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# Effects of caffeine and 3-isobutyl-1-methylxanthine on voltage-activated potassium currents in vertebrate neurones and secretory cells

Michelle A. Reiser, Theresa D'Souza & [1]Stuart E. Dryer

Program in Neuroscience, Department of Biological Science, Florida State University, Tallahassee, FL 32306-4075, USA

**1** The effects of caffeine and 3-isobutyl-1-methylxanthine (IBMX) on voltage-activated $K^+$ currents were examined by use of patch clamp recording techniques in dissociated chick autonomic ganglion neurones, chick pineal cells and rat anterior pituitary cells.

**2** In chick ciliary ganglion neurones, caffeine (0.1-10 mM) produced a robust blockade of delayed rectifier $K^+$ currents ($I_{DR}$). Blockade was rapid in onset and concentration- and voltage-dependent. Caffeine produced greater inhibition with larger depolarizing voltage pulses. Similar inhibition of $I_{DR}$ was observed in excised outside-out 'maxi-patches' indicating a direct effect on the $K^+$ channels. Caffeine also inhibited $I_{DR}$ in chick sympathetic neurones, chick pineal cells and rat anterior pituitary cells.

**3** Application of 10 mM caffeine caused inhibition of transient A-currents ($I_A$) in chick ciliary ganglion neurones. Inhibition of $I_A$ was voltage-dependent with greater inhibition observed at more positive command potentials. Application of 1 mM caffeine did not cause inhibition of $I_A$.

**4** Application of 1 mM IBMX, a structural analogue of caffeine, caused inhibition of $I_{DR}$ and $I_A$ in chick ciliary ganglion neurones. The voltage-dependence of the inhibition of both currents was qualitatively different from that observed with caffeine. The inhibitory effects of 1 mM IBMX and 10 mM caffeine on $I_{DR}$ and $I_A$ were additive.

**5** Direct inhibition of voltage-activated $K^+$ currents can potentially produce significant secondary effects on intracellular free $Ca^{2+}$. These results indicate that caution must be used in the design and interpretation of experiments in which millimolar concentrations of caffeine or IBMX are used in pharmacological studies of intracellular $Ca^{2+}$dynamics or other second messenger mechanisms.

**Keywords:** Caffeine; calcium stores; potassium currents; phosphodiesterase

## Introduction

The methylxanthines caffeine and 3-isobutyl-1-methylxanthine (IBMX) are widely used as experimental tools in pharmacological studies. There are four classes of caffeine actions that have been extensively described in the literature: caffeine is an antagonist of adenosine receptors, it is an inhibitor of certain cyclic nucleotide phosphodiesterases, and it modifies release of $Ca^{2+}$ from ryanodine- and inositol phosphate ($IP_3$)-sensitive intracellular stores (reviewed in Daly, 1993; Pozzan *et al.*, 1994; Erlich *et al.*, 1994). Caffeine has also been shown to inhibit agonist-stimulated $IP_3$ formation in some systems (Erlich *et al.*, 1994). These effects occur at different concentrations. Thus, millimolar concentrations of caffeine stimulate $Ca^{2+}$ release from ryanodine-sensitive internal stores (Erlich *et al.*, 1994). By contrast, inhibition of phosphodiesterase and blockade of adenosine receptors occur at 10 and 100 fold lower concentrations, respectively (Daly, 1993). The most common laboratory use of caffeine in recent years has been to probe the mechanisms controlling intracellular $Ca^{2+}$ dynamics, often in cultured or acutely isolated cells (Pozzan *et al.*, 1994; Erlich *et al.*, 1994). IBMX is a structurally related methylxanthine that shares some of the actions of caffeine (Daly, 1993). This compound is widely used as an inhibitor of various cyclic nucleotide phosphodiesterases. IBMX also interacts with certain adenosine receptors (Daly, 1993) but is not generally used to alter mobilization of intracellular $Ca^{2+}$ stores.

Most studies of internal $Ca^{2+}$ mobilization have used high caffeine concentrations, typically 5-20 mM. Because of this, it is reasonable to ask whether this drug has other actions that might confound the interpretation of at least some of these experiments. In this regard, there are data showing that millimolar concentrations of caffeine can inhibit voltage-dependent $K^+$ currents in mammalian ventricular myocytes (Sanchez-Chapula, 1992; Varro *et al.*, 1993) and vascular smooth muscle (Noack *et al.*, 1990) as well as voltage-activated $Na^+$ channels in ventricular myocytes (Habuchi *et al.*, 1991). To date, there have been no studies describing direct effects of caffeine on voltage-activated $K^+$ channels in neurones or secretory cells, even though this compound is widely used in studies of those cell types (reviewed by Pozzan *et al.*, 1994). However, millimolar concentrations of IBMX can inhibit a voltage-activated and tetrathylammonium-sensitive $K^+$ current in rat sensory neurones (Usachev *et al.*, 1995).

In the present study, we have characterized the effects of the methylxanthines caffeine and IBMX on voltage-activated $K^+$ currents in selected vertebrate neurones and secretory cells. The results indicate that both methylxanthines cause a direct inhibition of multiple voltage-activated $K^+$ currents in several cell types. Because these actions could cause significant secondary effects on intracellular $Ca^{2+}$ regulation, they need to be considered in the design and interpretation of experiments in which these compounds are used to probe second messenger dynamics.

## Methods

### *Cell isolation*

Embryonic chick ciliary and lumbar sympathetic neurones and pineal cells were isolated as described previously (Henderson & Dryer, 1992; Wisgirda & Dryer, 1994). Briefly, ciliary ganglia were dissected from embryonic day 13 (E13) chick embryos, lumbar sympathetic ganglia were dissected at E18, and pineal

[1] Author for correspondence.

glands were dissected at E20–21. Tissues were digested with collagenase, dissociated by 8–12 passes through a fire-polished sterile Pasteur pipette, and plated onto sterile poly-D-lysine-coated glass coverslips. Recordings were made within 4 h of plating. Cultures of rat anterior pituitary cells were prepared as described in detail elsewhere (Gorospe & Freeman, 1985). Briefly, anterior pituitary glands from randomly cycling female rats were digested with hyaluronidase and collagenase, dissociated by gentle trituration with a Pasteur pipette, rinsed, and further digested with pancreatin. Cells were washed, resuspended in cell culture medium, and plated onto poly-D-lysine-coated glass coverslips. Cells were maintained for 2–3 days in a sterile $CO_2$ incubator at 37°C prior to use in electrophysiological experiments.

### *Electrophysiology*

Whole-cell recordings were made at room temperature as described elsewhere (Wisgirda & Dryer, 1993). Electrodes were filled with a sterile solution consisting of (mM): KCl 120, $MgCl_2$ 2, HEPES/KOH 10, EGTA 10, and leupeptin 0.1 at a pH of 7.4. Pipette resistance was 2–6 MΩ. It was possible to compensate up to 90% of this series resistance without introducing oscillations into the current output of the clamp amplifier. Voltage commands and data analysis were performed by use of a personal computer equipped with commercially available software (PCLAMP, Axon Instruments). Cells were mounted in a 300 μl chamber and perfused at a 1.5–2.0 ml $min^{-1}$ in a $Ca^{2+}$-free saline consisting of (mM): NaCl 145, KCl 5.4, $MgCl_2$ 6.2, HEPES/NaOH 13, D-glucose 5 and tetrodotoxin 250 nM, pH 7.4. For experiments on $I_{DR}$, a series of 500 ms depolarizing steps were applied from a holding potential of −60 mV (Wisgirda & Dryer, 1993). The amplitude of $I_{DR}$ was measured at the end of the voltage steps. $I_A$ was always evoked from a holding potential of −120 mV in the presence of 10 mM external tetraethylammonium (TEA), which served to reduce contributions from $I_{DR}$. The amplitude of $I_A$ was determined at the peak of the current. All whole-cell currents were leak-subtracted (Wisgirda & Dryer, 1993). In some experiments, excised outside-out 'maxi' patches were obtained by slowly withdrawing the recording pipette after achieving a stable whole-cell recording and depolarizing pulses were applied from a holding potential of −60 mV (Dryer, 1991). Pseudo-macroscopic records were then obtained by averaging the responses to 4–8 depolarizing steps. These records were not leak subtracted. Methylxanthines were dissolved in normal bath saline and applied to cells or excised patches by whole-bath gravity fed perfusion from individual reservoirs controlled by valves. The dead-time of the perfusion system was 30–50 s. Caffeine, IBMX and tetrodotoxin were obtained from Sigma Chemical Company (St. Louis, MO, U.S.A.).

## Results

### *Effects of caffeine on $I_{DR}$ in chick ciliary ganglion neurones*

As described previously (Wisgirda & Dryer, 1993) application of depolarizing voltage steps from a holding potential of −60 mV in $Ca^{2+}$-free external salines containing 250 nM te-

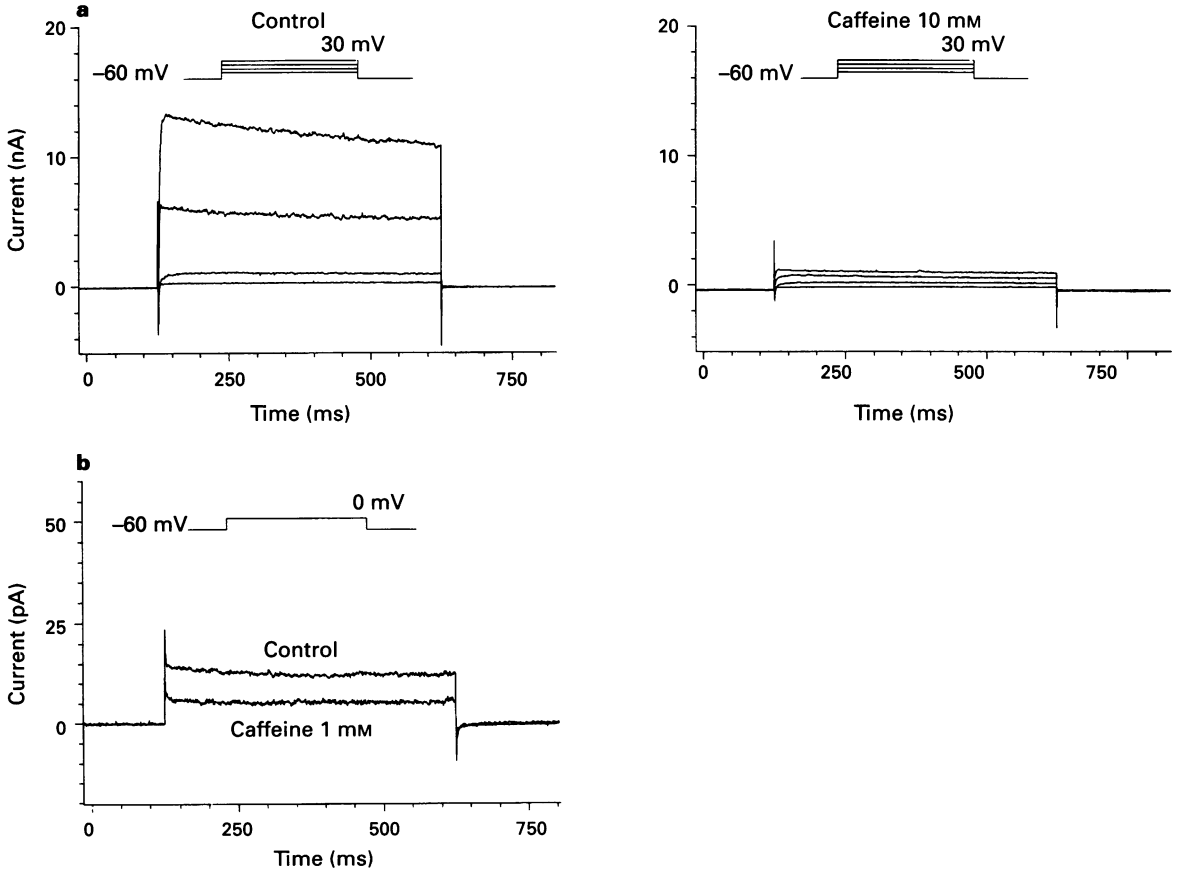


**Figure 1** Effects of caffeine on delayed rectifier $K^+$ currents in chick ciliary ganglion neurones. (a) Families of whole-cell currents evoked by depolarizing voltage steps from a holding potential of −60 mV. Left, currents evoked in normal external saline. Right, currents evoked after 60 s exposure to saline containing 10 mM caffeine. In this and subsequent figures, voltage protocols are shown as insets above the current traces. Note large reduction of slowly inactivating outward currents ($I_{DR}$). (b) Currents recorded in large excised outside-out patch. Traces shown are the average of five pseudomacroscopic currents. Currents were evoked before and after application of 1 mM caffeine.

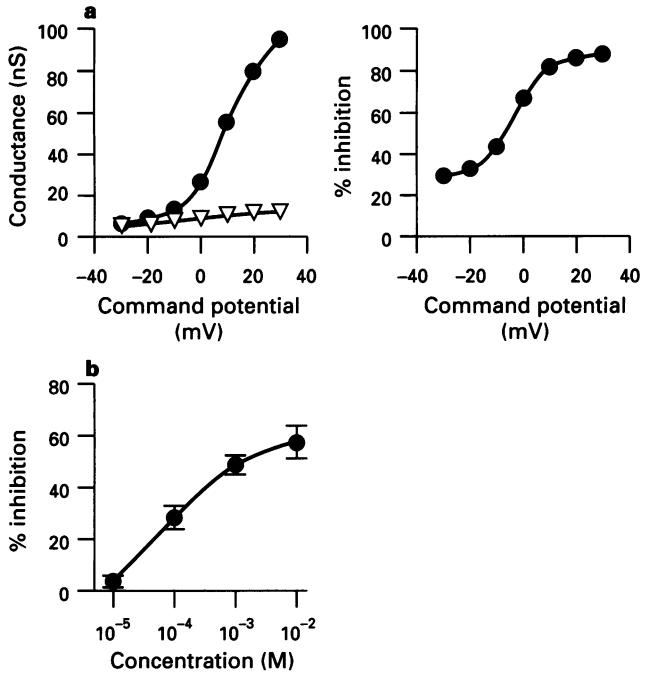


**Figure 2** Voltage- and concentration-dependence of caffeine effects on $I_{DR}$ in chick ciliary ganglion neurones. (a) Voltage-dependence of caffeine blockade. Data from cell shown in Figure 1a. Currents were measured at the end of the depolarizing voltage steps. Left, activation curves for $I_{DR}$ in the absence (●) and presence (△) of 10 mM caffeine. Curves are spline fits to the data. Right, inhibition of $I_{DR}$ plotted against command potential. (b) Plot of mean $I_{DR}$ inhibition vs. caffeine concentration in nine ciliary ganglion neurones. Vertical lines represent s.e.mean.

trodotoxin evoked large slowly inactivating outward currents ($I_{DR}$) in ciliary ganglion neurones. Currents measured at the end of the test pulse to +30 mV were markedly inhibited (50–90%) by bath application of 10 mM caffeine (Figure 1a). Inhibition was maximal in 0.5–2 min, and partial or full recovery was seen with 5–8 min of washing (not shown). Blockade of $I_{DR}$ could also be observed in excised outside-out 'maxi' patches (Figure 1b). The traces shown are the average of currents evoked by four steps to 0 mV from a holding potential of −60 mV. With this recording configuration, $K^+$ channels are isolated from soluble cytoplasmic regulatory molecules, suggesting a direct effect of caffeine on the channel molecules or associated subunits. It was not possible to discern the effects of caffeine on single $I_{DR}$ channels in excised maxi-patches because multiple channels were invariably present and discrete unitary openings were difficult to resolve. The effects of caffeine on whole-cell $I_{DR}$ were voltage-dependent, as a greater percentage of the current was inhibited with larger depolarizing voltage steps over a range of −30 to +30 mV (Figure 2a). Blockade of $I_{DR}$ was also observed in the presence of 5 mM external $Ca^{2+}$ (not shown). For those experiments, $Ca^{2+}$-activated $K^+$ currents were blocked by addition of 100 μM $Cd^{2+}$ to the external salines. The effects of caffeine on $I_{DR}$ were concentration-dependent (Figure 2b). Mean inhibition of close to 30% (measured at the end of the depolarizing voltage steps) was produced by 100 μM caffeine at command potentials of +30 mV, and inhibition increased at higher concentrations. Maximum blockade occurred with 10 mM caffeine. Thus, in ciliary ganglion neurones, caffeine is a more potent inhibitor of $I_{DR}$ than tetraethylammonium (TEA), which produces half-maximal blockade at 3–5 mM. Complete inhibition was never observed at any caffeine concentration tested. Application of 10 μM caffeine did not produce significant inhibition of $I_{DR}$ in chick ciliary ganglion neurones (Figure 2b).

### *Actions of caffeine on $I_{DR}$ in other cell types*

Caffeine inhibited $I_{DR}$ in three other cell types, including a second closely related population of neurones and two types of nonneuronal secretory cells. Application of 10 mM caffeine caused blockade of whole-cell $I_{DR}$ in dissociated chick sympathetic ganglion neurones, similar to that shown above for parasympathetic ciliary ganglion neurones (not shown). Application of 1–10 mM caffeine also caused a marked inhibition of $I_{DR}$ in rat cultured anterior pituitary cells, which are excitable (Figure 3a), and in nonexcitable chick pineal cells (Figure 3b). The characteristics of caffeine blockade in all three cell types were similar to those described above for ciliary ganglion neurones, even though there were differences in the kinetics of $I_{DR}$ in the different cell types. Moreover, in all cell types tested, substantial inhibition of $I_{DR}$ occurred at concentrations at or below those typically used to induce $Ca^{2+}$ release from ryanodine-sensitive internal stores. Caffeine never caused complete inhibition of $I_{DR}$ at any concentration or command potential tested.

### *Effects of caffeine on $I_A$ in chick ciliary ganglion neurones*

Chick ciliary ganglion neurones express a large tetraethylammonium (TEA)-resistant transient A-current that is easily observed when depolarizing pulses are applied from more negative holding potentials (Wisgirda & Dryer, 1993). For these experiments, $I_A$ was evoked from a holding potential of −120 mV in $Ca^{2+}$-free external saline containing 10 mM external TEA. Application of 10 mM caffeine caused a significant inhibition of peak $I_A$, although the mean inhibition at +30 mV (25±7%, mean±s.e.mean, $n=9$ cells) was somewhat less than that observed for $I_{DR}$ (55±7%, $n=9$ cells) at the same caffeine concentration (Figure 4a). Complete inhibition of $I_A$ was never observed and caffeine did not alter the time course of $I_A$ inactivation. As with $I_{DR}$, blockade of $I_A$ by 10 mM caffeine was voltage-dependent, with a greater percentage inhibition observed at more positive command potentials (Figure 4b). Application of 1 mM or 100 μM caffeine did not produce significant blockade of $I_A$ in chick ciliary ganglion neurones at any command potential (not shown). The effects of other concentrations of caffeine were not examined.

### *Effects of IBMX on voltage-activated $K^+$ currents in ciliary ganglion neurones*

Application of 1 mM IBMX caused inhibition of $I_{DR}$ (Figure 5a) and $I_A$ (Figure 5b) in chick ciliary ganglion neurones. Inhibition was rapid in onset (0.5–1.5 min) and partial recovery was observed with 5–8 min of washing. Mean $I_{DR}$ inhibition at the end of a test pulse to +30 mV was 27±6% ($n=9$ cells) and mean inhibition of peak $I_A$ measured at +30 mV was 28±8% ($n=8$ cells). IBMX did not alter the time course of $I_A$ inactivation. Application of 100 μM IBMX did not cause significant inhibition of either $I_A$ or $I_{DR}$ (not shown). IBMX precipitates from physiological salines at concentrations above 1 mM. The voltage-dependence of blockade of $I_{DR}$ and $I_A$ by 1 mM IBMX was qualitatively different from that produced by caffeine. In the case of $I_{DR}$, the percentage inhibition was a complex function of command potential (Figure 5c). The largest inhibition was consistently observed with either small or large depolarizing voltage steps. However, there was typically a region between −20 and −10 mV where there was much less inhibition. This complex voltage-dependence was a consistent observation. In five cells mean $I_{DR}$ inhibition was 37±6% at −40 mV, 25±8% at −30 mV, and 12±4% at −20 mV. In the case of $I_A$, inhibition of IBMX consistently showed little or no dependence on the command potential in six cells tested (Figure 5d). It should be noted that forskolin, a compound that causes activation of adenylate cyclase, does not cause inhibition of either of those currents in ciliary ganglion neurones (S.E. Dryer, unpublished observations) suggesting that

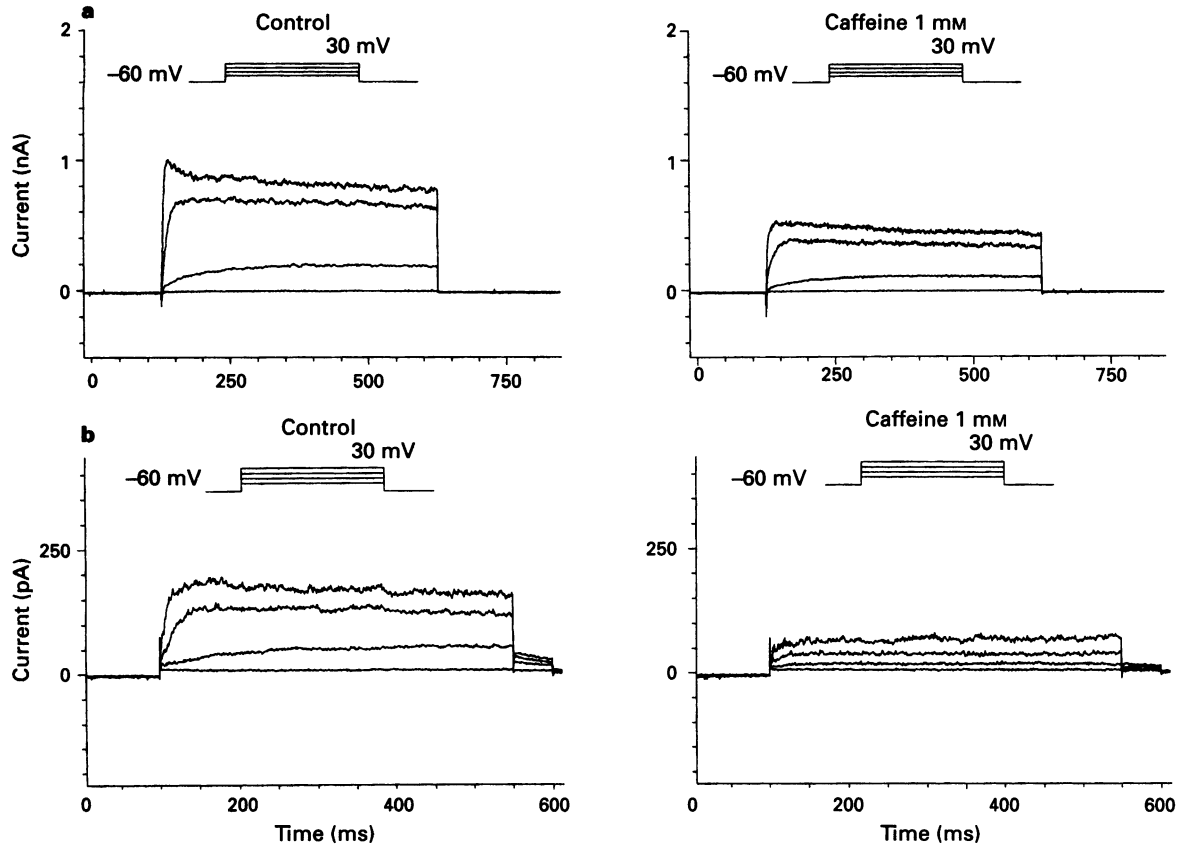


**Figure 3** Effects of caffeine on $I_{DR}$ in secretory cells. (a) Families of currents evoked in cultured rat anterior pituitary cells before (left) and 60 s after (right) application of 1 mM caffeine. (b) Families of currents evoked in dissociated chick pineal cells before (left) and 60 s after (right) application of 1 mM caffeine.

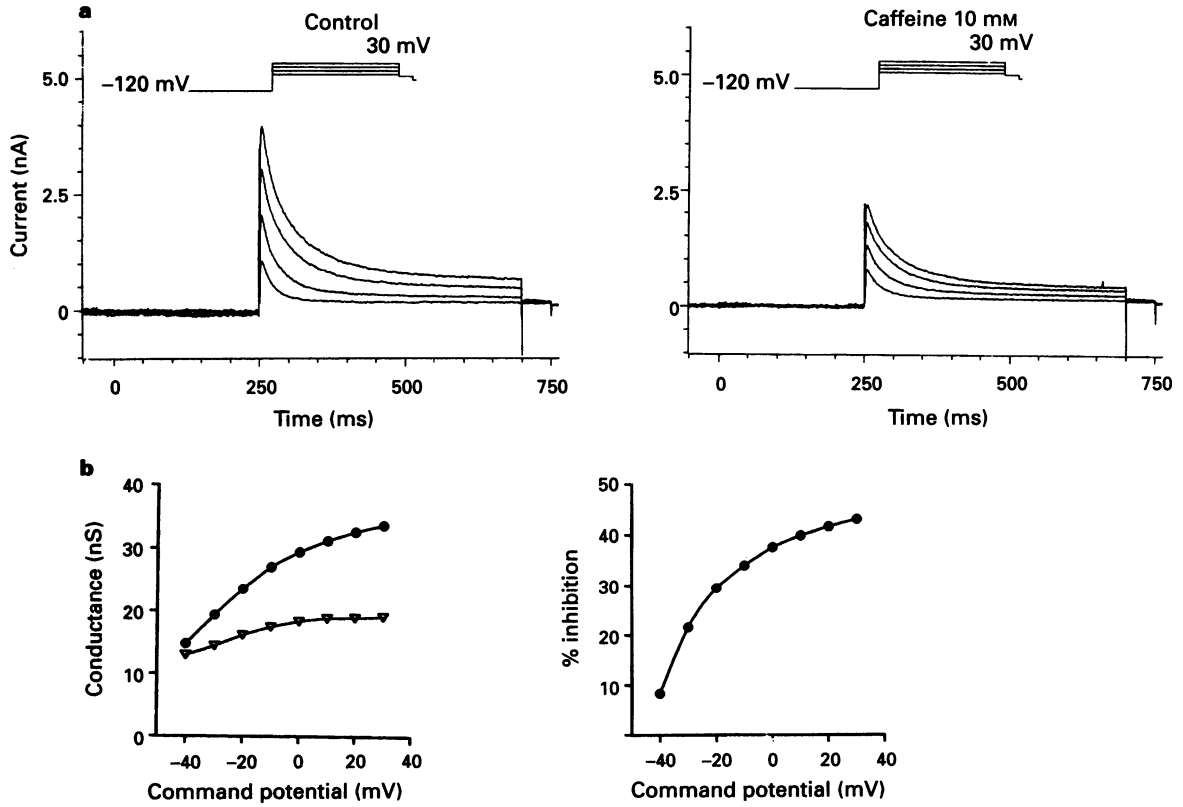


**Figure 4** Effects of caffeine on transient $K^+$ currents ($I_A$) in chick ciliary ganglion neurones. (a) Families of currents evoked from a holding potential of −120 mV in the presence of 10 mM external tetraethylammonium. Currents were evoked before (left) and after (right) application of 10 mM caffeine. (b) Voltage-dependence of caffeine blockade of $I_A$. Data from cell shown in (a). Left, activation curves of peak currents before (●) and after (△) application of 10 mM caffeine. Right, plot of $I_A$ inhibition vs. command potential. Curves are spline fits to the data.

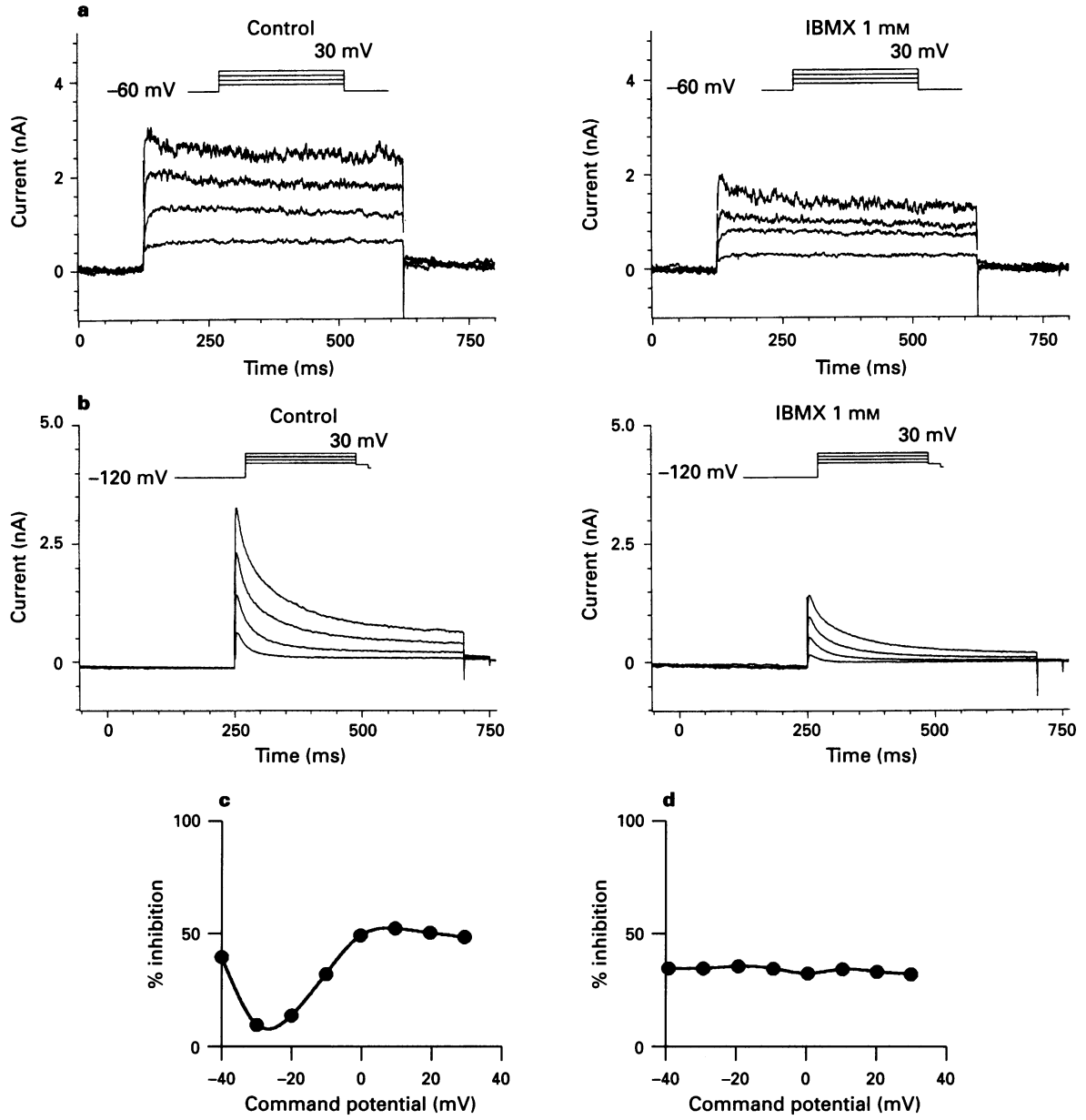


**Figure 5** Effects of 3-isobutyl-1-methyl xanthine (IBMX) on $I_{DR}$ and $I_A$ in chick ciliary ganglion neurones. (a) Families of currents ($I_{DR}$) evoked from a holding potential of −60 mV before (left) and 60 s after (right) application of 1 mM IBMX. (b) Families of currents ($I_A$) evoked from a holding potential of −120 mV in the presence of 10 mM tetraethylammonium. Currents were evoked before (left) and 90 s after (right) application of 1 mM IBMX. (c and d) Voltage-dependence of IBMX inhibition of $I_{DR}$ (c) and $I_A$ (d) in the chick ciliary ganglion neurones shown above. Curves are spline fits to the data. Note difference from the voltage-dependence of caffeine inhibition shown in Figures 2 and 4.

the inhibitory effects of IBMX are not caused by increases in cyclic AMP secondary to inhibition of cyclic nucleotide phosphodiesterases.

The differences in the voltage-dependence of IBMX and caffeine actions suggest that these compounds inhibit $K^+$ currents by binding to different sites. If so, IBMX should cause additional inhibition in the presence of a saturating concentration of caffeine. That proved to be the case. $I_A$ or $I_{DR}$ were evoked in chick ciliary ganglion neurones by use of the voltage clamp protocols described above and application of 10 mM caffeine caused inhibition of both currents as described above. Switching to saline containing 11 mM caffeine did not cause additional inhibition of either current (0±3% compared to 10 mM caffeine in the case of $I_{DR}$, 0±2% in the case of $I_A$, $n=6$ cells in each group, data not shown). By contrast, application of 1 mM IBMX in the presence of 10 mM caffeine caused greater inhibition of $I_{DR}$ (27±4%) and $I_A$ (31±7%) than that produced by 10 mM caffeine alone ($n=6$ cells in each group) (Figure 6).

## Discussion

In this study, we have found that millimolar concentrations of caffeine produce a robust inhibition of voltage-activated $K^+$ currents in neurones and secretory cells from two different vertebrate species. In ciliary ganglion neurones, significant inhibition was detected with 100 μM caffeine. A structurally related methylxanthine, IBMX, also caused inhibition of voltage-activated $K^+$ currents in chick ciliary ganglion neurones at millimolar concentrations. Previous studies have shown that millimolar concentrations of caffeine inhibit voltage-dependent

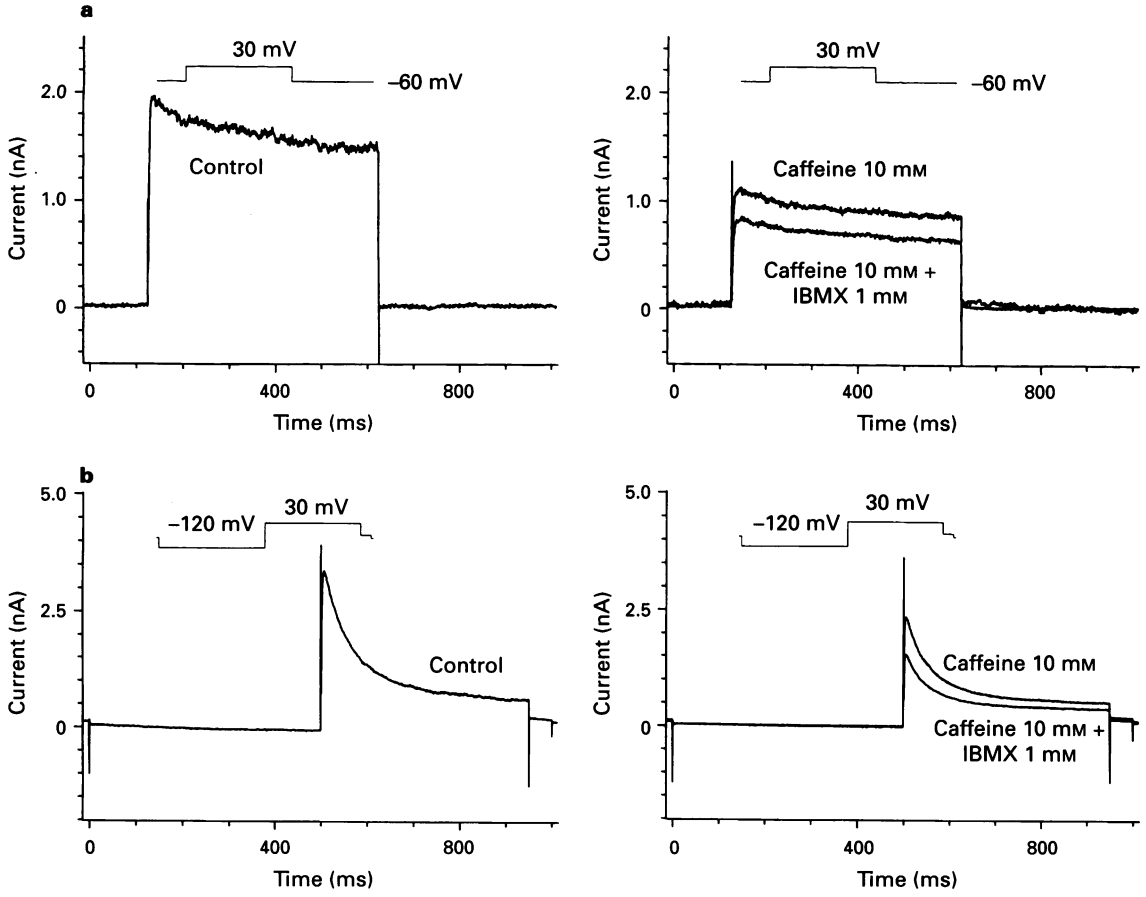


**Figure 6** Combined effects of caffeine and 3-isobutyl-1-methylxanthine (IBMX) on $I_{DR}$ and $I_A$ in chick ciliary ganglion neurones. (a) Delayed rectifier currents evoked by depolarizing steps to +30 mV from a holding potential of −60 mV before (left) and after application of 10 mM caffeine and after application of 1 mM IBMX in the presence of 10 mM caffeine, as indicated (right). (b) In a different cell, A-currents were evoked in the presence of 10 mM TEA by depolarizing voltage steps to +30 mV from a holding potential of −120 mV before (left) and after application of 10 mM caffeine and after application of 1 mM IBMX in the presence of 10 mM caffeine, as indicated (right). Note that 1 mM IBMX produces additional inhibition of both currents even in the presence of 10 mM caffeine.

$K^+$ currents in rat ventricular myocytes (Sanchez-Chapula, 1992; Varro *et al.*, 1993) and guinea-pig portal vein (Noack *et al.*, 1990), although these effects were not described in detail. IBMX has been shown to inhibit a voltage-activated tetraethylammonium-sensitive $K^+$ current in rat sensory neurones (Usachev *et al.*, 1995).

The available evidence strongly suggests that inhibition is a direct effect on the $K^+$ channels and is not secondary to methylxanthine effects on intracellular regulatory enzymes. First, the inhibitory effects described here and previously were rapid in onset and at least partially reversible within minutes. Second, caffeine causes a rapid blockade of $I_{DR}$ in excised outside-out 'maxi' patches. With this recording configuration, the patch membrane is isolated from soluble cytoplasmic regulatory molecules, including cyclic nucleotides, cyclic nucleotide phosphodiesterases, cyclic nucleotide-dependent protein kinases, and internal $Ca^{2+}$ stores. However, we cannot exclude the possibility that caffeine acts on membrane-bound regulatory proteins that remain associated with the excised patch membrane. Finally, caffeine and IBMX block multiple voltage-activated $K^+$ currents (in several different cell types in the case of caffeine). The biophysical properties of the caffeine-sensitive $K^+$ channels are clearly different in some of these cell types. For example, one of the caffeine-sensitive currents in ventricular myocytes is a $Ba^{2+}$-sensitive inward rectifier (Varro *et al.*, 1993). This suggests a direct effect on conserved pore regions of the channel molecules as it is unlikely that the same regulatory cascades could result in blockade of $K^+$ channels in all of these preparations (see below).

What is the mechanism of caffeine inhibition of voltage-activated $K^+$ currents? The available results suggest at least two non-mutually exclusive possibilities, which entail binding to sites that are either remote from the pore or within the pore. In the first case, caffeine may bind to regulatory domains on $K^+$ channel molecules, or accessory subunits, resulting in changes in the voltage-dependent probability of finding channels in the open state; this could occur by stabilization of closed or inactivated states of the channel molecules or by altering the conformation of the voltage sensors. This may be analogous to the actions of certain dihydropyridine compounds on the gating of L-type $Ca^{2+}$ channels (Bean, 1984). However, it seems unlikely that this could occur in several structurally diverse $K^+$ channels, as structural domains outside of the pore are not highly conserved in different $K^+$ channel subfamilies (Salkoff *et al.*, 1992). A more likely possibility is that caffeine may simply occlude pores in a manner similar to that of tetraethylammonium ions (Stanfield, 1983). The pore domains of different $K^+$ channel subfamilies tend to be highly conserved (Salkoff *et al.*, 1992). Voltage-dependence could arise if caffeine binding sites are only accessible in open channels. Voltage-dependent blockade could also occur if charged caffeine molecules bind to sites located within the transmembrane electric field (Hille, 1992), but it should be noted that at physiological pH only a small percentage of caffeine molecules are charged.

The inhibitory effects of 1 mM IBMX can be observed in the presence of saturating concentrations of caffeine (10 mM). This, together with the qualitatively different voltage-dependence of inhibition, suggests that the two methylxanthines bind to different sites on the channel molecules. The effects of IBMX on $I_A$ in ciliary ganglion neurones are essentially vol-

tage-independent. This could occur if IBMX molecules bind to sites accessible from either the open or closed states and/or are not affected by the transmembrane electrical field at their binding sites. In the case of $I_{DR}$, the complex voltage-dependence could arise if charged IBMX molecules have access to the pore from both the inner or outer face of the membrane and if asymmetric energy barriers limit access to a single IBMX binding site. Alternatively, there may be multiple IBMX binding sites on the channel molecules (Hille, 1992).

Regardless of the mechanism of blockade, these results have important implications for the use of caffeine or IBMX in laboratory experiments, especially in isolated or cultured cells. Caffeine has become one of the standard pharmacological tools for the study of $Ca^{2+}$ release from internal stores because it activates type-1 and type-2 ryanodine receptors (Erlich *et al.*, 1994; Pozzan *et al.*, 1994). Because of this, caffeine can evoke sustained increases in free cytoplasmic $Ca^{2+}$ in many cells. This can result in $Ca^{2+}$ oscillations in some cell types (reviewed by Pozzan *et al.*, 1994). Paradoxically, caffeine can also inhibit spontaneous and agonist-evoked intracellular $Ca^{2+}$ oscillations (D'Andrea *et al.*, 1993; Pozzan *et al.*, 1994).

These sorts of results are usually interpreted as either direct effects of caffeine on various $Ca^{2+}$ release channels coupled with mass-action interactions between various $Ca^{2+}$ release compartments, or in terms of inhibition by caffeine of agonist-stimulated $IP_3$ formation (Erlich *et al.*, 1994; Pozzan *et al.*, 1994). However, the present results indicate that caffeine will produce a substantial blockade of $K^+$ channels at concentrations less than or equal to those typically used to study intracellular $Ca^{2+}$ stores. This could become significant in the case of those cells, especially excitable cells, that use changes in membrane potential or action potentials as a part of their $Ca^{2+}$ regulatory apparatus. This is because partial inhibition of $K^+$ currents can increase resting excitability and spike duration thereby causing increases in intracellular $Ca^{2+}$. With greater inhibition of $K^+$ currents, repetitive firing and even spike initiation can be blocked, potentially resulting in gradual decreases in intracellular $Ca^{2+}$ over time.

There is reason to believe that this could have profound effects on $Ca^{2+}$ oscillations in some cell types. For example, rat chromaffin cells are only capable of exhibiting full-blown $Ca^{2+}$ oscillations over a narrow range of basal intracellular free $Ca^{2+}$ concentrations (D'Andrea *et al.*, 1993). In those cells, spontaneous $Ca^{2+}$ oscillations were blocked by elimination of $Ca^{2+}$ influx by EGTA or nitrendipine, or by large increases in $Ca^{2+}$ influx evoked by elevated external KCl. These results are consistent with recent multiple-pool theoretical models of $Ca^{2+}$ oscillations that predict that normal regulation can only occur over a restricted operative range of internal free $Ca^{2+}$ concentrations (Somogy & Stucki, 1991).

In summary, these results indicate that possible effects of caffeine and IBMX secondary to blockade of voltage-activated $K^+$ currents need to be carefully considered in the design and interpretation of experiments on mobilization of internal $Ca^{2+}$ stores, and second messenger dynamics, especially in excitable cells.

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## References

BEAN, B. (1984). Nitrendipine block of cardiac calcium channels: High affinity binding to the inactivated state. *Proc. Natl. Acad. Sci. U.S.A.*, **81**, 6388–6392.

DALY, J. (1993). Mechanisms of action of caffeine. In *Caffeine, Coffee, and Health*. ed. Garrattini, S., pp. 97–150. New York: Raven Press.

D'ANDREA, P., ZACCHETTI, D., MELDOLESI, J. & GROHOVAZ, F. (1993). Mechanism of $[Ca^{2+}]_i$ oscillations in rat chromaffin cells. *J. Biol. Chem.*, **268**, 15213–15220.

DRYER, S.E. (1991). $Na^+$-activated $K^+$ channels and voltage-evoked ionic currents in brain-stem and parasympathetic neurones of the chick. *J. Physiol.*, **435**, 513–552.

ERLICH, B., KAFTAN, E., BEZPROZVANNAYA, S. & BEZPROZVANNY, I. (1994). The pharmacology of intracellular $Ca^{2+}$ release channels. *Trends. Pharmacol. Sci.*, **15**, 145–149.

GOROSPE, W.C. & FREEMAN, M.E. (1985). Detection of prolactin inhibitory activity in uterine epithelial cell secretions and rat serum. *Endocrinology*, **116**, 1559–1564.

HABUCHI, Y., TANAKA, H., FURUKAWA, T. & TSUJIMURA, Y. (1991). Caffeine-induced block of $Na^+$ current in guinea pig ventricular cells. *Am. J. Physiol.*, **261**, H1855–1863.

HENDERSON, D. & DRYER, S.E. (1992). Voltage- and $Ca^+$-activated ionic currents in acutely dissociated cells of the chick pineal gland. *Brain Res.*, **572**, 182–189.

HILLE, B. (1992). *Ionic Channels of Excitable Membranes*, 2nd ed. pp. 390–422, Sunderland, MA: Sinauer Press.

NOACK, T., DEITMER, P. & GOLENHOFEN, K. (1990). Features of a calcium independent caffeine sensitive outward current in single smooth muscle cells from guinea pig portal vein. *Pflügers Arch.*, **416**, 467–469.

POZZAN, T., RIZUTTO, R., VOLPE, R. & MELDOLESSI, J. (1994). Molecular and cellular physiology of intracellular calcium stores. *Physiol. Rev.*, **74**, 595–636.

SALKOFF, L.B., BAKER, K., BUTLER, A., COVARRUBIAS, M., PAK, M.D. & WEI, A.G. (1992). An essential set of $K^+$ channels conserved in flies, mice, and humans. *Trends Neurosci.*, **15**, 161–166.

SANCHEZ-CHAPULA, J. (1992). Caffeine inhibits depolarization-activated outward currents in rat ventricular myocytes. *Eur. J. Pharmacol.*, **229**, 163–169.

SOMOGY, R. & STUCKI, J.W. (1991). Hormone-induced calcium oscillations in liver cells can be explained by a simple one pool model. *J. Biol. Chem.*, **266**, 11068–11077.

STANFIELD, P.R. (1983). Tetraethylammonium ions and the potassium permeability of excitable cells. *Rev. Physiol. Biochem. Pharmacol.*, **97**, 1–67.

USACHEV, Y., KOSTYUK, P. & VERKHRATSKY, A. (1995). 3-Isobutyl-1-methylxanthine affects potassium permeability in rat sensory neurons via pathways that are sensitive and insensitive to $[Ca^{2+}]_i$. *Pflügers Arch.*, **430**, 420–428.

VARRO, A., HESTER, S. & PAPP, J.G. (1993). Caffeine-induced decreases in the inward rectifier potassium and the inward calcium currents in rat ventricular myocytes. *Br. J. Pharmacol.*, **109**, 895–897.

WISGIRDA, M.E. & DRYER, S.E. (1993). Characteristics of multiple voltage-activated $K^+$ currents in acutely dissociated chick ciliary ganglion neurones. *J. Physiol.*, **470**, 171–189.

WISGIRDA, M.E. & DRYER, S.E. (1994). Functional dependence of $Ca^{2+}$-activated $K^+$ current on L- and N-type $Ca^{2+}$ channels: Differences between chick sympathetic and parasympathetic neurons suggest different regulatory mechanisms. *Proc. Natl. Acad. Sci. U.S.A.*, **91**, 2858–2862.

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