Voltage-activated Ca\textsuperscript{2+} channels in rat renal afferent and efferent myocytes: no evidence for the T-type Ca\textsuperscript{2+} current

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Early studies examining the effects of Ca\textsuperscript{2+} channel-blocking agents on renal haemodynamics suggested that such agents act selectively on pre-glomerular resistance, increasing P\textsubscript{GC}.\textsuperscript{3} This premise was confirmed as diverse approaches, developed to directly assess the effects of these agents on the renal microcirculation, demonstrated a preferential dilation of the AA, and no effect on the EA.\textsuperscript{4,5} Indeed, the EA appears to be unique, in that not only is this vessel insensitive to L-type Ca\textsuperscript{2+} blockers, but, with rare exceptions,\textsuperscript{6,7} depolarization does not cause vasoconstriction or Ca\textsuperscript{2+} entry.\textsuperscript{8,9} Similarly, hyperpolarization, which attenuates AA vasoconstriction, presumably by reducing the activities of voltage-activated Ca\textsuperscript{2+} channels, has no discernible effect on the EA.\textsuperscript{10}

Aims

Based on indirect methods, it has been suggested that both L- and T-type Ca\textsuperscript{2+} channels mediate signalling in the renal afferent arteriole and that T-type Ca\textsuperscript{2+} channels are involved in signalling in the efferent arteriole. However, Ca\textsuperscript{2+} currents have never been studied in these two vessels. Our study was initiated to directly determine the type of Ca\textsuperscript{2+} channels in these vessels for the first time, using patch clamp.

Methods

Native myocytes were obtained from individually isolated rat renal afferent and efferent arterioles and from rat tail arteries (TA). TA myocytes, which possess both L- and T-type Ca\textsuperscript{2+} currents, served as a positive control. Inward Ca\textsuperscript{2+} and Ba\textsuperscript{2+} currents (I\textsubscript{Ca} and I\textsubscript{Ba}) were measured in 1.5 mmol/L Ca\textsuperscript{2+} and 10 mmol/L Ba\textsuperscript{2+}, respectively, using the whole-cell configuration. By exploiting known differences in activation and inactivation characteristics and differing sensitivities to nifedipine and kurtoxin, the presence of both L- and T-type Ca\textsuperscript{2+} channels in TA myocytes was readily demonstrated. Afferent arteriolar myocytes exhibited relatively large I\textsubscript{Ca} densities (~20 ± 0.2 pA/pF) in physiological Ca\textsuperscript{2+} and the I\textsubscript{Ba} was 3.6-fold greater. These currents were blocked by nifedipine, but not by kurtoxin, and did not exhibit the activation and inactivation characteristics of T-type Ca\textsuperscript{2+} channels. Efferent arteriolar myocytes did not exhibit a discernible voltage-activated I\textsubscript{Ca} in physiological Ca\textsuperscript{2+}.

Conclusion

Our findings support the physiological role of L-type Ca\textsuperscript{2+} channels in the afferent, but not efferent, arteriole, but do not support the premise that functional T-type Ca\textsuperscript{2+} channels are present in either vessel.

Keywords

Renal microcirculation • L-type Ca\textsuperscript{2+} channels • T-type Ca\textsuperscript{2+} channels • Tail artery • Patch clamp • Afferent arteriole

1. Introduction

The renal afferent and efferent arterioles (EAs) regulate the inflow and outflow resistances of the glomerulus and thereby regulate the pressure within the intervening glomerular capillaries (P\textsubscript{GC}). Afferent arteriolar (AA) constriction reduces P\textsubscript{GC} and, for example, prevents the transmission of systemic hypertension to the glomerulus.\textsuperscript{1} A selective efferent vasoconstriction increases P\textsubscript{GC} and is essential in preserving glomerular filtration rate (GFR) when renal perfusion is compromised.\textsuperscript{2} Considering their differing roles and the physiological need for independent regulation, it is not surprising that these two vessels have quite distinct regulatory mechanisms. A key difference involves the types of Ca\textsuperscript{2+} entry mechanisms present in each vessel.
Molecular approaches confirmed that L-type Ca$^{2+}$ channel protein is preferentially expressed in the AA, but also suggested that additional species of voltage-activated Ca$^{2+}$ channels, including T- and N-type Ca$^{2+}$ channels, might be expressed in the both vessel types. With the advent of pharmacological agents capable of blocking such channels, it was subsequently shown that T/L-type channel blockers (e.g. mibebradil, efonidipine) reverse agonist-induced vasooconstriction of both afferent and EAs in contrast to dihydropyridines, which dilate only the AA (reviewed in Hayashi et al.). Such observations have led to a prevailing view that both L- and T-type Ca$^{2+}$ channels mediate signalling in the AA and that T-type Ca$^{2+}$ channels play an important role in the EA. Nevertheless a number of facts are inconsistent with this premise. These include the lack of effect of depolarization on the EA, the ability of T-type selective dihydropyridines to fully block responses of the AA and the low availability of T-type Ca$^{2+}$ channels at physiological membrane potentials of the unstimulated afferent and EA in situ (−40 to −45 mV). In this regard, it is important to note that although these agents do block T-type Ca$^{2+}$ channels they all have other actions that can affect vascular responses.

Do T-type Ca$^{2+}$ channels actually play an important role in the renal microcirculation or are the actions of these pharmacological agents due to other reported effects? These questions are critical in regard to our understanding of the control of the renal circulation and have important clinical implications. For example, it has been suggested that because T-type Ca$^{2+}$ channel blockers reverse effenter vasooconstriction, these agents are renal protective when compared with L-type Ca$^{2+}$ blockers which by increasing PGC promote hyper-tensive injury. These considerations point to importance of studies that directly evaluate the voltage-activated Ca$^{2+}$ channels that are present in the renal afferent and EAs using patch clamp. To our knowledge, the present study is the first to do so using single myocytes freshly dispersed from individually isolated afferent and EAs.

2. Methods


The characteristics of the Ca$^{2+}$ channels present in native renal AA and EA myocytes were evaluated using the whole-cell patch-clamp at room temperature (23 °C). Individual renal arterioles were isolated from the renal cortex (excluding the juxtaglomerular area) of normal male Sprague–Dawley rat kidneys using the gel-perfusion technique. Tissue was harvested under deep halothane anaesthesia (1.5–2% flow rate, assessed by the absence of the paw withdrawal reflex) and animals were euthanized by exsanguination. Only relaxed myocytes isolated from AA and EA as previously described were used in electrophysiological experiments. Myocytes from the rat tail artery (TA) were used as a positive control, as both T- and L-type Ca$^{2+}$ currents are consistently reported in these cells. TA myocytes were dispersed from the tissue with the enzyme solution containing 2 mg/mL of collagenase NB8, 200 U/mL of collagenolytic protease, 2 mg/mL of disase II, and 0.8 U/mL of elastase III (30–35 min, 37 °C). All types of isolated myocytes were studied under identical conditions. Electrophysiological recordings were performed as described previously.

Pipettes (resistance 6–12 MΩ) were filled with a solution containing (mmol/L): 120 Cs$^{+}$-methanesulfonate, 20 CsCl, 5 HEPES, 2 MgATP, 0.5 Na$_2$GTP, 5 EGTA, 0.3 MgCl$_2$, pH = 7.2. The external bath solution contained (mmol/L): 140 NaCl, 5 CsCl, 5 HEPES, 1 MgCl$_2$, 5 glucose, pH = 7.35. Ca$^{2+}$ (ICa), and Ba$^{2+}$ (IBa) currents were studied in 1.5 mmol/L CaCl$_2$ and 10 mmol/L BaCl$_2$, respectively. Current densities were calculated by correcting for leak current (offline) and then dividing by the cell capacitances (Cm).

Current–voltage (I–V) relationships for ICa and IBa were recorded from a holding potential of −80 mV to activate both the L- and T-type current components. Half-activation voltage ($V_1/2$) and the slope factor of activation ($k_a$) were obtained from normalized whole-cell I–V fits to the function: $[V_m - E_{a}]^2/(1 + exp([V_m - V_1/k_a])$, where $V_m$ is membrane potential; $E_a$ is the apparent reversal potential, and $g$ is the scaling factor. In 35 afferent myocytes, mean $E_R$ and $g$ were 44 ± 2 mV and 0.04 ± 0.004 mV$^{-1}$ (peak ICa) and 61 ± 1 mV and 0.03 ± 0.002 mV$^{-1}$ (peak IBa), respectively. In 32 TA myocytes, mean $E_a$ and $g$ were 53 ± 2 mV and 0.06 ± 0.01 mV$^{-1}$ (peak ICa) and 60 ± 2 mV and 0.02 ± 0.001 mV$^{-1}$ (peak IBa), respectively.

Ca$^{2+}$ channel availability was investigated by applying 500 ms conditioning voltages ($V_c$) (between −80 to +40 mV in 10 mV increments) followed by a test pulse (0 or −20 mV). Current amplitudes at the test pulse were normalized and fit to a modified Boltzmann function: $I(V_a)=I_0+I_1/[1+exp((V_a-V_1)/k_a)]$, where $V_a$ and $k_a$ are half-inactivation potential and the slope factor of inactivation, and $A$ is non-inactivating component of the current.

Data are expressed as the means using the standard error of the mean (SEM). Differences between means were evaluated by paired or unpaired Student’s t-test. For multiple measurements, analysis of variance followed by the Bonferroni post hoc test was applied to assess significance. Probabilities with $P < 0.05$ were considered significant.

3. Results

At physiologic levels of extracellular Ca$^{2+}$ (1.5 mmol/L), AA myocytes displayed a prominent ICa. The inward current exhibited a threshold near −30 mV (Figure 1A and C), typical of the L-type Ca$^{2+}$ channels reported in other vascular smooth muscle cells (VSMCs). ICa reached maximum near 10 mV and had the mean peak amplitude of $-8.9 \pm 1.3 \mu A$ (n = 36, Figure 1C). The properties of these channels were further studied in the presence of extracellular 10 mmol/L Ba$^{2+}$. It has been previously demonstrated that the L-type IBa recorded in this concentration of Ba$^{2+}$ is activated in a similar voltage range as ICa in physiological levels of calcium. Under these conditions, IBa reached its maximal at 20 mV and mean peak IBa was increased to $37.1 \pm 5.1 \mu A$ (n = 36, Figure 1B and C). The peak IBa significantly correlated with the size of afferent myocytes expressed as Cm ($P = 0.011$). As shown in Figure 1D, peak ICa and IBa densities were $-2 \pm 0.2$ and $-7.3 \pm 0.8 \mu A/pF$, respectively. This 3.6-fold increase in the current is typical for L-type Ca$^{2+}$ channels.

In this group of afferent myocytes, Cm ranged from 2.9 to 10.3 pF (mean 5.5 ± 0.3 pF, n = 36), being similar to 5.6 ± 0.2 pF (range 2.2–11 pF) obtained in 125 myocytes isolated from 87 AAs and 42 animals.

Notably, no measurable whole-cell inward current was observed in afferent myocytes at membrane potentials negative to −30 mV in either Ba$^{2+}$ or Ca$^{2+}$ (Figure 1D). Moreover, the small ICa and IBa seen at −20 mV (grey tracings in Figure 1A and B) did not exhibit temporal inactivation. Both features are characteristics of the L- and not T-type Ca$^{2+}$ channels. To further pursue this issue, we applied the same approach to myocytes isolated from the rat TA, a vessel known to express both the T- and L-type Ca$^{2+}$ channels.

Figure 2A shows representative IBa traces recorded from a TA myocyte using the same experimental protocol as shown in Figure 1. Mean I–V data comparing IBa (filled squares) and ICa (open squares) densities recorded in 32 TA myocytes are depicted in Figure 2B.
Although the general character of $I-V$ curves for afferent and TA myocytes were similar, modest differences were discerned. First, the peak $I_{Ba}$ density was significantly larger in the afferent myocytes ($27.3 \pm 0.8 \, \text{pA/pF, } n=36$) compared with the TA myocytes ($22.7 \pm 0.2 \, \text{pA/pF, } n=32, \, P=0.00005$), although TA myocytes ($C_m=30.9 \pm 1.4 \, \text{pF, } n=32$) were 5.5 times larger than afferent myocytes ($C_m=5.5 \pm 0.3 \, \text{pF, } n=36$). Secondly, switching from $Ca^{2+}$ to $Ba^{2+}$ produced a small, albeit significant, shift in the half-activation potential ($V_{a}$) of AA myocytes, whereas no such shift was detected in the TA myocytes (Figure 2C). Although the $V_{a}$ for the whole-cell $I_{Ba}$ of TA myocytes ($0.8 \pm 0.4 \, \text{mV}$) was significantly more negative than that of afferent myocytes ($3.4 \pm 0.5 \, \text{mV, } P=0.00003$) (Figure 2C), the difference was quite modest. Accordingly, the presence of different $Ca^{2+}$ channel types could not be clearly discerned based only on the difference in the activation of the composite whole-cell current as was demonstrated in some VSMCs.\textsuperscript{19–21}

In addition to a more negative $V_{a}$, $T$-type $Ca^{2+}$ currents exhibit rapid inactivation which is particularly prominent at negative voltages, where the $T$-component of the current is prevailed over the $L$-component. The comparison of the representative tracings depicted in grey in Figures 1A and 2A illustrates that $I_{Ba}$ at $-20 \, \text{mV}$ decreases within a 100 ms depolarizing step in the TA myocyte, but not in the afferent myocytes. To highlight this difference in the current kinetics, the mean $I_{Ca}$ and $I_{Ba}$ densities measured at $-20 \, \text{mV}$ in afferent and TA myocytes (leak-corrected off-line) are compared in Figure 2D and E, respectively. Both $I_{Ca}$ and $I_{Ba}$ display prominent inactivation in TA myocytes, but not in afferent myocytes.

To further evaluate if the observed difference in the kinetics of $I_{Ba}$ in afferent and TA myocytes represented differing contributions of $T$- and $L$-type channels, we used an availability protocol. Figure 3A and C depicts traces of $I_{Ba}$ recorded at the test potential of $0 \, \text{mV}$ following $0.5 \, \text{s}$ conditioning voltages between $-80$ and $40 \, \text{mV}$ for afferent and TA myocytes, respectively. When both $L$- and $T$-type $Ca^{2+}$ channels are present, both currents will contribute to the initial peak of the whole-cell $I_{Ba}$. At the end of $100 \, \text{ms}$ test pulse, however, the $L$-type current will dominate, as $T$-type channels are rapidly inactivated and their contribution to the whole-cell $I_{Ba}$ will thus be diminished (as seen in Figure 2E). Moreover, since $L$- and $T$-type channels exhibit marked differences in the voltage-dependency of inactivation with the $T$-type inactivating at more negative voltages than the $L$-type,\textsuperscript{17,19,22} the effects of depolarizing conditioning pulses on the peak $I_{Ba}$ will differ from their effects on $I_{Ba}$ measured at the end of the $100 \, \text{ms}$ test pulse, if both currents are present. To evaluate this difference in afferent myocytes, the peak $I_{Ba}$ and $I_{Ba}$ measured at the end of $100 \, \text{ms}$ test pulse were normalized and fitted with a modified Boltzmann function in each cell and the mean values are compared in
A significant leftward shift in the availability of the peak $I_{Na}$ at $-20$ mV. $C_m = 33.1$ pf. (B) $I-V$ relationships for the peak-subtracted peak $I_{Ca}$ (open square) and $I_{Na}$ (filled square) densities in rat tail arterial myocytes, $n = 32$. (C) Changes in the half-activation potential for $I_{Ca}$ and $I_{Na}$ in the two cell types (for renal afferent arteriole, $n = 35$; for TA, $n = 32$; see Section 2 for further details). (D) Comparison of the mean $I_{Ca}$ and $I_{Na}$ respectively, recorded at $-20$ mV in renal afferent arteriole (n = 38-39) and TA (n = 33) myocytes. Currents were leak-corrected offline, grey lines show SEM.

In contrast, TA myocytes subjected to the same protocol exhibited a significant leftward shift in the availability of the peak $I_{Na}$ ($V_h = -23.1 \pm 1.6$ mV) compared with that for $I_{Na}$ at 100 ms ($V_h = -10.7 \pm 1.1$ mV, $n = 12$, $P = 0.00002$, Figure 3D). Also, significant changes in $k_h$ ($11.4 \pm 0.6$ vs. $8.7 \pm 0.7$ mV, $P = 0.0033$) and in $A$ ($0.25 \pm 0.03$ vs. $0.36 \pm 0.03$, $P = 0.00001$) for the peak and 100 ms $I_{Na}$, respectively, were found. These effects strongly suggest the presence of at least two current components in TA myocytes; with the peak $I_{Na}$ containing both the T- and L-components while $I_{Na}$ at 100 ms is predominantly the L-type. The similarity between the half-inactivation potential for $I_{Na}$ at 100 ms in TA myocytes and that for $I_{Na}$ measured both at the peak and 100 ms in afferent myocytes supports the dominance of the L-type in the latter (compare Figure 3B and D). The analysis of $I_{Na}$ availability at $-20$ mV in TA myocytes (Figure 3D, filled triangles), where the T-component is dominant, shows even more striking difference with an over 30 mV negative shift in the availability ($V_h = -43.4 \pm 1.3$ mV, $k_h = 5.6 \pm 1.3$ mV, $A = 0.36 \pm 0.04$, $n = 9$) compared with that for $I_{Na}$ measured at 100 ms at 0 mV. The size of $I_{Na}$ at $-20$ mV in afferent myocytes was too small to derive meaningful availability dependence using this approach.

The presence of the fast inactivating T-type component can also be demonstrated by the analysis illustrated in Figure 3E. These data depict difference currents obtained by subtracting $I_{Na}$ at the test potential 0 mV following a conditioning step to $-20$ mV (when the current is predominantly L-type since the T-type is inactivated as illustrated in Figure 3D) from that obtained after a conditioning step to $-80$ (when both currents are available). As shown in Figure 3E, a fast-inactivating T-type current is readily demonstrated in the TA myocytes, but not in afferent myocytes. The non-inactivating difference current in both cell types is due to the partial inactivation of L-type channels at $-20$ mV (Figure 3B and D). Furthermore, the analysis of the current kinetics of the mean $I_{Na}$ densities measured at 500 ms conditioning pre-pulses to physiologically relevant voltages of $-40$, $-30$, and $-20$ mV in afferent (left) and TA (right) myocytes in Figure 3F clearly demonstrates fast inactivating kinetics of $I_{Na}$, a characteristic property of the T-type current, in TA, but not in afferent myocytes.

We next examined the effects of nifedipine, a selective L-type Ca$^{2+}$ channel blocker. Figure 4A and C compares the effect of 1 mmol/L nifedipine on the mean $I_{Ca}$ recorded at $-20$ mV, whereas Figure 4B and D compares the mean $I-V$s in afferent (n = 9) and TA (n = 13) myocytes. Note that in the afferent myocytes, nifedipine significantly eliminated $I_{Ca}$ inhibiting the current by $75 \pm 7\%$ ($P = 0.007$). In comparison, in TA myocytes the block of $I_{Ca}$ at $-20$ mV to nifedipine (Figure 4C) was significantly less ($27 \pm 4\%$, $P = 0.00005$). Moreover, the full $I-V$ plot of TA myocytes (Figure 4D) revealed a significant
blockade at more positive potentials, but clearly exhibited a component of the nifedipine-insensitive voltage-activated inward current. A comparison of the steady-state half-activation parameters disclosed a significant nifedipine-induced leftward shift in $V_a$ in TA myocytes, but not in afferent myocytes (Figure 4E). We were able to conduct the availability at 0 mV test potentials in two nifedipine-treated TA myocytes and found $V_h$ to be $-38.9$ and $-37.5$ mV, values similar to those seen in the absence of nifedipine at test potentials of $-20$ mV (Figure 3D).

Figure 5 illustrates the effects of 200 nmol/L kurtoxin, a selective inhibitor of T-type Ca$^{2+}$ channels, on afferent (A–D) and TA (E–H) myocytes. $I_{Ba}$ was monitored using repeated 100 ms steps from a holding potential of $-80$ to 0 mV, during control periods and following application of kurtoxin. Figure 5A and E depicts representative control tracings and tracings obtained after 5 min treatment in kurtoxin in the two cell types. In the afferent myocyte kurtoxin caused a small but similar decrease in both the peak and $I_{Ba}$ at 100 ms (Figure 5A), whereas in the TA myocyte the inhibitor completely blocked an initial rapidly inactivating component of $I_{Ba}$ but had little effect on $I_{Ba}$ at the end of step depolarization (Figure 5E). Figure 5B and F compares the mean kurtoxin-sensitive currents (control minus time controls without kurtoxin) in afferent and TA myocytes, respectively, illustrating the effects of kurtoxin and current run-down. Note, that an inactivating, kurtoxin-sensitive current was evident in the myocytes from TA, but not in myocytes from the AA. Figure 5G and H depicts the time-dependent effects of kurtoxin on the initial peak $I_{Ba}$ in both cell types, whereas Figure 5D and H shows its effect on the currents measured at 100 ms. To account for a run-down, data from time controls ($n = 4–5$) in which measurements were made in the absence of kurtoxin are depicted as filled symbols in these figures. In TA myocytes, kurtoxin significantly suppressed the peak current by $36.8 \pm 6.6\%$ ($P = 0.005$, Figure 5G), whereas the effect was seen on $I_{Ba}$ at the end of 100 ms was indistinguishable from the time controls (Figure 5H). No significant effects of kurtoxin were observed on either peak or 100 ms $I_{Ba}$ in afferent myocytes (Figure 5C and D).

The EA is an unusual blood vessel, in that contractile responses are not affected by selective L-type Ca$^{2+}$ channel blockers$^{4,23}$ or by hyperpolarization.$^{11}$ Similarly, depolarization has no effect on tone or calcium entry in this vessel.$^{8–10}$ Conversely, based on responses to non-selective agents that preferentially block T-type channels, it has been suggested that T-type Ca$^{2+}$ channels contribute to the activation of the EA.$^{13,23–25}$ In this study, we were able to obtain the first
measurements of $I_{Ca}$ and $I_{Ba}$ in myocytes from isolated EAs. We could not, however, maintain the whole-cell access long enough statistically to compare full $I-V$s in both conditions as in afferent myocytes. Therefore, the whole-cell currents were compared at a test potential to 0 mV. The current traces were leak-subtracted offline, corrected for the cell size, averaged and compared in Figure 6 with the currents in afferent myocytes recorded under identical conditions. In 1.5 mmol/L $Ca^{2+}$, no measurable inward current was detected in efferent myocytes (Figure 6A). With barium as the charge carrier, a small inward current was observed (Figure 6C). However, similar to $I_{Ca}$, it was significantly smaller compared with the afferent myocytes ($-0.5 \pm 0.4$, $n = 5$, vs. $-3.5 \pm 0.4$ pA/pF, $n = 36$) (Figure 6D). Notably, as in afferent myocytes, the small $I_{Ba}$ seen in efferent myocytes did not display time-dependent inactivation (Figure 6C).

4. Discussion

The present study is the first to demonstrate and characterize voltage-activated $Ca^{2+}$ channel currents in myocytes isolated from native AA and EA using direct patch-clamp techniques. AA myocytes exhibited a relatively high density of L-type $Ca^{2+}$ currents and these currents could readily be measured in physiological $Ca^{2+}$. In contrast, efferent myocytes did not exhibit a measurable voltage-activated inward current under physiological $Ca^{2+}$ conditions. These differences are unlikely due to difference in cell size and different rate of cell dialysis as the cell size (measured as $C_m$) was similar in both types of arterioles, but was 5-fold less than that of TA myocytes which had significantly smaller $I_{Ca}$ and $I_{Ba}$ density than AA myocytes. We could not demonstrate the presence of a measurable T-type channel.
Ca\(^{2+}\) current in myocytes from either the AA or EA. These findings support the results of previous studies demonstrating the differing sensitivities of the AA and EA to specific L-type Ca\(^{2+}\) channel-blocking agents.\(^3\) – 6,26 Conversely, our findings do not support the premise that T-type Ca\(^{2+}\) channels directly contribute to the activation of either vessel. A number of laboratories have demonstrated that non-selective T-type channel inhibitors, such as mibefradil, efonidipine, or pimozide, are capable of blocking contractile responses of both AA and EA (see review Hayashi et al.\(^13\)) and it is suggested that these channels contribute to the activation of both vessels. It must be emphasized, however, that this premise is supported by indirect observations and relies exclusively on contractile responses. In contrast, our conclusions are based on the direct measurements of Ca\(^{2+}\) channel currents in single myocytes. Nevertheless, when comparing the results between the intact preparations and enzymatically isolated cells, one could question whether ion channels remain intact in isolated myocytes.

To address this possibility, we conducted parallel studies in myocytes isolated from the rat TA using the same cocktail of enzymes. Although the function of the rat TA differs from that of the renal arterioles, TA myocytes are the only vascular cell type, where the presence of both L- and T-type currents has been consistently documented,\(^16,17\) thus this tissue represented a critical positive control. Another important argument could be that the expression of functional Ca\(^{2+}\) channels varies between different cells. For example, previous studies found T-type currents only in limited number of VSMCs isolated from the same vessel,\(^16,17,19\) including conduit vessels of the kidney.\(^21\) To address this concern, all myocytes isolated from TA and AAAs and studied with the same protocol were included in our comparative analysis in contrast to previous studies in which exemplary cells expressing either T- or L-type currents were normally compared.

The electrophysiological and pharmacological approaches we used were sufficient to demonstrate the presence of both T- and L-type Ca\(^{2+}\) currents in myocytes isolated from the rat TA, but not in similarly treated populations of afferent myocytes. Using the key biophysical properties of the T-type Ca\(^{2+}\) channels, such as fast inactivating kinetics and more negative voltage range of their activation and inactivation, our analysis clearly demonstrates the presence of all these features in TA myocytes, but not in afferent myocytes (Figures 2 and 3). The electrophysiological evidence is fully supported by the pharmacological separation of the two type Ca\(^{2+}\) currents in TA myocytes using the selective L-type Ca\(^{2+}\) channel inhibitor, nifedipine, and a relatively selective T-type blocker, kurtoxin, which at 200 nmol/L does not significantly affect the L-type currents\(^27\) (Figures 4 and 5). Furthermore, a significant leftward shift in the steady-state activation dependency for I\(_{\text{Ba}}\) remaining in 1 µmol/L nifedipine in TA, but not in afferent myocytes (Figure 4E) provides further argument against the presence of the T-type currents in the renal afferent myocytes.

Although we did not observe T-type Ca\(^{2+}\) currents in these vessels, the expression of the Ca\(_{\text{v}}\)3.1 and Ca\(_{\text{v}}\)3.2 genes of the
T-type Ca\(^{2+}\) channels has been demonstrated in the rat renal AAs isolated from the superficial regions of the renal cortex or from juxtamedullary nephrons and in the outer medullary vasa recta.\(^{12}\) We, therefore, cannot rule out the presence of a small, beyond the electrophysiological detection level, T-type current in the AA. However, it would be difficult to directly compare protein expression between the two cell types using immunocytochemistry without understanding molecular nature of the T-type Ca\(^{2+}\) channels in TA myocytes which has not been established, and is outside the scope of this study. Furthermore, immunocytochemical analysis using antiCaV3.1, antiCaV3.2, and antiCaV3.3 antibodies in vascular SMCs has proved to be inconclusive.\(^{28,29}\) Functionally, we found that the L-type Ca\(^{2+}\) current is rather prominent in AA myocytes with the peak \(I_{\text{Ca}}\) density measured at physiological Ca\(^{2+}\) was approximately twice that of TA myocytes (Figures 1 and 2). The size of the current in TA myocytes is comparable with that recorded in other types of arterial myocytes at physiological levels of Ca\(^{2+}\).\(^{19,30,31}\) Similarly, the presence of L-type, but the absence of detectable T-type Ca\(^{2+}\) currents has also been reported in smooth muscle like vasa recta pericytes.\(^{32}\) Based on our experimental evidence, we therefore suggest that L-type Ca\(^{2+}\) channels contribute more importantly to depolarization-induced activation of these vessels. This view is also consistent with the observation that contractile responses and depolarization-induced Ca\(^{2+}\) influx of the AA are completely blocked by selective L-type antagonists.\(^{4,8,23}\)

We did not observe a significant voltage-activated inward current in EA myocytes in physiological Ca\(^{2+}\), although minute \(I_{\text{Ba}}\) (\(\sim7\)-fold smaller than in afferent myocytes) was detected (Figure 6). This finding is consistent with the lack of effect of potassium-induced depolarization on tone\(^{6,10}\) and ineffectiveness of selective L-type blockers on this vessel\(^{6}\) as well as with the lack of their effect on intracellular [Ca\(^{2+}\)] or Ca\(^{2+}\) influx\(^{8}\). Also, no L-type protein was detected in cortical EAs.\(^{12}\) One can only speculate on the nature of the Ca\(^{2+}\) entry in the EA. Previous studies have shown that, unlike the AA, angiotensin II-induced vasoconstriction in the EA is not dependent on membrane depolarization.\(^{15}\) Moreover, using the fura-2/manganese quench technique, we have previously shown that angiotensin II activates a voltage-independent and nifedipine-insensitive Ca\(^{2+}\) entry in this vessel.\(^{8}\) The molecular mechanisms and the type(s) of ion channels involved are however not known and require further focused studies.

In the mouse conversely to the rat, Poulsen et al.\(^{7}\) recently reported that potassium-induced depolarization elicits a transient vasoconstriction in isolated EAs. Since our studies and those cited above were conducted using rat arterioles, the possibility of species differences in the expression of Ca\(^{2+}\) channels in the EA cannot be ruled out. We also cannot exclude a possibility that afferent and EAs from juxtamedullary nephrons may express L- and T-type channels in different proportions compared with the superficial cortical arterioles studied in this article, thus explaining a greater sensitivity of perfused juxtamedullary arterioles to non-specific T-type channel blockers such as mibebradil or pimozide.\(^{24}\) Alternatively, the action of at least some inhibitors may be explained by their action elsewhere due to their lack of specificity via, for example, effects on endothelial

**Figure 6** Calcium channel currents in rat renal efferent arterioles. (A and C) Comparison of the mean \(I_{\text{Ca}}\) and \(I_{\text{Ba}}\) densities recorded at 0 mV in myocytes isolated from efferent and afferent arterioles as indicated. Grey lines show SEM. (B and D) Statistical comparison of the peak densities for \(I_{\text{Ca}}\) and \(I_{\text{Ba}}\), respectively, in the efferent and afferent myocytes.
channels. To our knowledge, the renal microvascular effects of kurotoxin, which is considered to be the most specific T-type \( Ca^{2+} \) channel blocker as our study also suggests, have not been investigated. Accordingly, the possibility that these agents affect the renal microcirculation by actions other than a T-type \( Ca^{2+} \) channel blockade also merits consideration. A final resolution of this issue awaits further investigations.

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**References**