Developmental changes in the functional characteristics and expression of voltage-gated \( K^+ \) channel currents in rat aortic myocytes

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Abstract

Objective: Active control of the arterial diameter by vascular smooth muscle is one of the principle mechanisms by which vessels adapt to a significant rise in blood pressure after birth. Although voltage-gated \( K^+ \) (Kv) channels play an important role in the regulation of excitation–contraction coupling in arteries, very little is known about postnatal modification of Kv channels. We therefore investigated changes in the functional characteristics and expression of Kv channels in rat aortic myocytes (RAMs) during early postnatal development.

Methods: Kv currents (\( I_{Kv} \)) were investigated in single smooth muscle cells freshly dispersed from neonatal (1–3 days) and adult Wistar rat thoracic aorta using the whole-cell patch clamp technique.

Results: \( I_{Kv} \) in neonates had significantly faster activation kinetics and was inactivated at more positive voltages than \( I_{Kv} \) in adults (half-inactivation potential \( 24\pm2 \) and \( 40\pm3 \) mV and slope factor \( 4.2\pm0.4 \) and \( 11.1\pm0.5 \) mV, respectively). No difference in the steady state activation was found. \( I_{Kv} \) in neonates was insensitive to a high concentration of tetraethylammonium (TEA, 10 mM) but blocked 4-aminopyridine (4-AP, IC\(_{50}\)=0.5\pm0.1 mM), whereas \( I_{Kv} \) in adult RAMs was almost completely abolished by 10 mM TEA and was relatively insensitive to low concentrations of 4-AP. \( I_{Kv} \) in both age groups was insensitive to charybdotoxin (300 nM) or \( \alpha \)-dendrotoxin (200 nM). Immunoblot analysis showed that the expression of Kv1.2 \( \beta \)-protein decreased and Kv2.1 increased with development.

Conclusion: Significant changes in functional characteristics of the native \( I_{Kv} \) and the expression of particular Kv channel proteins occurred during postnatal vascular development. These changes could play an important role in adaptation to extraterine life. © 2002 Elsevier Science B.V. All rights reserved.

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1. Introduction

After birth, blood vessels undergo rapid structural alterations in order to maintain cardiovascular homeostasis in the face of a significant rise in blood pressure [1]. Active control of the arterial diameter by vascular smooth muscle (VSM) is also likely to play a pivotal role and functional studies have reported altered contractility during development [2,3]. Relatively little is known, however, of analogous changes in membrane mechanisms which control \( Ca^{2+} \) homeostasis in VSM.

\( K^+ \) channels play an important role in the regulation of VSM contractility [4]. Investigation of \( K^+ \) channels during development of the cardiovascular system has hitherto focused predominantly on cardiac tissue, with significant alterations being observed [5–7]. Observations in the vasculature are largely limited to pulmonary circulation [8–10]. Ovine foetal pulmonary myocytes predominantly
2.2. Electrophysiological recordings

Cells were placed in a chamber of volume 50–100 μl and were continually superfused (~1 ml/min) with PSS or test solution via a five-barrel pipette [14]. Whole cell K⁺ currents were recorded at room temperature using the standard patch-clamp technique (Axopatch 200B amplifier and PCLAMP 6 software, Axon Instruments). Glass micropipettes filled with pipette solution had a resistance range of 3–5 MΩ. At the beginning of each experiment, the capacitive transient in response to 10 mV hyperpolarizing step depolarisation (filtered at 50 kHz and sampled at 200 kHz) was recorded and cell membrane capacitance (Cm) calculated from the area under the capacitive transient. Time constants of the capacitive transient decay were 25–60 times faster in neonatal (0.06±0.005 ms, n=42) and adult (0.15±0.01 ms, n=71) RAMs than changes in the activation kinetics of K⁺ currents and, therefore, capacitance transients were not compensated. Calculated average series resistance was similar in neonatal (13.6±0.9 MΩ, n=42) and adult (13.7±0.8 MΩ, n=71) RAMs. Since the whole-cell current was small (average amplitude <500 pA at +80 mV in PSS) in both neonatal and adult myocytes, the calculated maximum voltage error was <7 mV and therefore neglected. In all voltage protocols used cells were held at –80 mV and stimulated at 0.1 Hz.

2.3. Western blot analyses

Aorta segments were placed in cold lysis buffer containing a protease inhibitor cocktail (Complete, Boehringer Mannheim) and homogenised (24,000 rpm, 2×1 min; Ultra-Turrax T25 homogeniser, Janke & Kunkel, IKA-Labortechnik). Cell lysate was then agitated slowly for 1 h and debris removed by centrifugation (2500 C; room temperature). Bound antibody was detected by the ECL method. Western blots were probed overnight (37 °C) with either anti-Kv1.1 (1:1000), anti-Kv1.2 (1:1000), anti-Kv1.5 (1:500) or anti-Kv2.1 (1:1000) antibodies (Alomone, Israel) in 1% milk in PBS-T. After incubation (37 °C; 15–18 min neonates, 30 min adults), blots were gently triturated in three consecutive volumes of Ca²⁺-free EGTA–PSS. The last two volumes were combined, filtered through 95-μm nylon mesh and centrifuged (1100 g for 5 min). Cells were then resuspended in PSS and maintained at +4 °C for use on the same day.
method (Amersham) and analysed using QUANTISCAN software (Biosoft, UK).

2.4. Composition of solutions and materials

PSS (mM): 130 NaCl, 5 KCl, 1.5 CaCl$_2$, 1.2 MgCl$_2$, 10 HEPES and 10 glucose, pH 7.2; Ca$^{2+}$-free PSS: same composition, but omitting CaCl$_2$; pipette solution (mM): 110 KCl, 10 NaCl, 5 mgATP, 10 HEPES, 10 EGTA and 0.5 CaCl$_2$ (estimated free [Ca$^{2+}$]=8 mM), pH 7.2; lysis buffer (mM): 50 Tris–Cl (pH 7.5), 250 NaCl, 5 EDTA, 5 DTT, 10 NaF, and 0.1% v/v Igepal. All chemicals and enzymes, except charybdotoxin (ChTx, Peninsula Labs., USA), were purchased from Sigma or BDH Merck.

2.5. Data analysis and statistics

Data analysis, statistics and curve fitting were performed with PCLAMP6, Origin 4.1 (Microcal Software) and Microsoft EXCEL computer software. Data are presented as mean±S.E.M. Significance was determined using unpaired Student’s $t$-test and differences were deemed significant at $P<0.05$ unless stated otherwise.

3. Results

3.1. Outward $K^+$ currents in neonatal and adult RAMs

Fig. 1A shows a family of whole cell outward $K^+$ currents recorded from a representative neonatal RAM in response to 300 ms step membrane depolarisations. In normal PSS current appeared at membrane potentials positive to $-30$ mV, rapidly reached maximum amplitude and then slowly decayed during maintained depolarisation. At membrane potentials above $+60$ mV small fluctuations superimposed on the outward current were often observed. Application of 1 $\mu$M paxilline, a selective inhibitor of BK$_{ca}$ channels [15], did not significantly affect the outward $K^+$ current, but eliminated fluctuations (Fig. 1A and B). The amplitude of the outward $K^+$ current measured at test potential $+60$ mV in PSS ranged between 25 and 480 pA (mean $131\pm25$ pA) in 24 neonatal myocytes.

The outward $K^+$ current in adult RAMs, although activated at similar membrane voltages, was characterised by larger fluctuations at positive membrane potentials (Fig. 1C). Suppression of the current by 1 $\mu$M paxilline was greater than in neonatal cells, being blocked by $35\pm3\%$ ($n=10$), when measured at $+60$ mV, in contrast to $6\pm4\%$ ($n=6$) in neonatal myocytes ($P<0.0002$). At more negative potentials paxilline was less effective (e.g. $17\pm5\%$ at $+20$ mV compared with inhibition at $+60$ mV, $P<0.002$, paired $t$ test) (Fig. 1D) suggesting that the relative contribution of BK$_{ca}$ channels to the whole cell outward current is larger in adult than in neonatal RAMs.

At $+60$ mV in PSS, the net outward current in adult myocytes ranged between 40 and 1160 pA (mean value $239\pm38$ pA, $n=35$), approximately twice that of neonates ($P<0.04$). However, because the adult myocytes ($C_m=11.0\pm0.6$ pF, $n=72$) were more than twice the size of
neonatal cells ($C_m = 4.6 \pm 0.2 \, \text{pF}$, $n=42$, $P<0.0001$), the current amplitude corrected for cell size was significantly smaller in adult ($18 \pm 1 \, \text{pA/pF}$, $n=35$) than neonatal ($25 \pm 4 \, \text{pA/pF}$, $n=24$, $P<0.05$) RAMs.

3.2. Comparison of the effect of $K^+$ channel inhibitors on $K^+$ currents in neonatal and adult RAMs

Fig. 2 illustrates the effect of 1 and 10 mM TEA and 5 mM 4-AP on the whole cell $K^+$ currents in neonatal and adult RAMs using the voltage protocol described above. In neonatal RAMs (Fig. 2Ab) the effect of 1 mM TEA (a concentration which should eliminate most BK$_{ca}$) was similar (13.5% inhibition at +60 mV; $n=20$) to that observed with 1 mM paxilline (compare Figs. 2B and 1B). In adult myocytes, the inhibitory effect of 1 mM TEA was significantly larger at positive voltages ($51 \pm 3\%$ inhibition at +60 mV, $n=25$, $P<0.001$) than that which occurred with paxilline but, as with paxilline, the degree of block was decreased at negative potentials (Fig. 2D). The addition of 300 nM ChTx, another potent inhibitor of BK$_{ca}$ to PSS reduced the current amplitude at +60 mV in adult RAMs to a similar degree (28±8%, $n=7$) as with paxilline ($P>0.05$; not shown), suggesting that the greater effect of 1 mM TEA in adult RAMs is chiefly due to an increased sensitivity of $I_{Kv}$ to this inhibitor, as described below.

An increase of TEA concentration to 10 mM, however, caused a significantly larger decrease in the outward current amplitude in adults (Fig. 2Cc) than in neonates (Fig. 2Ac). Inhibition of the current at +60 mV was 82±2% in eight adult RAMs in comparison to 30±5% in 20 neonatal RAMs ($P<0.0001$). Further addition of 5 mM 4-AP completely eliminated $K^+$ currents in neonates (Fig. 2Ad and B) and had little additional effect on the residual current in adult RAMs (Fig. 2Cd and D).

The current remaining in the presence of both TEA and 4-AP was small and showed an almost linear dependence on membrane voltage in both cell types (Fig. 2B and D), most likely representing a small ‘leak’ current. The mean slope resistance measured in the linear range (between −90 and −60 mV) of the $I-V$ relationship was not significantly different in neonatal (17±3 GΩ, $n=16$) and adult (8±1 GΩ, $n=35$) RAMs.

Fig. 3 compares the differential sensitivity of $I_{Kv}$ to 4-AP in neonatal and adult RAMs studied in the presence of 1 μM paxilline. $I_{Kv}$ was potently inhibited by the drug with $IC_{50}=0.5 \pm 0.1$ mM in neonates, whereas in adults $I_{Kv}$ was blocked only by 43.0±8.6% by 20 mM 4-AP.

3.3. Activation and inactivation of $I_{Kv}$ in neonatal and adult RAMs

Since it appeared that the sensitivity of $I_{Kv}$ to TEA and 4-AP, under conditions when the residual BK$_{ca}$ currents were eliminated, differed in neonatal and adult myocytes, the biophysical characteristics of $I_{Kv}$ in these cells in the presence of 1 μM paxilline were considered in more detail.

As shown in Figs. 1 and 2, the rate of $I_{Kv}$ onset at the beginning of the membrane depolarisation appeared to be higher in neonatal than in adult RAMs. Therefore, voltage-dependence of $I_{Kv}$ activation was quantified by measurement of the onset of $I_{Kv}$ activation between −20 and +80 mV. This fitted well to a single exponential function in both cell types (Fig. 4A). The rate of activation for $I_{Kv}$ between 0 and +80 mV was 3–14 times lower in adult than in neonatal cells ($P<0.003$, Fig. 4B).

Voltage-dependent inactivation of $I_{Kv}$ was investigated using the voltage protocol described in Fig. 5. In neonatal RAMs $I_{Kv}$ was relatively stable between −100 and −50 mV but decreased rapidly between −40 and −10 mV and was completely inactivated at potentials above −10 mV (Fig. 5Aa and BC). In adult RAMs $I_{Kv}$ inactivation began at more negative potentials (between −80 and −60 mV),
achieving maximum inhibition between −10 and 0 mV (Fig. 5Ab and CO). Interestingly, further increasing the conditioning potential resulted in a progressive increase in the current amplitude during the test pulse in adults, but not in neonates (Fig. 5A and B). The normalised $I_{Kv}$, fitted to the Boltzmann function (Fig. 5B), demonstrated a significant negative shift in voltage-dependence of inactivation in adult RAMs compared to neonatal cells ($V_{0.5} = −23.6±1.8$ and $−39.6±3.3$ mV respectively, $P<0.003$). The slope of inactivation for $I_{Kv}$ in adults ($k = 11.1±0.5$ mV, $n = 8$) was also less than half that of neonates ($k = 4.2±0.4$ mV, $n = 6$, $P<0.0001$). Whilst the fraction $A$ of the non-inactivating current remaining at positive potentials tended to be larger in adults ($0.35±0.3$, $n = 8$) than in neonates ($0.27±0.02$, $n = 6$, $P<0.004$, one-tailed $t$ test), accurate comparison was complicated by a tendency for the current in adult RAMs to increase at these voltages.

In contrast to $I_{Kv}$ availabilities no significant differences in voltage-dependence of steady-state activation of $I_{Kv}$ calculated from $I−V$ relationships were observed (Fig. 5C). The mean half-activation potential and slope factor were $1.1±2.2$ and $14.3±0.7$ mV ($n = 6$) in neonatal and $5.2±3.3$ and $15.2±0.8$ mV ($n = 10$) in adult RAMs, respectively.

3.4. Effect of α-dendrotoxin and charybdotoxin on $I_{Kv}$

Some TEA-sensitive delayed rectifier currents are additionally characterised by sensitivity to nanomolar concentrations of certain toxins, including ChTx and α-dendrotoxin (DTx) [16]. Therefore, the effects of 300 nM ChTx and 200 nM DTx on $I_{Kv}$, measured at $+60$ mV, were investigated under conditions when BK was blocked by either $1$ μM paxilline or $1$ mM TEA. Neither ChTx nor DTx inhibited $I_{Kv}$ in neonatal and adult RAMs ($P>0.05$; Fig. 6).

3.5. Developmental changes in the expression of Kv1.2, Kv1.5 and Kv2.1 channel proteins in rat aorta

The differences in the $I_{Kv}$ characteristics of neonatal and adult RAMs could be indicative of differential expression of the Kv channel isoforms. We have focused on three Kv α-subunits, Kv1.2, Kv1.5 and Kv2.1, which encode a delayed rectifier K+ current similar to the native $I_{Kv}$ in neonates and adults. To study the expression of Kv α-subunits, Western blot analysis was performed on total protein isolated from rat aorta, heart and brain (positive control). These were loaded on the same gel and developed under identical conditions (Fig. 7A). The expression of Kv1.2 was significantly decreased and that of Kv2.1 significantly increased with development (Fig. 7B). For Kv1.5 two bands, (molecular masses of 95 and 76 kDa) were detected in rat heart. Both were specific to binding of the anti-Kv1.5 antibody, as they were not detected when the primary antibody was preincubated with the corresponding antigen (not shown). No Kv1.5 bands were detectable in neonatal aorta, and only a 95 kDa protein was apparent in the adult aorta. Qualitatively similar results were obtained in another two experiments.

4. Discussion

4.1. Isolation and characterisation of voltage-gated K+ currents in neonatal and adult RAMs

Despite using a low Ca2+-containing pipette solution,
Fig. 4. Differences in the kinetics of \( I_{\text{Na}} \) activation. (A and B) Time course of the onset of \( I_{\text{Na}} \) between \(-20\) and \(+80\) mV (20 mV increment) in neonatal (1-day-old, \( C_m=4.2\) pF) and adult (\( C_m=13.6\) pF) RAMs, respectively. Smooth lines show monoexponential fit with time constant (\( \tau \)) equal to 54.1, 8.2, 4.8, 2.8, 2.6 and 1.9 ms in neonatal and 61.8, 29.3, 18.7, 10, 9.8 and 8.7 ms in adult myocyte at \(-20, 0, +20, +40, +60\) and \(+80\) mV, respectively. (C) Voltage dependence of the mean \( \tau \) in six neonatal (○) and ten adult (●) RAMs (0.003>P>0.0001 between 0 and \(+80\) mV).

Fig. 5. Availability and steady-state activation of \( I_{\text{Na}} \) in neonatal and adult RAMs. Availability of \( I_{\text{Na}} \) was measured using a two-pulse protocol with 10 s conditioning potentials (\( V_c \)) and 120 ms test potential to +60 mV, as shown in (A). Interpulse interval 10 ms. (Aa and Ab) \( I_{\text{Na}} \) recorded during the test pulse after \( V_c \) varied from \(-100\) and \(+30\) mV in 10 mV increment in neonatal (1-day-old, \( C_m=8.6\) pF) and adult (\( C_m=22.6\) pF) RAMs, respectively. Arrows indicate \( I_{\text{Na}} \) recorded after \( V_c \) equal to \(+30\) (a) and \(-10\) (b) mV. (B) Dependence of the normalised \( I_{\text{Na}} \) on \( V_c \). The current amplitude was normalised with respect to the mean current measured between \(-100\) and \(-70\) mV in six neonatal (○) and \(-100\) and \(-90\) mV in eight adult (●) RAMs. The mean data were then fit according to the Boltzmann equation (solid lines)

\[
I_{k} = \frac{1-A}{1+\exp\left(\left(V-V_{1/2}\right)/k\right)} + A
\]

where \( V_{1/2} \), the half-inactivation potential, was equal to \(-23\) and \(-39\) mV (vertical dashed lines), \( k \), the slope factor, was 4.7 and 11.9 mV and \( A \), the noninactivating component, was 0.28 and 0.33 for neonates and adults, respectively. (C) Steady-state activation for \( I_{\text{Na}} \) calculated from \( I-V \) relationships shown in Fig. 1 and corrected for a residual leak current calculated from the mean slope resistance measured as described in the text. Solid lines were drawn according to the Boltzmann function described above with \( A = 0 \) and half-activation potentials equal to 1.3 and 4.9 mV and the slope factors of 14.8 and 15.7 mV in neonatal and adult RAMs, respectively.
The differences in electrophysiological and pharmacological properties in \( I_{Kv} \) in neonatal and adult RAMs could explain the differences in the sensitivity of contraction of intact rat aorta to 4-AP and TEA described previously by Gomez et al. [12]. However, in contrast to our findings voltage-dependent inactivation and 4-AP sensitivity of \( I_{Kv} \) in neonatal and adult RAMs were found to be similar [12]. Differing experimental conditions may provide an explanation. Thus, we directly blocked the residual BK with paxilline, whilst a Ca\(^{2+}\)-free PSS containing 2 mM Co\(^{2+}\) to block Ca\(^{2+}\) entry and BK currents was employed in [12]. We avoided the use of divalent ions as they can directly affect \( I_{Kv} \); at least in adult RAMs we found that addition of 0.2 mM Cd\(^{2+}\) shifted the \( I_{Kv} \) availability by ~10 mV to more positive voltages (\( V_{0.5} = -30 \pm 2 \text{ mV} \)) and significantly increased the slope \( (k = 8 \pm 1 \text{ mV}, n = 5, P < 0.03) \) compared to that measured in control solutions.

4.2. Possible molecular equivalents of \( I_{Kv} \) in neonatal and adult RAMs

The different properties of \( I_{Kv} \) in adult and neonate suggested possible alterations in expression of the Kv channel proteins. In both cell types \( I_{Kv} \) was characterised by a relatively low rate of inactivation and can be classified as a delayed rectifier current. Genes, which encode slowly-inactivated delayed rectifiers, corresponding to Kv1.1, Kv1.2, Kv1.5, Kv1.6, Kv2.1 and to Kv3.1b channels have previously been found in VSM [17–19]. Kv1.1, Kv1.6 and Kv3.1b homomultimers, which encode a rapid delayed rectifier current similar to that observed in neonatal RAMs, are inhibited by low concentrations of TEA (<1 mM) and, in addition, Kv1.1 and Kv1.6 channel currents are also blocked by nanomolar concentrations of DTx [20,16]. These pharmacological characteristics are however not shared with \( I_{Kv} \) of neonatal RAMs. Moreover, no detectable expression of Kv1.1 \( \alpha \)-protein in both neonatal and adult aorta and Kv3.1b expression in adult tissue was found (not shown). Currents of Kv1.2 and Kv1.5 channels however are TEA-insensitive (in common with neonatal \( I_{Kv} \)) but unlike the neonatal \( I_{Kv} \) currents through Kv1.2 homomultimers are inhibited by DTx and ChTx [21,22]. The neonatal \( I_{Kv} \), therefore would seem to have characteristics more similar to the Kv1.5 channel. However it also shares characteristics with a Kv1.2/Kv1.5 heteromultimer since DTx and ChTx sensitivity is known to be lost in this heteromultimer. Indeed, the presence of only one Kv1.5 \( \alpha \)-subunit in the tetramer is sufficient for this loss of toxin sensitivity [21]. The loss of the toxin sensitivity may be unique for Kv1.2/Kv1.5 heteromultimers as e.g. Kv1.1/Kv1.5 heteromultimers retain sensitivity to DTx [23].

Kv2.1 channel currents are characterised by a relatively slow kinetic of activation and demonstrate a moderate sensitivity to TEA with half block between 1 and 10 mM [24,19,25]. In addition, a U-shape dependence of inactiva-
tion observed in adult RAMs was previously demonstrated for Kv2.1 channels [26]. These properties of Kv2.1 match well those of the $I_{Ks}$ in adult RAMs. Also relevant, an increased Kv2.1 gene and protein expression with postnatal development has been reported in cultured ovine pulmonary myocytes [10].

If the predictions above, based on electrophysiological and pharmacological characteristics are correct, we would expect the expression of Kv1.2 and Kv1.5 $\alpha$-proteins to decrease and the expression of Kv2.1 $\alpha$-subunit to increase during postnatal development. Indeed, increased expression of Kv2.1 protein and a reciprocal decrease in expression of Kv1.2 protein was observed from neonate to adult. However, one anomaly occurred as we were not able to detect the Kv1.5 $\alpha$-protein in neonatal tissue, whereas the corresponding 95 kDa band was apparent in adult rat aorta. This was unlikely to be due to protein degradation in the neonatal tissue since the isolation procedure was performed on ice or at +4 °C in the presence of protease inhibitors. Secondly, the absence of signal was unlikely to
result from use of an inappropriate antibody since, in preliminary experiments, using adult tissue, qualitatively similar results were obtained using anti-Kv1.5 antibody from three sources (Alomone, Chemicon and Upstate Biotechnology). Neither was unequal loading of proteins likely to be contributory as no significant differences between lanes was found in Coumassie blue stained gels run in parallel experiments (standard marker proteins, e.g. β-actin could not be used because expression of both α- and β-actins changes during vascular development [27]). Furthermore, postnatal changes in Kv channel expression observed in the heart corresponded to the developmental changes in the expression of Kv1.2 and Kv2.1 channels previously described [6,28].

Whilst it is possible that the level of expression of the Kv1.5 α-subunit is low in neonatal RAMs and below the level of detection, this cannot explain the appearance of the 95 kDa Kv1.5 band in adult aorta without a corresponding current. Possible contamination with endothelial cells cannot be excluded, but the relative cell mass is small compared to smooth muscle and therefore the contribution to Kv proteins should be small. Also, Kv channels are not predominant in endothelial cells [29]. Nerve cells, which increase in number with development, may, theoretically, contribute to the 95 kDa band as both ~90 and 65 kDa Kv1.5 bands were found in cultured Schwann cells [30].

In the whole rat heart 95 and 76 kDa bands were detected (Fig. 7A) and the density of both increased with development. In the mouse heart, at least three alternatively spliced Kv1.5 isoforms have been found [31]. Interestingly, the carboxyl-truncated isoform was not functional, but inhibited the expression of the functionally active Kv1.5 isoform [31]. If this were to occur in the rat aorta it could provide an explanation for the expression of the Kv channel without any functional current. Studies of differential expression of these isoforms may provide an answer to this apparent paradox.

### 4.3. Functional relevance of developmental changes in \( I_{Kv} \)

Appreciation of developmental changes in ion channels may impact upon investigations of the ‘foetal programming’ hypothesis [32] which suggests that adulthood cardiovascular disease can arise from permanent modification of the cardiovascular system in utero. Arrested development of the normal pathways of control of vascular contractility e.g. the \( K^+ \) channels described here, or permanent alteration of function, could lead to ultimate failure of cardiovascular homeostasis. We have recently described abnormalities in vascular function of the offspring of animals subjected to dietary modification in pregnancy [33,34] and it would be of considerable interest to determine whether abnormal development of \( K^+ \) channels may play a role in the abnormalities observed.

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### References


