RAPID REPORT

Calcium-induced calcium release regulates action potential generation in guinea-pig sympathetic neurones

Sarah A. Locknar, Karen L. Barstow, John D. Tompkins, Laura A. Merriam and Rodney L. Parsons

Department of Anatomy and Neurobiology, University of Vermont College of Medicine, Burlington, VT 05405, USA

Experiments were done using guinea-pig sympathetic neurones dissociated from the stellate ganglia to establish whether calcium-induced calcium release (CICR) modulated action potential (AP) generation in mammalian neurones. Using measurements of intracellular calcium ($[Ca^{2+}]_i$) with the Ca^{2+}-sensitive dye fluo-3, we demonstrated that 10 mM caffeine activated ryanodine receptors and caused a rise in $[Ca^{2+}]_i$ in both Ca^{2+}-containing and Ca^{2+}-deficient solutions. We also demonstrated that combined treatment with caffeine and 1 μM thapsigargin or caffeine and 20 μM ryanodine blocked subsequent caffeine-induced elevations of $[Ca^{2+}]_i$. Treatment with thapsigargin, ryanodine or 200 μM Cd^{2+} to disrupt CICR decreased the latency to AP generation during 400 ms depolarizing current ramps using the perforated patch whole cell patch clamp in current clamp mode. Treatment with 500 μM tetraethylammonium also decreased the latency to AP generation during depolarizing current ramps in control cells, but not in cells pretreated with thapsigargin to deplete internal Ca^{2+} stores. In summary, we propose that an outward current, carried at least in part through BK channels, is activated by CICR at membrane voltages approaching the threshold for AP initiation and that this current opposed depolarizing current ramps applied to guinea-pig sympathetic stellate neurones.

(Received 10 December 2003; accepted after revision 14 January 2004; first published online 14 January 2004)
Corresponding author R. L. Parsons: Department of Anatomy and Neurobiology, University of Vermont College of Medicine, Burlington, VT 05405, USA. Email: Rodney.Parsons@uvm.edu

Calcium-induced calcium release (CICR) is a mechanism by which calcium (Ca^{2+}) influx can stimulate Ca^{2+} release from internal stores (Fabiato & Fabiato, 1975). Although first demonstrated and extensively studied in muscle cells, CICR also occurs in neurones (Marrion & Adams, 1992; Kuba, 1994; Verkhratsky & Shmigol, 1996; Cohen *et al.* 1997; Berridge, 1998). Neurones, like many other cell types, express Ca^{2+}-sensitive ryanodine receptors (RyRs) in their smooth endoplasmic reticulum (ER) membrane. Binding of Ca^{2+} to RyRs activates a Ca^{2+}-permeable channel allowing Ca^{2+} to exit from the ER and enter the cytosol (Kuba, 1994). Although CICR has been pharmacologically activated in neurones with caffeine, only a few physiological roles for CICR in neurones have been identified (Kawai & Watanabe, 1989; Sah & McLachlan, 1991; Jobling *et al.* 1993; Moore *et al.* 1998).

Previously, we demonstrated in mudpuppy parasympathetic neurones that CICR stimulates spontaneous miniature hyperpolarizations (in current clamp) and spontaneous miniature outward currents (SMOCs, in voltage clamp), which are initiated by simultaneous activation of approximately 20 large conductance, Ca^{2+}- and voltage-activated potassium (BK) channels (Merriam *et al.* 1999; Scornik *et al.* 2001). More recently, we showed that a CICR-activated mechanism regulates the latency to action potential (AP) generation during depolarizing current ramps in mudpuppy cardiac neurones (Parsons *et al.* 2002). This CICR-mediated modulation of AP latency was reduced significantly or eliminated by conditions that: (a) depleted the caffeine-sensitive intracellular Ca^{2+} stores in the ER (b) reduced Ca^{2+} influx through voltage-dependent Ca^{2+} channels (VDCCs), or (c) blocked BK channels (Parsons *et al.* 2002). We proposed that when membrane depolarizations approach the threshold for AP generation, Ca^{2+} influx through VDCCs initiates CICR. The Ca^{2+} released from internal stores raises the intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) in domains between the ER and the plasma membrane to levels high enough ($\geq$ 40 μM, Scornik *et al.* 2001) to activate membrane outward currents that decrease the effectiveness of the depolarizing current (Parsons *et al.* 2002).

© The Physiological Society 2004
DOI: 10.1113.jphysiol.2003.059485

These recent observations in amphibian autonomic neurones demonstrate a previously undefined role for CICR. CICR can act as an amplification mechanism for Ca^{2+}, activating a subpopulation of BK channels, not directly activated by Ca^{2+} influx, that act to prolong the latency to AP generation. However, it has not been reported whether this regulatory role of CICR exists in mammalian autonomic neurones. The present study, in dissociated guinea-pig stellate sympathetic neurones, demonstrates that caffeine activates RyRs and causes a rise in $[Ca^{2+}]_i$, verifying the presence of CICR. Furthermore, pharmacological disruption of CICR decreased the latency to AP generation produced by depolarizing current ramps. These observations indicated that this novel CICR-mediated mechanism can regulate AP generation in guinea-pig sympathetic stellate neurones as well.

Methods

All experiments were performed on sympathetic neurones enzymatically dissociated from the guinea-pig stellate ganglia. Guinea-pigs were killed by halothane overdose (halothane vapour from 1 ml of a 100% halothane solution) followed by exsanguination using animal procedures that were approved by the University of Vermont Institutional Animal Care and Use Committee. Experiments were performed in buffered physiological solution, at 33–35°C, containing (mM): 121 NaCl, 5.9 KCl, 2.5 $CaCl_2$, 1.2 $MgCl_2$, 26 Na-Hepes, 8 glucose, pH 7.3.

Electrophysiological methods

The perforated patch configuration of the whole cell patch clamp technique (Horn & Marty, 1988) was used to measure membrane voltage with the current clamp bridge mode of an Axoclamp 2 A/Digidata 1200/pCLAMP 6.0.3 acquisition system (Axon Instruments, Union City, CA, USA). The pipette solution contained: 140 mM potassium aspartate, 30 mM KCl, 5 mM $MgCl_2$, 10 mM Hepes-KOH, pH 7.2, and the patch pipettes were backfilled with 0.2 mg ml^{-1} amphotericin B (Sigma, St Louis, MO, USA).

To measure the latency to AP generation, 400 ms depolarizing current ramps were applied prior to and following drug treatment, with control and test results obtained from the same cell. To compare results prior to and following drug treatment, the membrane potential was electrotonically set between –55 and –60 mV, which is the range of resting membrane potentials (RMP) published for guinea-pig sympathetic neurones at physiological temperatures (Cassell *et al.* 1986). The average RMP of the dissociated stellate neurones measured following seal formation, but prior to warming to 33–35°C, was –56 ± 0.9 mV ($n = 25$ cells). All cells used had RMPs > –48 mV at 19–21°C and exhibited overshooting APs. The duration of the current ramp was maintained at 400 ms, but the rate of depolarization was adjusted under control conditions to allow for a decrease or increase in latency to the first AP. The latency was determined as the time interval from onset of the current ramp to the point at which the rising phase of the AP crossed 0 mV (Parsons *et al.* 2002). In some experiments, brief (1–2 ms) suprathreshold depolarizing current pulses were applied to generate single APs.

$[Ca^+]_i$ measurements

Changes in $[Ca^{2+}]_i$ were assessed from variations in fluo-3 fluorescence intensity (Barstow *et al.* 2004). Cells were loaded with fluo-3 AM with the AM-ester cleavage step performed in a 37°C incubator. During Ca^{2+} imaging, tetrodotoxin (TTX, 0.3 μM) was included in the bath solution to eliminate spontaneous action potential generation. Images were acquired at 0.33 Hz with a bath flow of ~1 ml min^{-1} using a Noran Oz confocal microscope (Middleton, WI, USA). An average brightness over time plot was constructed for a defined intracellular area with the plots corrected for dye bleaching using a single or double exponential decay algorithm (Microcal Origin 7.0, Northampton, MA, USA) and normalized to this decay curve to give a fluo-3 fluorescence ratio (F/F_o). Any increase in F/F_o greater than the average background fluorescence plus 4 times the standard deviation was considered to be a caffeine-induced change in fluorescence.

Drugs

All drugs were obtained from commercial sources: thapsigargin (1 μM), ryanodine (20 μM) were from Calbiochem (La Jolla, CA, USA), pluronic F-127 and fluo-3-AM were from Molecular Probes (Eugene, OR, USA); caffeine (10 mM), tetraethylammonium (TEA, 500 μM) and tetrodotoxin (0.3 μM) were from Sigma. Thapsigargin, fluo3-AM, and pluronic F-127 were diluted each day from frozen aliquots of concentrated dimethylsulfoxide (DMSO) stock solutions. For a vehicle control, DMSO was added at the final concentration to the control solution. Details about individual experiments can be found in the figure legends.

Data analysis

Control and test results were averaged from different cells and the averaged values from a number of cells were

© The Physiological Society 2004

expressed as the mean ± S.E.M. of the control or test group. Data were analysed with the Student's paired t test with $P < 0.05$ considered statistically significant.

Results

Caffeine causes a rise in $[Ca^{+}]_i$ in dissociated guinea-pig stellate neurones

Caffeine sensitizes RyRs so that Ca^{2+} can be dumped from intracellular stores at ambient $[Ca^{2+}]_i$ (McPherson *et al.* 1991) and is commonly used to test whether functional RyRs exist in neurones (Smith *et al.* 1983; Kuba, 1994). Consequently, we determined whether 10 mM caffeine caused a rise in $[Ca^{2+}]_i$ in the dissociated stellate neurones using measurements of fluo-3 fluorescence. In the initial series of experiments, the fluo-3 fluorescence ratio (F/F_o) was measured with cells maintained in normal Ca^{2+}-containing solution. A caffeine-induced increase in F/F_o was observed in 37 of 39 cells. However, the pattern of $[Ca^{2+}]_i$ increase differed between cells. In most cells (34 of 39), caffeine elicited a rapid Ca^{2+} transient that peaked within 6 s (Fig. 1*B* and *C*). This transient ranged in size from $F/F_o = 1.1$–3.2 (average $= 1.6 \pm 0.1$, Fig. 1*A*). In 17 of 34 of these cells, a second Ca^{2+} transient was seen, and in three of those cells, a third Ca^{2+} transient was

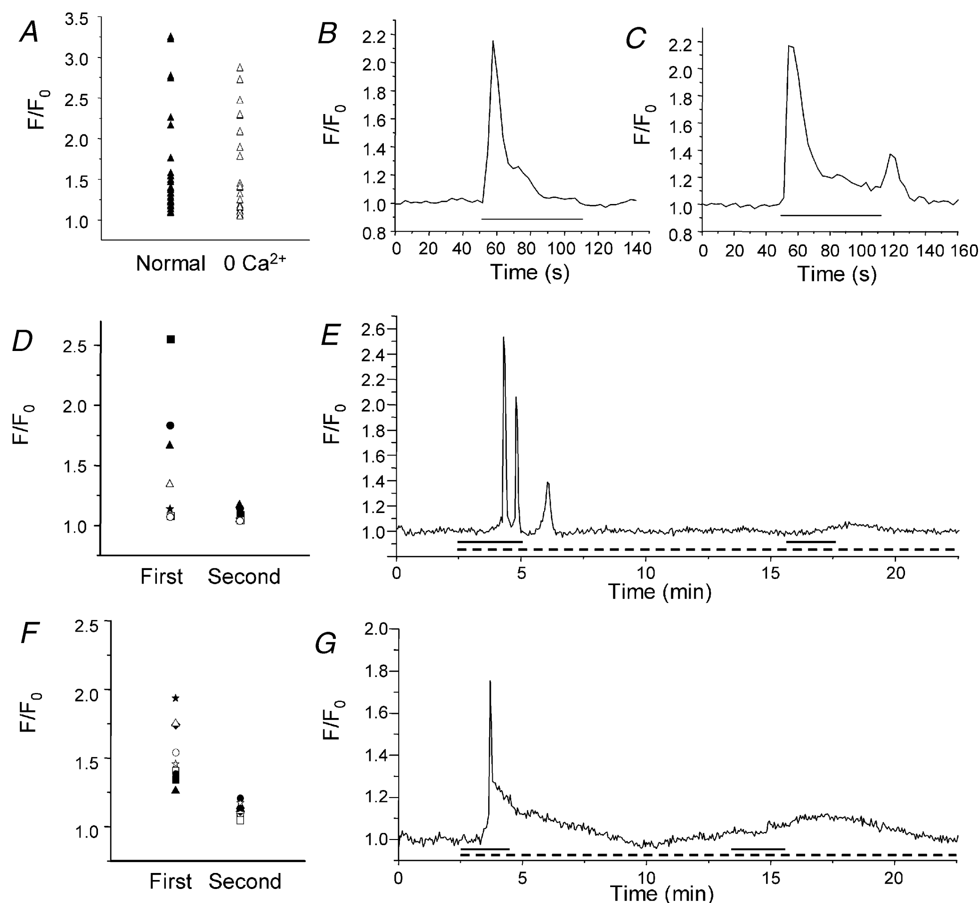

Figure 1. Caffeine induces Ca^{2+} release in guinea-pig stellate neurones
A, the peak increase in fluo-3 fluorescence (F/F_0) in response to 10 mM caffeine for cells maintained in Ca^{2+}-containing or Ca^{2+}-deficient solution. *B* and *C*, examples of $[Ca^{2+}]_i$ response to caffeine in normal Ca^{2+} solution. *B*, this cell showed both a fast response (peak) and a slow response (shoulder). *C*, another example showing two peaks with the second response arising on the shoulder during caffeine washout. *D*–*G*, $[Ca^{2+}]_i$ response to subsequent applications of 10 mM caffeine during treatment with 1 μM thapsigargin (*D*, *E*) or 20 μM ryanodine (*F*, *G*) in normal Ca^{2+} solution. The first caffeine application caused a Ca^{2+} transient whereas a second application of caffeine presented 10 min later was ineffective. *E* and *G*, example recordings illustrate caffeine-induced elevations of $[Ca^{2+}]_i$ were effectively eliminated by sustained thapsigargin (*E*) or ryanodine treatment (*G*). The continuous lines below the traces indicate the application of 10 mM caffeine. The dashed lines indicate the application of 1 μM thapsigargin (*E*) or 20 μM ryanodine (*G*).

© The Physiological Society 2004

seen. In many cells, transient responses were observed after washout of caffeine had begun (Fig. 1*C*). The other type of Ca^{2+} response developed slowly, reached a plateau and then returned to baseline. In 3 of 37 cells this was the only change in F/F_o that occurred, but it also was observed as a shoulder in 13 of 34 cells that exhibited Ca^{2+} transients (Fig. 1*B*).

Hoesch *et al.* (2001) reported for rabbit nodose neurones that caffeine activated a Ca^{2+}-permeable non-selective cationic conductance and that Ca^{2+} influx through these channels contributed to a caffeine-induced rise in $[Ca^{2+}]_i$. However, they found that brief exposure to a Ca^{2+}-deficient, Mg^{2+}-substituted solution prior to and during the caffeine challenge eliminated any rise in $[Ca^{2+}]_i$ associated with this pathway. To ensure that the caffeine-induced rise in $[Ca^{2+}]_i$ observed in guinea-pig stellate neurones was due to CICR, we tested the response to caffeine in cells that were exposed to a Ca^{2+}-deficient solution, in which Ca^{2+} was replaced with Mg^{2+}, for approximately one minute prior to and during caffeine challenge. A caffeine-induced rise in $[Ca^{2+}]_i$ was noted in 22 of 25 cells. Unlike the responses described above, all cells with a measurable caffeine response showed an initial Ca^{2+} transient (22 of 25). Once again, F/F_o for the first Ca^{2+} transient ranged widely from 1.1 to 2.9 (mean = 1.7 ± 0.1) (Fig. 1*A*). In 11 of 22 cells, a second Ca^{2+} transient was seen, and in two cells, four Ca^{2+} transients were observed. Two cells also exhibited the slow Ca^{2+} response following the transients. None of the cells exposed to the Ca^{2+}-deficient solution showed the slow Ca^{2+} response alone.

In seven other cells we tested whether treatment with thapsigargin along with caffeine effectively depleted the caffeine-sensitive Ca^{2+} stores. Thapsigargin treatment inhibits the ER Ca^{2+}-ATPase, resulting in depletion of Ca^{2+} stores (Thomas & Hanley, 1994). Application of thapsigargin and caffeine initiated a rise in intracellular Ca^{2+} similar to that described above with average F/F_o = 1.5 ± 0.2 (Fig. 1*D*). The second caffeine application produced no significant elevation of F/F_o (1.06 ± 0.01) (Fig. 1*E*). These results demonstrated that the initial caffeine–thapsigargin treatment effectively depleted caffeine-sensitive Ca^{2+} stores.

We completed similar experiments in nine additional cells to test whether exposure to caffeine and 20 μM ryanodine inhibited a subsequent caffeine-induced elevation of $[Ca^{2+}]_i$. Ryanodine in micromolar concentrations can either block the Ca^{2+} release channel or lock it open in a subconductance state (Meissner, 1994). The initial ryanodine and caffeine application initiated a Ca^{2+} response similar to those described above with average F/F_o = 1.6 ± 0.1 (Fig. 1*F*). However, the decay of the fluorescence signal was consistently prolonged following washout of caffeine. The second caffeine application produced a slowly developing increase in F/F_o with the peak value equal to 1.14 ± 0.02 (Fig. 1*G*). These results demonstrated that the initial caffeine–ryanodine treatment effectively reduced subsequent caffeine-induced Ca^{2+} release from internal stores.

The above series of experiments with thapsigargin and caffeine or ryanodine and caffeine illustrate that CICR is effectively blocked by two different methods. In the case of thapsigargin and caffeine, the intracellular Ca^{2+} stores become depleted and are unable to refill. In the case of ryanodine and caffeine, the RyRs are blocked and limit passage of Ca^{2+} from the ER into the cytoplasm. All further experiments in this study that utilize thapsigargin or ryanodine are also pretreated with 10 mM caffeine for several minutes to effectively eliminate CICR.

Treatment with thapsigargin, ryanodine or Cd^{2+} decreased the latency to AP generation

We next determined whether inhibition of CICR affected the latency to AP generation produced during depolarizing current ramps. A series of 400 ms depolarizing current ramps were applied before and during drug exposure. In the presence of thapsigargin, the latency to AP generation was decreased by 37 ± 3% (n = 5 cells; Fig. 2*A*). In the complementary set of experiments, the latency to AP generation was significantly reduced by 29 ± 3% (n = 4 cells) during treatment with ryanodine (Fig. 2*B*).

CICR is activated by Ca^{2+} influx through VDCCs (Kuba, 1994; Berridge, 1998). Treatment with the VDCC blocker Cd^{2+}, which reduces Ca^{2+} influx and thus decreases CICR, also significantly decreased the latency to AP generation in four cells by 33 ± 6% (Fig. 2*C*).

AP configuration is not altered by thapsigargin or ryanodine treatment

CICR-activated Ca^{2+}-dependent K^+ conductances can contribute to AP repolarization and/or to generation of the hyperpolarizing after potential (HAP) in autonomic neurones (Kawai & Watanabe, 1989; Sah & McLachlan, 1991; Jobling *et al.* 1993; Moore *et al.* 1998; Akita & Kuba, 2000). Consequently, we compared AP configuration prior to and after inhibition of CICR by thapsigargin and ryanodine. Neither treatment (n = 4 cells exposed to thapsigargin and three cells exposed to ryanodine) affected the configuration of the AP or HAP amplitude

© The Physiological Society 2004

in dissociated guinea-pig stellate neurones. An example recording from a thapsigargin-treated cell is shown in Fig. 3*A*. Thus, CICR-activated K^+ conductances apparently did not participate in AP repolarization or HAP generation in these neurones.

TEA decreased the latency to AP generation, but only when CICR was intact

We also tested whether activation of BK channels contributed to the CICR modulation of AP generation during depolarizing current ramps. TEA was used at a concentration of 500 μM, which should block a significant portion of the BK channels (Franciolini *et al.* 2001). First, we determined the latency to AP generation in seven cells prior to and during exposure to TEA. After 5 min in TEA, the latency to AP generation was decreased by 29 ± 8% (Fig. 4*A*). Second, we tested in six other cells whether the effect of TEA on the latency to AP generation required that CICR be intact. Following depletion of Ca^{2+} stores by thapsigargin treatment, TEA had no effect on the latency to AP generation (Fig. 4*B*). These observations demonstrated that the TEA-sensitive channels affecting latency were activated by CICR.

Since BK channels can contribute to AP repolarization in autonomic neurones (Adams & Harper, 1995), we tested whether exposure to 500 μM TEA slowed AP repolarization. TEA increased AP duration by 25 ± 7% (measured at –30 mV) in seven cells (for example, see Fig. 3*B*). This effect of TEA on AP configuration was similar to that reported for rat intracardiac neurones (Franciolini *et al.* 2001).

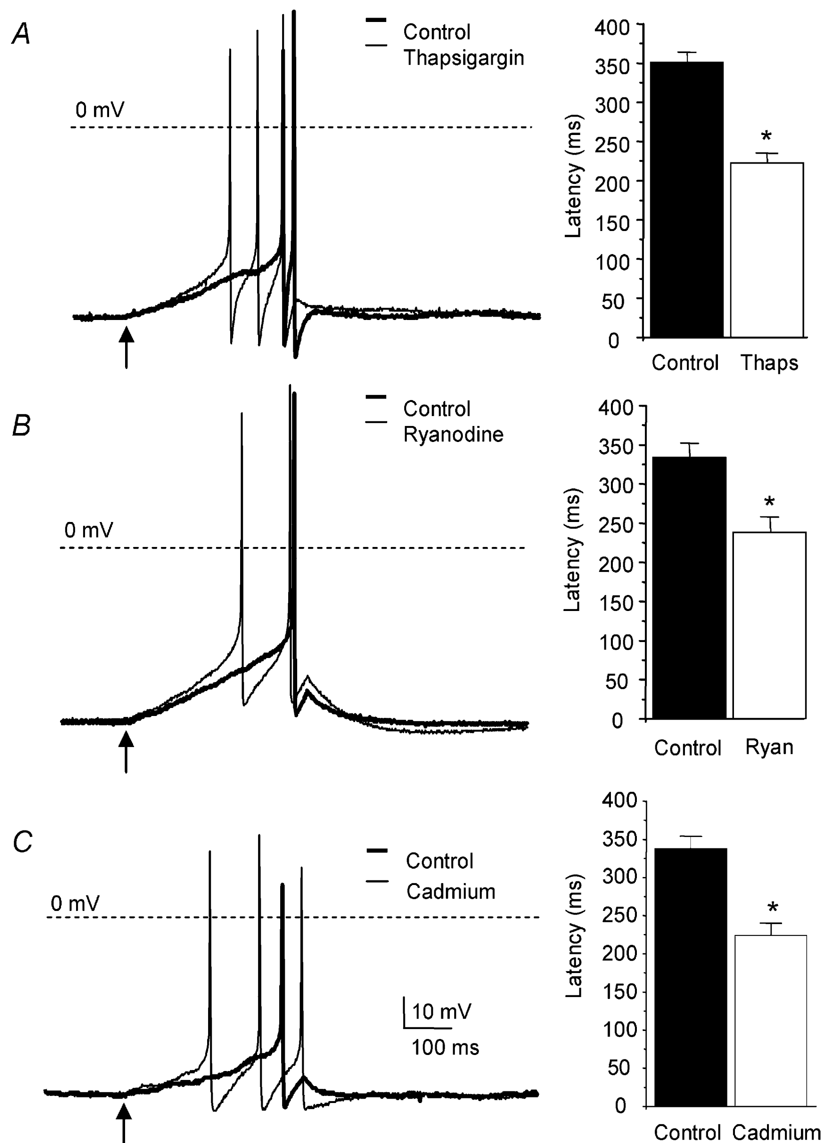

Figure 2. Disruption of CICR decreases latency to AP generation
Left panels, AP generation during 400 ms depolarizing ramps prior to (dark trace) and during pharmacological treatment (light trace) with 1 μM thapsigargin (*A*), 20 μM ryanodine (*B*) or 200 μM Cd^{2+} (*C*) to disrupt CICR. In *A* and *B*, the cells were challenged with 10 mM caffeine for 3 min during the initial period of exposure to thapsigargin or ryanodine. The arrows indicate the onset of the depolarizing ramp. Right panels, the bar graphs show the latency to AP generation before and during thapsigargin ($n = 5$; *A*), ryanodine ($n = 4$; *B*) or Cd^{2+} ($n = 6$; *C*). Asterisks indicate significant difference between control and test conditions.

© The Physiological Society 2004

To ensure that the inhibition of BK channels by TEA was not affected by thapsigargin treatment, we tested the effect of TEA on AP duration in cells pretreated with thapsigargin. TEA prolonged AP duration by 38 ± 7% (measured at –30 mV) in the six cells pretreated with thapsigargin (data not shown). Thus, an increase in AP duration in TEA occurred in both control cells and cells pretreated with thapsigargin, demonstrating that the ability of TEA to inhibit BK channels was not affected by depletion of internal Ca^{2+} stores.

Discussion

Three key observations were made in the present study. First, caffeine initiated a rise in $[Ca^{2+}]_i$ in guinea-pig stellate neurones, suggesting the presence of functional ryanodine receptors. Second, interruption of CICR decreased the latency to AP generation during depolarizing current ramps, indicating that CICR-activated outward currents can modulate neuronal excitability in guinea-pig sympathetic stellate neurones. Third, exposure to TEA decreased the latency to AP generation, but only when CICR was intact. This suggests that CICR-activated BK channels contributed to the outward current opposing the depolarizing current ramp.

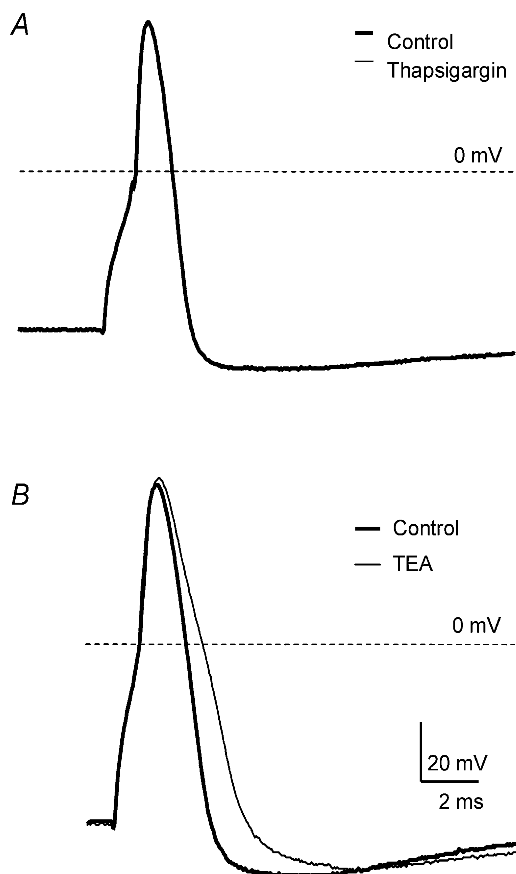

Figure 3. TEA treatment, but not thapsigargin treatment, alters AP configuration

APs recorded from 2 different cells prior to and during exposure to 1 μM thapsigargin (*A*), or 500 μM TEA (*B*). To deplete internal Ca^{2+} stores, 10 mM caffeine was present during the initial exposure to thapsigargin.

The results presented here for guinea-pig stellate neurones are similar to those reported previously for mudpuppy cardiac neurones (Parsons *et al.* 2002). With both cell types, disruption of CICR, either by depletion of internal Ca^{2+} stores or reduction of Ca^{2+} influx through VDCCs, and inhibition of BK channels decreased the latency to AP generation by a depolarizing current ramp.

Caffeine caused an increase in F/F_o, suggesting that caffeine initiated a transient rise in $[Ca^{2+}]_i$ in the majority of guinea-pig sympathetic stellate cells. Furthermore, the caffeine-induced rise in $[Ca^{2+}]_i$ was similar in Ca^{2+}-containing and Ca^{2+}-deficient solutions. We conclude that caffeine stimulated Ca^{2+} release from internal stores and that influx of Ca^{2+} through caffeine-activated ion channels in the plasma membrane did not contribute to the transient elevation of $[Ca^{2+}]_i$.

Co-application of caffeine with either thapsigargin or ryanodine caused an initial rise in $[Ca^{2+}]_i$ and substantially reduced the rise in $[Ca^{2+}]_i$ produced by a second caffeine application. Although the declining phase of the initial Ca^{2+} transient was different in cells exposed to thapsigargin or ryanodine, both procedures effectively minimized CICR. The effects of ryanodine on the release channel are concentration dependent and at lower concentrations ryanodine locks the release channel in a subconductance state (Meissner, 1994). Thus, we suggest the slow decline of the Ca^{2+} transient may represent Ca^{2+} movements through channels with reduced conductance.

In this study, we used pharmacological manipulations to inhibit CICR by three different mechanisms: (i) exposure to thapsigargin to deplete internal Ca^{2+} stores, (ii) exposure to ryanodine to block the release channel and/or deplete internal Ca^{2+} stores and (iii) exposure to Cd^{2+} to block Ca^{2+} influx through VDCCs. All three decreased the latency to AP generation during depolarizing current ramps. Thus, we suggest that disruption of CICR eliminated an outward current that normally opposed the depolarizing ramp current in untreated cells.

Previously, other investigators have reported in different autonomic neurones that CICR-activated K^+ conductances can contribute to AP repolarization and/or to generation of the HAP (Kawai & Watanabe, 1989; Sah & McLachlan, 1991; Jobling *et al.* 1993; Moore

© The Physiological Society 2004

et al. 1998; Akita & Kuba, 2000). However, neither thapsigargin nor ryanodine treatment affected the rate of AP repolarization or HAP amplitude in the dissociated guinea-pig stellate neurones. Thus, CICR-activated Ca^{2+}-dependent K^+ conductances apparently did not participate in AP repolarization or HAP generation in these neurones.

Previously, in mudpuppy parasympathetic cardiac neurones we found that inhibition of BK channels by iberiotoxin reduced the latency to AP generation during depolarizing current ramps (Parsons *et al.* 2002). We concluded that BK currents contributed to the CICR-activated outward current that opposed the depolarizing ramp in these amphibian neurones. The present results with TEA, a specific blocker of BK channels at low concentrations, suggest that a CICR-activated BK current also contributed to the outward current opposing the depolarizing ramp current in guinea-pig stellate neurones. This effect of TEA on the latency to AP generation was eliminated when CICR was disrupted by thapsigargin. Thus, the rise in $[Ca^{2+}]_i$ due to Ca^{2+} influx through VDCCs when the cells were depolarized to the AP threshold was insufficient to directly activate BK currents that opposed the depolarizing ramp current. Rather, rises of $[Ca^{2+}]_i$ in local domains near BK channels mediated by CICR apparently were required. This mechanism is comparable to that thought to occur in the mudpuppy parasympathetic neurones (Parsons *et al.* 2002). In contrast, the BK channels that are involved in AP repolarization were activated directly by Ca^{2+} influx through nearby VDCCs (Adams *et al.* 1982; MacDermott & Weight, 1982) because the TEA-induced slowing of AP repolarization occurred in cells pretreated with thapsigargin to deplete internal Ca^{2+} stores.

Conclusions and implications

Many neurones, including mammalian intracardiac neurones (Xi-Moy & Dun, 1995; Cuevas *et al.* 1997),

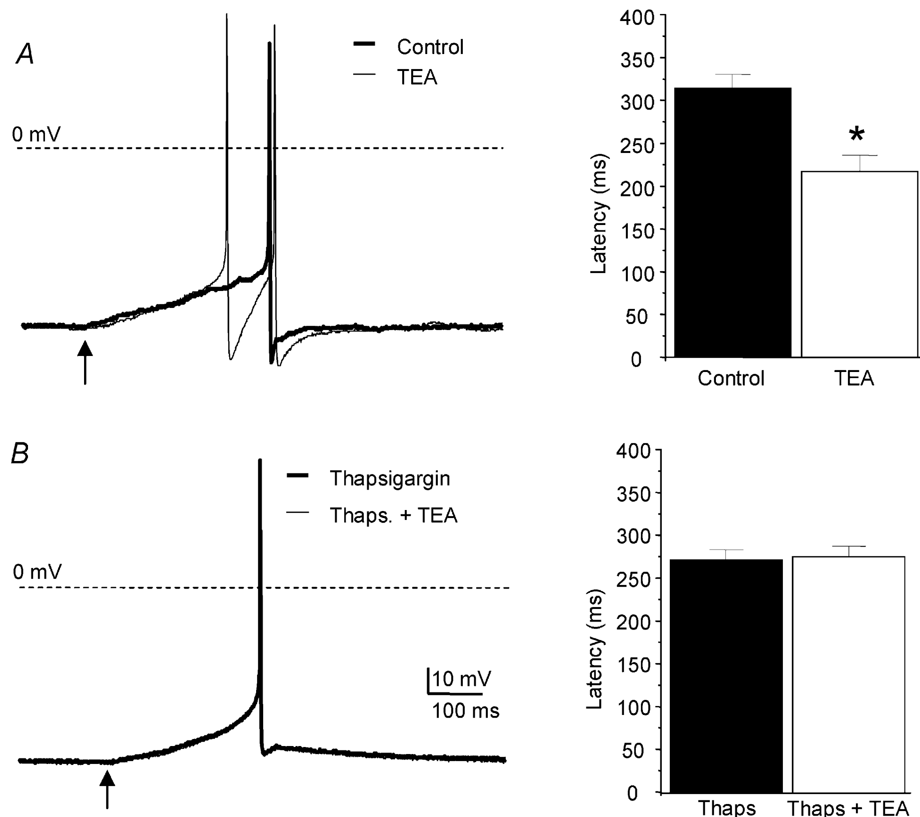

Figure 4. TEA-induced decrease in the latency to AP generation requires CICR
Left panels, AP generation during 400 ms depolarizing ramps prior to (dark trace) and during treatment with 500 μM TEA (light trace). *A*, latency to AP generation is decreased following treatment with TEA. *B*, TEA did not affect the latency to AP generation in cells that were pretreated with 1 μM thapsigargin and 10 mM caffeine to deplete internal Ca^{2+} stores. Right panels, the bar graphs show the average latency to AP generation before and during TEA application ($n = 7$; *A*) and before and during TEA application for 6 cells pretreated with thapsigargin (*B*). The asterisk indicates significant difference between control and test conditions.

© The Physiological Society 2004

express the M-current, which is a non-inactivating, depolarization-activated K^+ conductance that opposes depolarizing stimuli and regulates excitability (Brown, 1988). Preliminary results from our lab using guinea-pig intracardiac neurones showed that CICR inhibition by thapsigargin–caffeine treatment did not affect the latency to AP generation during depolarizing ramps (n = 5 cells, K. L. Barstow & R. L. Parsons, unpublished observations). Neither guinea-pig stellate cells (Gilbert *et al.* 1998) nor mudpuppy cardiac neurones (R. L. Parsons and L. A. Merriam, unpublished observations) appear to express M-currents or express M-currents in only a small subpopulation of cells. We suggest that in these two neurone types a CICR-activated outward current, rather than a depolarization-activated M-current, modulates the efficiency of depolarizing current ramps to generate APs. CICR activation of outward currents near the threshold potential for AP generation may therefore be either an alternative or additional mechanism whereby neuronal excitability is modulated.

References

Adams PR, Constanti A, Brown DA & Clark RB (1982). Intracellular Ca^{2+} activates a fast voltage-sensitive K^+ current in vertebrate sympathetic neurones. *Nature* **296**, 746–749.

Adams DJ & Harper AA (1995). Electrophysiological properties of autonomic ganglion neurons. In *The Autonomic Nervous System*, ed. Burnstock G, pp. 153–212, Harwood Academic Publishers, Reading, UK.

Akita T & Kuba K (2000). Functional triads consisting of ryanodine receptors, Ca^{2+} channels, and Ca^{2+}-activated K^+ channels in bullfrog sympathetic neurons. Plastic modulation of action potential. *J General Physiol* **116**, 697–720.

Barstow KL, Locknar SA, Merriam LA & Parsons RL (2004). The modulation of action potential generation by calcium-induced calcium release is enhanced by mitochondrial inhibitors in mudpuppy parasympathetic neurons. *Neuroscience* (in press).

Berridge MJ (1998). Neuronal calcium signaling. *Neuron* **21**, 13–26.

Brown D (1988). M-currents: an update. *Trends Neurosci* **11**, 294–299.

Cassell JF, Clark AL & McLachlan EM (1986). Characteristics of phasic and tonic sympathetic ganglion cells of the guinea-pig. *J Physiol* **372**, 457–483.

Cohen AS, Moore KA, Bangalore R, Jafri MS, Weinreich D & Kao JPY (1997). Ca^{2+}-induced Ca^{2+} release mediates Ca^{2+} transients evoked by single action potentials in rabbit vagal afferent neurones. *J Physiol* **499**, 315–328.

Cuevas J, Harper AA, Trequattrini C & Adams DJ (1997). Passive and active membrane properties of isolated rat intracardiac neurons: regulation by H- and M-currents. *J Neurophysiol* **78**, 1890–1902.

Fabiato A & Fabiato F (1975). Contractions induced by a calcium-triggered release of calcium from the sarcoplasmic reticulum of single skinned cardiac cells. *J Physiol* **249**, 469–495.

Franciolini F, Hogg R, Catacuzzeno L, Petris A, Trequattrini C & Adams DJ (2001). Large-conductance calcium-activated potassium channels in rat neonatal intracardiac ganglion neurons. *Pflugers Arch* **441**, 629–638.

Gilbert R, Ryan JS, Horackova M, Smith FM & Kelly ME (1998). Actions of substance P on membrane potential and ionic currents in guinea pig stellate ganglion neurons. *Am J Physiol* **274** (*Cell Physiol* **43**), C892–C903.

Hoesch RE, Weinreich D & Kao JP (2001). A novel Ca^{2+} influx pathway in mammalian primary sensory neurons is activated by caffeine. *J Neurophysiol* **86**, 190–196.

Horn R & Marty A (1988). Muscarinic activation of ionic currents measured by a new whole-cell recording method. *J General Physiol* **92**, 145–159.

Jobling P, McLachlan EM & Sah P (1993). Calcium induced calcium release is involved in the afterhyperpolarization in one class of guinea pig sympathetic neuron. *J Auton Nerv System* **42**, 251–258.

Kawai T & Watanabe M (1989). Effects of ryanodine on the spike after-hyperpolarization in sympathetic neurones of the rat superior cervical ganglion. *Pflugers Arch* **413**, 470–475.

Kuba K (1994). Ca^{2+}-induced Ca^{2+} release in neurones. *Jpn J Physiol* **44**, 613–650.

MacDermott AB & Weight FF (1982). Action potential repolarization may involve a transient, Ca^{2+}-sensitive outward current in a vertebrate neurone. *Nature* **300**, 185–188.

McPherson PS, Kim Y-K, Valdivia H, Knudson CM, Takekura H, Franzini-Armstrong C, Coronado R & Campbell KP (1991). The brain ryanodine receptor: a caffeine-sensitive calcium release channel. *Neuron* **7**, 17–25.

Marrion NV & Adams PR (1992). Release of intracellular calcium and modulation of membrane currents by caffeine in bull-frog sympathetic neurones. *J Physiol* **445**, 515–535.

Meissner G (1994). Ryanodine receptor/Ca^{2+} release channels and their regulation by endogenous effectors. *Annu Rev Physiol* **56**, 485–508.

Merriam LA, Scornik FS & Parsons RL (1999). Ca^{2+}-induced Ca^{2+} release activates spontaneous miniature outward currents (SMOCs) in parasympathetic cardiac neurons. *J Neurophysiol* **82**, 540–550.

Moore KA, Cohen AS, Kao JPY & Weinreich D (1998). Ca^{2+}-induced Ca^{2+} release mediates a slow post-spike hyperpolarization in rabbit vagal afferent neurons. *J Neurophysiol* **79**, 688–694.

© The Physiological Society 2004

Parsons RL, Barstow KL & Scornik FS (2002). Spontaneous miniature hyperpolarizations affect threshold for action potential generation in mudpuppy cardiac neurons. *J Neurophysiol* **88**, 1119–1127.

Sah P & McLachlan EM (1991). Ca^{2+}- activated K^+ currents underlying the afterhyperpolarization in guinea pig vagal neurons: a role for Ca^{2+}-activated Ca^{2+} release. *Neuron* **7**, 257–264.

Scornik FS, Merriam LA & Parsons RL (2001). Number of K_{Ca} channels underlying spontaneous miniature outward currents (SMOCs) in mudpuppy cardiac neurons. *J Neurophysiol* **85**, 54–60.

Smith SJ, MacDermott AB & Weight FF (1983). Detection of intracellular Ca^{2+} transients in sympathetic neurones using arsenazo III. *Nature* **304**, 350–352.

Thomas D & Hanley MR (1994). Pharmacological tools for perturbing intracellular calcium storage. In *A Practical Guide to the Study of Calcium in Living Cells*, ed. Nuccitelli R, pp. 65–89. Academic Press, New York.

Verkhratsky A & Shmigol A (1996). Calcium-induced calcium release in neurones. *Cell Calcium* **19**, 1–14.

Xi-Moy SX & Dun NJ (1995). Potassium currents in adult rat intracardiac neurons. *J Physiol* **486**, 15–31.

Acknowledgements

This work was supported by NIH grant HL65481 and NSF Grant IBN-007641. The Noran Laser Scanning confocal microscope is housed in the Imaging Core supported by NIH COBRE Grant 1 P20 RR-16435. JDT is supported by NIH Training Grant HL07594.

© The Physiological Society 2004