazdi et al., 2007), where they play an important role in the regulation of neuronal excitability. Although the physiological roles of calcium-dependent potassium channels have been well documented, their functional contribution to the pathophysiology of neurological diseases (e.g., ataxia) is still poorly understood. Here to assess the possible functional contribution of SK channels to the altered intrinsic electrical properties of Purkinje neurons in a rat model of cerebellar ataxia induced by 3-acetylpyridine (3-AP) (Janahmadi et al. 2009), the effect of UCL 1684, a potent SK channel blocker, was examined. 3-AP, which is a neurotoxin

and a niacinamide receptor antagonist, it is known to be an effective agent that selectively destroys inferior olive neurons, the major source of the climbing fibres innervating the cerebellar Purkinje neurons (Balaban 1985; Torres-Aleman et al. 1996; Caddy and Vozeh 1997; Seoane et al., 2005). As a result, the cerebellar cortex loses its climbing fibre input, and the rats become ataxic (Llinás et al., 1975). It is well-documented that the climbing input to a Purkinje neuron is essential for normal cerebellar function because it controls the intrinsic properties of the output of PCs (Cerminara and Rawson 2004; McKay et al., 2007).

Methods

Fourteen Wistar rats (3 to 4 weeks old) were used in this study. The rats were divided into two groups consisted of 8 rats in control group and 6 rats in 3-AP treated group. All animals (were housed at 22°C and maintained on a 12:12 h light/dark cycle with free access to food and water. All procedures for the maintenance and the use of the experimental animals were approved by the Institutional Ethics Committee (IEC) at the University of Shahid Beheshti Medical Sciences. To induce ataxia, rats were given a single i.p. 65 mg/kg dose of 3-acetylpyridine (Sigma-Aldrich) dissolved in physiological saline (Janahmadi et al., 2009). Our previous results showed that there was no significant difference between the behavioural responses in control (untreated) and vehicle (saline treated) groups (Janahmadi et al., 2009; Goudarzi et al., 2010), therefore, statistical comparison was performed between control (untreated) and 3-AP treated groups. The dose of neurotoxin was chosen based on previous studies demonstrating that it caused a severe motor impairment and induced cerebellar ataxia (Janahmadi et al., 2009). Next, the electrophysiological recordings were done at day 0 (pre-test) and one day after ataxia induction.

Electrophysiology

Animals were deeply anesthetised by inhalation of ether and then decapitated. The rat brains were rapidly removed and the cerebellar vermis was then dissected and placed in ice-cold artificial cerebrospinal fluid (ACSF) containing (in mM): 124 NaCl, 5 KCl, 1.2 KH_2PO_4, 1.3 $MgSO_4$, 2.4 $CaCl_2$, 26 $NaHCO_3$ and 10 glucose, bubbled continuously with carbogen gas (95% oxygen and 5% carbon dioxide) to adjust the pH to 7.4. Parasagittal slices (300 μm thick) were cut from the vermis of rats using a vibroslicer (752M, Campden Instruments Ltd, UK). The slices were incubated at 36°C for >30 min and then stored at room temperature.

Whole cell patch current clamp recording

After recovery (>1 h) at 22 to 25°C, a single slice was transferred to a submerged chamber mounted on the stage of an upright microscope (Olympus; BX 51W) and continuously superfused with oxygenated ACSF. The flow rate was kept at 1 to 2 ml/min using a peristaltic pump (Hugo Sachs Electronik, Ismatec, Germany). To study the intrinsic firing characteristics of Purkinje neurons, 1 mM kynurenic acid and 100 μM picrotoxin, the blockers of ionotropic glutamate (Stone, 1993) and GABA (Yoon et al., 1993) receptors, respectively, were added to the recording ACSF. Whole cell patch clamp recordings using a Multiclamp 700B amplifier (Molecular devices, Axon Instruments, Foster City, CA) in the current clamp mode were performed on the cerebellar Purkinje cells and were digitised with a Digidata computer interface (Axon Instruments). Neurons were visually identified by their shape and location using infrared differential interface contrast (IR-DIC) video microscopy with a 60x water immersion objective. The images were detected with an IR-sensitive CCD camera (Hamamatsu, ORSA, Japan) and displayed on a monitor.

Spontaneous activity was monitored with a whole cell patch pipette (resistance 3-6 MΩ) pulled from borosilicate glass using a PC-10 puller (Narishige, Japan) and filled with a solution containing (in mM): 135 potassium methyl sulphate (KMeSO4), 10 KCl, 10 HEPES, 1 $MgCl_2$, 2 Na_2ATP, and 0.4 Na_2GTP (pH 7.2, adjusted with KOH; 290 mosm). After establishment of the GΩ seal, the whole cell configuration was achieved simply by the application of a brief suction to break through the membrane. Cells with a seal < 1 GΩ before the rupture of the membrane were discarded, and the test seal function was constantly monitored throughout the recording to ensure that the seal was stable. In addition, the series resistance (typically <15 MΩ) was checked for stability during the experiments. The signals were filtered at 10 kHz and sampled at 20 kHz using Clampex 9 software (Axon Instruments).

The membrane properties and action potential parameters were measured, including the firing regularity, action potential half-width, time to peak, and after hyperpolarisation (AHP) amplitude. In the current clamp mode, the instantaneous firing frequency and spike frequency adaptation (SFA) ratio were also measured. The regularity of the firing was assessed using the coefficient of variation (CV) of the interspike intervals, which was calculated as the ratio of the standard deviation to the mean. The action potential half-width was the time difference between

the rising and falling phase of an action potential, measured at 50% of the amplitude of the spike. The instantaneous frequency was calculated as the inverse of the first interspike interval for the trains of action potential that were elicited by the injection of depolarising current pulses (1600 ms duration, 0.1 nA and 0.5 nA). The SFA ratio is equal to $F_{initial}/F_{final}$, where $F_{initial}$ is the first instantaneous frequency, calculated from the first interspike interval, and F_{final} is the final instantaneous frequency, calculated from the last interspike interval (Venance and Glowinski, 2003). The amplitude of the AHP was measured from the baseline (-60 mV) to the peak of the AHP. The resting input resistance was measured from the slope of a linear fit of the current-voltage curve of current-clamp recordings. The action potential properties were measured and compared while holding the cell's membrane potential at -60 mV.

All chemicals and drugs were obtained from Sigma (UK). Drugs were stored in a stock from which working solutions were prepared fresh every day. The doses of the drugs were chosen based on preliminary experiments using values from the literature (Daniel et al., 2004; Loewenstein et al., 2005, Moldavan et al., 2006).

Statistical Analysis

Data are presented as the mean ± SEM. Statistical comparisons were carried out using Student's *t* test or a two-way analysis of variance (ANOVA) followed by a Tukey HSD post hoc test. A $p \leq 0.05$ was considered significant.

Results

3-acetylpyridine induced profound alterations in the intrinsic membrane properties of Purkinje neurons

The present study examined how the intrinsic properties of Purkinje neurons were affected by 3-AP, a neurotoxin. Whole cell current clamp recordings were made from a total of 45 cerebellar Purkinje neurons in 14 rats; twenty-five neurons were recorded from eight control rats and twenty neurons were recorded from six rats treated with 3-AP.

In the control condition, Purkinje cells displayed a regular firing pattern (Figure 1Ai) with a mean input resistance of 73.35 ± 0.45 MΩ (Figure 2A) and mean coefficient of variation (CV) of

the interspike interval of 0.07 ± 0.008 (n=25; Figure 2B). In contrast, Purkinje neurons from 3-AP-treated rats discharged with an irregular pattern of activity, as evidenced by a significant increase in the CV (0.18 ± 0.03, n=15; Figs. 1Bi & 2B), and exhibited much larger input resistance (81.51 ± 3.96 MΩ, n=20, P<0.01; Figure 2A). The duration of the action potential at half the maximal amplitude was shortened significantly (from 0.72 ± 0.03 ms to 0.54 ± 0.001 ms, P<0.05; Figure 2C), and the time to peak was significantly increased (0.53 ± 0.02 ms in control rats and 0.65 ± 0.05 ms in 3-AP treated rats, p<0.05; Figure 2C) by 3-AP treatment. However, treatment did not affect the peak amplitude of the action potentials (52.16 ± 3.2 mV in control and 50.61± 2.7 mV in ataxic groups; Figure 2D) but did significantly reduce the AHP amplitude (-5.82 ± 0.25 mV and -4.5 ± 0.2 mV in control and ataxic groups, respectively, p<0.01; Figures 1C&2D).

Next, the evoked firing characteristics of PCs were investigated. With a membrane potential of --60 mV, trains of action potentials were elicited in both the control and ataxic conditions when the weak (0.1 nA) and strong (0.5 nA) depolarising current pulses (1600 ms duration) were injected. The reciprocal of the interspike interval (1/first ISI) between the first two action potentials was used to compare the initial instantaneous firing frequency of PCs in response to the pulses of depolarising current. In comparison to the control condition, treatment with 3-AP caused a significant increase in the initial discharge frequency during a train of action potentials that was evoked by injecting a strong depolarising pulse (0.5 nA, p<0.001, n=15, Figure 3A). The value of this parameter in control rats was 95.42 ± 5.17 Hz and 135.89 ± 6.09 Hz in the ataxic group. However, there was no statistically significant difference between the control and 3-AP-treated groups in response to weak (0.1 nA) depolarising current injections (Figure 3A).

In addition, to further quantify the effect of 3-AP treatment on the responses evoked by depolarising current injections, the spike frequency adaptation ratio was calculated. In ataxic rats, the mean SFA ratio calculated for the trains of action potentials evoked by 0.5 nA current steps was significantly lower (40%, P<0.05) than in control groups (from 2.18 ± 0.33 to 1.35 ± 0.07; Figure 3B), and therefore, the frequency of the action potential discharge was increased in Purkinje neurons from the ataxic group (Figure 3C).

Contribution of small conductance K^+ channels to intrinsic properties of cerebellar Purkinje neurons: A comparison between ataxic and control conditions

To determine the functional role of SK channels and their contribution to the intrinsic properties of PCs, the effect of UCL1684, a high-affinity blocker of SK channels (SK1-3), was assessed both in the control and ataxic conditions. In control Purkinje cells, a bath application of UCL1684 (60 nM) caused the firing pattern to become irregular (Figure 1Aii), and a marked reduction in the firing regularity was observed when the firing coefficient of variation (a useful parameter to describe the firing regularity) of PCs was compared to the control condition. This difference was evidenced by a significant increase in the CV (0.23 ± 0. 05, $p<0.001$; Figure 2B). A blockade of the SK channels also increased the membrane input resistance (77.03 ± 1.47 MΩ, $P<0.05$; Figure 2A). The firing activity in the Purkinje neurons from ataxic rats became very regular (Figure 1Bii), and the coefficient of variation of the interspike interval was significantly decreased (0.09 ± 0.01, $p<0.05$; Figure 2B); however, the mean membrane resistance remained unchanged in the presence of UCL 1684. Alterations in the parameters of the action potentials upon application of UCL 1684 were also explored. In the control condition, UCL 1684 significantly increased the time to peak of the action potentials (0.77 ± 0.04 ms, $P<0.001$, Figure 2C) and the half width of the action potentials (0.87 ± 0.02 ms, $p<0.01$; Figure 2C) but significantly decreased both the peak amplitude of the action potentials (35.41± 3.4 mV, $p<0.001$) and the AHP amplitude (-4.41 ± 0.1 mV, $p<0.001$; Figure 2D). However, the blockage of the SK channels by UCL 1684 neither significantly changed the time to peak nor affected the peak amplitude of the action potentials in the ataxic rats (Figs. 2C &D), but it did cause a significant increase in the duration of the action potentials (0.79 ± 0.002 ms, $p<0.05$; Figure 2C) and a significant reduction in the AHP amplitude (-3.29 ± 0.17 mV, $p<0.01$; Figure 2D).

Next, we examined whether UCL 1684 could differentially affect the evoked firing responses of PCs in the control and ataxic conditions. The application of UCL 1684 significantly increased the initial firing frequencies in the trains of action potentials elicited by injection with a strong depolarising current pulse (0.5 nA) but not in response to a weak (0.1 nA) current step, in both control (160.86 ± 20.1, $p<0.05$) or ataxic (159.24 ± 8.03, $p<0.05$; Figure 3A) rats.

Furthermore, UCL 1684 (60 nM) in Purkinje neurons from control but not ataxic rats significantly reduced the spike frequency adaptation (from 2.18 ± 0.33 to 1.34 ± 0.16, $p<0.05$), thereby enhancing Purkinje neuronal excitability (Figures 3B-D)

Discussion

The present study examined the functional consequences of alterations in the intrinsic properties of Purkinje neurons in an animal model of ataxia induced by neurotoxin 3-AP. The electrophysiological findings demonstrate that, in ataxic rats, plastic changes in the intrinsic electrophysiological properties of Purkinje neurons were produced. These changes were manifested as an increase in the firing irregularity that was accompanied by a significant increase in CV_{ISI}, a decrease in the time to peak and a significant decrease in the half width and the AHP amplitude. Both the decrease in the firing precision and the AHP amplitude could be attributed to the suppression of SK potassium channels (Hallworth et al., 2003). This decrease was also associated with a significant increase in the initial instantaneous frequency and a decrease in the SFA ratio in response to the strong depolarising pulse. To further determine the possible mechanism(s) underlying such alterations in the intrinsic electrophysiological behaviour of PCs, the effects of SK channel blockade on the intrinsic properties of PCs were investigated. The data of the present work indicated that the blockage of SK channels by UCL 1684 (60 nM) produced effects on control Purkinje neurons that were almost entirely distinct from those recorded in ataxic rats; the blockade of these channels disrupted the precision of the firing in control PCs, but it decreased the irregularity of the firing pattern while restoring the precision of the firing in PCs from ataxic rats, as evidenced by a significant increase and decrease in CVs, respectively. The action potential parameters and the evoked firing characteristics of control PCs appeared to be more profoundly affected by SK channel blockade than did those in the ataxic condition.

Previous studies have demonstrated that PCs are capable of firing intrinsically even in the absence of synaptic inputs (Llinás & Sugimori, 1980; Häusser &Clark, 1997; Raman and Bean, 1999; Womack & Khodakhah, 2002); however, distinct synaptic inputs are necessary for producing distinct neuronal output responses. Purkinje neurons receive two excitatory inputs from a climbing fibre and additional input from many parallel fibres. Olivary climbing fibre discharge plays an important role in regulating cerebellar function by controlling the intrinsic properties of PCs (Cerminara and Rawson, 2004; McKay et al., 2007, Janahmadi et al., 2009).

Inactivating or chemically destroying the inferior olive/climbing fibre system has been shown to result in marked modifications in the spike firing behaviour of Purkinje cells (Colin et al., 1981, Montarolo et al., 1982; Cerminara and Rawson, 2004; Janahmadi et al., 2009). In agreement with previously published data, here, we observed a marked alteration in the intrinsic excitability of Purkinje cells from ataxic rats. Neuronal intrinsic excitability plays a critical role in the transition of synaptic inputs to the particular output function; hence, alterations in the intrinsic properties of neuronal cells may profoundly affect the functioning neuronal circuits. We have recently demonstrated that abolition of the inferior olivary climbing fibre using neurotoxin 3-AP caused changes in the intrinsic firing pattern of PCs, which was associated with a decrease in the precision of firing, and that pre-treatment with combined riluzole and 3-AP restored the normal intrinsic properties (Janahmadi et al., 2009), thereby improving the rats' motor performance. It was assumed that the neuroprotective action of riluzole was due to the opening of intrinsic SK channels. It has also been previously shown that the precision of pacemaking in PCs is maintained mainly by K_{Ca} channels (Womack & Khodakhah, 2003; Womack et al., 2004); thus, reduction of K_{Ca} channel activity may be the main cause of firing irregularity in the PCs of ataxic mice (Sausbier et al., 2004; Walter et al., 2006). Nevertheless, the functional contribution of K^+ channels, particularly SK channels, to the intrinsic electrophysiological properties of PCs in the ataxic condition has not yet been fully determined.

A marked alteration in the first instantaneous firing frequency, and thus the interspike interval, in response to the strong current pulse (0.5 nA), but not a weak step, following 3-AP treatment indicates that SK channels, which are important for controlling early spike frequency adaptation, have undergone functional changes. Therefore, the effects of potent SK channels blockers on the intrinsic electrophysiological properties of PCs in control and ataxic conditions were assessed.

Possible ionic mechanisms underlying plastic changes in the intrinsic properties of PCs following 3-AP induced ataxia

Ataxia is a neurological disease characterised by a lack of balance and incoordination, and decreased neuronal firing precision may be an underlying mechanism (Shakkottai et al., 2004; Sausbier et al., 2004; Walter et al., 2006).To determine the functional role of SK channels in firing irregularity, the effects of UCL 1684, a potent SK blocker (Dunn, 1999), on intrinsic electrophysiological properties of PCs were compared between control and ataxic conditions. The blockade of SK channels induced firing irregularity, changed the action potential shape,

suppressed the AHP amplitude, increased both the half width and the firing frequency and decreased the SFA ratio in the control condition. Blockage of these channels also produced similar effects on the half width, the AHP amplitude and the initial firing frequency of PCs in the ataxic group. However, in ataxic rats, blocking of the SK channels led to an increase in the firing precision but left the SFA ratio unchanged. These findings suggest the differential contribution of SK channels to the intrinsic excitability of PCs in normal and ataxic conditions, possibly because of plastic alterations in the intrinsic properties of the PCs that occurred in the ataxic condition. Cerebellar Purkinje neurons express Ca^{2+} -activated K^{+} channels on their soma and dendrites (Gahwiler &Llano, 1989; Knaus et al., 1996; Jacquin & Gruol, 1999; Cingolani et al., 2002), which are activated by Ca^{2+} influx through P/Q type voltage gated calcium channels (Vergara et al., 1998; Womack et al., 2004). BK channels contribute to the electrophysiological properties of PCs, including action potential repolarisation and fast AHP that follows a single action potential (Edgerton & Reinhart, 2003; McKay & Turner, 2004; Womack & Khodakhah, 2003, 2004). In contrast, SK channels play a pivotal role in shaping the neuronal firing pattern (Wolfart et al., 2001; Cloues and Sather, 2003) and in regulating spike frequency adaptation (Yen et al., 1999; Pedarzani et al., 2005; Brosh et al., 2006; Vatanparast & Janahmadi, 2009) in many nerve cells, including PCs. SK channels, which are voltage-insensitive and blocked by apamin, a bee venom toxin (Hallworth et al., 2003; Sah, 1996), play a role in setting the intrinsic firing frequency (Edgerton and Reinhart, 2003) and contribute to a slow AHP current that may last several seconds following bursts of action potentials (Bowden et al., 2001; Sotcker et al., 1999; Marrion and Tavalin, 1998). These channels are active at membrane potentials that are close to the cell resting potential in mature PCs and participate in the regulation of neuronal hyperexcitability and burst firing (Haghdoost et al., 2007).

Considering the suppressive effect of UCL 1684 on the AHP amplitude, an increase in the neuronal excitability and a decrease in the regularity of firing were expected in the control group; however, in the ataxic condition, blockage of the SK channels decreased the firing regularity and induced an irregular firing pattern in the PCs. These results suggest that SK channels play an important role in regulating the firing behaviour: the dysfunction of these channels potentially may contribute to the disruption of the normal firing behaviour of PCs seen in ataxic rats. It is believed that the AHP enhances the precision of firing (Deister et al., 2009); therefore, the firing irregularity observed in the presence of UCL 1684 in control PCs could be due to the blockage of

the SK channel, which is responsible for the action potential AHP. Apamin, a selective blocker of SK channels, has also been reported to induce an irregular firing pattern in dopaminergic neurons of the substantia nigra (Lovejoy et al., 2001) and midbrain (Ji et al., 2009).

In addition, UCL 1684 significantly decreased the SFA ratio in the control condition but not in the ataxic condition, suggesting that SK channels play a significant role in affecting early spike frequency adaptation in normal PCs; this result may reflect the down-regulation of SK channels in the ataxic condition. Several mechanisms have been proposed to underlie early SFA, including a slow activation of outward currents, a slow reduction in inward currents, the summation of the AHP, and a reduction in the availability of fast Na^+ channels (Miles et al., 2005; Gu et al., 2007; Vatanparast & Janahmadi, 2009). Here a reduction in both the peak amplitude of the action potential and the AHP amplitude and an increase in the time of peak imply that a reduction in Na^+ channel availability and/or summation of the AHP could be the most likely underlying mechanisms of SFA in the control condition, but not in the ataxic condition. However, further voltage clamp study is needed to address this issue. There is also evidence supporting the involvement of the AHP in the SFA phenomenon in other cell types, where blockage of AHP conductance leads to reductions in spike frequency adaptation (Madison & Nicoll, 1984).

Conclusions

In conclusion, the present data strongly support the idea that cerebellar ataxia, induced by neurotoxin 3-AP, led to profound changes in the intrinsic properties of Purkinje neurons and altered the functional characteristics of potassium channels, possibly Ca^{2+}-activated K^+ channels, which could be the key mechanisms underlying this intrinsic plasticity. It could be proposed that such alterations might be related to the motor impairment observed in 3-AP-treated (ataxic) rats.

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Figures Legend

Fig. 1

Profound changes in the intrinsic firing properties of Purkinje neurons were induced in an animal model of ataxia by injection with 3-AP.

A(i), somatic whole cell patch clamp recording of spontaneous intrinsic firing under the control condition and A(ii) in the presence of UCL 1684 (60 nM). The regularity of firing is shown on an expanded time scale (lower traces). B(i), intrinsic spontaneous firing pattern of PCs recorded from 3-AP treated rats and B(ii) following the application of UCL 1684 (60 nM). The change in firing precision is shown on an expanded time scale (lower traces). (C-E) Superimposed traces from the control, control+UCL1684, 3-AP and 3-AP+UCL1684 conditions.

Fig. 2

UCL 1684 differentially influences the electrophysiological properties of PCs from normal and ataxic rats.

(A) Effects of 3-AP treatment and SK channel blockage on membrane input resistance. (B) Summary data of the mean coefficient of variation of spontaneous action potentials under the control condition and the 3-AP and UCL 1684 treatments. The effects of 3-AP treatment and SK channel blockage by UCL 1684 (60 nM) (C) on the half width and the time to peak of the action potential and (D) on the AHP amplitude and the peak amplitude of action potentials.
*, **,***, significantly different ($P<0.05$, $P<0.01$, $P<0.001$) from control; +, ++, significant different ($P<0.05$, $P<0.01$) from the 3-AP treated group

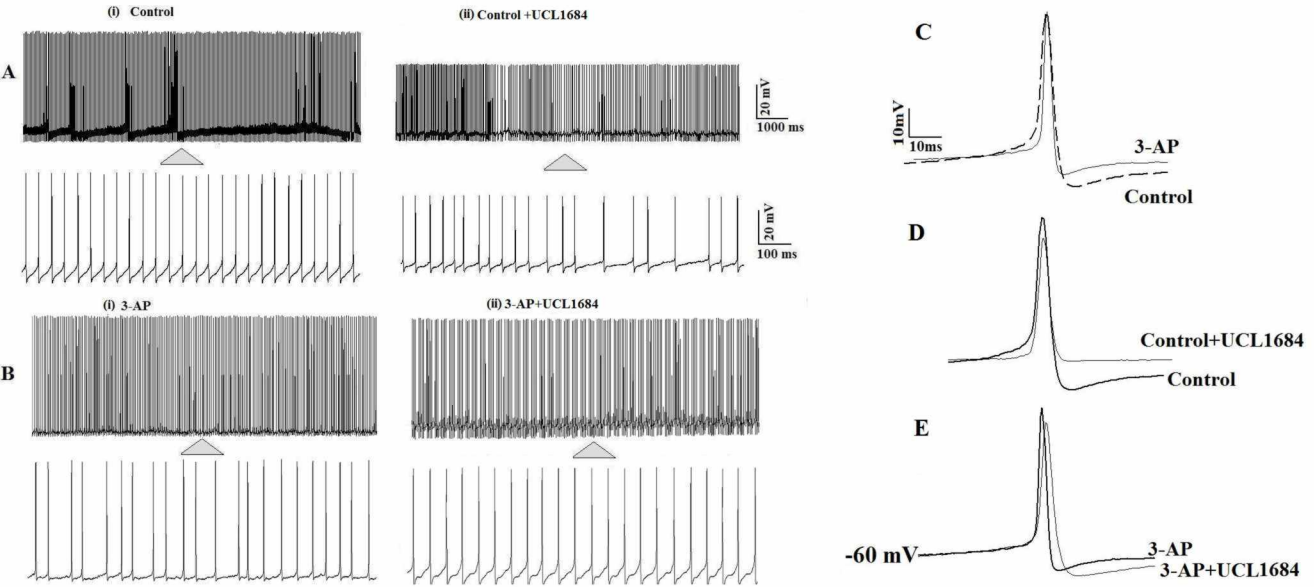
Fig. 3

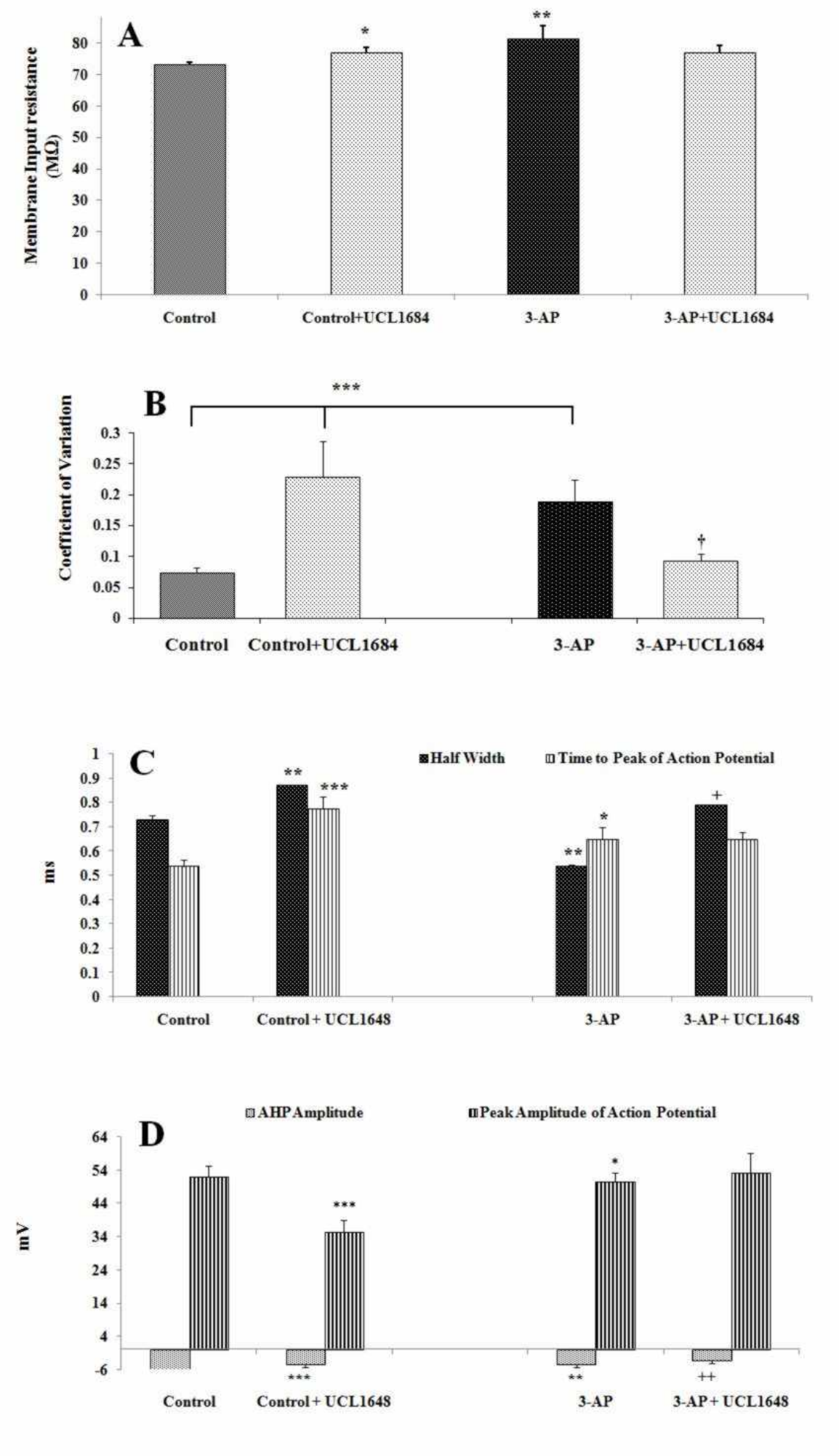
Effects of 3-AP treatment and UCL 1684 application on evoked firing responses of Purkinje neurons

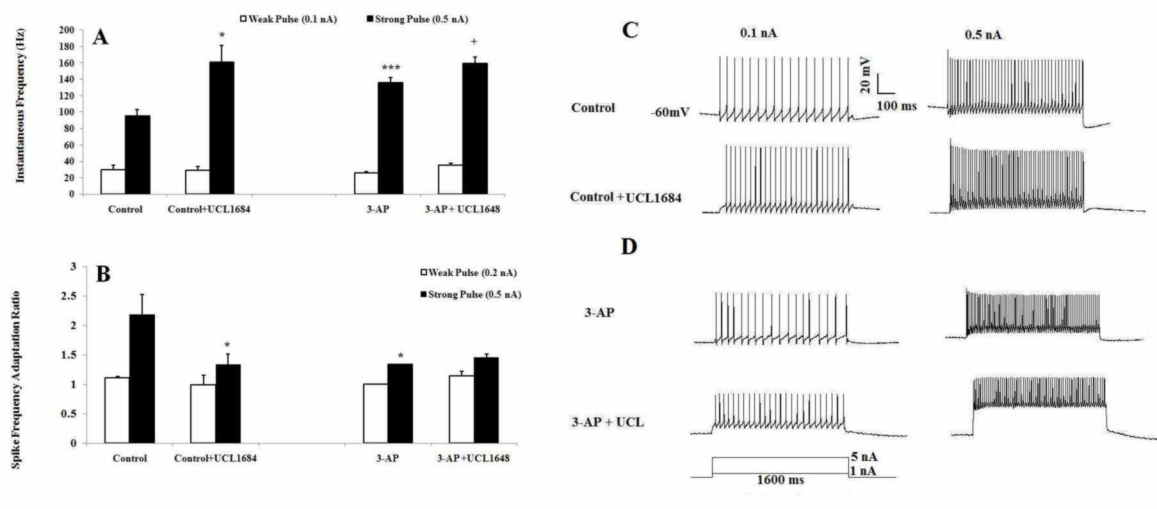
(A) Histogram showing the first instantaneous firing frequency (1/first ISI) for low and high frequency discharges evoked by weak (0.1 nA) and strong (0.5 nA) depolarising current pulses (1600 ms) , both in the control and ataxic conditions before and after UCL 1684 treatments. (B) Comparison of the spike frequency adaptation (SFA) ratio before and after a bath application of

UCL 1684 (60 nM) in the control and ataxic conditions. (C & D) Representative traces illustrating the differences in action potential firing recorded from Purkinje neurons in control and ataxic groups when elicited by depolarising current injections (0.1 nA, left and 0.5 nA, right) before and after SK channel blockage by UCL1684.

*, **, ***, significantly different ($p<0.05$, $p<0.01$, $p<0.001$) from controls; +, significant difference ($p<0.05$) from 3-AP treated (ataxic) groups.


(i) Control
(ii) Control +UCL1684
A
20 mV
1000 ms
20 mV
100 ms
(i) 3-AP
(ii) 3-AP+UCL1684
B
C
10mV
10ms
3-AP
Control
D
Control+UCL1684
Control
E
-60 mV
3-AP
3-AP+UCL1684


A
Membrane Input resistance (MΩ)
0
10
20
30
40
50
60
70
80
*
**
Control
Control+UCL1684
3-AP
3-AP+UCL1684
B
Coefficient of Variation
0
0.05
0.1
0.15
0.2
0.25
0.3
***
†
Control
Control+UCL1684
3-AP
3-AP+UCL1684
C
ms
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
Half Width
Time to Peak of Action Potential
**
***
**
*
+
Control
Control + UCL1648
3-AP
3-AP + UCL1648
D
mV
-6
4
14
24
34
44
54
64
AHP Amplitude
Peak Amplitude of Action Potential
***
***
*
**
++
Control
Control + UCL1648
3-AP
3-AP + UCL1648


A
Weak Pulse (0.1 nA)
Strong Pulse (0.5 nA)
Instantaneous Frequency (Hz)
200
180
160
140
120
100
80
60
40
20
0
*
***
+
Control
Control+UCL1684
3-AP
3-AP+ UCL1648
B
Weak Pulse (0.2 nA)
Strong Pulse (0.5 nA)
Spike Frequency Adaptation Ratio
3
2.5
2
1.5
1
0.5
0
*
*
Control
Control+UCL1684
3-AP
3-AP+UCL1648
C
0.1 nA
0.5 nA
20 mV
100 ms
Control
-60mV
Control +UCL1684
D
3-AP
3-AP + UCL
5 nA
1 nA
1600 ms